Analysis of subgingival microbiota and IL-1β, TNF-α and CX3CL1 levels in gingival crevicular fluid of fixed dental prostheses

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Prosthetic biomaterials can affect the composition of the subgingival microbiota and consequently the production of proinflammatory cytokines, causing damage to the periodontium. A total of 40 patients were divided into two groups: 20 with monolithic zirconia (MZ) prostheses and 20 with porcelain fused to metal (PFM) with nickel-chromium (Ni-Cr) alloy prostheses. Subgingival plaque and gingival crevicular fluid samples were taken. The Checkerboard technique for DNA-DNA hybridization and the enzyme-linked immunosorbent assay technique were performed. Teeth with MZ presented a lower percentage of bleeding on probing and tooth mobility compared to teeth with PFM with Ni-Cr alloy. Prosthodontic teeth harbored higher total levels of the 18 bacterial species than non-prosthodontic teeth. There was a higher prevalence of *S. gordonii* and *V. parvula* species in PFM with Ni-Cr alloy compared to MZ. There was an increase in IL-1β, TNF-α and CX3CL1 levels in PFM with Ni-Cr alloy compared to MZ. MZ is a candidate biomaterial with fewer negative effects on the periodontium, allowing for longer prostheses longevity in the mouth.

Keywords: Dental fixed prostheses, Subgingival microbiota, Immune markers, Gingival crevicular fluid

INTRODUCTION

Periodontitis is a chronic infection induced by the constant challenge of a polymicrobial dysbiotic film and in the presence of a dysregulated immune response in a genetically susceptible host[1]. It is considered the sixth most common osteolytic disease affecting humans[2], has a prevalence of 62.3% and in it is most severe form affects 23.6% of the world's population[3,4]. This disease can impair the quality of life of individuals, especially in a very advanced stage that ensures tooth loss resulting in an impact on the patient´s economy and oral health[5].

A fixed dental prostheses with poor marginal and internal fit (>120 μm) may generate increased dentobacterial plaque deposition causing tissue damage[6,7]. In addition, prosthetic biomaterials can affect biofilm formation due to their chemical composition and physical characteristics such as the presence of rough and irregular surfaces, surface free energy, and metal ion release[8]. Also, the use of a poor cementation technique of the prostheses leads to the formation of biofilms that adhere between the margin of the restoration and the tooth surface further favoring decay[9,10]. For this reason, the careful choice of these biomaterials by the clinician is an important part of avoiding prosthetic treatment failure[11,12]. The use of monolithic zirconia (MZ) prostheses has increased in recent years, and this is because they are more durable, more esthetic biomaterials, require very minimal preparation and have a high fracture and flexural strenght, which allows them to be more biocompatible with the tissues that support the tooth[13]. On the other hand, porcelain-fused-to-metal (PFM) prostheses are more economical and are usually placed in cases where there is little tooth structure[14,15] however, scientific evidence has shown that they could produce local allergic reactions[16] and induce changes in the composition of the subgingival microbiota[17].

Although there are more than 500 bacterial species colonizing the gingival sulcus, only a small portion of these microorganisms can trigger the destruction of periodontal tissues[18]. Socransky and Haffe first grouped periodontopathogenic species into several complexes. The blue, yellow, purple and green complexes are formed by primary colonizers and compatible in health, the orange complex is constituted by bridging colonizers and the red complex by late colonizers such as *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola* whose virulence factors trigger the host immune response[19].

Gingival crevicular fluid (GCF) is a transudate located in the gingival sulcus and is in direct contact with the prosthetic restoration. In fact, it is a useful tool for the detection and monitoring of periodontitis because of its easy collection[20]. In fixed dental prostheses, seventeen different inflammatory mediators have been studied to date in GCF in order to know and evaluate

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the inflammatory changes that occur as a consequence of their use and thereby improve their clinical application in patients requiring this type of treatment[21]. Among them IL-1β and TNF-α are proinflammatory cytokines whose main function lies in the induction of osteoclastic activity, inhibition of osteoblastic activity and secretion of other cytokines and chemokines such as fractalkine-CX3CL1, which can act as a cell adhesion molecule and as a potent chemoattractant of inflammatory cells (monocytes, lymphocytes, dendritic cells and macrophages), as well as stimulates preosteoclast migration and causes osteoclastogenesis creating a vicious cycle that accelerates disease development[22,23].

The overall objective of the present study was to evaluate the effects of MZ and PFM prostheses on the composition of the subgingival microbiota and the levels of IL-1β, TNF-α and CX3CL1 in GCF. It is hypothesized that MZ prostheses, being more biocompatible with the tissues that support the teeth, produce less change in the composition of the subgingival microbiota and thus a decrease in the levels of proinflammatory cytokines compared to PFM prostheses. This of great interest to determine which biomaterial retains less bacteria and induces a lower inflammatory response and thus have a therapeutic alternative that maintains the periodontal health of the individual, considering that a healthy periodontium is the key to the success of a prosthetic treatment[24,25].

MATERIALS AND METHODS

Study design and approval by the bioethics committee

A cross-sectional study was carried out in the Implantology and Oral Rehabilitation Postgraduate Clinics of the Faculty of Dentistry of the Autonomous University of Guerrero (UAGro). The laboratory analysis was carried out in the Laboratory of Microbiology Research of the Faculty of Chemical-Biological Sciences of the UAGro and in the Laboratory of Molecular Genetics of the Division of Graduate Studies and Research of the Faculty of Dentistry of the UNAM (LGM-FO-UNAM), from August 2022 to June 2023. The study was approved by the UAGro ethics committee (approval CB-002/22). The details of the study were explained to each participant in accordance with the Declaration of Helsinki.

Participants and study groups

Patients were informed about the objective and procedures of the study. The anonymity of each of the subjects and their right not to participate was respected, while those patients who agreed to participate in the study were asked to sign the informed consent form and were also given a free dental cleaning. The inclusion criteria were patients of both genders, aged between 18 and 85 years, residents of the State of Guerrero, with fixed dental prostheses of MZ and PFM with nickel-chromium (Ni-Cr) alloy more than 1 year and less than 5 years old, with at least one unrestored tooth. In addition, all dental preparations and cementation of the prostheses were performed by a single operator specialized in oral prosthetics and implantology. The prostheses complied with four prosthetic parameters: The first was that the finishing line of the dental preparation was of the chamfer type. The second is the location of the prosthetic margin in relation to the marginal gingival ridge which is of the subgingival type. The third is its fabrication method, which in the case of PFM prostheses was by conventional method, while in the case of MZ was by CAD/CAM system, both in the same certified dental laboratory. And the fourth was that the MZ prostheses were cemented with U200 self-adhesive resin cement (RelyX™ U200, 3M ESPE, Maplewood, MN, USA); this type of dental luting cement provides an optimal chemical and micromechanical bond with all-ceramic restorations (zirconia, disilicate, feldspar-based ceramics) and is also characterized by high compressive/tensile strength, low solubility, and excellent color and esthetic stability[9,10,26]. While the PFM prostheses were cemented with type 1 glass ionomer (GC Fuji I® Glass Ionomer Luting Cement, GC, Tokyo, Japan); this type of dental luting cement provides ideal chemical bonding for metal-ceramic restorations, creates minimal film thickness, therefore removal of excess material is easy, and releases fluoride ions, which makes it suitable for preventing dental caries[10,27,28]. In both cases, dental luting cements have antimicrobial activity[29,30], as well as low chances of microleakage, are easy to apply, provide adequate working and curing time, have optimal wettability and sufficient viscosity for complete prolongation[26-28].

Exclusion criteria were patients who were under antibiotic treatment at the time of the study or three months prior to the study, who were on prolonged anti-inflammatory or immunosuppressive treatment, with decompensated systemic diseases, pregnant women, current smokers, patients with orthodontic appliances and with aggressive periodontitis.

Periodontal clinical evaluation

The patients were evaluated by manual probing using a Goldman Fox/Williams (DTX-HUPGF/W6, Hu-Friedy, Chicago, IL, USA) type periodontal probe by a calibrated operator and considering the following clinical parameters: Probing depth (PD), gingival migration (GM), clinical attachment loss (CAL), bleeding on probing (BOP) and tooth mobility (TM). Radiographic bone loss (RBL) was determined by analysis of dentoalveolar projections using a radiovisiograph (NanoPix 2, Vetesa, Dental Supplies, Jiangsu, China).

A general diagnosis of all the teeth and an individual diagnosis of the teeth with prostheses and their natural contralaterals teeth, without prostheses (control group) were performed. The plaque index (PI) was obtained using the O' Leary index[31]. For PD, GM and CAL, the average of six sites per tooth was obtained by summing by vestibular and lingual/palatal the three measurements (mesial, middle and distal) and then divided by the total number of measurements. The percentage of BOP was determined by summing the teeth that bled on probing, then multiplied by 100 and divided by the total number

of teeth. The average TM was obtained by summing each of the values and then dividing by the total number of teeth. In this way, patients were classified according to the new 2017 AAP/EFP classification of periodontal and peri-implant diseases. For subclassification of periodontitis stage (I, II, III and IV), BOP and CAL were considered. Periodontal grade (A, B and C) is the evidence of rapid progression and is estimated with direct or indirect evidence of progression rate in three categories: Slow, moderate and rapid progression and was evaluated with the percentage of RBL/age ratio[32].

Sampling of GCF and subgingival plaque

In the same patient the tooth with MZ or PFM fixed prostheses and a tooth without restoration (control tooth) were selected, preferably contralateral to the prostheses tooth, if it was not found with the contralateral tooth or had restoration, it was moved distally or mesially in search of the next tooth without restoration for sampling.

A relative isolation was performed with cotton rolls, drying of the tooth surface and removal of the supragingival plaque with great caution not to generate bleeding with a CK6 curette (CM42045, HuFriedy), without touching the gingival margin. The GCF was collected with sterile Periopaper (Gingival Fluid Collection Strips, ORAFLOW, Smithtown, NY, USA) by inserting the paper strip into the gingival sulcus 1 to 2 mm for 30 s. Subsequently, each of the periopapers were removed and placed on the Periotron 8000 sensors (Model 8010, ORAFLOW) to determine the number of Periotron units collected and their conversion to microliters[33]. The samples were then placed in Eppendorf tubes containing 100 μL each of sterile 0.9% saline. Finally, the tubes were placed in cold storage at −80°C for further processing.

Subsequently, a subgingival plaque sample was taken with a Gracey mini-five #11/12 octagonal handle #2 curette (CM41707, HuFriedy), taking a minimal portion of plaque from the mesiobuccal side of each of the teeth with fixed prostheses and their contralateral natural teeth, and placed in individual 1.5 mL microcentrifuge tubes with 150 μL of TE buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 7.6). To these tubes 100 μL of NaOH (0.5 M) was added and the sample was dispersed. Finally, the vials were stored at −20°C until further processing[34].

Microbiological evaluation

The checkerboard technique for DNA-DNA hybridizations was performed at the LGM-FO-UNAM. Eighteen digoxigenin-labeled full-length genomic DNA probes were prepared from the following bacterial species: *Actinomyces georgiae, Actinomyces naeslundii, Streptococcus anginosus, Streptococcus gordonii, Veillonella parvula, Capnocytophaga gingivalis, Capnocytophaga sputigena, Campylobacter rectus, Eubacterium nodatum, Fusobacterium nucleatum subsp. nucleatum, Fusobacterium periodonticum, Prevotella intermedia, Prevotella nigrescens, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans stp. b* and *Cutibacterium acnes*. The evaluation of the number of bacteria in the samples was performed by comparing the signals obtained with those generated by microbiological standards containing 10^5 and 10^6 cells of each species[34].

Determination of IL-1β, TNF-α and CX3CL1 levels in GCF

Prior to the assay, GCF samples were eluted from paper strips at 4°C by vortexing for 30 min. The tubes were centrifuged at 15,000 rpm, for 5 min at 4°C and the supernatant was recovered in sterile Eppendorf tubes. The samples were then placed in cold storage at −80°C for further analysis[35]. Cytokine levels in GCF were measured by sandwich enzyme-linked immunosorbent assay (ELISA). We use the following kits from the same brand (R&D Systems, Minneapolis, MN, USA): Human TNF-α QuantikineTM HS ELISA (HSTA00E), Human IL-1beta/IL-1F2 QuantikineTM HS ELISA (HSLB00D) and Human CX3CL1 QuantikineTM HS ELISA (DCX310). The sensitivity and range of these assay kits were 0.049 pg/mL and 0.2 to 10 pg/mL, 0.063 pg/mL and 0.1 to 8 pg/mL, 0.072 ng/mL and 0.2 to 10 ng/mL respectively. Aliquots of 50 μL and 100 μL were prepared respectively and the levels of TNF-α, IL-1β and CX3CL1 in GCF were determined following the manufacturer's instructions.

Statistical analysis

The STATA V.15 statistical program was used, considering a value of $p \leq 0.05$ as significant. The normality of the data was examined using the Shapiro-Wilk test. Some data had a normal distribution and others did not have a normal distribution, so a parametric analysis was performed using the Student's *t*-test and a nonparametric analysis using the Mann-Whitney *U* test. For the comparison of qualitative variables, the χ^2 test and Fisher's Exact test were used. Finally, Spearman's correlation coefficient was used to analyze the correlation between inflammatory mediators and bacterial levels.

RESULTS

Sociodemographic and clinical features

Forty patients were selected for the study according to the inclusion and exclusion criteria, *i.e.*, 20 patients with MZ prostheses and 20 patients with PFM prostheses with Ni-Cr alloy. It was found that in general, all the patients presented generalized periodontitis, however, when the diagnosis was made by restored or control teeth (without prostheses), some were found with gingivitis and others with periodontitis, none of them healthy. The sociodemographic and clinical characteristics of the population studied are shown in Table 1. It was observed that in the group of patients with PFM prostheses with Ni-Cr alloy there was a higher prevalence of females compared to the group of patients with MZ prostheses ($p \leq 0.05$). There was also observed that in the group of patients with MZ prostheses there was a higher socioeconomic status compared to the group of patients with PFM prostheses ($p \leq 0.05$). When analyzing the GCF volume, a higher GCF volume was observed in teeth

Table 1 Sociodemographic and clinical characteristics of the study groups (*N*=40)

Subjects Details	Total *n*=40	MZ *n*=20	PFM *n*=20	Value of *p*
Age (years)	55.92±11.88	55.7±11.90	56.15±12.09	0.90*
Gender, *n* (%)				<0.05****
Male	14(35)	10(50)	4(20)	
Female	26(65)	10(50)	16(80)	
Education, *n* (%)				0.82****
No education	1(2.5)	—	1(5)	
Elementary school	5(12.5)	2(10)	3(15)	
Junior high school	2(5)	1(5)	1(5)	
Senior high school	—	—	—	
College	32(80)	17(85)	15(75)	
Socioeconomic status (%)				<0.05****
Low	—	—	—	
Medium	20(50)	4(20)	16(80)	
High	20(50)	16(80)	4(20)	
Periodontal condition (%)				
Stages				0.32***
Stage I	—	—	—	
Stage II	15(37.50)	9(45)	6(30)	
Stage III	25(62.50)	11(55)	14(70)	
Stage IV	—	—	—	
Grades				0.92****
Grade A	9(22.50)	5(25)	4(20)	
Grade B	27(67.50)	13(65)	14(70)	
Grade C	4(10)	2(10)	2(10)	
Volume of GCF collected (μL)	0.4(0.5–0.6)	0.4(0.3–0.5)	0.5(0.5–0.6)	<0.05**
Clinical parameters				
Plaque index (%)	51.65(50–59.45)	50(50–60.65)	55.75(50–60.65)	0.12**
Bleeding on probing (%)	55.75±24.35	48.41±20.49	63.10±26.16	<0.05*
Probing depth (mm)	4.48(4.15–5.16)	4.50(4.25–4.93)	4.34(4.1–5.2)	0.87**
Clinical attachment level (mm)	5.14±1.40	5.02±1.33	5.25±1.50	0.61*
Tooth mobility (mm)	0.27(0.125–0.565)	0.185(0.075–0.37)	0.385(0.205–0.71)	<0.05**
Radiographic parameters				
Bone resorption (%)	23.82(15.26–30.30)	22.12(13.89–28.84)	24.935(16.21–34.72)	0.55**

Data were reported with mean±standard deviation, median (p-25–p75) and *n* (%). *p* Values were reported by Student's *t*-test *, Mann-Whitney *U* test **, χ^2 *** and Fisher's exact test ****. Considering a value of $p \leq 0.05$*.
MZ: Monolithic zirconia, PFM: Porcelain fused to metal.

with PFM prostheses with Ni-Cr alloy compared to MZ prostheses ($p \leq 0.05$). In relation to the general clinical parameters, patients with MZ prostheses presented a lower percentage of BOP and less TM than patients with PFM prostheses with Ni-Cr alloy ($p \leq 0.05$).

Table 2 presents the site-specific clinical and radiographic characteristics. A higher prevalence of periodontitis was observed in teeth that had been restored with PFM prostheses with Ni-Cr alloy compared to teeth with MZ prostheses, but without statistical significance ($p \leq 0.337$). We also found a lower prevalence of BOP in teeth with MZ prostheses compared to their control teeth ($p \leq 0.05$) and a higher BOP in teeth with PFM prostheses with Ni-Cr alloy compared to teeth with MZ prostheses and even with their control teeth ($p \leq 0.05$).

Analysis of subgingival microbiota in fixed dental prostheses

Teeth with MZ and PFM prostheses with Ni-Cr alloy harbored higher total levels of the 18 bacterial species compared to control teeth ($p \leq 0.05$). Among both types of biomaterials, PFM prostheses with Ni-Cr alloy harbored higher total levels of bacterial species than MZ

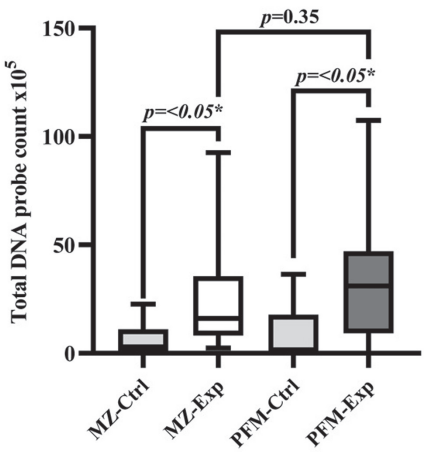

Fig. 1 Total bacterial levels by group. Differences were determined using the Mann Whitney *U* test. Considering a value of $p \leq 0.05$* as significant. MZ-Ctrl: monolithic zirconia-control, MZ-Exp: monolithic zirconia-experimental, PFM-Ctrl: porcelain fused to metal-control, PFM-Exp: porcelain fused to metal-experimental.

Table 2 Clinical and radiographic characteristics of teeth restored with monolithic zirconia and porcelain Fused to Metal prostheses their restoration free contralateral teeth (*N*=80)

Features	Experimental	MZ (*n*=40) Tooth natural	Value of *p*	Experimental	PFM (*n*=40) Tooth natural	Value of *p*	MZ *vs* PFM (*n*=40) Value of *p*
Periodontal condition							
Gingivitis (%)	10(50)	6(30)	0.245***	7(35)	6(30)	0.245***	0.337**
Periodontitis (%)	10(50)	14(70)		13(65)	14(70)		
Clinical parameters							
Plaque index (%)	51.25±18.97	55±20.83	0.555*	53.75±9.15	56.25±13.75	0.502*	0.606*
Bleeding on probing (%)							
Yes	10(50)	14(70)	0.021***	17(85)	9(45)	0.021****	0.05****
No	10(50)	6(30)		3(15)	11(55)		
Depth at probing (mm)	2.16(1.83–2.83)	2.16(1.99–2.49)	0.945**	2.33(2-3.16)	2(1.99–2.34)	0.086**	0.646**
Clinical attachment level (mm)	2(1.83–2.74)	2.66(1.66–349)	0.371**	2.32(1.74–3)	2.41(1.66–3.74)	0.386**	0.647**
Tooth mobility (%)							
Mild	15(75)	18(90)	0.560****	10(50)	12(60)	0.560****	0.191****
Moderate	5(25)	2(10)		9(45)	7(35)		
Severe	—	—		1(5)	1(5)		
Radiographic parameters							
Bone resorption (%)	1.66(0–5.42)	6.04(0–10.18)	0.075**	2.13(0–8.93)	3.29(0–10.6)	0.363**	0.535**

Data were reported with mean±standard deviation, median (p-25–p75) and *n* (%). *p* Values were reported by Student's *t*-test *, Mann-Whitney *U* test **, χ^2 *** and Fisher's exact test ****. Considering a value of $p \leq 0.05$*.
MZ: monolithic zirconia, PFM: porcelain fused to metal.

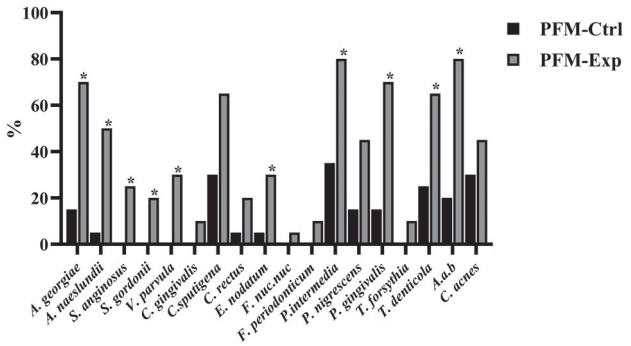

Fig. 2 Frequency of bacterial species between PFM-Ctrl *vs* PFM-Exp.
Differences were calculated using Fisher's exact test and χ^2 considering a value of $p \leq 0.05$* as significant. PFM-Ctrl: porcelain fused to metal-control, PFM-Exp: porcelain fused to metal-experimental.

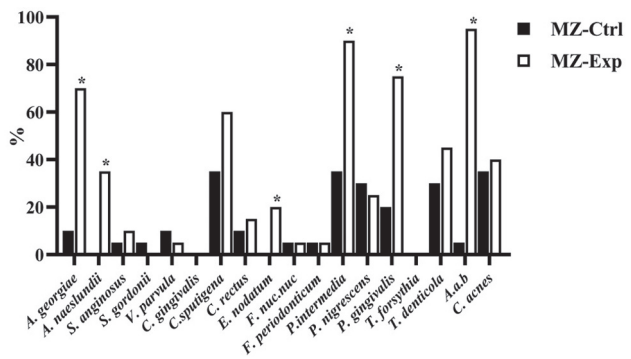

Fig. 3 Frequency of bacterial species between MZ-Ctrl *vs* MZ-Exp.
Differences were calculated using Fisher's exact test and χ^2 considering a value of $p \leq 0.05$* as significant. MZ-Ctrl: monolithic zirconia-control, MZ-Exp: monolithic zirconia-experimental.

prostheses, but without statistical significance (p=0.35) (Fig. 1).

On the other hand, a higher prevalence of *A. georgiae, A. naeslundii, S. anginosus, S. gordonii, V. parvula, P. intermedia, E. nodatum, P. gingivalis, T. denticola* and *A.a.b* species was also found in teeth with PFM prostheses with Ni-Cr alloy compared to control teeth ($p \leq 0.05$) (Fig. 2), while a higher prevalence of the aforementioned species with the exception of *S. anginosus, S. gordonii, V. parvula* and *T. denticola* was also found in teeth with MZ prostheses compared to control teeth ($p \leq 0.05$) (Fig. 3).

The overall frequency of the 18 bacterial species was higher in teeth with PFM prostheses with Ni-Cr alloy compared to teeth with MZ prostheses but without statistical significance (p=0.33). However, there was a higher prevalence of *S. gordonii* and *V. parvula* species ($p \leq 0.05$) in PFM prostheses with Ni-Cr alloy compared to teeth with MZ prostheses (Fig. 4).

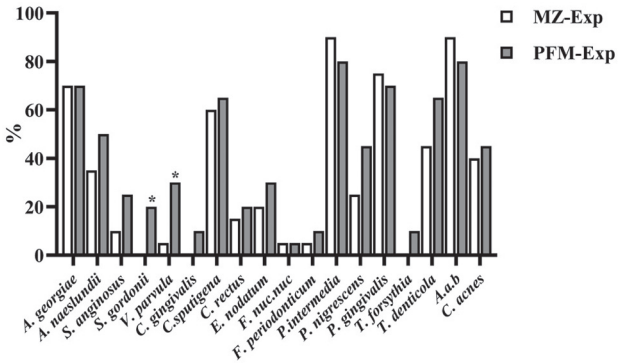

Fig. 4 Frequency of bacterial species between MZ-Exp *vs* PFM-Exp.
Differences were calculated using Fisher's exact test and χ^2 considering a value of $p \leq 0.05$* as significant. MZ-Exp: monolithic zirconia-experimental, PFM-Exp: porcelain fused to metal-experimental.

Analysis of IL-1β, TNF-α and CX3CL1 levels in GCF in fixed dental prostheses

Inflammatory markers in GCF were evaluated in both study groups. Teeth with PFM prostheses with Ni-Cr alloy presented an increase in IL-1β levels (17±1.80 pg/mL) compared to teeth with MZ prostheses (16±2.66 pg/mL) but without statistical significance (p=0.491) (Fig. 5-panel A1). On the other hand, when grouping by periodontal condition (gingivitis and periodontitis) we found that in teeth restored with MZ prostheses and gingivitis presented an increase in TNF-α levels (14.1±0.99 pg/mL) compared to control teeth with the same periodontal condition (12.8±0.60 pg/mL) ($p \leq 0.05$) (Fig. 5-panel B2). Also, we found that teeth with PFM prostheses with Ni-Cr alloy and periodontitis presented an increase in CX3CL1 levels (5.1±1.53 ng/mL) compared to teeth with MZ prostheses (4.2±1.53 ng/mL), but without statistical significance (p=0.071) (Fig. 5-Panel C1), while by periodontal condition we found an increase in CX3CL1 levels (5.76±1.83 ng/mL) compared to control teeth with the same periodontal condition (4.57±0.93 ng/mL) ($p \leq 0.05$) (Fig. 5-panel C3) and also compared to teeth with MZ prostheses with the same periodontal condition (3.9±0.87 ng/mL) ($p \leq 0.05$) (Fig. 5-panel C4).

Correlation of cytokines with subgingival microbiota in fixed dental prostheses

We looked for correlations between inflammatory markers with bacterial levels (bacterial counts) of the most periodontopathogenic species, mainly of the orange and red complexes in teeth that were restored with prostheses of both types (MZ and PFM), we found that, in patients with PFM prostheses with Ni-Cr alloy, *P. nigrescens* correlated positively with TNF-α levels (r=0.56; $p \leq 0.05$), as did *T. denticola* (r=0.434; $p \leq 0.05$). On the other hand, in patients with MZ prostheses, *A.a.b* correlated positively with TNF-α levels (r=0.43; $p \leq 0.05$) (Table 3).

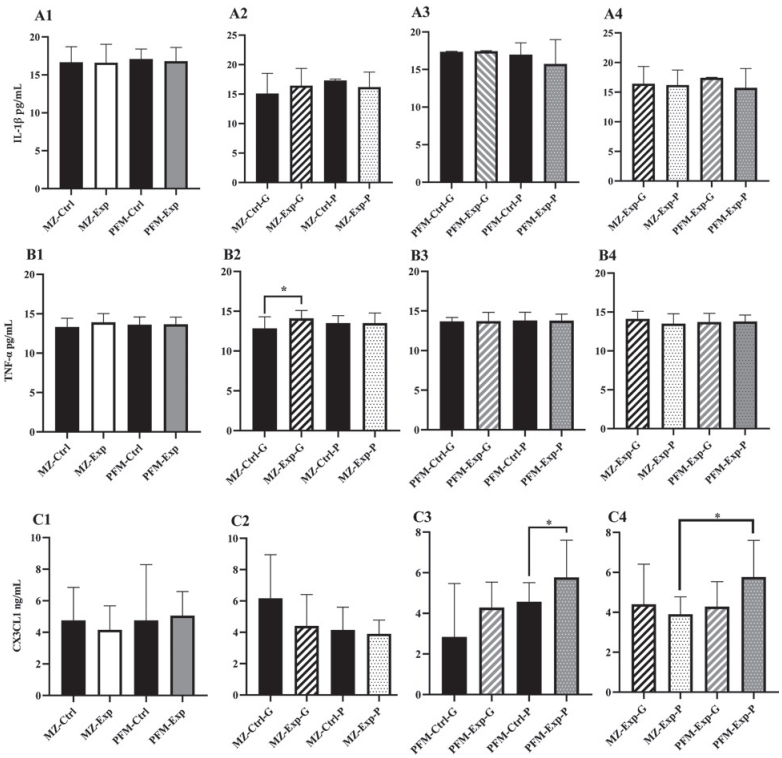

Fig. 5 Levels of IL-1β, TNF-α and CX3CL1 in GCF in teeth with monolithic zirconia and porcelain-fused-to-metal prostheses in different periodontal condition.
Differences were calculated using Student's *t*-test considering a value of $p \leq 0.05$* as significant. MZ-Ctrl: monolithic zirconia-control, MZ-Exp: monolithic zirconia-experimental, PFM-Ctrl: porcelain fused to metal-control, PFM-Exp: porcelain fused to metal-experimental. MZ-Ctrl-G: monolithic zirconia-control-gingivitis, MZ-Exp-G: monolithic zirconia-experimental-gingivitis, MZ-Ctrl-P: monolithic zirconia-control-periodontitis, MZ-Exp-P: monolithic zirconia-experimental-periodontitis, PFM-Ctrl-G: porcelain fused to metal-control-gingivitis, PFM-Exp-G: porcelain fused to metal-experimental-gingivitis, PFM-Ctrl-P: porcelain fused to metal-control-periodontitis, PFM-Exp-P: porcelain fused to metal-experimental-periodontitis.

DISCUSSION

Traditionally, the gold standard for the placement of a fixed dental prostheses is PFM restorations. However, currently in countries such as the United States and Germany, dentists widely recommend the use of fixed dental prostheses of one to three MZ units followed by PFM prostheses, because they present better esthetics and greater biocompatibility with the supporting tissues of the teeth[36,37].

In the overall periodontal evaluation of the present study subjects with MZ prostheses presented a lower BOP % and lower TM than subjects with PFM prostheses with Ni-Cr alloy ($p \leq 0.05$). It is well documented that BOP and TM are indicators of risk and severity of periodontal disease. BOP is indicative of gingival inflammation and TM of increased attachment loss[38]. Alrahlah *et al.* evaluated the effects of ceramic lumineers on clinical and inflammatory parameters, they observed that at four weeks after placement of the restorations the PI %, BOP % and GCF volume increased transiently, indicating the onset of gingival inflammation, however at twenty-four weeks, these parameters normalized, suggesting that the clinical application of these restorations for esthetic rehabilitation is a viable option with minimal risks of compromising periodontal health[39]. These results suggest that MZ prostheses produce a lower inflammatory response and thus less damage to periodontal tissues favoring a faster clinical recovery compared to PFM prostheses with Ni-Cr alloy.

On the other hand, in addition to natural teeth, dental prostheses are substrates for the formation of biofilms[8], in this sense, once a fixed dental prostheses is placed in the mouth, the surface is coated with an acquired film formed by salivary glycoproteins and immunoglobulins, this layer provides a series of receptors that facilitate the adhesion and colonization of microorganisms, subsequently the bacteria aggregate, proliferate and grow until they become a mature film that adheres firmly to these surfaces[40]. We found that teeth with prostheses of both types harbored higher total levels of the 18 bacterial species than natural (non-prosthetic) teeth (($p \leq 0.05$), which is due to the fact that prosthetic restored teeth have a marginal and internal fit[7] corresponding to the space between the tooth preparation termination line and the prosthetic

Table 3 Correlation between inflammatory mediators and levels of periodontopathogenic species

	P. intermedia	*P. nigrescens*	*P. gingivalis*	*T. forsythia*	*T. denticola*	*A.a.b*
MZ (n=20)						
IL-1β						
Value of rho	0.049	0.169	0.039	0.148	0.178	−0.008
Value of p	0.835	0.475	0.868	0.533	0.450	0.971
TNF-α						
Value of rho	−0.146	0.012	0.207	0.270	0.004	0.434
Value of p	0.148	0.959	0.382	0.249	0.984	0.05*
CX3CL1						
Value of rho	−0.063	−0.003	−0.032	0.374	−0.077	0.015
Value of p	0.790	0.987	0.893	0.103	0.746	0.948
PFM (n=20)						
IL-1β						
Value of rho	−0.013	−0.214	−0.052	0.148	0.178	0.014
Value of p	0.956	0.363	0.826	0.749	0.524	0.951
TNF-α						
Value of rho	0.336	0.561	0.377	0.334	0.434	0.187
Value of p	0.148	0.01*	0.100	0.149	0.05*	0.429
CX3CL1						
Value of rho	0.127	−0.313	0.161	−0.023	−0.323	0.185
Value of p	0.591	0.178	0.496	0.922	0.746	0.433

Spearman correlation analysis, Considering a value of $p \leq 0.05$*.
IL-1β: interleukin-1 Beta, TNF-α: tumor necrosis factor alpha, CX3CL1: fractalkine, MZ: monolithic zirconia, PFM: porcelain fused to metal.

margin. Under normal conditions it is accepted that this space should not be greater than 120 μm, however, when there is a marginal discrepancy (>120 μm), this results in biofilm deposition[41], in comparison with the biological interface of natural teeth called cemento-enamel junction, which despite having irregularities on its surface the degree of biofilm formation is lower[42].

In the microbiological analysis, we found a higher prevalence of the species *A. georgiae, A. naeslundii, S. anginosus, S. gordonii, V. parvula, P. intermedia, E. nodatum, P. gingivalis, T. denticola* and *A.a.b*, in teeth with PFM prostheses with Ni-Cr alloy compared to their contralateral natural teeth ($p \leq 0.05$), although some of these species are commensal, *P. gingivalis, T. denticola* and *A.a.b* is highly pathogenic to periodontal tissues, furthermore these results are comparable with a study published by Rademacher *et al.* who investigated whether PFM prostheses influence the composition of the subgingival microbiome and observed a higher species richness in PFM prostheses compared to their natural teeth. At the phylum level, they found a higher prevalence of Bacteroidetes and Spirochaetes in restored sites showing bleeding on probing while at the genus level, a higher prevalence of *Prevotella* and *Treponema*[17]. In a very similar way Passariello *et al.* evaluated the composition of the subgingival microbiota in teeth with metal-ceramic prostheses with different periodontal condition and observed that in teeth with periodontitis there is a significant increase of species *C. rectus, E. saphenum, M. timidum, P. gingivalis, P. intermedia, P. tannerae, S. exigua* and *T. forsythia* in comparison with their natural teeth[43].

The biofilm bacteria that accumulate on the misaligned prosthetic margins, lower the pH by producing different bacterial metabolites (LPS, acids, sulfur and ammonia) that dissolve the surface oxides of dental alloys, which reduces the corrosion resistance and consequently the release of metal ions, producing on the one hand:

1) Rough and irregular surfaces that provide favorable interfaces for bacterial colonization, protecting bacteria against shear forces during their initial reversible bonding and biofilm formation[8].

Consequently, studies have shown a higher prevalence of species of: *P. gingivalis*, *P. intermedia* and *T. forsythia* in metal pontics from sites with inflamed gingiva compared to non-metal ceramic pontics with the same periodontal condition[44]. Higher colony forming unit counts of *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia* and *C. albicans* species have also been found in materials such as polymethylmethacrylate and titanium compared to zirconium oxide[45]. Zirconium has also been shown to accumulate less bacteria than titanium both in quantity and in the presence of recognized potential

periodontopathogens such as *P. gingivalis*, and other primary colonizing species such as *S. mutans*, *A. viscosus* and *A. naeslundii*. Thus, biofilm formation on different types of dental alloys and ceramics depends on the genus and species of the microorganism[46]. However, scientific evidence indicates that zirconia is better in terms of lower retention and accumulation of periodontopathogens[6,11,47].

2) Metal ions released in the microenvironment interact with cells (bacteria, keratinocytes, neutrophils, macrophages, lymphocytes) and soluble molecules present in the gingival sulcus and can cause a number of adverse effects such as inflammation (through the release of proinflammatory cytokines such as IL-1β and TNF-α), oxidative stress, genomic instability and chromosomal damage[48,49].

It has been shown that base metal alloys such as Cr-Co show increased biofilm accumulation compared to zirconia and feldspar-based porcelain[50]. Likewise, it has also been shown that Cr-Co alloys developed more corrosion pitting and viable microbial cells than titanium alloys[51]. While Ni alloys induced elevated levels of cell toxicity compared to Cr-Co alloys[52]. It has also been shown that, Ni-Cr alloys are less corrosion resistant and show lower biocompatibility, *i.e.*, higher cytotoxicity and cell growth inhibition[53]. Even exposure of Ni-Cr alloys to *E. coli* LPS, in an acidic environment (pH <5) decreases their corrosion resistance. Therefore, corrosion resistance is a fundamental characteristic of dental alloys to show better biocompatibility with tooth supporting tissues[54].

On the other hand, Heboyan *et al.* analyzed the composition of microbiota in the GCF in teeth with metal-ceramic and zirconia prostheses fabricated by conventional and CAD/CAM methods. They observed that the best results both qualitative and quantitative composition of microflora in the gingival sulcus were achieved in subjects with zirconia prostheses using CAD/CAM technology[47]. In our study, we found that a higher prevalence of the above mentioned species with the exception of *S. anginosus, S. gordonii, V. parvula* and *T. denticola* was also found in MZ prostheses compared to their contralateral natural teeth ($p \leq 0.05$). This finding is the first to be reported in the literature because there is no study comparing the composition of the microbiota in teeth with MZ prostheses compared to their natural restoration-free contralaterals, suggesting that this type of prostheses is more biocompatible and less retentive than PFM prostheses with Ni-Cr alloy.

We found a higher prevalence of *S. gordonii* and *V. parvula* species ($p \leq 0.05$) in teeth with PFM prostheses with Ni-Cr alloy compared to teeth with MZ prostheses. *S. gordonii* is a Gram-positive, facultative anaerobic, commensal, opportunistic bacterium, which resides mainly in the mucosa of the oral cavity and upper airways. It can cause diseases such as apical periodontitis and infective endocarditis[19,55]. It is part of the yellow complex as part of the primary colonizers[19] and expresses cell wall proteins such as adhesins, which facilitate its binding on platelets, erythrocytes, monocytes and dendritic cells producing acute immune responses[55]. On the other hand, *V. parvula* is a Gram-negative, anaerobic, commensal and opportunistic bacterium, inhabiting mainly the mucosa of the oral cavity and gastrointestinal tract. Its prevalence has been increased in diseases such as vertebral osteomyelitis, Sjögren's syndrome and in type 2 diabetes mellitus[56]. It is part of the purple complex[19] and also like *S. gordonii* is a primary colonizer. Both bacteria can coaggregate with other oral microorganisms contributing to the development of periodontitis. In fact, Sakanaka *et al.* demonstrated that the food web in oral biofilm ecosystems affects their maturation process specifically, the cross-feeding of ornithine by *S. gordonii* that induces putrescine production by *F. nucleatum* and *V. parvula* that produces lysine and induces cadaverine production, these polyamines favor the overgrowth and habitat expansion of *P. gingivalis* providing increased pathogenicity in dental biofilms[57]. These results suggest that the presence of Ni-Cr alloys in PFM prostheses favor a greater accumulation of *S. gordonii* and *V. parvula* that promote the formation of a nutrient-rich microenvironment, exploited by bridging and late colonizers resulting in a cooperative metabolism within oral biofilms that may tip the balance towards periodontitis.

In relation to inflammatory mediators, we unexpectedly found that teeth with MZ prostheses and gingivitis presented increased levels of TNF-α ($p \leq 0.05$) compared to their controls. TNF-α is a proinflammatory cytokine that positively regulates to receptor activator of nuclear factor κB ligand by promoting osteoclastogenesis, leading to alveolar bone loss[21-23]. Furthermore, by Spearman correlation, we observed that in patients with MZ prostheses presented a positive correlation of TNF-α with bacterial levels of *A. actinomycetemcomitans* and in patients with PFM prostheses with Ni-Cr alloy, a positive correlation with *P. nigrescens* and *T. denticola* bacteria was demonstrated. Our findings are probably due to a higher prevalence of *A. actinomycetemcomitans* in teeth with MZ prostheses compared to PFM, however in teeth with PFM prostheses with alloy Ni-Cr there was a higher prevalence of *P. nigrescens* and *T. denticola* two periodontopathogenic species that could be exerting greater damage due to the presence of their virulence factors interacting with the pattern recognition receptors of host cells. TNF-α levels have been shown to transiently increase after placement of a metal-free ceramic restoration and after four weeks normalize, indicating faster recovery of gingival tissue[39]. However, contrary to our study, some authors have observed that TNF-α levels increase in teeth with Chromium-Cobalt and Ni-Cr alloy metal-ceramic prostheses compared with zirconia prostheses and with their natural contralateral teeth, indicating that this type of restorations could be inducing a more damaging effect on periodontal tissues[58].

Our analysis on CX3CL1 levels allowed us to observe that this chemokine was increased in teeth with PFM prostheses with Ni-Cr alloy and that in turn had periodontitis compared to their controls ($p \leq 0.05$) and

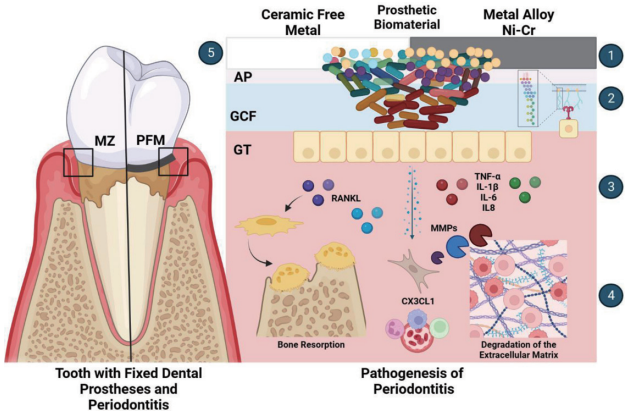

Fig. 6 Effects of different prosthetic biomaterials on the periodontium.
1) The presence of a metal alloy (Ni-Cr) in a prosthetic restoration accumulates a greater amount of bacteria (*S. gordonii* and *V. parvula*) in the area promoting polymicrobial dysbiosis. 2) Virulence factors such as LPS from periodontopathogenic bacteria interact with the PRRs of the keratinocytes of the junctional epithelium favoring the release of proinflammatory cytokines such as 3) TNF-α and IL-1β that act on gingival fibroblasts and leukocytes increasing the expression of MMPs that degrade the extracellular matrix, as well as RANKL that produces osteoclastogenesis and chemokines such as 4) CX3CL1 that is a potent chemoattractant, which recruits more immune cells promoting a vicious cycle that accelerates disease development. 5) Metal-free ceramic biomaterials such as monolithic zirconia can also aggravate the periodontal condition, but in a milder form, since they have a better marginal and internal fit and present fewer irregularities on their surface, which greatly improves the degree of biofilm formation, *i.e.*, there is less retention of bacteria and therefore less production of inflammatory mediators, which reduces damage to the periodontium. MZ: monolithic zirconia; PFM: porcelain fused to metal; Ni-Cr: nickel-chromium; GT: gingival tissue; GCF: gingival crevicular fluid; AP: acquired pellicle; LPS: lipopolysaccharides; PRRs: pattern recognition receptor; RANKL: receptor activator of nuclear factor κB ligand; TNF-α: tumor necrosis factor; IL-1β: interleukin 1 beta; CX3CL1: fractalkine; IL-6: interleukin 6; IL-8: interleukin 8; MMPs: matrix metalloproteases. www.biorender.com (accessed on 9 may 2023)

to teeth with MZ prostheses with the same periodontal condition ($p \leq 0.05$). CX3CL1 is a potent chemoattractant that mediates leukocyte adhesion to the site of inflammation and destruction of gingival tissue[59]. CX3CL1 has been shown to be increased in patients with periodontitis and other autoimmune diseases such as rheumatoid arthritis[60-64], however, this is the first study to evaluate the levels of this chemokine in teeth restored with MZ and PFM prostheses with Ni-Cr alloy. Likewise, our findings could suggest that having a higher amount of periodontopathogenic bacteria on Ni-Cr alloy PFM prosthetic crows would favor a higher production of this chemokine that attracts other immune cells to the contact site between the prostheses and the gingival tissue, further perpetuating the inflammatory state.

We recognize several limitations in our study. The sample size could be larger, although it was sufficient to find some significant differences between the study groups. Also, despite taking care of the cementation protocol and given that prosthetic margins were below the gingival margin, the difference in the type of cement present in the tens of micrometers between the abutment teeth and the prostheses could surely affect the results of this study and is a situation that should not be ignored. The inclusion of healthy patients with prosthetic restorations could highlight the inflammatory process. In addition, creating a form of follow-up by planning a longitudinal study, where changes in the periodontium before and after the placement of different prosthetic materials could be very relevant to analyze the gradual changes that occur in the recovery of the supporting tissues of the teeth.

Clinical application

The use of PFM prostheses with Ni-Cr alloy induce changes in the composition of the subgingival microbiota producing a more dysbiotic biofilm with a high prevalence of periodontopathogenic bacteria which in turn lead to an increase in the levels of proinflammatory cytokines, further favoring the deterioration of the tissues that support the tooth (Fig. 6). In addition, we propose the use of MZ prostheses as candidate prosthetic biomaterials for oral rehabilitation with less negative effects on the periodontal condition, which will allow a longer durability of the prostheses in the mouth.

CONCLUSIONS

Recognizing that the sample size was small to generalize the results, for this study we concluded that the periodontal condition according to clinical parameters was better in teeth restored with MZ prostheses compared to PFM with Ni-Cr alloy. In general, teeth restored with a fixed dental prostheses will always accumulate more bacteria than natural teeth and will therefore be predisposed to develop periodontitis. However, between the two types of prosthetic biomaterials evaluated, we observed that, in teeth restored with PFM crowns with Ni-Cr alloy, there was a greater accumulation and retention of bacteria compared to teeth restored with MZ crowns. Likewise, the use of PFM crowns prosthetic with Ni-Cr alloy increased the levels of IL-1β, TNF-α and CX3CL1 causing greater damage to the periodontium.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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