Comparison of peri-implant submucosal microbiota in arches with zirconia or titanium implant-supported fixed complete dental prostheses (IFCDP): study protocol for a randomized controlled trial

CURRENT STATUS: Under Review

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Subject Areas

*Translational Medicine*  *Internal Medicine*

Keywords

*Dental implants, Edentulous, Microbiota, Bio-complication, Zirconia, Titanium*
Abstract

• **Background:** Implant-supported prostheses are applied in edentulous patients with high success rate. However, the incidence rate of biological complications namely peri-implant mucositis and peri-implantitis increases year by year after the placement of prostheses. Pathogenic bacteria accumulated adjacent to prostheses is proved to be the main reason of biological complication. Titanium, one of the classical materials for implant-supported prostheses, performs well in the aspect of biocompatibility and ease maintenance, but is still susceptible for biofilm formation. Zirconia, emerging as an appealing substitute, not only has comparable properties, but presents different surface properties influencing oral bacteria adhesion. However, scarce scientific research proves its direct effect on oral bacteria flora. Study exploring the different effects of material properties on biofilm formation and composition might provide a clue on this topic.

• **Methods:** The proposed study is designed as a 5-year randomized controlled trial. We plan to enroll 36 edentulous (maxilla and/or mandible) patients seeking of full-arch fixed implant-supported prostheses. The participants will be randomly divided into two groups. Group 1: participants will be restored with zirconia frameworks with ceramic veneering; Group 2: participants will be restored with titanium frameworks with acrylic resin veneering. Ten reexaminations will be completed at the end of this 5-year trial. Mucosal conditions around the implants will be recorded every six months after restoration. Peri-implant submucosal plaque will be collected at each reexamination, and bacteria flora analysis will be performed with 16S rRNA gene sequencing technology, to compare the differences in microbial diversity between two groups. X-ray examination will be applied every 12 months as a key index to evaluate the marginal bone level around implants.

• **Discussion:** The current study aims to explore the oral microbiology of patients restored with zirconia ceramic frameworks and those restored with titanium frameworks. By evaluating and comparing the features of the microbiota and the mucosal condition around two different materials, whether zirconia to be a recommendable material for fixed implant-supported prostheses could be figured out. This study aims to provide a tangible decision-making suggestion for full arch implant-supported prosthesis in edentulous patients.

• **Trial registration:** International Clinical Trials Registry Platform (ICTRP), ID: ChiCTR2000029470. Registered on 2 February 2020.

Background

Nowadays, full-arch fixed implant-supported prostheses has shown satisfactory clinical result in edentulous patients, with a long-term implant survival nearing 100% [1]. However, biological complications after the placement of implant-supported fixed complete dental prostheses (IFCDP) occur continuously over time [2]. Previous studies have indicated that peri-implant mucosa inflammation is associated with plaque accumulation [3, 4] around implants and prosthesis. Investigations on the bacterial adhesion on titanium, the most commonly used materials in daily practice, has revealed that the corrosion of titanium will increase the plaque accumulation [5]. Zirconia ceramic frameworks, with or without ceramic veneering, is considered as a promising material with good bio-compatibility and mechanical property [6]. Therefore, many studies are investigating the bacterial adhesion on zirconia discs/abutments and compared it with titanium [7–11]. Results from in vitro studies are inclined to consider zirconia to be more bacterial estrangement. However, related direct evaluation in vivo of bacterial controlling around implants is controversial. Grabner-Schreiber et al has found that higher bacterial counts are associated with titanium discs compared with zirconia [12]. Scarano et al also showed that the bacterial adhesion to pure titanium surfaces was significantly more when compared with the zirconium oxide surfaces [13]. While Egawa et al found that the bacterial adherence on titanium was comparable to that on the zirconia surface if the surface roughness of both materials had mirror-like flat textures [14].

The controversial results may be caused by the difference of study design. First of all, material surface roughness is a key influence factor on bacterial colonization [15–17]. Therefore, statistics of bacterial adhesion on materials without uniform surface roughness are not comparable [7-13]. Secondly, studies carried out in
partial edentulism cases could not exclude the influence from flora of remaining teeth on peri-implant microbiota, which might disturb the comparison of peri-implant microbiota between different groups [11–13]. To eliminate these interference factor, a study focused on full-arch restoration with materials with equal smoothness could be close to qualified. A previous study recruited twenty edentulous subjects to investigate early material colonization around zirconia and titanium abutments before final restorations. Its result has declared no difference in early bacterial colonization between two materials. Since this observation period was so short that only three month samplings were collected [8], a study is needed to explore whether there might be a different performance of frameworks after final restoration. Considering that bacterial colonization might be influenced by aging of zirconia [18] and corrosion of titanium in long-term use [5], comparing study with longer observation period should be advocated.

Therefore, there is a need for a randomized controlled study with sufficient sample and long-term observation period to provide direct clinical evidence about the effect of prostheses' surface properties on submucosal microbiota in full-arch implant-treated patients. The aim of the current study is to compare the clinical conditions and peri-implant microbiota of edentulous patients restored with titanium-based prostheses and zirconia-based prostheses.

**Objectives**

The aim of the study is: (1) to investigate and compare the implant survival rates and biological complication rate between groups with two different restorative materials on full-arch fixed implant-supported rehabilitation: zirconia frameworks with ceramic veneering and titanium frameworks with acrylic resin veneering; (2) to compare the effect of two different restorative materials on microbiota diversity of the full-arch fixed implant-supported restoration: zirconia frameworks with ceramic veneering versus titanium frameworks with acrylic resin veneering.

**Method/design**

**Design and setting of the study**

The proposed study is designed as a pragmatic randomized controlled trial with a 5-year follow-up. Consolidated Standards of Reporting Trials (CONSORT) diagram is demonstrated in Table 1. We plan to enroll 36 edentulous patients (maxilla and/or mandible) in need of dental implant prosthesis. The treatment protocol is to rehabilitate these patients with full-arch fixed implant-supported prosthesis with placement of four to eight implants in the maxilla and/or mandible. The restorative materials will be randomly divided into two groups: zirconia frameworks with ceramic veneering (Fig. 1) versus titanium frameworks with acrylic resin veneering (Fig. 2). The trial will last for five years and patients will be followed up per six months. During each visit, clinical examination, surface roughness test and submucosa plaque collection will be complete and biological and mechanical complications will be treated. X-ray assessment will be performed per year. Table 2 shows the participant timeline.

**Table 1 Consolidated Standards of Reporting Trials (CONSORT) diagram**
Table 2
Participant Timeline

| TIMEPOINT | T0   | T1   | T2   | Month 0 | Month 6 | Month 12 | Month 18 | Month 24 | Month 30 | Month 36 | Month 42 | Month 48 | Month 54 | Month 60 |
|-----------|------|------|------|---------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| ENROLLMENT: |      |      |      |         |         |          |          |          |          |          |          |          |          |          |
| Eligibility screen | X    |      |      |         |         |          |          |          |          |          |          |          |          |          |
| Informed consent | X    |      |      |         |         |          |          |          |          |          |          |          |          |          |
| Interventions | Final restoration: titanium | Final restoration: zirconia | Implant survival rate | Peri-implant plaque index | Bleeding on probing | Suppuration | Probing depth |
|---------------|-----------------------------|-----------------------------|----------------------|--------------------------|-------------------|------------|---------------|
| Impression   | X                           |                             |                      |                          |                   |            |               |
| Allocation   |                             | X                           |                      |                          |                   |            |               |
| Interactions |                             |                             |                      |                          |                   |            |               |
The primary outcome variable is the difference between two groups for implant survival rate, peri-implant plaque index, peri-implant mucosal conditions, marginal bone resorption, peri-implant submucosal bacteria species. The secondary outcome variable is the difference of mechanical complication rates and surface roughnesses between two groups.

Ethical considerations

The proposed study is designed as a prospective single-center, randomized controlled trial. The trial has been approved by the Ethics Committee of Stomatology School and Hospital of Peking University, China (PKUSSIRB-202054027). In addition, the study has been registered in Clinical-Trials.gov and the identifier number is ChiCTR2000029470.

The systematic health condition of all the participants will be recorded. Before the implant surgery, all patients will receive clinical and radiographic assessment. All patients will also be required to receive oral hygiene instructions before implant surgery.

Recruitment

The recruitment of the participants will be initiated in May 2020 at the Stomatology School and Hospital of Peking University, China. Approximately 50 edentulous patients received full-arch implant-supported prosthesis every year in this hospital. By issuing announcements in the registered area and waiting area, it is expected that 36 eligible participants will be recruited within 2 years. Eligible patients will receive the study protocols and consent forms. Subjects will be included in the study until they sign the consent forms.

Inclusion criteria

1) Edentulous jaw

(2) General health, ASA I ~ II classification of American Society of Anesthesiologists

(3) Age over 22 years old

(4) Good oral hygiene and good compliance

(5) Signing an informed written consent form

Exclusion criteria
(1) Age under 22
(2) Poor oral hygiene and uncontrolled periodontitis
(3) Antibiotics use in the past 3 months
(4) Systemic disease: uncontrolled diabetes mellitus, cardiovascular disease, disease of immune system, blood disorders such as coagulation disorders, severe osteoporosis
(5) Long-term medication users: steroid, anti-epileptic drug, biophosphonates
(6) HIV infection, Hepatitis B, syphilis
(7) Bruxism
(8) Smoker
(9) Uncontrolled infection in the area intended for implant placement or other areas
(10) Maxillofacial tumor
(11) Face-neck radiotherapy
(12) Mental illness or high expectations
(13) Unable to sign informed consent
(14) Antibiotic use within the past 3 months

Interventions

The treatment protocol aims to rehabilitate these patients with full-arch fixed implant-supported prosthesis with placement of four to eight implants in the maxilla and/or mandible. The restorative materials will be randomly divided into two groups: zirconia ceramic frameworks with or without ceramic veneering versus titanium frameworks with acrylic resin veneering. Before prosthesis fitting, standard polishing procedure (GB/T 6060.2-2006) and examination will be done to ensure the same surface roughness between groups. The surface roughnesses of all specimens are measured with the help of a profilometer (Mitutoyo Surftest SJ-401; Mitutoyo Corp), according to a previous study [19].

Primary and secondary outcome variables

The primary outcome variable is the difference between two groups in implant survival rate, peri-implant plaque index, peri-implant mucosal tissue conditions, marginal bone resorption, peri-implant submucosal bacteria species. The secondary outcome variable is the difference of mechanical complication rates and surface roughnesses between two groups.

Clinical assessment

Clinical examination will be recorded at baseline (immediately after prosthesis placement) and every 6 months after final prosthesis.

The following parameters will be evaluated: peri-implant plaque index (PLI), Bleeding Index (BI), suppuration (0/1) and probing depth (PD). PLI, suppuration and PD is evaluated at four sites per implant (mesiobuccal, buccal, distobuccal and lingual/palatal) [20]. The PD will be measured to the nearest mm using a plastic graded probe (Hu-Friedy Manufacturing, Chicago, IL, USA). Bleeding Index (BI) is recorded at two sites per implant (buccal and lingual).

PLI: 0—no plaque in the gingival margin area; 1—thin plaque on the tooth surface of the gingival margin area, but it is not visible on inspection, if the side of the probe tip is used to scrape plaque; 2—Medium amount of
plaque can be seen on the adjacent surface; 3—A large amount of soft dirt can be seen in the gingival sulcus or the gingival margin area and the adjacent surface.

BI: 0—absence of inflammation and absence of bleeding on probing; 1—mild marginal inflammation (slight change in color, little change in texture of any portion of but not the entire marginal or papillary gingival unit) and absence of bleeding on probing; 2—mild marginal inflammation (criteria as above but involving the entire marginal or papillary gingival unit) and spotting gingival sulcus bleeding on probing; 3—moderate inflammation (glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit) and linear gingival sulcus bleeding on probing; 4—severe inflammation (marked redness, edema, and/or hypertrophy of the marginal or papillary gingival unit) and bleeding on probing over the sulcus.

The two examiners will be trained and calibrated prior to and during the trial to achieve maximum reproducibility in the measurements [21]. For continuous periodontal clinical parameters (PD), the standard error of measurement will be evaluated. The average level of agreement between two examiners will be considered satisfactory when greater than 90% (Kappa test) for the other clinical variables.

To evaluate mechanical complication, an analysis variable will be used [22]. If no alterations are present, a “Alpha” classification will be attributed; if minor chipping occurs—not requiring any intervention besides polishing or recontouring without the need for prosthesis retrieval—the prosthesis will be recorded as “Bravo”; a “Charlie” classification will be attributed when the occurrence of major chipping, need for prosthesis retrieval and laboratory intervention; and finally, a “Delta” classification indicates fracture of the framework is present.

X-ray assessment
Marginal bone loss (MBL): immediately after final prosthesis, periapical radiograph will be taken for each implant. Periapical radiographs will also be taken per year after final prosthesis. For standardization, a paralleling technique will be conducted using an intramural digital system (Digora Toto, Soredex, Finland). A software program (Kodak Dental Imaging 6.1, Carestream Health®, Rochester, NY, USA) will be used to do radiographic analysis. The crestal bone level CBL will be measured as the vertical distance between 2 mm below the implant–abutment interface and the most crestal part of the alveolar bone [23, 24]. MBL will be measured mesially and distally for each implant. In each group, peri-implant MBL will be measured to the nearest mm.

The peri-implantitis lesions (PI) are defined as having PPD ≥ 5 mm, with positive suppuration or BI ≥ 1 and radiographic evidence of bone loss (> 2 mm) or according to consensus [25]. The peri-implant mucositis lesions (PM) are defined as being positive for suppuration or BI ≥ 1 and at the same time, no radiographic evidence of bone loss. The healthy implant sites (HI) are determined as having probing depths ≤ 4 mm, BI = 0, with no radiographic evidence for bone loss.

The rates of peri-implantitis and peri-implant mucositis will be calculated 1 year, 3 years and 5 years after final restoration.

Laboratory assessment

1. Microbiological monitoring

1.1 Sample collection
Sulcus sampling will be performed immediately before prosthetic treatment and every 6 months after final prostheses. Before sampling, antimicrobial mouth wash should not be used within the past 48 hours, and the patient should not take food within the past 1 hr. Prior to sampling, clinical sites will be isolated and dried. If there is any supragingival/supramucosal plaque and calculus, they will be carefully removed. Submucosal plaque around one single implant will be sampled by inserting 4 sterile paper points (No. 30) into the base of the sulcus or pocket for 20 s. The paper points will be placed in labeled Eppendorf tubes and frozen, and transport to the laboratory for the subsequent extraction of DNA. 4 paper points around one single implant will be analyzed together as a unit.

1.2 Processing of microbiological samples
Bacterial identification and classification using 16S rRNA (ribosomal Ribonucleic Acid) gene sequencing technology-Polymerase Chain Reaction (PCR) samples (Sequencing of V1 and V3), library preparations, library quality inspections, and quantifications will be performed on qualified DeoxyriboNucleic Acid (DNA) samples of the oral cavity microorganisms, and the identified TAG sequences will be used for sample differentiation. Qualified libraries will be sequenced by the Illumina Hiseq 2500 high-throughput sequencing platform. Paired-End (PE) reads obtained by Hiseq / Miseq sequencing are spliced into one sequence, and the target sequence is subjected to quality control filtering. The filtered sequence is compared with a reference database, and the chimeric sequence is removed to obtain the final optimized sequence. Operational taxonomic units (OTU) cluster analysis and species classification annotations are based on optimized sequences, diversity index analysis is based on OTU clustering results, species structure analysis and species difference analysis are based on taxonomic information. Beta diversity analysis, principal co-ordinates analysis (PCoA analysis), and Linear Discriminant Analysis (LDA) Effect Size analysis will be used to compare differences in microbial diversity and differences in significant microbial species between two different groups.

2. Surface roughness assessment

The surface roughness will be measured using a profilometer (Mitutoyo Surftest SJ-401; Mitutoyo Corp) [19]. The tests will be performed before prosthetic delivery and every 6 months after final prostheses. To standardize the surface roughness measurements, 6 points are tested, which are located at mesial buccal, buccal, distal buccal, distal lingual, lingual, and mesial lingual around each abutment. For each point, measurements and analysis will be repeated twice. The reproducibility will be assessed by calculating the intraclass correlation coefficient (ICC) with a confidence interval of 95%.

Randomization, allocation and blinding

The subjects will be randomly divided into two groups according to the prosthetic materials: group 1: titanium framework with acrylic resin veneering; group 2: zirconia framework with /without ceramic veneering. The allocation of patients will be randomized using computer-generated permuted block randomization, and the allocation ratio will be 1:1. Randomization will be performed by sealed envelopes that will be opened after final impression being taken.

Sample analysis of microbiota in laboratory will be blinded after assignment to interventions. Each sample has a number associated with an allocation sequence, dental position information, and acquisition time information. The analyst of the PCR laboratory does not know the source of the sample. Interim statistical analysis will be done 1 year and 3 years after final prostheses. Final statistical analysis will be done at the end of this trial. The analyst is blinded to both the allocation of the patients and the interim analysis results.

Sample size

Sample size has been calculated by NCSS-PASS software according to previous studies. At 5 years, peri-implant bone loss in metal-resin/metal-ceramic IFCDP was 0.9 mm (standard deviation [SD] 0.4 mm), peri-implant bone loss in ceramic IFCDP was 0.6 mm, (standard deviation [SD] 0.1 mm) [26]. In our study, the criterion for significance is set at $\alpha = 0.05$ (type I error) and at $\beta = 0.10$ (type II error). The analysis is two-tailed. Assuming the dropout rate at 15%, in order to determine if there is a difference in the amount of bone loss between the two groups, 18 cases per group and 36 cases in total are finally determined. Another study included twenty edentulous subjects who received two mandibular implants. The abutments were either Titanium or zirconium dioxide (non-submerged implant placement, within-subject comparison, left-right randomization). After 3 months, mean absolute counts (mean) for P. gingivalis from titanium abutment versus zirconia abutment were 1000000(SD:0) versus 64000(SD:36770). Mean absolute counts (mean) for P. Intermedia from titanium abutment versus zirconia abutment were 600088(SD:952117) versus 3600089(SD:804935) [8]. In this study, the criterion for significance will be set at $\alpha = 0.05$ (type I error) and at $\beta = 0.10$ (type II error). The analysis will be two-tailed. Assuming the dropout rate at 15%, in order to determine if there is a difference in the amount of P. gingivalis and P. Intermedia between the two groups, 3 patients per group and 6 patients in total will be required.

In sum, the sample size is determined to be 36 in total and 18 per group.
**Statistical analysis**

All the statistical computations will be handled by the Statistical Package for Social Sciences software (SPSS, version 19.0 for Mac, SPSS Inc., Chicago, IL, USA).

1. **Clinical monitoring and X-ray assessment**

Continuous variables will be described by mean +/- standard deviation or median. Grade and quantitative data will be described by percentage. Age and other basic information between group 1 and group 2 will be compared by independent t test. Gender, implant survival rates, peri-implantitis rates and peri-implant mucositis rates between test group and control group will be compared by Chi-square test. PD and X-ray indices between group 1 and group 2 will be compared by independent t test. Generalized estimating equation (GEE) tests will be used to evaluate the differences of PLI and BI between two groups and among in follow-ups. Actual p-values are reported with significant differences accepted at 0.05.

2. **Surface roughness assessment**

Roughnesses between group 1 and group 2 will be compared by independent t test. Actual p-values are reported with significant differences accepted at 0.05.

3. **Microbiological monitoring**

The mean counts ($\times 10^5$) of individual bacterial species and the percentage of the total DNA probe will be calculated initially in each implant, then per subject and averaged across patients between groups. Periodontal pathogens include *P. gingivalis*, *F. nucleatum* subspecies, *P. intermedia* and etc. The proportions for the species will be distributed into the six complexes and the “other” group, as proposed by Socransky et al [27]. Differences between group1 and group 2 for microbiologic parameters will be sought using the Wilcoxon signed rank test. Adjustments for multiple comparisons [28] will be performed when the bacterial species are evaluated simultaneously. The level of significance will be set at 5%.

Alpha and beta diversity analyses will be performed using Primer7 and QIIME2 [29, 30]. Alpha diversity, Shannon's diversity index of both species number and their distribution, Margalef's index of numbers, and Pielou's index of evenness of distribution [31, 32, 33] will be analyzed, and the significance of the differences between groups will be derived using an unpaired Student's t test. Beta diversity analysis includes visualization of data at multiple taxonomic levels, with unweighted and weighted UniFrac distance metrics so to generate PCoA plots [34]. Analysis of similarity (ANOSIM) tests will be performed to determine whether microbial communities are significantly different between groups. White's non-parametric test will be applied to test the differences in specific microbiota taxonomic abundance between the groups, with a cutoff of 0.005 false discovery rate using the software package STAMP [35].

**Dissemination of results**

The results of the trial will be published in international peer-reviewed journals. A summary of the study results will also be recorded at ClinicalTrials.gov to allow general access to obtain findings.

**Interim analyses**

Interim statistical analysis will be done 1 year and 3 years after final prostheses. The analyst will be blinded to the allocation of the patients and will submit the analysis results to data and safety monitoring board DSMB. DSMB will announce an early close as long as DSMB find the drop-out implants or patients exceed 20% of the enrolled implants or patients.

**Discussion**

The current study focuses on the effect of prosthetic material property on peri-implant microbiota. The authors
hypothesize that the corrosion and aging of framework may have a crucial role in the selection of the bacteria that adhere to the pellicles on the surface of the prostheses. Considering that the biofilm and the microbiota might further influence the long-term peri-implant mucous condition and bone loss, our data will widen our view of the etiology of peri-implant diseases, promote updated treatment strategies and prevent peri-implant diseases.

Specifically, by controlling surface roughness and analyzing submucosal microbiota, our research targeted at full-arch implant-treated patients is about to provide scientific evidence of material property difference between zirconia and titanium in the term of influencing biofilm formation. Since previous study indicated a roughness of Ra of 0.2 µm was the threshold for maximum reduction of bacterial adhesion on abutment surfaces [36, 37], a national standard of 0.025 µm is set for all the prostheses in this study. In vivo study found that there was no universally optimum roughness that can prevent adhesion of all bacterial species[38], and surface charge, surface energy, surface topography, and material stiffness would all influence the bacterial response, thus we use clinical index to evaluate which material is more susceptible to biofilm formation. To compare the pathogenicity of plaque in two groups, flora analysis is crucial. One limitation is that although the surface roughness of two kinds of framework is polished to the same roughness in time of restoration, we could not prevent the surface roughness of the two materials from changing due to long term use. On the one hand, the oral cavity is an aggressive environment. Material surface in the mouth are covered by the salivary pellicle (up to 1,000 nm thick [39]), which can also alter the nanotopography of restorative material [40], thereby greatly influence surface roughness. Mechanical stimulus, temperature, and pH conditions can also favor additional bacterial adhesion to the prostheses and alter material surface characteristics. On the other hand, during the implementation of this trial, regular periodontal maintenance such as air-polishing is planned to carry out. It is unknown whether surface roughness will be altered by these procedures. Furthermore, zirconia frameworks with ceramic veneering has different mechanical properties with titanium frameworks veneered with acrylic resin. Literature revealed that technical complications occurs occasionally though with a low incidence [41, 42]. For titanium framework, 19.4% incidence of wear and 0.7% incidence of fracture were observed, while for zirconia 7.3% incidence of wear and 5.9% incidence of fracture were reported [43]. Considering the possible roughness changing caused by chipping, further analysis with chipping incidence included is needed.

The results of the trial will support a tangible decision-making for dental implant prostheses in edentulous patients.

**Trial status**

The trial was registered at Clinicaltrials.org and the study is open for recruitment. The recruitment of the participants will be initiated in May 2020 and will be completed in May 2022. Study Protocol version (2st edition, 2020.03.11).

**Abbreviations**

BI: Bleeding Index; CAL: Clinical Attachment Level; DNA: Deoxyribo Nucleic Acid; mm: Millimeters; min: Minutes; OHI: Oral Hygiene Instruction; PD: Probing Depth; RCT: Randomized clinical Trial; SPIRIT: Standard Protocol Items: Recommendations for interventional Studies; PI: peri-implantitis; PM: peri-implant mucositis; DSMB: data and safety monitoring board; CBL: crestal bone level; EP: Eppendorf; IFCDP: implant-supported fixed complete dental prostheses; MBL: Marginal bone loss; OTU: operational taxonomic units; PCoA: principal co-ordinates analysis; LDA: Linear Discriminant Analysis; PCR: Polymerase Chain Reaction; rRNA (ribosomal Ribonucleic Acid); DNA: DeoxyriboNucleic Acid; ANOSIM: Analysis of similarity.

**Declarations**

**Competing interests**
The authors declare no potential competing interests with respect to the authorship and/or publication of this article.

**Funding**

The authors declare that this study is being supported by a research grant from the new medical technology program of stomatological hospital of Beijing university (number: PKUSSNT-19B11). The fund is used to pay for ethical application fees and page fees.

**Authors' contributions:**

The clinical study is being conducted thanks to the contribution of JY: she designed the trial and she will be the general study coordinator. She also participated in the development of this manuscript together with PJ. J L is in charge of the recruitment of the participants. ZY is responsible for clinical evaluation, microbiota sample collection and maintenance of the participants. CZ will do microbiological samples processing and analysis. QL will perform X-ray measurement and do interim statistical analysis at year 1 and year 3. QL will be blinded to the allocation of the patients. He will submit the analysis results to JH. JH, together with other specials: ZL and LT, will consist data and safety monitoring board (DSMB). DSMB will communicate with JY as long as DSMB group found this trial could be early terminated. PJ will perform X-ray measurement and do final statistical analysis at the end of this trial, and she will be blinded to both the allocation of the patients and to the interim analysis results from QL. She also participated in the development of this manuscript. PJ and JY contribute equally to this article. Other authors were invited to comment on the paper and approved the final draft.

**Acknowledgements**

We would like to give thanks to all of our participants, who volunteered their time to take part in this study.

**Availability of data and materials**

The authors declare that the study protocol was registered in February 2020 at ClinicalTrials.gov with registration number ChiCTR2000029470. The data from the current study will be available in the following ClinicalTrials.gov web site: http://www.chictr.org.cn/searchproj.aspx?title=&officialName=&subjectid=&secondaryid=&applier=&studyleader=&ethicalcommitteesanction=&sponsor=&studyailment=&studyarea=&studyfindings=&studyapproval=&studyoverseas=&studycompleteness=&measure=&intercode=&sourceofspends=&createyear=0&isuploadrf=&whetherpublic=&btngo=btn&verifycode=&page=1

**Ethical Approval and consent to participate**

The proposed study is designed as a prospective single-center, randomized controlled trial. The trial has been approved by the Ethics Committee of Stomatology School and Hospital of Peking University, China (PKUSSIRB-202054027). In addition, the study has been registered in Clinical-Trials.gov and the identifier number is ChiCTR2000029470. Eligible patients will receive the study protocols and informed consents. Subjects will be included in the study until they sign the informed consents.

**Consent for publication**

Not applicable.

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Figure 1

Zirconia framework with ceramic veneering (group 1). Figure 1a: Zirconia framework;
Figure 2
Zirconia framework with ceramic veneering (group 1). Figure 1b: Zirconia framework with ceramic veneering
Figure 3

Titanium framework with acrylic resin veneering (group 2). Figure 2a: Titanium framework;
Titanium framework with acrylic resin veneering (group 2). Figure 2b: Titanium framework with acrylic resin veneering

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