Metagenomic Assessment of Different Interventions for Treatment of Chronic Periodontitis: A Systematic Review and Meta-Analysis

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Appendix A

Excluded studies by full text

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>REASON OF EXCLUSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (Badar et al., 2019)</td>
<td>No metagenomics testing (ongoing study)</td>
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<tr>
<td>2. (Flemming et al., 2011)</td>
<td>No metagenomics testing</td>
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<td>3. (Grzech-Leśniak, Gaspiric and Sculean, 2019)</td>
<td>No metagenomics testing</td>
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<td>4. (Leonhardt et al., 2007)</td>
<td>No metagenomics testing</td>
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<td>5. (Sajedinejad et al., 2018)</td>
<td>No metagenomics testing</td>
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<td>6. (Sanz-sánchez et al., 2015)</td>
<td>No metagenomics testing</td>
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<td>7. (Ramich et al., 2014)</td>
<td>No metagenomics testing</td>
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<tr>
<td>8. (RAO, 2008)</td>
<td>No metagenomics testing</td>
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<tr>
<td>9. (Yilmaz et al., 2012)</td>
<td>No metagenomics testing</td>
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<tr>
<td>10. (Herrero et al., 2016)</td>
<td>In vitro study</td>
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<td>11. (Kovtun et al., 2012)</td>
<td>In vitro study</td>
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<tr>
<td>12. (Lee et al., 2015)</td>
<td>In vitro study</td>
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<td>13. (Li et al., 2018)</td>
<td>In vitro and animal study</td>
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<td>14. (Morelli et al., 2017)</td>
<td>In vitro study</td>
</tr>
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<td>15. (Yamada et al., 2018)</td>
<td>In vitro and animal study</td>
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<tr>
<td>16. (Zupančič et al., 2018)</td>
<td>In vitro study</td>
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<tr>
<td>17. (‘NCT02633345’, 2015)</td>
<td>Participants not having periodontitis (Ongoing study)</td>
</tr>
<tr>
<td>18. (Adams et al., 2017)</td>
<td>Participants not having periodontitis</td>
</tr>
<tr>
<td>19. (Rafiek et al., 2019)</td>
<td>Not all participants had periodontitis</td>
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<tr>
<td>20. (Schwarzberg et al., 2014)</td>
<td>Not all participants had periodontitis</td>
</tr>
<tr>
<td>21. (Teng et al., 2016)</td>
<td>Participants not having periodontitis</td>
</tr>
<tr>
<td>22. (Galimanas, 2014)</td>
<td>Observational study: Case-control study</td>
</tr>
<tr>
<td>23. (Mason, 2016)</td>
<td>Observational study: Cross-sectional study</td>
</tr>
<tr>
<td>24. (Momn, 2017)</td>
<td>Observational study: Case series and in vitro study for probiotics on cell line</td>
</tr>
<tr>
<td>25. (Pozhitkov et al., 2015)</td>
<td>Observational study: Cross-sectional study</td>
</tr>
<tr>
<td>26. (Belstrom et al., 2018)</td>
<td>Included smokers</td>
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<td>27. (Bizzarro et al., 2016)</td>
<td>Included smokers</td>
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<tr>
<td>28. (Valenza et al., 2009)</td>
<td>Included smokers</td>
</tr>
<tr>
<td>29. (Chen et al., 2018)</td>
<td>Assessed only salivary sample</td>
</tr>
<tr>
<td>30. (Szafranski, Winkel and Stiesch, 2017)</td>
<td>Review article</td>
</tr>
</tbody>
</table>
REFERENCES:


Appendix B

1. Characteristics of included studies
   (Califf et al., 2017)

| Methods | Study design: non-randomized clinical trial  
          | Conducted in: USA  
          | Number of centers: 1, graduate periodontology clinic at the Ostrow School of Dentistry of the University of Southern California (USC)  
          | Recruitment period: Not specified  
          | Trial Registry: Not specified  
          | Funding source: Not specified  |
|---------|--------------------------------------------------|
| Participants | Number: 34 patients; 17 per group  
              | Inclusion criteria: Patients with at least four separate teeth with a pocket depth of ≥6 mm  |
| Interventions | Group T: 15 ml of a fresh solution of 0.25% sodium hypochlorite rinse. Clorox regular bleach (The Clorox Company, Oakland, CA) diluted with tap water served as the source of 0.25% sodium hypochlorite.  
              | Group Ctl: 15 ml of water rinse  
              | Participants were asked to rinse their mouths twice weekly for 30 s. Participants were also instructed in conventional oral hygiene, but they received no subgingival or supragingival scaling prior to the study.  |
| Outcomes | 1. 16S rRNA metagenome analysis by NGS  
          | 2. Shotgun metagenomics analysis  
          | 3. Metabolite extraction and analysis by ultraperformance liquid chromatography (UPLC) system  
          | Sampling technique:  
          | • Microbiological samples from 3- to 12-mm-deep periodontal pockets and from supragingival sites at all three study visits  
          | • three teeth were sampled per patient per time point with a sterile Gracey curette per sampled tooth. Samples from individual teeth were analyzed separately.  
          | • supragingival sampling, one sample pooled from three teeth with the heaviest plaque accumulation  
          | • After sampling, the paper points were stored in 200 μl of 1x phosphate-buffered saline solution and frozen immediately at -80°C  
          | Microbiome analysis platform:  
          | Assessed by Illumina MiSeq platform + shotgun sequencing  
          | Sequence data were analyzed with QIIME version 1.9  
          | Follow up at baseline (visit 1), at day 14 (visit 2), and at month 3  |

2. (Chang, 2012)

| Methods | Study design: before-and-after study (preliminary of Shi et al 2018)  
          | Conducted in: Los Angeles, USA  
          | Number of centers: not specified  
          | Recruitment period: not specified  
          | Trial Registry: not specified  
          | Funding source: NIH/NIDCR PHS Grants RC1DE020298 and 1R01DE021574, and NIH PHS Grant No. U54HG004968.  |
| Participants | Number: 4 patients  
              | Inclusion criteria:  
              | • Healthy subjects with generalized moderate to severe chronic periodontitis  
              | Exclusion criteria:  
              | • Subjects with a history of antibiotic therapy in the past 6 months  
              | • any history of smoking or diabetes  |
| Interventions | Conventional periodontal therapy including scaling and root planing as well as oral hygiene instructions  |
### Outcomes

1. Clinical parameters:
   - a. gingival index,
   - b. recession,
   - c. pocket depth
   - d. bleeding on probing
2. Microbiological results:
   - a. subgingival community profile
   - b. abundance

### Sampling technique:
- subgingival plaque samples were taken from 4 sites from each patient
- The subgingival sample obtained with a sterile curette (Hu-Friedy Mfg. Co., Inc., Chicago, IL).
- The sampled plaque was suspended directly in 300 μl of ATL buffer (Qiagen, Inc., Valencia, CA) containing 0.25 ml of 0.1 mm glass beads (BioSpec Products, Inc., Bartlesville, OK) and immediately transported to the laboratory

### Microbiological sample analysis:
1. Shotgun sequencing Illumina GAIIx sequencing platform (Illumina, Inc., San Diego, CA)
2. 16S rRNA clone library using sanger sequencing (ABI 3730xl sequencer)
3. Reference genome alignment sequencing analyses

### Follow up
- at baseline and 4-6 weeks

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### Methods

**Study design:** RCT

**Conducted in:** Germany

**Number of centers:** multicenter: Medizinische Fakultät der Humboldt Universität Berlin (Charité) [Berlin]; Universitätsklinikum Carl Gustav Carus, Zentrum für Zahn-, Mund- und Kieferheilkunde [Dresden]; Zentrum der Zahn-, Mund- und Kieferheilkunde (Carolinum), Poliklinik für Parodontologie [Frankfurt]; Justus-Liebig-Universität Gießen, Poliklinik für Parodontologie [Gießen]; Universitätsklinikum Greifswald, Poliklinik für Zahnerhaltung, Parodontologie und Kinderzahnheilkunde [Greifswald]; Universitätsklinikum Heidelberg, Poliklinik für Zahnerhaltungskunde, Sektion Parodontologie [Heidelberg]; University Hospital Muenster, Dept. of Periodontology [Muenster]; Universität Würzburg, Poliklinik für Parodontologie [Würzburg]

**Recruitment period:** October 2008-December 2011

**Trial Registry:** NCT00707369

**Funding source:** DFG grant: EH 365/1-1

### Participants

**Number:** 96 patients

**Inclusion criteria:**
1. patients with untreated chronic periodontitis (localized: <30% and generalized: ≥ 30% of teeth with moderate: ≥ 3mm to <5mm and severe: ≥ 5mm attachment loss.
2. non-smoking patients with localized severe and generalized moderate chronic periodontitis

**Exclusion criteria:**
1. if they show confirmed or assumed allergies or hyper-sensitive skin reactions against amoxicillin (or other penicillins or other ingredients of Amoxicillin-ratiopharm® 500mg), metronidazole (or other 5-nitroimidazoles and ingredients of Flagyl® 400mg), systemic diseases or conditions, or show confirmed lactose intolerance;
2. have Down's syndrome;
3. known AIDS/HIV;
4. regularly take systemic medication affecting the periodontal conditions, e.g. phenytoin, nifedipine, and/or steroid drugs;
5. professional periodontal therapy during 6 months prior to baseline;
6. require antibiotic treatment for dental appointments;
7. are undergoing or require extensive dental or orthodontic treatment;
8. are pregnant or breastfeeding;
9. have rampant caries;
10. any oral or extraoral piercing in or around the oral cavity with ornaments or accessory jewelry;
11. are dental students or dental professionals;
12. have participated in a clinical dental trial in the six months preceding the study;
13. cognitive deficits.

### Interventions

**Group A:** mechanical debridement + 500 mg amoxicillin and 400 mg metronidazole three times daily for 7 days (antibiotic)

**Group P:** mechanical debridement and placebo (placebo)

Supportive periodontal therapy was performed in three months intervals over a 24-months period
Outcomes

1. clinical variables:
   a. %PPD: percentage of tooth sites with pocket depth ≥5 mm
   b. %BOP: percentage of tooth sites with bleeding on probing
   c. %RAL, percentage of tooth sites with further relative attachment loss of ≥1.3mm between baseline and 2 months after therapy;
2. microbiome variables
   a. Richness
   b. Evenness
   c. Diversity
   d. Dissimilarity

Sampling technique:
- Subgingival specimens from 4 teeth with a probing depth of ≥6 mm, one in each quadrant.
- One sterile paper point (ISO45, Roeko Dental, Langenau, Germany) was inserted for 10 seconds in each site and all paper points were removed and pooled in one sterile collection tube
- Samples were stored at −20°C until further use.

Microbiome analysis platform:
- DNA Purified by QiaAmp Mini DNA-Isolation Kit (Qiagen, Hilden, Germany)
- Sequencing by Illumina MiSeq sequencing
- Sequence processing by R language environment v.3.4.3 and RStudio v.1.0.153, following the DADA2 workflow
- Follow up 2 months

4. (Hagenfeld et al., 2019) for (Harks et al., 2016)

Methods

Study design: RCT
Conducted in: Germany

Number of centers: two centers: Dept. of Periodontology and Restorative Dentistry, University Hospital, Muenster, Germany, and Dept. of Periodontology, University Hospital, Wuerzburg, Germany.
Recruitment period: March 2011- September 2011
Trial Registry: NCT02697539
Funding source: Kurt Wolff GmbH, Bielefeld, Germany

Participants

Number: The main study included 70 patients (sample analysis of a previously published study Harks et al)
Hagefeld et al analyzed samples from only 41 patients

Inclusion criteria:
- Patients suffering from untreated localized mild-to-moderate chronic periodontitis
- pocket probing depths (PPD) of ≥4 mm at a minimum of 4 teeth (except third molars).
- Age range: 18-75 years.
- Patients must have had at least 10 natural teeth (except third molars)
- nonsmokers

Exclusion criteria:
- known systemic diseases that may influence the periodontal conditions
- regular consumption of drugs that may interfere with periodontal conditions.
- Patients undergoing or requiring extensive dental or
  • orthodontic treatment,
  • pregnant or breastfeeding
- patients undergoing professional periodontal therapy during the 6 months prior to baseline
- patients with periodontal pockets ≥6 mm in more than 2 sextants.
- Group 1: 35 randomised, 34 analyzed (1 loss to follow up)
- Group 2: 35 randomised, 33 analyzed (2 loss to follow up)

For hagenfeld samples from:
- Group1: 21 patients and Group 2: 21 patients

Interventions

Group 1: zinc-substituted carbonated hydroxyapatite dentifrice (mHA group, BioRepair, Wolff, Bielefeld, Germany)
Group 2: dentifrice containing an amine fluoride/stannous fluoride (AmF/SnF2 group, Meridol, CP GABA, Hamburg, Germany)
- no further oral hygiene instructions
- After 4 weeks, mechanical periodontal therapy was performed according to the at baseline recorded clinical measurements. All patients were advised to keep brushing their teeth exclusively with the originally provided toothpaste
Outcomes

1. clinical variables: (outcomes of the original article Harks et al)
   a. PFR (plaque formation rate)
   b. Gingival index (GI)
   c. Plaque index (PI)
   d. Bleeding on probing (BOP)
   e. Pocket probing depth (PPD)
   f. Recession depth (REC)
   g. AL (Attachment loss)

2. microbiome variables
   a. Ribosomal sequence variants (RSV)
   b. Diversity

Sampling technique:
- supragingival plaque (buccal/lingual and interproximal) from 4 sites per sample tooth and 2 subgingival specimens, one in each quadrant.
- One sterile paper point (ISO 45 ISO 45, Roeko, Ulm Germany) was inserted for 10 seconds in each site and all paper points were removed and pooled in in transport tubes containing 500 μl Ringer-Glycerin Solution,
- Samples were stored in liquid nitrogen until further use. (Harks et al)
- Hagenfeld stated samples were stored at −20°C

Microbiome analysis platform:
- DNA Purified by QiaAmp Mini DNA-Isolation Kit (Qiagen, Hilden, Germany)
- Sequencing by Illumina MiSeq sequencing
- Sequence processing by R language environment v.3.4.3 and RStudio v.1.0.153, following the DADA2 workflow

Follow up at Baseline, 4 weeks and 12 weeks

5. (Jünemann et al., 2012)

Methods

Study design: RCT pilot study of Hagenfeld et al, 2018
Conducted in: Germany
Number of centers: multi-center
Recruitment period:
Trial Registry: ISRCTN64254080 (NCT00707369)
Funding source: a grant of the German Federal Ministry of Education and Research (BMBF) in the framework of the ParoPhylo project (0313801N).

Participants

Number: 4 patients (2 per group)
Inclusion criteria:
- non-smokers,
- generalized
- severe chronic periodontitis, i.e. more than 38% of sites with pocket
- probing depths of 6 mm or more

Interventions

Experimental (Ex): mechanical debridement + 500 mg amoxicillin and 400 mg metronidazole three times daily for 7 days.
Control (Co): mechanical debridement + placebo

Outcomes

Same outcomes as Hagefeld et al, 2018
Same sampling technique as Hagefeld et al, 2018
Microbiome analysis platform:
purified with the QiaAmp Mini DNA-Isolation Kit (Qiagen, Hilden, Germany).
Sequencing of the amplicon libraries was carried out on the Ion Torrent Personal Genome Machine (PGM) system using the Ion Sequencing 200 kit (all Life Technologies)
Sequence processing by UCHIME algorithm and statistical software suite R v 2.9.10 and the vegan R-package
Follow up at 2 months

6. (Nakano et al., 2017)

Methods

Study design: RCT
Conducted in: Japan
Number of centers: 1: Showa University School of Dentistry, Tokyo, Japan
Recruitment period: not specified
Trial Registry: UMIN000015706
Funding source: research grants from Morinaga Milk Industry.
### Participants

**Number:** 46 participants

**Inclusion criteria:**

- Adults aged 65 years and older with tongue coating.

**Exclusion criteria:**

1. Eating pureed and finely-chopped meals;
2. Receiving parenteral nutrition;
3. Receiving treatment for dental disease (except adjustment of dentures, oral hygiene instructions);
4. History of allergy to milk;
5. Having received antibiotic treatment in the past 1 month, or expected to receive it in the near future;
6. Using oral care products for prevention of oral malodor or improvement of oral hygiene;
7. Regular consumption of LF or LPO-containing food or oral care products;
8. Presence of exacerbating diseases of the liver, kidney, heart, lung, gastro-intestine, blood, endocrine system, and metabolic system.

**Test:** 24 participants: 1 lost to follow up, 3 violated the eligibility criteria

**Placebo:** 22, 5 violated the eligibility criteria.

### Interventions

**Test Group:** Tablets contain 80 mg of LF+LPO powder (Lactoferrin + lactoperoxidase) (Orabarrier; Morinaga Milk Industry, Tokyo, Japan): 20 mg of LF, 2.6 mg of LPO and 2.6 mg of glucose oxidase. LF and LPO had been purified from bovine milk. Glucose oxidase was obtained from Penicillium chrysogenum. The LF+LPO powder also contained glucose and pH-adjusting agents that support the effects of the active ingredients

**Placebo Group:** The placebo tablets contained dextrin and coloring materials instead of LF+LPO powder.

Participants were asked to suck a tablet after every meal for 8 weeks. They were instructed not to change their oral hygiene regimens throughout the study period.

### Outcomes

**1. clinical variables**

a. O’Leary’s plaque control record (O’Leary’s PCR)

b. Probing pocket depth (PPD)

c. Bleeding on probing (BOP)

d. VSC, volatile sulfur compound in oral air analyzed with a portable gas chromatography device (OralChroma; FIS, Itami, Japan)

**2. microbiological variables:**

a. Diversity indices (Shannon index, Chao1, number of observed species [number of OTUs] and PD whole tree) and distances between samples (UniFrac distance)

b. Microbiota composition

c. Abundance

**Sampling technique:**

- Tongue coating and supragingival plaque were collected using sterile swabs (Puritan Medical Products, Guilford, ME, USA) under constant pressure
- The swabs were suspended in 1mL sterile saline stored in vials

**Microbiome analysis platform:**

- Bacterial DNA was extracted using a commercial kit (QIAamp; Qiagen, Hilden, Germany)
- Amplified using PCR with a TaKaRa Ex Taq HS kit (TaKaRa Bio, Shiga, Japan)
- QIAquick 96 PCR Purification Kit (Qiagen)
- Sequencing by Illumina MiSeq:
- Analysis processing by QIIME software package version 1.8.0 (25, 26) and BLAST program (ver. 2.2.22)

**Follow up** at baseline, 4 and 8 weeks

### Methods

**Study design:** RCT

**Conducted in:** Brazil

**Number of centers:** 1: Graduate Periodontal Clinic of the Piracicaba Dental School, Piracicaba, Brazil

**Recruitment period:** March to October 2008

**Trial Registry:** NCT02907528

**Funding source:** grants from Sao Paulo Research Foundation (FAPESP) (process 2013/50389-1), Coordination for the Improvement of Higher Education Personnel (CAPES), and National Institute of Dental and Craniofacial Research (NIDCR). NIDCR grant T32-DE014320. The sequencing assay was supported through NIDCR grant R01-DE022579

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7. (Queiroz et al., 2017)
### Participants

**Number:** 41 patients  
**Inclusion criteria:**  
- Systemically healthy  
- Non-smokers  
- ≥35 years of age  
- With a buccal Class II furcation defect on mandibular molars due to Chronic Periodontitis, with:  
  1) A horizontal furcation probing depth (PD) >4 mm;  
  2) Bleeding on probing;  
  3) <1 mm of gingival recession after non-surgical therapy;  
  4) >2 mm of keratinized gingiva; and  
  5) <2 mm of interproximal bone loss were identified  
**Exclusion criteria:**  
1) Pregnant or lactating;  
2) Had received antibiotics in the last 3 months or required antibiotics prior to dental therapy;  
3) Were taking prescribed anti-inflammatory agents.

### Interventions

1) **BONE group:** bone substitute consisting of b-TCP/HA (beta tricalcium phosphate/hydroxyapatite)  
2) **BONE+EMD group:** mixture of EMD (Enamel Matrix derivative) and bone substitute consisting of b-TCP/HA; and  
3) **EMD group.**

### Outcomes

**Microbiological analysis of subgingival plaque sample**  
**Sampling technique:**  
- Sterile paper points were inserted to the base of the furcation defect for 30 seconds,  
- Placed in sterile tubes containing 300 mL of Tris–EDTA 0.1 mM and immediately stored at -20°C.  
**Microbiome analysis platform:**  
- DNA purification kit MiniAmp kit, QIAGEN, Valencia, CA  
- Multiplexed bacterial tag-encoded FLX amplicon pyrosequencing  
- Results were visualized with the Python library matplotlib as well as open source programs for statistical analysis and data visualization (R project and Interactive Tree of Life)  
Samples were collected at baseline and at the 3- and 6-month re-evaluation visits.

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### 8. (Shi et al., 2015)

**Methods**  
**Study design:** Controlled Before-and-after study *(Full study of Chang, 2012)*  
**Conducted in:** USA  
**Number of centers:** UCLA  
**Recruitment period:** 2009-2010  
**Trial Registry:** Not specified  
**Funding source:** NIH/NIDCR grants RC1 DE020298 and R01 DE021574.  

**Participants**  
**Number:** 12 patients  
**Inclusion criteria:**  
- Adult volunteers with chronic periodontitis  
- Systemically healthy  
- The age of the patients ranged from 37 to 65 years with an average age of 53 years  
**Exclusion criteria:**  
- Subjects with a history of antibiotic treatment in the past 6 months, history of smoking, or diabetes were excluded from the study.  

**Interventions**  
- Initial periodontal therapy: scaling and root planing (SRP), and oral hygiene instructions  
- Follow up: baseline, 4 and 19 weeks (on average 60 days)

**Outcomes**  
- Same outcomes as Chang, 2012  
- Same sampling technique except that sampling was performed for only 2 affected tooth sites rather than 4 in Chang, 2012  
- Same metagenomics analysis using Shotgun Illumina platform.  
- But included seven subgingival samples of healthy individuals from the Human Microbiome Project  
- The study included diseased, resolved and healthy groups.

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### 9. (Yamanaka et al., 2012)

**Methods**  
**Study design:** Before and after study  
**Conducted in:** Japan  
**Number of centers:** 1: YA Dental Clinic in Yonago, Tottori, Japan  
**Recruitment period:** Not specified  
**Trial Registry:** IRCT 138904284413 N1  
**Funding source:** Grants-in Aid for Young Scientist 23792517 (T.T.) and by Grants-in Aid for Scientific Research 20192403 (Y.Y.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
### Participants

<table>
<thead>
<tr>
<th>Participants</th>
<th>Number: 19 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion criteria:</strong></td>
<td></td>
</tr>
<tr>
<td>• All subjects had at least 19 teeth.</td>
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<tr>
<td>• Generally healthy adults,</td>
<td></td>
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<tr>
<td>• With no use of antibiotics or periodontal surgery during the preceding 6 months or during the periodontal therapy.</td>
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</tr>
</tbody>
</table>

### Interventions

| Interventions | Periodontal therapy including scaling, curettage, tooth brushing instruction, and professional mechanical tooth cleaning, but not surgical intervention or antibiotics. |

### Outcomes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Bacterial composition of saliva and supragingival plaque samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sampling technique:</strong></td>
<td></td>
</tr>
<tr>
<td>1. Stimulated saliva samples</td>
<td></td>
</tr>
<tr>
<td>2. Sterile curettes were used to collect supragingival plaque from all tooth surfaces</td>
<td></td>
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<tr>
<td>3. Samples were stored at -30°C until further</td>
<td></td>
</tr>
<tr>
<td><strong>Metagenomic analysis:</strong></td>
<td></td>
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<tr>
<td>Pyrosequencing (FLX instrument of Roche)</td>
<td></td>
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<tr>
<td><strong>Follow up:</strong></td>
<td>at baseline and approximately 2 years after the first sample collection</td>
</tr>
</tbody>
</table>

### 1. ChiCTR-IOR-16008194

### Characteristics of ongoing studies

<table>
<thead>
<tr>
<th>Study name</th>
<th>Probiotic modulation of oral ecology.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methods</strong></td>
<td>Study design: case-control, for cases, cross-over study</td>
</tr>
<tr>
<td></td>
<td>Conducted in: Hong Kong</td>
</tr>
<tr>
<td></td>
<td>Number of centers: Faculty of Dentistry, University of Hong Kong</td>
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<tr>
<td></td>
<td>Funding source: Faculty of Dentistry, University of Hong Kong</td>
</tr>
<tr>
<td><strong>Participants</strong></td>
<td>Number: 14</td>
</tr>
<tr>
<td></td>
<td>Inclusion criteria:</td>
</tr>
<tr>
<td>1. Systemically healthy,</td>
<td></td>
</tr>
<tr>
<td>2. Non-smoker,</td>
<td></td>
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<tr>
<td>3. Adult subjects (30-65 years)</td>
<td></td>
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<tr>
<td></td>
<td>Exclusion criteria:</td>
</tr>
<tr>
<td>1. A recent history of trauma or tooth extractions;</td>
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<tr>
<td>2. Pregnant or lactating females;</td>
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<tr>
<td>3. Diagnosis of periimplantitis (PD &gt;=5mm with signs of supporting bone loss);</td>
<td></td>
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<tr>
<td>4. Implant sites treated with augmentation procedures;</td>
<td></td>
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<tr>
<td>5. Subjects who underwent antibiotic therapy within past 3 months;</td>
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<tr>
<td>6. Radiation therapy in the head and neck area, HIV, Tuberculosis, hepatitis or other infectious diseases or uncontrolled diabetes, ischaemic heart disease, thyroid disorders or psychological problems.</td>
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<tr>
<td><strong>Interventions</strong></td>
<td>Group1: Diagnosed as having generalized chronic periodontitis (CP);</td>
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<tr>
<td></td>
<td>Group2: Diagnosed as periodontally healthy controls (H);</td>
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<tr>
<td></td>
<td>Group3: Partially edentulous and have received implant therapy, with periimplant health and ready to receive a definitive prosthesis.</td>
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<tr>
<td></td>
<td>Intervention: Probiotic supplement containing Lactobacillus strains; DSM 17938 and ATCC PTA 5289 (ProdentisTM, BioGaia, Lund Sweden) is regulated as food supplement in Hong Kong</td>
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<tr>
<td></td>
<td>Control: placebo</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td>microbiome analysis from saliva and plaque samples around teeth and implants</td>
</tr>
<tr>
<td><strong>Starting date</strong></td>
<td>1/5/2016</td>
</tr>
<tr>
<td><strong>Contact information</strong></td>
<td>Principal investigator: Aneesha Acharya. Oral Rehabilitation Department (Implant Dentistry), The Prince Philip Dental Hospital, 34- Hospital Road, Hong Kong. <strong>Telephone:</strong> +85251307181. <strong>Email:</strong> <a href="mailto:aneesha.a2@gmail.com">aneesha.a2@gmail.com</a></td>
</tr>
</tbody>
</table>
Appendix C
Risk of bias assessment
NON- RCTs:
The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool
(Califf et al., 2017)

<table>
<thead>
<tr>
<th>Bias domain</th>
<th>Signalling questions</th>
<th>Elaboration</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias due to confounding</td>
<td>1.1 Is there potential for confounding of the effect of intervention in this study?</td>
<td>In rare situations, such as when studying harms that are very unlikely to be related to factors that influence treatment decisions, no confounding is expected and the study can be considered to be at low risk of bias due to confounding, equivalent to a fully randomized trial. There is no Ni (No information) option for this signalling question.</td>
<td>Y/PY/PN/N</td>
</tr>
<tr>
<td></td>
<td>If N/PN to 1.1: the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2. Was the analysis based on splitting participants’ follow up time according to intervention received?</td>
<td>If participants could switch between intervention groups then associations between intervention and outcome may be biased by time-varying confounding. This occurs when prognostic factors influence switches between intended interventions.</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td></td>
<td>If N/PN, answer questions relating to baseline confounding (1.4 to 1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>If Y/PY, proceed to question 1.3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome?</td>
<td>If intervention switches are unrelated to the outcome, for example when the outcome is an unexpected harm, then time-varying confounding will not be present and only control for baseline confounding is required.</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td></td>
<td>If N/PN, answer questions relating to baseline confounding (1.4 to 1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>If Y/PY, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questions relating to baseline confounding only</td>
<td>1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?</td>
<td>Appropriate methods to control for measured confounders include stratification, regression, matching, standardization, and inverse probability weighting. They may control for individual variables or for the estimated propensity score. Inverse probability weighting is based on a function of the propensity score. Each method depends on the assumption that there is no unmeasured or residual confounding.</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td></td>
<td>1.5. If Y/PY to 1.4:Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?</td>
<td>Appropriate control of confounding requires that the variables adjusted for are valid and reliable measures of the confounding domains. For some topics, a list of valid and reliable measures of confounding domains will be specified in the review protocol but for others such a list may not be available. Study authors may cite references to support the use of a particular measure. If authors control for confounding variables with no indication of their validity or reliability pay attention to the subjectivity of the measure. Subjective measures (e.g. based on self-report) may have lower validity and reliability than objective measures such as lab findings.</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td></td>
<td>1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?</td>
<td>Controlling for post-intervention variables that are affected by intervention is not appropriate. Controlling for mediating variables estimates the direct effect of intervention and may introduce bias. Controlling for common effects of intervention and outcome introduces bias.</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
</tbody>
</table>
### Bias domain

<table>
<thead>
<tr>
<th>Question</th>
<th>Signalling questions</th>
<th>Elaboration</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Questions relating to baseline and time-varying confounding</strong></td>
<td></td>
<td></td>
<td><strong>NA/Y/PY/PN/N/ NI</strong></td>
</tr>
<tr>
<td>1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?</td>
<td>Adjustment for time-varying confounding is necessary to estimate the effect of starting and adhering to intervention, in both randomized trials and NRSI. Appropriate methods include those based on inverse probability weighting. Standard regression models that include time-updated confounders may be problematic if time-varying confounding is present.</td>
<td></td>
<td><strong>NA/Y/PY/PN/N/ NI</strong></td>
</tr>
<tr>
<td>1.8. If Y/PY to 1.7: Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?</td>
<td>See 1.5 above.</td>
<td></td>
<td><strong>NA/Y/PY/PN/N/ NI</strong></td>
</tr>
<tr>
<td><strong>Risk of bias judgement</strong></td>
<td>See Table 1.</td>
<td></td>
<td><strong>Low / Moderate / Serious / Critical / NI</strong></td>
</tr>
</tbody>
</table>

#### Optional: What is the predicted direction of bias due to confounding?

Can the true effect estimate be predicted to be greater or less than the estimated effect in the study because one or more of the important confounding domains was not controlled for? Answering this question will be based on expert knowledge and results in other studies and therefore can only be completed after all of the studies in the body of evidence have been reviewed. Consider the potential effect of each of the unmeasured domains and whether all important confounding domains not controlled for in the analysis would be likely to change the estimate in the same direction, or if one important confounding domain that was not controlled for in the analysis is likely to have a dominant impact.

**Favours experimental / Favours comparator / Unpredictable**

### Bias in selection of participants into the study

<table>
<thead>
<tr>
<th>Question</th>
<th>Signalling questions</th>
<th>Elaboration</th>
<th>Response options</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?</td>
<td>This domain is concerned only with selection into the study based on participant characteristics observed after the start of intervention. Selection based on characteristics observed before the start of intervention can be addressed by controlling for imbalances between experimental intervention and comparator groups in baseline characteristics that are prognostic for the outcome (baseline confounding). Selection bias occurs when selection is related to an effect of either intervention or a cause of intervention and an effect of either the outcome or a cause of the outcome. Therefore, the result is at risk of selection bias if selection into the study is related to both the intervention and the outcome</td>
<td></td>
<td><strong>Y/PY/PN/N/ NI</strong></td>
</tr>
<tr>
<td>2.2. If Y/PY to 2.1: were the post-intervention variables that influenced selection likely to be associated with intervention?</td>
<td></td>
<td></td>
<td><strong>NA/Y/PY/PN/N/ NI</strong></td>
</tr>
<tr>
<td>2.3 If Y/PY to 2.2: were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?</td>
<td></td>
<td></td>
<td><strong>NA/Y/PY/PN/N/ NI</strong></td>
</tr>
<tr>
<td>2.4. Do start of follow-up and start of intervention coincide for most participants?</td>
<td>If participants are not followed from the start of the intervention then a period of follow up has been excluded, and individuals who experienced the outcome soon after intervention will be missing from analyses. This problem may occur when prevalent, rather than new (incident), users of the intervention are included in analyses.</td>
<td></td>
<td><strong>Y/PY/PN/N/ NI</strong></td>
</tr>
</tbody>
</table>
2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?

It is in principle possible to correct for selection biases, for example by using inverse probability weights to create a pseudo-population in which the selection bias has been removed, or by modelling the distributions of the missing participants or follow up times and outcome events and including them using missing data methodology. However such methods are rarely used and the answer to this question will usually be “No”.

Risk of bias judgement

See Table 1. Low / Moderate / Serious / Critical / NI

Optional: What is the predicted direction of bias due to selection of participants into the study?

If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.

Favours experimental / Favours comparator / C) Towards null / Away from null /// Unpredictable

Bias in classification of interventions

3.1 Were intervention groups clearly defined?

A pre-requisite for an appropriate comparison of interventions is that the interventions are well defined. Ambiguity in the definition may lead to bias in the classification of participants. For individual-level interventions, criteria for considering individuals to have received each intervention should be clear and explicit, covering issues such as type, setting, dose, frequency, intensity and/or timing of intervention. For population-level interventions (e.g. measures to control air pollution), the question relates to whether the population is clearly defined, and the answer is likely to be ‘Yes’.

Y/PY/PN/NI

3.2 Was the information used to define intervention groups recorded at the start of the intervention?

In general, if information about interventions received is available from sources that could not have been affected by subsequent outcomes, then differential misclassification of intervention status is unlikely. Collection of the information at the time of the intervention makes it easier to avoid such misclassification. For population-level interventions (e.g. measures to control air pollution), the answer to this question is likely to be ‘Yes’.

Y/PY/PN/NI

3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?

Collection of the information at the time of the intervention may not be sufficient to avoid bias. The way in which the data are collected for the purposes of the NRSI should also avoid misclassification.

Y/PY/PN/NI

Risk of bias judgement

See Table 1. Low / Moderate / Serious / Critical / NI

Optional: What is the predicted direction of bias due to measurement of outcomes or interventions?

If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.

Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable

Bias due to deviations from intended interventions

If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2

4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?

Deviations that happen in usual practice following the intervention (for example, cessation of a drug intervention because of acute toxicity) are part of the intended intervention and therefore do not lead to bias in the effect of assignment to intervention. Deviations may arise due to expectations of a difference between intervention and comparator (for example because participants feel unlucky to have been assigned to the comparator group and therefore seek the active intervention, or components of it, or other interventions). Such deviations are not part of usual practice, so may lead to biased effect estimates. However these are not expected in observational studies of individuals in routine care.

Y/PY/PN/NI
4.2. If Y/PY to 4.1: Were these deviations from intended intervention unbalanced between groups and likely to have affected the outcome? Deviations from intended interventions that do not reflect usual practice will be important if they affect the outcome, but not otherwise. Furthermore, bias will arise only if there is imbalance in the deviations across the two groups.

| If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6 |
|---|---|---|
| 4.3. Were important co-interventions balanced across intervention groups? | Risk of bias will be higher if unplanned co-interventions were implemented in a way that would bias the estimated effect of intervention. Co-interventions will be important if they affect the outcome, but not otherwise. Bias will arise only if there is imbalance in such co-interventions between the intervention groups. Consider the co-interventions, including any pre-specified co-interventions, that are likely to affect the outcome and to have been administered in this study. Consider whether these co-interventions are balanced between intervention groups. | Y/PY/N/N/NI |
| 4.4. Was the intervention implemented successfully for most participants? | Risk of bias will be higher if the intervention was not implemented as intended by, for example, the health care professionals delivering care during the trial. Consider whether implementation of the intervention was successful for most participants. | Y/PY/N/N/NI |
| 4.5. Did study participants adhere to the assigned intervention regimen? | Risk of bias will be higher if participants did not adhere to the intervention as intended. Lack of adherence includes imperfect compliance, cessation of intervention, crossovers to the comparator intervention and switches to another active intervention. Consider available information on the proportion of study participants who continued with their assigned intervention throughout follow up, and answer ‘No’ or ‘Probably No’ if this proportion is high enough to raise concerns. Answer ‘Yes’ for studies of interventions that are administered once, so that imperfect adherence is not possible. We distinguish between analyses where follow-up time after interventions switches (including cessation of intervention) is assigned to (1) the new intervention or (2) the original intervention. (1) is addressed under time-varying confounding, and should not be considered further here. | Y/PY/N/N/NI |

4.6. If N/PN to 4.3, 4.4 or 4.5: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention? It is possible to conduct an analysis that corrects for some types of deviation from the intended intervention. Examples of appropriate analysis strategies include inverse probability weighting or instrumental variable estimation. It is possible that a paper reports such an analysis without reporting information on the deviations from intended intervention, but it would be hard to judge such an analysis to be appropriate in the absence of such information. Specialist advice may be needed to assess studies that used these approaches. If everyone in one group received a co-intervention, adjustments cannot be made to overcome this. NA

| Risk of bias judgment | See Table 2 | LOW |

Optional: What is the predicted direction of bias due to deviations from the intended interventions? If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.

<p>| Bias due to missing data | 5.1 Were outcome data available for all, or nearly all, participants? | “Nearly all” should be interpreted as “enough to be confident of the findings”, and a suitable proportion depends on the context. In some situations, availability of data from 95% (or possibly 90%) of the participants may be sufficient, providing that events of interest are reasonably common in both intervention groups. One aspect of this is that review authors would ideally try and locate an analysis plan for the study. | Y/PY/N/N/NI |
|---|---|---|
| 5.2 Were participants excluded due to missing data on intervention status? | Missing intervention status may be a problem. This requires that the intended study sample is clear, which it may not be in practice. | Y/PY/N/N/NI |</p>
<table>
<thead>
<tr>
<th>Section</th>
<th>Question</th>
<th>Y/P/Y/PY/PN/N/NI</th>
<th>NA/Y/PY/PN/N/NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>Were participants excluded due to missing data on other variables needed for the analysis?</td>
<td>Y/P/Y/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td>5.4</td>
<td>If PN/N to 5.1, or YPY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?</td>
<td>NA/Y/PY/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td>5.5</td>
<td>If PN/N to 5.1, or YPY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?</td>
<td>NA/Y/PY/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
</tbody>
</table>

**Risk of bias judgement**

<table>
<thead>
<tr>
<th>Option</th>
<th>See Table 2</th>
<th>Low / Moderate / Serious / Critical / NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optional: What is the predicted direction of bias due to missing data?</td>
<td>If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.</td>
<td>Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable</td>
</tr>
</tbody>
</table>

**Bias in measurement of outcomes**

<table>
<thead>
<tr>
<th>Section</th>
<th>Question</th>
<th>Y/P/Y/PN/N/NI</th>
<th>NA/Y/PY/PN/N/NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Could the outcome measure have been influenced by knowledge of the intervention received?</td>
<td>Y/P/Y/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td>6.2</td>
<td>Were outcome assessors aware of the intervention received by study participants?</td>
<td>Y/P/Y/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td>6.3</td>
<td>Were the methods of outcome assessment comparable across intervention groups?</td>
<td>Y/P/Y/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
<tr>
<td>6.4</td>
<td>Were any systematic errors in measurement of the outcome related to intervention received?</td>
<td>Y/P/Y/PN/N/NI</td>
<td>NA/Y/PY/PN/N/NI</td>
</tr>
</tbody>
</table>

**Risk of bias judgement**

<table>
<thead>
<tr>
<th>Option</th>
<th>See Table 2</th>
<th>Low / Moderate / Serious / Critical / NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optional: What is the predicted direction of bias due to measurement of outcomes?</td>
<td>If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.</td>
<td>Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable</td>
</tr>
</tbody>
</table>
### Bias in selection of the reported result

<table>
<thead>
<tr>
<th>Question</th>
<th>Y/PY/PN/NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the reported effect estimate likely to be selected, on the basis of the results, from...?</td>
<td></td>
</tr>
<tr>
<td>For a specified outcome domain, it is possible to generate multiple effect estimates for different measurements. If multiple measurements were made, but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
<td></td>
</tr>
<tr>
<td>7.1 ... multiple outcome measurements within the outcome domain?</td>
<td></td>
</tr>
<tr>
<td>Because of the limitations of using data from non-randomized studies for analyses of effectiveness (need to control confounding, substantial missing data, etc), analysts may implement different analytic methods to address these limitations. Examples include unadjusted and adjusted models; use of final value vs change from baseline vs analysis of covariance; different transformations of variables; a continuously scaled outcome converted to categorical data with different cut-points; different sets of covariates used for adjustment; and different analytic strategies for dealing with missing data. Application of such methods generates multiple estimates of the effect of the intervention versus the comparator on the outcome. If the analyst does not pre-specify the methods to be applied, and multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
<td></td>
</tr>
<tr>
<td>7.2 ... multiple analyses of the intervention-outcome relationship?</td>
<td></td>
</tr>
<tr>
<td>Particularly with large cohorts often available from routine data sources, it is possible to generate multiple effect estimates for different subgroups or simply to omit varying proportions of the original cohort. If multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
<td></td>
</tr>
<tr>
<td>7.3 ... different subgroups?</td>
<td></td>
</tr>
<tr>
<td>Particularly with large cohorts often available from routine data sources, it is possible to generate multiple effect estimates for different subgroups or simply to omit varying proportions of the original cohort. If multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.</td>
<td></td>
</tr>
</tbody>
</table>

### Risk of bias judgement

<table>
<thead>
<tr>
<th>Question</th>
<th>Y/PY/PN/NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of bias judgement</td>
<td></td>
</tr>
<tr>
<td>See Table 2</td>
<td></td>
</tr>
<tr>
<td>Optional: What is the predicted direction of bias due to selection of the reported result?</td>
<td></td>
</tr>
<tr>
<td>If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.</td>
<td></td>
</tr>
</tbody>
</table>

### Overall bias

<table>
<thead>
<tr>
<th>Overall bias</th>
<th>Risk of bias judgement</th>
<th>Low / Moderate / Serious / Critical / NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optional: What is the overall predicted direction of bias for this outcome?</td>
<td>2 Somewhat likely</td>
<td></td>
</tr>
<tr>
<td>3 Not likely</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Can’t tell</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### FOR BEFORE AND AFTER STUDIES

**QUALITY ASSESSMENT TOOL OR QUANTITATIVE STUDIES**

1. *(Chang, 2012)*

**COMPONENT RATINGS**

**A) SELECTION BIAS**

(Q1) Are the individuals selected to participate in the study likely to be representative of the target population?

1 Very likely

(Q2) What percentage of selected individuals agreed to participate?

1 80 - 100% agreement

2 60 – 79% agreement

3 less than 60% agreement

4 Not applicable

5 Can’t tell

<table>
<thead>
<tr>
<th>RATE THIS SECTION</th>
<th>STRONG</th>
<th>MODERATE</th>
<th>WEAK</th>
</tr>
</thead>
<tbody>
<tr>
<td>See dictionary</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

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B) STUDY DESIGN

Indicate the study design

1 Randomized controlled trial
2 Controlled clinical trial
3 Cohort analytic (two group pre + post)
4 Case-control
5 Cohort (one group pre + post (before and after))
6 Interrupted time series
7 Other specify __________________________
8 Can’t tell

Was the study described as randomized? If NO, go to Component C.

No
Yes

If Yes, was the method of randomization described? (See dictionary)

No
Yes

If Yes, was the method appropriate? (See dictionary)

No
Yes

(D) BLINDING

(Q1) Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants?

1 Yes
2 No
3 Can’t tell

(Q2) Were the study participants aware of the research question?

1 Yes
2 No
3 Can’t tell

C) CONFOUNDERS

(Q1) Were there important differences between groups prior to the intervention?

1 Yes
2 No
3 Can’t tell

The following are examples of confounders:

1 Race
2 Sex
3 Marital status/family
4 Age
5 SES (income or class)
6 Education
7 Health status
8 Pre-intervention score on outcome measure

(Q2) If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g. stratification, matching) or analysis)?

1 80 – 100% (most)
2 60 – 79% (some)
3 Less than 60% (few or none)
4 Can’t Tell

E) DATA COLLECTION METHODS

(Q1) Were data collection tools shown to be valid?

1 Yes
2 No
3 Can’t tell

(Q2) Were data collection tools shown to be reliable?

1 Yes
2 No
3 Can’t tell
F) WITHDRAWALS AND DROP-OUTS

(Q1) Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?

1 Yes
2 No
3 Can’t tell
4 Not Applicable (i.e. one time surveys or interviews)

(Q2) Indicate the percentage of participants completing the study. (If the percentage differs by groups, record the lowest).

1 80-100%
2 60-79%
3 Less than 60%
4 Can’t tell
5 Not Applicable (i.e. Retrospective case-control)

G) INTERVENTION INTEGRITY

(Q1) What percentage of participants received the allocated intervention or exposure of interest?

1 80-100%
2 60-79%
3 Less than 60%
4 Can’t tell

(Q2) Was the consistency of the intervention measured?

1 Yes
2 No
3 Can’t tell

(Q3) Is it likely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?

4 Yes
5 No
6 Can’t tell

H) ANALYSES

(Q1) Indicate the unit of allocation (circle one)

community organization/institution practice/office individual

(Q2) Indicate the unit of analysis (circle one)

community organization/institution practice/office individual

(Q3) Are the statistical methods appropriate for the study design?

1 Yes
2 No
3 Can’t tell

(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

1 Yes
2 No
3 Can’t tell

GLOBAL RATING

Please transcribe the information from the gray boxes on pages 1-4 onto this page. See dictionary on how to rate this section.

GLOBAL RATING FOR THIS PAPER (circle one):

1 STRONG (no WEAK ratings)
2 MODERATE (one WEAK rating)
3 WEEK (two or more WEAK ratings)
With both reviewers discussing the ratings:

Is there a discrepancy between the two reviewers with respect to the component (A-F) ratings?

No  Yes

If yes, indicate the reason for the discrepancy

1 Oversight
2 Differences in interpretation of criteria
3 Differences in interpretation of study

Final decision of both reviewers (circle one):

1 STRONG
2 MODERATE
3 WEAK

1. (Shi et al., 2015)

COMPONENT RATINGS

A) SELECTION BIAS

(Q1) Are the individuals selected to participate in the study likely to be representative of the target population?

1 Very likely
2 Somewhat likely
3 Not likely
4 Can’t tell

(Q2) What percentage of selected individuals agreed to participate?

1 80 - 100% agreement
2 60 – 79% agreement
3 less than 60% agreement
4 Not applicable
5 Can’t tell

RATE THIS SECTION  STRONG  MODERATE  WEAK
See dictionary  1  2  3

B) STUDY DESIGN

Indicate the study design

1 Randomized controlled trial
2 Controlled clinical trial
3 Cohort analytic (two group pre + post)

4 Case-control
5 Cohort (one group pre + post (before and after))
6 Interrupted time series
7 Other specify __________________________
8 Can’t tell

Was the study described as randomized? If NO, go to Component C.

No  Yes

If Yes, was the method of randomization described? (See dictionary)

No  Yes

If Yes, was the method appropriate? (See dictionary)

No  Yes

C) CONFOUNDERS

(Q1) Were there important differences between groups prior to the intervention? 1 Yes

2 No

3 Can’t tell

The following are examples of confounders:

1 Race
2 Sex
3 Marital status/family
4 Age
5 SES (income or class)
6 Education
7 Health status
8 Pre-intervention score on outcome measure

(Q2) If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g. stratification, matching) or analysis)?

1 80 – 100% (most)
2 60 – 79% (some)
3 Less than 60% (few or none)
D) BLINDING
(Q1) Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants?
1 Yes
2 No
3 Can’t tell

(Q2) Were the study participants aware of the research question?
1 Yes
2 No
3 Can’t tell

E) DATA COLLECTION METHODS
(Q1) Were data collection tools shown to be valid?
1 Yes
2 No
3 Can’t tell

(Q2) Were data collection tools shown to be reliable?
1 Yes
2 No
3 Can’t tell

F) WITHDRAWALS AND DROP-OUTS
(Q1) Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?
1 Yes
2 No
3 Can’t tell
4 Not Applicable (i.e. one time surveys or interviews)

(Q2) Indicate the percentage of participants completing the study. (If the percentage differs by groups, record the lowest).
1 80-100%
2 60-79%
3 Less than 60%
4 Can’t tell
5 Not Applicable (i.e. Retrospective case-control)

G) INTERVENTION INTEGRITY
(Q1) What percentage of participants received the allocated intervention or exposure of interest?
1 80-100%
2 60-79%
3 Less than 60%
4 Can’t tell

(Q2) Was the consistency of the intervention measured?
1 Yes
2 No
3 Can’t tell

(Q3) Is it likely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?
4 Yes
5 No
6 Can’t tell

I) ANALYSES
(Q1) Indicate the unit of allocation (circle one)
Community organization/institution practice/office
individual

(Q2) Indicate the unit of analysis (circle one)
Community organization/institution practice/office
individual

(Q3) Are the statistical methods appropriate for the study design?
1 Yes
2 No
3 Can’t tell

(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?
1 Yes
2 No
4 Can’t tell

GLOBAL RATING

COMPONENT RATINGS

Please transcribe the information from the gray boxes on pages 1-4 onto this page. See dictionary on how to rate this section.

<table>
<thead>
<tr>
<th>A</th>
<th>SELECTION BIAS</th>
<th>STRONG</th>
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<tr>
<td></td>
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<tr>
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</table>

GLOBAL RATING FOR THIS PAPER (circle one):
1 STRONG (no WEAK ratings)
2 MODERATE (one WEAK rating)
3 WEAK (two or more WEAK ratings)

With both reviewers discussing the ratings:

Is there a discrepancy between the two reviewers with respect to the component (A-F) ratings?

No Yes

If yes, indicate the reason for the discrepancy
1 Oversight
2 Differences in interpretation of criteria
3 Differences in interpretation of study

Other biases:
The reviewers agreed upon lowering the risk of bias in this study by 1 grade as the authors did not provide any information about one of the outcomes (the clinical condition) and did not provide any numbers indicating the microbiological outcomes. They also only included in the analysis the samples of periodontal pockets that showed clinical improvement.

Final decision of both reviewers (circle one):
1 STRONG
2. WEAK

2. (Yamanaka et al. 2012)

COMPONENT RATINGS

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1. Yes
2. No
3. Can’t tell
1. (Hagenfeld et al., 2018)

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<tr>
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<th>Support for Judgment</th>
</tr>
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| Incomplete outcome data (attrition bias)  | Low risk          | Quote: "After repetition, still 6 post-treatment samples and 1 pre-treatment sample showed an insufficient quality and were thus removed from further analysis. To maintain pairwise comparisons, the related 7 paired samples were also excluded from further analysis."
| Selective reporting (reporting bias)      | Low risk          | Comment: all outcomes were reported                        |
| Other bias                                | Low risk          | Comment: No other sources of bias identified               |

2. (Hagenfeld et al., 2019) and (Harks et al., 2016)

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<td>Quote: &quot;Participants were randomly assigned to the test or the control group by the use of computerized center-specific randomization lists. Quad-block randomization lists were generated for each center by a statistician who was not involved in any other aspect of the trial.&quot;</td>
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<td>Quote: &quot;Randomization lists were stored exclusively at the study centers. Randomization was performed by 2 study nurses who were not involved in measurements or treatment of the participating patients.&quot;</td>
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| Blinding of participants and personnel (performance bias) | Unclear risk      | Quote: "the blinded dentifrices"
"randomized, double-blind"
Comment: no further details at either articles |
| Blinding of outcome assessment (detection bias) | Unclear risk      | Comment: not mentioned                                    |
| Incomplete outcome data (attrition bias)  | High risk         | Comment: The main study of (Harks et al., 2016) randomized 35 patients for each group, from which a balanced loss to follow up at the end of the study occurred (1 patient vs 2 patients). In the study of (Hagenfeld et al., 2019), only samples from 41 patients (20 patients in Group 1 vs 21 patients in Group 2) were included with no justification of the difference in number of participants |
| Selective reporting (reporting bias)      | Low risk          | Comment: all outcomes were reported                        |
| Other bias                                | Low risk          | Comment: No other sources of bias identified               |

3. (Jünemann et al., 2012)

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4. (Nakano et al., 2017)

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<td>Quote: &quot;Participants were randomly assigned to receive either placebo or test tablets&quot;</td>
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| Quote: "These placebo and test tablets were identical in weight, texture and appearance and were of readily soluble perforated type to prevent asphyxia and aspiration."
| Comment: not mentioned                                               |
| Blinding of outcome assessment (detection bias)                      | Unclear risk      |                      |
| Incomplete outcome data (attrition bias)                             | High risk         |                      |
| Quote: "Five participants in the placebo group and three in the test group failed to comply with the suggested intake rate (less than 75%). Therefore, 37 subjects with full analysis sets were included in the efficacy analysis."
| Comment: patients who violated the eligibility criteria were excluded from analysis rather than performing an intention to treat analysis at least for the clinical variables. |
| Selective reporting (reporting bias)                                 | Low risk          |                      |
| Comment: all outcomes were reported                                  |
| Other bias                                                           | Low risk          |                      |
| Comment: No other sources of bias identified                         |

5.(Queiroz et al., 2017)

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| Quote: "Patients were randomized to the three groups described below"
| Comment: No further details.                                         |
| Allocation concealment (selection bias)                              | Low risk          |                      |
| Quote:"Patients were randomized to the three groups described below and treatment for each buccal furcation defect was revealed after flap elevation and root/ defect debridement"
| Comment: blinding of the personnel is not possible due to difference in surgical procedure. No information about the participants' blinding |
| Blinding of participants and personnel (performance bias)            | Low risk          |                      |
| Blinding of outcome assessment (detection bias)                      | Unclear risk      |                      |
| Comment: Not mentioned                                               |
| Incomplete outcome data (attrition bias)                             | Low risk          |                      |
| Comment: No losses to follow up                                      |
| Selective reporting (reporting bias)                                 | Low risk          |                      |
| Comment: all outcomes were reported                                  |
| Other bias                                                           | Low risk          |                      |
| Comment: No other sources of bias identified                         |