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Modified minimally invasive surgical technique plus Bio Oss Collagen® for regenerative therapy of isolated interdental intrabony defect: study protocol for a randomized controlled trial

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Abstract

Introduction

Periodontal regeneration surgery has been widely used to deal with intrabony defects. Modified minimally invasive surgical technique (M-MIST) is designed to deal with the isolated interdental intrabony defects, which has achieved satisfactory periodontal

regenerative effect. Bio Oss Collagen® as a bioactive material has been applied to periodontal regeneration, and it is similar to human cancellous bone, with the ability to promote bone formation, meanwhile it has very good plasticity and spatial stability. The combination of different materials and techniques has become a research hotspot in recent years. By combining the superiority of regeneration technology and materials, better regeneration effect can be achieved. This study is planned to search the difference between M-MIST plus Bio Oss Collagen® and M-MIST only in regeneration therapy of intrabony defects.

Methods and Analysis: The present research is designed as a two group parallel randomized controlled trial. The needed number of participants is 40. The patients will be randomly assigned into two groups for further periodontal regenerative surgery. Test group: M-MIST plus Bio Oss Collagen®. Control group: M-MIST only. After 12 months follow up, the measurement indexes will be recorded, which include clinical attachment gain, radiographic intrabony defect depth change, as the primary results, and secondary outcomes are full mouth plaque scores, probing depth, full mouth bleeding scores, gingival recession, mobility, height of the gingival papilla, and visual analog scale. The paired samples t test will be applied to detect any difference between baseline and 1 year registrations. A general linear model will be performed to study the relationship between the second outcome and primary outcome.

Ethics and Dissemination: The present research has gotten approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002). Data of the present research will be registered in the International Clinical Trials Registry Platform. Additionally, we will disseminate the results through scientific dental journals.

Trial registration number: ChiCTR-2000030851.

Protocol version: Protocol Version 3, 05.03.2020.

Strengths and limitations of this study:

The present trial will be the first research to compare the periodontal regenerative effect of M-MIST plus Bio Oss Collagen® and M-MIST only in isolated interdental intrabony defects.

The research may provide another therapeutic choice for intrabony defects.

This trial is a randomised, examiner-blind clinical trial with long time follow-up.

The participants may withdraw during the 1 year follow-up.

Key words: Intrabony defect, periodontal regenerative therapy, Bio Oss Collagen®, modified minimally invasive surgical technique.

INTROCDUTION

Periodontal diseases are inflammatory diseases with high incidence around teeth leading to loss of periodontal attachment and alveolar bone, even to tooth loss.[1,2] It is reported that periodontitis was one of the most universal diseases around the world.[3] Scaling and root planning is the essential therapy for every patient.[4] However, for tooth with deep periodontal pockets, it could not achieve more therapeutic effect than surgical therapy does.[5] After periodontal initial therapy, the residual deep pockets were often associated with intrabony defects, which could be identified as a clinical challenge.[6] The intrabony defects may result in unmanageable inflammation, and even to ultimately tooth loss.[7] Therefore, intrabony defects are generally regarded as surgical indications.[8]

Periodontal regeneration surgery has been widely applied to dealing with intrabony defects

with the purpose of reformation of periodontal attachment to save the involved teeth better.[9-12] In the past 20 years, the development of periodontal regeneration therapy is mainly reflected in two aspects. Firstly, surgical design and techniques have been paid attention to, especially the minimally invasive surgery techniques.[13-18] Secondly, great progress has been made in regenerative materials, such as enamel matrix derivative (EMD),[16] demineralized freeze-dried bone allograft (DFDBA),[17] recombinant human platelet-derived growth factor BB (rhPDGF-BB),[18] Bio Oss Collagen®.[19] These methods had more advantages than simple flap debridement in increasing clinical attachment and decreasing probing depth of the affected teeth.[20,21] However, common complications, such as the exposure of barrier membrane and embedded material, contributed to the poor clinical results of periodontal regeneration surgery.[22]

To solve this problem, many periodontal surgical designs and techniques have been proposed and continually improved.[23-26] As early as in 1995, Harrel and Ress[23] suggested applying minimally invasive surgery to periodontal surgical treatment, whose key points were small incision, small flap, and reduction of damage of soft and hard tissues. In 1995 and 1999, Cortellini and Tonetti[24,25] put forward the papilla preservation techniques, which preserved the interdental soft tissues as completely as possible, and isolated the operative area from the oral environment. On this basis, in order to further improve the surgical effect, the concept of minimally invasive surgery had been proposed and improved gradually. In 2007, Cortellini and Tonetti[26] proposed a minimally invasive surgical technique (MIST) to deal with periodontal intrabony defects for more periodontal tissue regeneration. This technique was designed to reduce the surgical trauma, the operative time, and the postoperative discomfort.[26] A number of clinical studies[14,27] confirmed its effectiveness and advantages. In

2009, Cortellini and Tonetti[15] further designed a modified minimally invasive surgical technique (M-MIST). This method only opened the buccal tiny flap to ensure adequate blood supply, tighter primary wound closure and lower risk of bacterial infection.

The combination of different materials and techniques has become a research hotspot in recent years.[28] By combining the advantages of regeneration technology and materials, better regeneration results can be achieved.[29] M-MIST only opens a small flap in the buccal side to get a minimal interdental passage, saving the palatal soft tissue.[15] Bio Oss Collagen® is similar to human cancellous bone, with the ability to promote bone formation.[30] Bio Oss Collagen® with very good plasticity and spatial stability,[31] is suitable for the small field of vision resulted by M-MIST. Therefore, the present study is planned to research the therapeutic effect of M-MIST plus Bio Oss Collagen® and M-MIST only in the periodontal tissue regeneration of isolated interdental intrabony defects. The presumption was raised that the combination of M-MIST and Bio Oss Collagen® would lead to a better result in the periodontal regeneration effect of intrabony defects than M-MIST alone. The practicability, applicability and extensibility of the combination will be investigated in the present study. The primary outcomes were clinical attachment gain and radiographic bone filling intrabony defect, which would be recorded by clinical examination and periapical radiographs. The clinical attachment gain and bone filling represent the periodontal tissue regeneration.[15] The secondary outcomes were probing depth, full mouth plaque scores, full mouth bleeding scores, gingival recession, mobility, height of the gingival papilla, and visual analog scale. These indexes represent the inflammatory situation of periodontal tissue.[26] The present article described the design of the trial.

METHODS AND ANALYSIS

This research is a randomized controlled trial with two parallel groups. It will be carried out on the basis of the World Medical Association Declaration of Helsinki. Patients who are involved in stage Ⅲ or Ⅳ periodontitis,[32] and need periodontal regenerative treatment for isolated intrabony defects are the potential participants. The experimentation of this research will be conducted at Peking University School and Hospital of Stomatology (Beijing, China). The present research has gotten approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002) and registered in the International Clinical Trials Registry Platform under the ID: ChiCTR-2000030851. The research framework is showed in Figure 1.

Participants selection

All participants come from the Periodontology Department, Peking University School and Hospital of Stomatology. At re-evaluation after periodontal initial therapy, if the subject is found to have isolated intrabony defects, he or she will be informed about the study. The potential subjects will receive information about the research plan to get a thorough understanding. The subjects will be incorporated into this trial only after their signature of the consent form. The personal information of the participants will be confidentially stored in our department.

As regard to the patients levels, the following criteria should be met: (1) Age 18 to 75 years; (2) Patients with stage Ⅲ or Ⅳ periodontitis at least 2 months after periodontal initial

therapy; (3) Good compliance; (4) Good oral hygiene; (5) The full mouth plaque score and full mouth bleeding score are both less than 30%. As for the morphological requirements of the intrabony defect, it should be isolated intrabony defect with more than 3mm depth, combined with more than 5mm probing depth and attachment loss. In addition, the intrabony defect should not exceed the lingual surface of root. The morphology of the intrabony defect will be detected during the operation and finally determined whether to be enrolled in the trial. About the dental standards, the associated tooth should maintain normal pulp vitality. Or it has received root canal therapy for more than 6 months. The intrabony defect existing around the anterior teeth, the premolars, or at the mesial side of mandibular first molars will be included to avoid the influence of furcation involvement. Patients with tumor, uncontrolled systemic disease, history of receiving antibiotics in the past 3 months will be excluded. The affected teeth with 3 degree mobility, furcation involvement, acute periapical inflammation, and root fractures will not be enrolled.

Randomization and blinding

The random sequence is produced through the random number table and the assignment is saved in a sealed envelope. A research worker who does not know the trial process will be in charge of the randomization. All subjects will be randomly assigned into two groups. Test group: M-MIST plus Bio Oss Collagen®. Control group: M-MIST. All surgical operations will be conducted by an experienced therapist in the dark of the assignment. Another two members in the dark of the research schedule will respectively take charge of the clinical examination and statistical analysis.

Interventions

The enrolled participants will receive periodontal clinical examination and take periapical radiographs. The surgical process will be performed as described in the literature[15]: if the width of the top of the gingival papilla is 2 mm or narrower, the simplified papilla preservation flap[25] will be performed to interdental papilla; if that is wider than 2 mm, the modified papilla preservation technique[24] will be used. Then the incision will extend along the gingival sulcus at the buccal side of the two adjacent teeth. Then a buccal flap will be opened to expose 1 to 2 mm buccal bone crest. With miniature blades and mini curettes, the granulation tissue will be taken out. The roots will be carefully planned with curettes. If the depth of the intrabony defect is 3 mm or deeper and the defect does not wrap to the lingual side of the tooth, the site will be finally enrolled in further examination and statistical analysis. If the intrabony defects do not meet the inclusion criteria, the surgery will be continued, but such defects will not be enrolled in further examination and statistical analysis. After thorough debridement and careful rinse with saline, the intrabony defect will be filled with Bio Oss Collagen®, abreast the buccal bone crest, in the test group. In the control group, intrabony defects will not be treated with any other materials. Finally, a single modified internal mattress suture will be performed to close the wound. After the surgery, if the tooth appears 2 to 3 degree mobility, the tooth will be splinted timely. The patients will be required to rinse with a 0.2% chlorhexidine solution for 1 week. The suture will be removed 1week after the surgery.

The surgeries will be performed at the Periodontology Department, Peking University

School and Hospital of Stomatology. An experienced periodontist with the help of operating microscope will conduct all operations.

Examination

At the baseline, all the enrolled subjects will receive periodontal examination by a calibrated research worker. Relative periodontal indexes will include full mouth plaque score,[26] clinical attachment loss of the involved teeth,[27] full mouth bleeding score,[26] gingival recession of the involved teeth,[12] mobility of the involved teeth,[26] probing depth of the involved teeth,[27] height of the gingival papilla,[27] periapical films of the interdental site.[26] During the surgery, the defect anatomy including the depth and number of walls of the intrabony defects will be examined by the operator. The patients will receive phone-call for re-examinations at 1 week, 1, 3, 6, 9, and 12 months after the surgery. At 1, 3, 6, 9, and 12 months after the surgery, full mouth plaque score and height of the gingival papilla will be recorded by the same calibrated examiner. At 12 months after the treatment, full mouth plaque score, clinical attachment gain of the involved teeth, gingival recession, mobility, full mouth bleeding score, height of the gingival papilla, probing depth of the involved teeth will be examined by the same examiner and periapical films of the defect associated site will be taken. In addition, subjects will finish a visual analog scale to evaluate the discomfort at 1 week after treatment. During the follow up, complications will be recorded once they happen and treated accordingly. At re-evaluation, if the oral hygiene is not good, we will clean the teeth by supragingival scaling and reinforcement oral hygiene instruction. All the data will be recorded in the periodontal examination charts and be registered and stored in computer. There are no data monitoring

committee in our hospital, in order to insure the correction and completion of data, two different researchers will take charge of the management and storage of data.

Sample size

The needed numbed of participants was calculated according to the formula:

$$n1 = n2 = 2\left[\frac{\sigma(Z_{\alpha/2} + Z_{\beta})}{\delta}\right]^2$$

On the basis the previous literatures,[15,33] the difference of clinical attachment gain using Bio Oss Collagen® or not (δ) was about 1.5 mm and the standard deviation (σ) was about 1.4 mm. We set the power of test (β) as 90% and the inspection level (α) as 0.05. After calculation, 18 subjects will be needed for each group. Suppose that the rate of lost to follow up is around 10%, 20 subjects will be needed for each group. Ultimately, at least 40 subjects will be needed in all.

Statistical analysis

One patient is identified as a data unit. If two or more infrabony defects go through the surgery in one patient, only one defect nearest to the midline will be enrolled.[34] The paired samples t test will be applied to detect any difference between baseline and 1 year registrations. A general linear model will be performed to study the relationship between the defect depth, number of bone walls, full mouth plaque score and full mouth bleeding score with clinical attachment gain, probing depth reduction, and radiographic intrabony defect depth change. The level of statistically significant difference will be installed at 0.05.

Statistical analysis will be computed using SPSS v26.0 software.

Withdrawal

Participants will be told that they can quit the research at any moment. The withdrawal will not influence their seeking for help from periodontists in our department in the future.

Dissemination of data

Data of the present research will be registered in the International Clinical Trials Registry Platform. Additionally, we will disseminate the results through scientific dental journals.

DISCUSSION

For isolated intrabony defect, M-MIST could be an effective method, with an average clinical attachment gain of 4.8 mm at 1 year after the surgery.[15] It allows access to root surface with only the buccal flap opening, which is minimal invasive and further enhances wound stability. But when bioactive materials, such as END and rhPDGF-BB, were used combined with M-MIST, periodontal regenerative effects were not better.[35,36] EMD might not be an ideal bioactive material in dealing with wide defects.[37] Bio Oss Collagen® is a bovine derived xenograft containing profuse collagen, which can fit into different types of defects.[31] The present research is designed to study the efficacy and potential applicability of M-MIST combined with Bio Oss Collagen® for periodontal tissue regeneration of periodontal

intrabony defects. If the results of M-MIST plus Bio Oss Collagen® turned out to be better than those of M-MIST alone in terms of radiographic and clinical defect reductions, Bio Oss Collagen® might be suggested as a combined application with M-MIST for periodontal tissue regeneration of intrabony defects.

TRIAL STATUS

The trial protocol received ethics approval in March 2020 and was registered at International Clinical Trials Registry Platform under the identifier number ChiCTR-2000030851 on 15 March 2020. The trial will begin after COVID-19 pandemic being controlled and is planned to be completed in October 2021.

Acknowledgements

Not applicable

Contributors

H-DZ, K-NL, C-RZ and J-XH proposed the concept of this work. C-RZ, K-NL and J-XH designed the trial. C-RZ and H-DZ drafted the document. Z-GY, L-LM and YH revised the part of randomization and calculation of sample size. K-NL and J-XH reviewed and finalized the manuscript. All authors agreed the final version.

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Competing interests

The authors declare that there is no conflict of interest

Patient and public involvement

There were no patients or the public associated with the design or dissemination scheme of this work.

Patient consent for publication

Not required.

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Figure 1. The framework of the trial and CONSORT flow chart

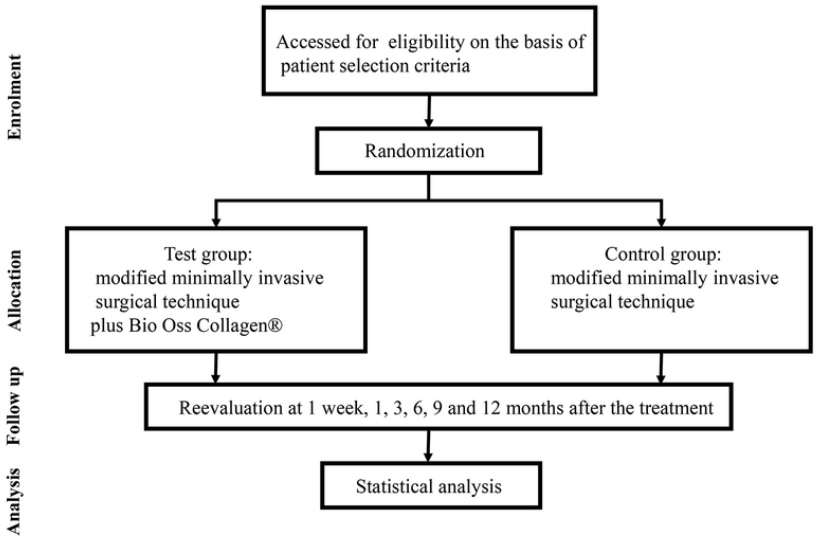

Figure 1. The framework of the trial and CONSORT flow chart

80x45mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions,and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5,6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7-10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7,8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	7
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Modified minimally invasive surgical technique plus Bio-Oss® Collagen for regenerative therapy of isolated interdental intrabony defects: study protocol for a randomised controlled trial

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Modified minimally invasive surgical technique plus Bio-Oss® Collagen for regenerative therapy of isolated interdental intrabony defects: study protocol for a randomised controlled trial

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Abstract

Introduction

Periodontal regeneration surgery has been widely used to deal with intrabony defects. Modified minimally invasive surgical technique (M-MIST) is designed to deal with isolated

interdental intrabony defects, having achieved satisfactory periodontal regenerative effect. Bio-Oss® Collagen, as a bioactive material, has been applied to periodontal regeneration. It is similar to human cancellous bone, with the ability to promote bone formation; further, it has exceptional plasticity and spatial stability. The combination of different materials and techniques has become a research hotspot in recent years. By combining the superiority of regeneration technology and materials, better regenerative effect can be achieved. This study will search for differences between M-MIST combined with Bio-Oss® Collagen and M-MIST exclusively in regeneration therapy for intrabony defects.

Methods and Analysis: The present research is designed as a two group parallel randomised controlled trial. The needed number of participants is 40. The patients will be randomly assigned to two groups, 20 participants in each group, for further periodontal regenerative surgery. Test group: M-MIST plus Bio-Oss® Collagen. Control group: M-MIST. After 12 months, the measurement indexes will be recorded; these will include clinical attachment gain and radiographic intrabony defect depth change, as the primary results, and secondary outcomes of full-mouth plaque scores, probing depth, full-mouth bleeding scores, gingival recession, mobility, gingival papilla height, and visual analogue scale (VAS). The paired samples *t*-test will be applied to detect any difference between baseline and one year registrations. A general linear model will be performed to study the relationship between the second and the primary outcome.

Ethics and Dissemination: The present research has received approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002). Data of the present research will be registered with the International Clinical Trials Registry Platform. Additionally, we will disseminate the results

through scientific dental journals.

Trial registration number: ChiCTR-2000030851.

Protocol version: Protocol Version 4, 07.14.2020.

Strengths and limitations of this study:

This trial is designed as a randomised, examiner-blind clinical trial.

The trial will be the first clinical study to compare the periodontal regenerative effect of M-MIST plus Bio-Oss® Collagen and M-MIST exclusively in isolated interdental intrabony defects.

The results of this trial might provide a new option for periodontal regeneration of isolated interdental intrabony defects.

The follow-up in the trial will last for one year.

The outcome of this study will not be applied to patients with systemic disease.

Key words: Intrabony defect; periodontal regenerative therapy; Bio-Oss® Collagen; modified minimally invasive surgical technique.

INTRODUCTION

Periodontal disease is an inflammatory disease, with a high incidence around the teeth. It can lead to loss of periodontal attachment and alveolar bone, and even to tooth loss.[1,2] Periodontal disease is caused by multiple factors, of which the initial factor is the interaction between the biofilm and the immune response.[3] Periodontal pathogens play a key role in the pathogenesis and development of periodontal and systemic diseases, including diabetes and cardiovascular

diseases, which can influence the development of periodontal disease.[4] It is reported that periodontitis is one of the most universal diseases around the world.[5] Scaling and root planning (SRP) is the essential therapy for every patient.[6] However, for teeth with deep periodontal pockets, SRP cannot not achieve a more therapeutic effect than surgery.[7] After periodontal initial therapy, the residual deep pockets have often been associated with intrabony defects, identified as a clinical challenge.[8] The intrabony defects may result in unmanageable inflammation, and ultimately to tooth loss.[9] Therefore, intrabony defects are generally regarded as surgical indications.[10]

Periodontal regeneration surgery has been widely applied to deal with intrabony defects with the purpose of reforming the periodontal attachment in hopes of saving the involved teeth.[11-14] In the past 20 years, the development of periodontal regeneration therapy has been reflected mainly in two aspects. First, surgical design and techniques have been studied, especially minimally invasive surgery.[15-20] Second, considerable progress has been made in regenerative materials, such as enamel matrix derivative (EMD),[18] demineralized freeze-dried bone allograft (DFDBA),[19] recombinant human platelet-derived growth factor BB (rhPDGF-BB),[20] and spongy bone with collagen (Bio-Oss® Collagen).[21] These methods offer more advantages than simple flap debridement in increasing clinical attachment and decreasing probing depth of the affected teeth.[22,23] However, common complications, such as the exposure of barrier membrane and embedded material, have contributed to poor clinical results of periodontal regeneration surgery.[24]

To solve this problem, many periodontal surgical designs and techniques have been proposed and continually improved on.[25-28] As early as in 1995, Harrel and Ress[25] suggested applying minimally invasive surgery to periodontal surgical treatment, whose key points were

small incision, small flap, and reduction of damage to soft and hard tissues. In 1995 and 1999, Cortellini[26,27] proposed the papilla preservation techniques to preserve interdental soft tissues as completely as possible and isolate the operative area from the oral environment. On this basis, to further improve the surgical effect, the concept of minimally invasive surgery was proposed and gradually improved upon. In 2007, Cortellini and Tonetti[28] proposed a minimally invasive surgical technique (MIST) to deal with periodontal intrabony defects for more periodontal tissue regeneration. This technique was designed to reduce surgical trauma, operative time, and postoperative discomfort.[28] A number of clinical studies[16,29] have confirmed its effectiveness and advantages of the technique. In 2009, Cortellini and Tonetti[17] further designed a modified minimally invasive surgical technique (M-MIST). This method only opened the tiny buccal flap to ensure adequate blood supply, tighter primary wound closure and lower risk of bacterial infection.

In recent years, the combination of different materials and techniques has become a research hotspot.[30] By combining the advantages of regeneration technology and materials, better regenerative results can be achieved.[31] M-MIST only opens a small flap on the buccal side to achieve minimal interdental passage, saving the palatal soft tissue.[17] Bio-Oss® Collagen is similar to human cancellous bone, with the ability to promote bone formation.[32] Bio-Oss® Collagen, with outstanding plasticity and spatial stability,[33] is suitable for the small field of vision resulting from M-MIST. Therefore, we plan to research the therapeutic effect of M-MIST combined with Bio-Oss® Collagen and the use of M-MIST exclusively for periodontal tissue regeneration of isolated interdental intrabony defects. The presumption was raised that combining M-MIST and Bio-Oss® Collagen would lead to a better result in periodontally regenerating intrabony defects than M-MIST alone. The practicability,

applicability and extensibility of the combination will be investigated in the present study. The primary outcomes were clinical attachment gain and radiographic bone-filling the intrabony defect, which would be recorded by clinical examination, periapical radiographs and cone beam computed tomography. The clinical attachment gain and bone filling represent periodontal tissue regeneration.[17] The secondary outcomes were probing depth, full-mouth plaque scores, full-mouth bleeding scores, gingival recession, mobility, height of the gingival papilla, and visual analogue scale (VAS). These indexes represent the inflammatory characteristics of periodontal tissue.[28] The present article describes the design of the trial.

METHODS AND ANALYSIS

This research is a randomised controlled trial with two parallel groups. It will be carried out according to the basis of the World Medical Association Declaration of Helsinki. Patients with stage Ⅲ or Ⅳ periodontitis,[34] (details in the supplemental material) needing periodontal regenerative treatment for isolated intrabony defects are the potential subjects. This study will be undertaken at Peking University School and Hospital of Stomatology (Beijing, China). The present research has received approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002), and it is registered with the International Clinical Trials Registry Platform (ID: ChiCTR-2000030851). The research framework is shown in Figure 1.

Participant selection

All participants will come from the Periodontology Department at Peking University School and Hospital of Stomatology. At re-evaluation after periodontal initial therapy, if the subject is found to have isolated intrabony defects, he or she will be informed about the study. The potential subjects will receive information about the research. The subjects will be incorporated into this trial only after their signature has been obtained. The personal information of the consent form will be confidentially stored in our department.

As regards the patient characteristics, the following criteria should be met: (1) age 18 to 75 years; (2) both genders will be considered for selection in the study; (3) patients with stage Ⅲ or Ⅳ periodontitis at least two months after periodontal initial therapy; (4) good compliance; (5) good oral hygiene; (6) full-mouth plaque score and full-mouth bleeding score each less than 30%; (7) systemically healthy. The intrabony defect should be an isolated intrabony defect of more than 3 mm in depth, combined with more than a 5 mm probing depth and attachment loss. In addition, the intrabony defect should not exceed in area the lingual surface area of the root. The morphology of the intrabony defect will be detected during the operation and finally determined whether the patient would be enrolled in the trial. The associated tooth should either maintain normal pulp vitality or it should have undergone root canal therapy for at least six months. The intrabony defect existing around the anterior teeth, the premolars or at the mesial side of mandibular first molars will be included to avoid the influence of furcation involvement. Patients with tumours, systemic diseases, or a history of receiving antibiotics in the past three months will be excluded. The affected teeth with 3° mobility, furcation involvement, acute periapical inflammation, or root fractures will not be enrolled.

Randomisation and blinding

The random sequence is produced through a random number table and the assignment is saved in a sealed envelope. A research worker unaware of the trial process will be in charge of the randomisation. All subjects will be randomly assigned to two groups. Test group: M-MIST combined with Bio-Oss® Collagen. Control group: M-MIST. All surgical operations will be performed by an experienced therapist in the dark of the assignment. Another two members in the dark of the research plan will respectively take charge of the clinical examination and statistical analysis.

Interventions

The enrolled participants will receive a periodontal clinical examination. They will have periapical radiographs and cone beam computed tomography taken. The surgical process will be performed as described in the literature[17]: if the width of the top of the gingival papilla is 2 mm or narrower, the simplified papilla preservation flap[27] will be performed; if it is wider than 2 mm, the modified papilla preservation technique[26] will be used. Then the incision will extend along the gingival sulcus on the buccal side of the two adjacent teeth, and a buccal flap will be reflected to expose 1 to 2 mm of buccal bone crest. With miniature blades and mini curettes, the granulation tissue will be curetted and the roots will be carefully planned with curettes. If the depth of the intrabony defect is 3 mm or more and the defect does not contain a lingual intrabony component, the site will be finally enrolled for further examination and statistical analysis. If the intrabony defects do not meet the inclusion

criteria, the surgery will be continued, but such defects will not be enrolled for further examination or statistical analysis. After thorough debridement and careful rinsing with saline solution, the intrabony defect will be filled with Bio-Oss® Collagen, level with the buccal bone crest, in the test group. In the control group, intrabony defects will not be treated with any other materials. Finally, a vertical mattress suture will be performed to close the wound. Periapical films of the defect associated site will be taken immediately after the surgery. If the tooth appears to have 2 to 3° mobility after the surgery, the tooth will be promptly splinted. The patients will be required to rinse with a 0.2% chlorhexidine solution for one week, and the suture will be removed one week after surgery.

The surgeries will be performed at the Periodontology Department, Peking University School and Hospital of Stomatology. An experienced periodontist with the help of an operating microscope will perform all operations.

Examination

At baseline, all the enrolled subjects will receive a periodontal examination by two experienced research professionals who have passed the inter-examiner agreement exam. Relative periodontal indexes will include full-mouth plaque score,[28] clinical attachment loss of the involved teeth,[29] full-mouth bleeding score,[28] gingival recession of the involved teeth,[14] mobility of the involved teeth,[28] probing depth of the involved teeth,[29] height of the gingival papilla,[29] periapical films and cone beam computed tomography of the interdental site.[28] During the surgery, the defect anatomy, including the depth and number of walls of the intrabony defects, will be examined by the operator. The patients will receive a phone-call for

re-examinations at 1 week and 1, 3, 6, 9, and 12 months post-surgery. At 1, 3, 6, 9, and 12 months post-surgery, a full-mouth plaque score and gingival papilla height will be recorded by the same calibrated examiners. At 12 months post-treatment, a full-mouth plaque score, clinical attachment gain of the involved teeth, gingival recession, mobility, full-mouth bleeding score, gingival papilla height, probing depth of the involved teeth will be examined by the same examiners. Periapical films and cone beam computed tomography of the defect associated site will be taken 6 and 12 months post-surgery. In addition, subjects will finish a VAS to evaluate the discomfort after 1 week of treatment. During the follow-up, complications will be recorded and treated once they happen. At re-evaluation, if the oral hygiene is deficient, we will clean the teeth by supragingival scaling and reinforcing oral hygiene instruction. All the data will be recorded in the periodontal examination charts and be registered and stored in the computer. There is no data monitoring committee in our hospital, so to ensure the correction and integrity of data, two different researchers will take charge of the management and storage of data.

Sample size

The needed number of participants was calculated according to the formula:

$$n1 = n2 = 2\left[\frac{\sigma(Z_{\alpha/2} + Z_{\beta})}{\delta}\right]^2$$

On the basis of previous literature,[17,35] the difference of clinical attachment gain using Bio-Oss® Collagen or not (δ) was about 1.5 mm and the standard deviation (σ) was about 1.4 mm. The power of test (β) is set as 10% and the inspection level (α) is set as 0.05. After calculation, 18 subjects will be needed for each group. Suppose that the rate of subjects lost

during follow up is around 10%, 20 subjects will be needed for each group. Ultimately, at least 40 subjects will be needed in all.

Statistical analysis

One patient will be identified as a data unit. If two or more intrabony defects go through the surgery in one patient, only one defect nearest to the midline will be enrolled.[36] The paired samples *t*-test will be applied to detect any difference between baseline and one year registrations. A general linear model will be performed to study the relationship between the defect depth, number of bony walls, full-mouth plaque score and full-mouth bleeding score with clinical attachment gain, probing depth reduction and radiographic intrabony defect depth change. The level of statistically significant difference will be set at 0.05. Statistical analysis will be done using SPSS version 26.0 software.

Withdrawal

Participants will be told that they can quit the research at any time. The withdrawal will not influence their receiving help from departmental periodontists in the future.

Dissemination of data

Data from the present research will be registered with the International Clinical Trials Registry Platform. Additionally, we will disseminate the results through scientific dental

journals.

DISCUSSION

For an isolated intrabony defect, M-MIST could be an effective treatment method, with an average clinical attachment gain of 4.8 mm at 1 year post-surgery.[17] It allows access to the root surface with only the buccal flap opening, which is minimally invasive and further enhances wound stability. However, when bioactive materials, such as EMD and rhPDGF-BB, were used combined with M-MIST, periodontal regenerative effects were not better.[37,38] EMD might not be an ideal bioactive material in dealing with wide defects.[39] Bio-Oss® Collagen is a bovine derived xenograft containing profuse collagen that can fit into different types of defects.[33] The present research is designed to study the effectiveness and potential applicability of M-MIST combined with Bio-Oss® Collagen for tissue regeneration of periodontal intrabony defects. If the results of M-MIST combined with Bio-Oss® Collagen turned out to be better than those of M-MIST alone in terms of radiographic and clinical defect improvement, Bio-Oss® Collagen might be considered as a combined application with M-MIST for periodontal tissue regeneration of intrabony defects.

TRIAL STATUS

The trial protocol received ethics approval in March 2020 and was registered at the International Clinical Trials Registry Platform (ID: ChiCTR-2000030851) on March 15, 2020. The trial will begin after the COVID-19 pandemic has been controlled; it is scheduled

to be completed in October, 2021.

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Contributors

H-DZ, K-NL, C-RZ and J-XH proposed the concept of this work. C-RZ, K-NL and J-XH designed the trial. C-RZ and H-DZ drafted the document. Z-GY, L-LM and YH revised the part on randomisation and calculation of the sample size. K-NL and J-XH reviewed and finalised the manuscript. All authors agreed to the final version.

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Competing interests

The authors declare that there is no conflict of interest

Patient and public involvement

Neither patients nor the public were associated with either the design or dissemination scheme of this work.

Patient consent for publication

Not required.

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Figure 1. Framework of the trial and CONSORT flow chart

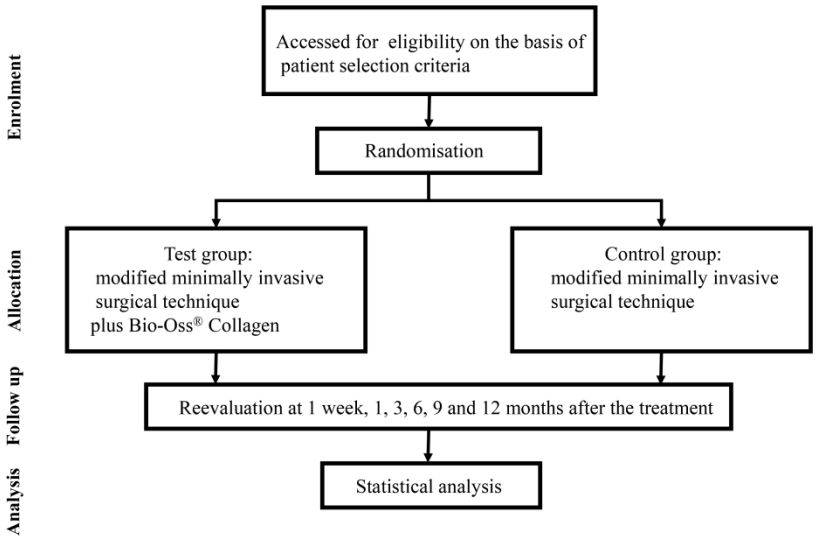

Figure 1. Framework of the trial and CONSORT flow chart

The stages of periodontitis

doi: 10.1002/JPER.18-0006

TABLE 3 Periodontitis stage – Please see text and appendix A (in online *Journal of Periodontology*) for explanation

Periodontitis stage		Stage I	Stage II	Stage III	Stage IV
Severity	Interdental CAL at site of greatest loss	1 to 2 mm	3 to 4 mm	≥5 mm	≥5 mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (15% to 33%)	Extending to mid-third of root and beyond	Extending to mid-third of root and beyond
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local	Maximum probing depth ≤4 mm Mostly horizontal bone loss	Maximum probing depth ≤5 mm Mostly horizontal bone loss	In addition to stage II complexity: Probing depth ≥6 mm Vertical bone loss ≥3 mm Furcation involvement Class II or III Moderate ridge defect	In addition to stage III complexity: Need for complex rehabilitation due to: Masticatory dysfunction Secondary occlusal trauma (tooth mobility degree ≥2) Severe ridge defect Bite collapse, drifting, flaring Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	Add to stage as descriptor	For each stage, describe extent as localized (<30% of teeth involved), generalized, or molar/incisor pattern			

The initial stage should be determined using CAL; if not available then RBL should be used. Information on tooth loss that can be attributed primarily to periodontitis – if available – may modify stage definition. This is the case even in the absence of complexity factors. Complexity factors may shift the stage to a higher level, for example furcation II or III would shift to either stage III or IV irrespective of CAL. The distinction between stage III and stage IV is primarily based on complexity factors. For example, a high level of tooth mobility and/or posterior bite collapse would indicate a stage IV diagnosis. For any given case only some, not all, complexity factors may be present, however, in general it only takes one complexity factor to shift the diagnosis to a higher stage. It should be emphasized that these case definitions are guidelines that should be applied using sound clinical judgment to arrive at the most appropriate clinical diagnosis.

For post-treatment patients CAL and RBL are still the primary stage determinants. If a stage-shifting complexity factor(s) is eliminated by treatment, the stage should not retrogress to a lower stage since the original stage complexity factor should always be considered in maintenance phase management.

CAL = clinical attachment loss; RBL = radiographic bone loss.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions,and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5,6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7-10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7,8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	7
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Modified minimally invasive surgical technique plus Bio-Oss® Collagen for regenerative therapy of isolated interdental intrabony defects: study protocol for a randomised controlled trial

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Modified minimally invasive surgical technique plus Bio-Oss® Collagen for regenerative therapy of isolated interdental intrabony defects: study protocol for a randomised controlled trial

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Abstract

Introduction

Periodontal regeneration surgery has been widely used to deal with intrabony defects. Modified minimally invasive surgical technique (M-MIST) is designed to deal with isolated

interdental intrabony defects, having achieved satisfactory periodontal regenerative effect. Bio-Oss® Collagen, as a bioactive material, has been applied to periodontal regeneration. It is similar to human cancellous bone, with the ability to promote bone formation; further, it has exceptional plasticity and spatial stability. The combination of different materials and techniques has become a research hotspot in recent years. By combining the superiority of regeneration technology and materials, better regenerative effect can be achieved. This study will search for differences between M-MIST combined with Bio-Oss® Collagen and M-MIST exclusively in regeneration therapy for intrabony defects.

Methods and Analysis: The present research is designed as a two group parallel randomised controlled trial. The total number of patients is 40. The patients will be randomly assigned to two groups, 20 participants in each group, for further periodontal regenerative surgery. Test group: M-MIST plus Bio-Oss® Collagen. Control group: M-MIST. After 12 months, the measurement indices will be recorded; these will include clinical attachment gain and radiographic intrabony defect depth change, as the primary results, and secondary outcomes of full-mouth plaque scores, probing depth, full-mouth bleeding scores, gingival recession, mobility, gingival papilla height, and visual analogue scale (VAS). The paired samples *t*-test will be applied to detect any difference between baseline and one year registrations. A general linear model will be performed to study the relationship between the second and the primary outcome.

Ethics and Dissemination: The present research has received approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002). Data of the present research will be registered with the International Clinical Trials Registry Platform. Additionally, we will disseminate the results

through scientific dental journals.

Trial registration number: ChiCTR-2000030851.

Protocol version: Protocol Version 4, 07.14.2020.

Strengths and limitations of this study:

This trial is designed as a randomised, examiner-blind clinical trial.

The trial will be the first clinical study to compare the periodontal regenerative effect of M-MIST plus Bio-Oss® Collagen and M-MIST exclusively in isolated interdental intrabony defects.

The follow-up in the trial will last for one year.

The outcome of this study will not be applied to patients with systemic disease.

Key words: Intrabony defect; periodontal regenerative therapy; Bio-Oss® Collagen; modified minimally invasive surgical technique.

INTRODUCTION

Periodontal disease is an inflammatory disease, with a high incidence around the teeth. It can lead to loss of periodontal attachment and alveolar bone, and even to tooth loss.[1,2] Periodontal disease is caused by multiple factors, of which the initial factor is the interaction between the biofilm and the immune response.[3] Periodontal pathogens play a key role in the pathogenesis and development of periodontal and systemic diseases, including diabetes and cardiovascular diseases, which can influence the development of periodontal disease.[4] It is reported that periodontitis is one of the most universal diseases around the world.[5] Scaling and root

planning (SRP) is the essential therapy for every patient.[6] However, for teeth with deep periodontal pockets, SRP cannot not achieve a more therapeutic effect than surgery.[7] After periodontal initial therapy, the residual deep pockets have often been associated with intrabony defects, identified as a clinical challenge.[8] The intrabony defects may result in unmanageable inflammation, and ultimately to tooth loss.[9] Therefore, intrabony defects are generally regarded as surgical indications.[10]

Periodontal regeneration surgery has been widely applied to deal with intrabony defects with the purpose of reforming the periodontal attachment in hopes of saving the involved teeth.[11-14] In the past 20 years, the development of periodontal regeneration therapy has been reflected mainly in two aspects. First, surgical design and techniques have been studied, especially minimally invasive surgery.[15-20] Second, considerable progress has been made in regenerative materials, such as enamel matrix derivative (EMD),[18] demineralized freeze-dried bone allograft (DFDBA),[19] recombinant human platelet-derived growth factor BB (rhPDGF-BB),[20] and spongy bone with collagen (Bio-Oss® Collagen).[21] These methods offer more advantages than simple flap debridement in increasing clinical attachment and decreasing probing depth of the affected teeth.[22,23] However, common complications, such as the exposure of barrier membrane and embedded material, have contributed to poor clinical results of periodontal regeneration surgery.[24]

To solve this problem, many periodontal surgical designs and techniques have been proposed and continually improved on.[25-28] As early as in 1995, Harrel and Ress[25] suggested applying minimally invasive surgery to periodontal surgical treatment, whose key points were small incision, small flap, and reduction of damage to soft and hard tissues. In 1995 and 1999, Cortellini[26,27] proposed the papilla preservation techniques to preserve interdental soft

tissues as completely as possible and isolate the operative area from the oral environment. On this basis, to further improve the surgical effect, the concept of minimally invasive surgery was proposed and gradually improved upon. In 2007, Cortellini and Tonetti[28] proposed a minimally invasive surgical technique (MIST) to deal with periodontal intrabony defects for more periodontal tissue regeneration. This technique was designed to reduce surgical trauma, operative time, and postoperative discomfort.[28] A number of clinical studies[16,29] have confirmed its effectiveness and advantages of the technique. In 2009, Cortellini and Tonetti[17] further designed a modified minimally invasive surgical technique (M-MIST). This method only opened the tiny buccal flap to ensure adequate blood supply, tighter primary wound closure and lower risk of bacterial infection.

In recent years, the combination of different materials and techniques has become a research hotspot.[30] By combining the advantages of regeneration technology and materials, better regenerative results can be achieved.[31] M-MIST only opens a small flap on the buccal side to achieve minimal interdental passage, saving the palatal soft tissue.[17] Bio-Oss® Collagen is similar to human cancellous bone, with the ability to promote bone formation.[32] Bio-Oss® Collagen, with outstanding plasticity and spatial stability,[33] is suitable for the small field of vision resulting from M-MIST. Therefore, we plan to research the therapeutic effect of M-MIST combined with Bio-Oss® Collagen and the use of M-MIST exclusively for periodontal tissue regeneration of isolated interdental intrabony defects. The presumption was raised that combining M-MIST and Bio-Oss® Collagen would lead to a better result in periodontally regenerating intrabony defects than M-MIST alone. The practicability, applicability and extensibility of the combination will be investigated in the present study. The primary outcomes were clinical attachment gain and radiographic bone-filling the

intrabony defect, which would be recorded by clinical examination, periapical radiographs and cone beam computed tomography. The clinical attachment gain and bone filling represent periodontal tissue regeneration.[17] The secondary outcomes were probing depth, full-mouth plaque scores, full-mouth bleeding scores, gingival recession, mobility, height of the gingival papilla, and visual analogue scale (VAS). These indices represent the inflammatory characteristics of periodontal tissue.[28] The present article describes the design of the trial.

METHODS AND ANALYSIS

This research is a randomised controlled trial with two parallel groups. It will be carried out according to the basis of the World Medical Association Declaration of Helsinki. Patients with stage Ⅲ or Ⅳ periodontitis,[34] (details in the supplemental material 1) needing periodontal regenerative treatment for isolated intrabony defects are the potential subjects. This study will be undertaken at Peking University School and Hospital of Stomatology (Beijing, China). The present research has received approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002), and it is registered with the International Clinical Trials Registry Platform (ID: ChiCTR-2000030851). The research framework is shown in Figure 1.

Participant selection

All participants will come from the Periodontology Department at Peking University School

and Hospital of Stomatology. At re-evaluation after periodontal initial therapy, if the subject is found to have isolated intrabony defects, he or she will be informed about the study. The potential subjects will receive information about the research. The subjects will be incorporated into this trial only after their signature has been obtained. (informed consent in the supplemental material 2) The personal information of the consent form will be confidentially stored in our department.

As regards the patient characteristics, the following criteria should be met: (1) age 18 to 75 years; (2) both genders will be considered for selection in the study; (3) patients with stage Ⅲ or Ⅳ periodontitis at least two months after periodontal initial therapy; (4) good compliance; (5) good oral hygiene; (6) full-mouth plaque score and full-mouth bleeding score each less than 30%; (7) systemically healthy. The intrabony defect should be an isolated intrabony defect of more than 3 mm in depth, combined with more than a 5 mm probing depth and attachment loss. In addition, the intrabony defect should not exceed in area the lingual surface area of the root. The morphology of the intrabony defect will be detected during the operation and finally determined whether the patient would be enrolled in the trial. The associated tooth should either maintain normal pulp vitality or it should have undergone root canal therapy for at least six months. The intrabony defect existing around the anterior teeth, the premolars or at the mesial side of mandibular first molars will be included to avoid the influence of furcation involvement. Patients with tumours, systemic diseases, or a history of receiving antibiotics in the past three months will be excluded. The affected teeth with 3° mobility, furcation involvement, acute periapical inflammation, or root fractures will not be enrolled.

Randomisation and blinding

The random sequence is produced through a random number table and the assignment is saved in a sealed envelope. A research worker unaware of the trial process will be in charge of the randomisation. All subjects will be randomly assigned to two groups. Test group: M-MIST combined with Bio-Oss® Collagen. Control group: M-MIST. All surgical operations will be performed by an experienced therapist in the dark of the assignment. Another two members in the dark of the research plan will respectively take charge of the clinical examination and statistical analysis.

Interventions

The enrolled participants will receive a periodontal clinical examination. They will have periapical radiographs and cone beam computed tomography taken. The surgical process will be performed as described in the literature[17]: if the width of the top of the gingival papilla is 2 mm or narrower, the simplified papilla preservation flap[27] will be performed; if it is wider than 2 mm, the modified papilla preservation technique[26] will be used. Then the incision will extend along the gingival sulcus on the buccal side of the two adjacent teeth, and a buccal flap will be reflected to expose 1 to 2 mm of buccal bone crest. With miniature blades and mini curettes, the granulation tissue will be curetted and the roots will be carefully planned with curettes. If the depth of the intrabony defect is 3 mm or more and the defect does not contain a lingual intrabony component, the site will be finally enrolled for further examination and statistical analysis. If the intrabony defects do not meet the inclusion criteria, the surgery will be continued, but such defects will not be enrolled for further

examination or statistical analysis. After thorough debridement and careful rinsing with saline solution, the intrabony defect will be filled with Bio-Oss® Collagen, level with the buccal bone crest, in the test group. In the control group, intrabony defects will not be treated with any other materials. Finally, a vertical mattress suture will be performed to close the wound. Periapical films of the defect associated site will be taken immediately after the surgery. If the tooth appears to have 2 to 3° mobility after the surgery, the tooth will be promptly splinted. The patients will be required to rinse with a 0.2% chlorhexidine solution for one week, and the suture will be removed one week after surgery.

The surgeries will be performed at the Periodontology Department, Peking University School and Hospital of Stomatology. An experienced periodontist with the help of an operating microscope will perform all operations.

Examination

At baseline, all the enrolled subjects will receive a periodontal examination by two experienced research professionals who have passed the inter-examiner agreement exam. Relative periodontal indexes will include full-mouth plaque score,[28] clinical attachment loss of the involved teeth,[29] full-mouth bleeding score,[28] gingival recession of the involved teeth,[14] mobility of the involved teeth,[28] probing depth of the involved teeth,[29] height of the gingival papilla,[29] periapical films and cone beam computed tomography of the interdental site.[28] During the surgery, the defect anatomy, including the depth and number of walls of the intrabony defects, will be examined by the operator. The patients will receive a phone-call for re-examinations at 1 week and 1, 3, 6, 9, and 12 months post-surgery. At 1, 3, 6, 9, and 12

months post-surgery, a full-mouth plaque score and gingival papilla height will be recorded by the same calibrated examiners. At 12 months post-treatment, a full-mouth plaque score, clinical attachment gain of the involved teeth, gingival recession, mobility, full-mouth bleeding score, gingival papilla height, probing depth of the involved teeth will be examined by the same examiners. Periapical films and cone beam computed tomography of the defect associated site will be taken 6 and 12 months post-surgery. In addition, subjects will finish a VAS to evaluate the discomfort after 1 week of treatment. During the follow-up, complications will be recorded and treated once they happen. At re-evaluation, if the oral hygiene is deficient, we will clean the teeth by supragingival scaling and reinforcing oral hygiene instruction. All the data will be recorded in the periodontal examination charts and be registered and stored in the computer. There is no data monitoring committee in our hospital, so to ensure the correction and integrity of data, two different researchers will take charge of the management and storage of data.

Sample size

The needed number of participants was calculated according to the formula:

$$n1 = n2 = 2\left[\frac{\sigma(Z_{\alpha/2} + Z_{\beta})}{\delta}\right]^2$$

On the basis of previous literature,[17,35] the difference of clinical attachment gain using Bio-Oss® Collagen or not (δ) was about 1.5 mm and the standard deviation (σ) was about 1.4 mm. The power of test (β) is set as 10% and the inspection level (α) is set as 0.05. After calculation, 18 subjects will be needed for each group. Suppose that the rate of subjects lost during follow up is around 10%, 20 subjects will be needed for each group. Ultimately, at

least 40 subjects will be needed in all.

Statistical analysis

One patient will be identified as a data unit. If two or more intrabony defects go through the surgery in one patient, only one defect nearest to the midline will be enrolled.[36] The paired samples *t*-test will be applied to detect any difference between baseline and one year registrations. A general linear model will be performed to study the relationship between the defect depth, number of bony walls, full-mouth plaque score and full-mouth bleeding score with clinical attachment gain, probing depth reduction and radiographic intrabony defect depth change. The level of statistically significant difference will be set at 0.05. Statistical analysis will be done using SPSS version 26.0 software.

Withdrawal

Participants will be told that they can quit the research at any time. The withdrawal will not influence their receiving help from departmental periodontists in the future.

Dissemination of data

Data from the present research will be registered with the International Clinical Trials Registry Platform. Additionally, we will disseminate the results through scientific dental journals.

DISCUSSION

For an isolated intrabony defect, M-MIST could be an effective treatment method, with an average clinical attachment gain of 4.8 mm at 1 year post-surgery.[17] It allows access to the root surface with only the buccal flap opening, which is minimally invasive and further enhances wound stability. However, when bioactive materials, such as EMD and rhPDGF-BB, were used combined with M-MIST, periodontal regenerative effects were not better.[37,38] EMD might not be an ideal bioactive material in dealing with wide defects.[39] Bio-Oss® Collagen is a bovine derived xenograft containing profuse collagen that can fit into different types of defects.[33] The present research is designed to study the effectiveness and potential applicability of M-MIST combined with Bio-Oss® Collagen for tissue regeneration of periodontal intrabony defects. If the results of M-MIST combined with Bio-Oss® Collagen turned out to be better than those of M-MIST alone in terms of radiographic and clinical defect improvement, Bio-Oss® Collagen might be considered as a combined application with M-MIST for periodontal tissue regeneration of intrabony defects.

Ethics and Dissemination:

The present research has received approval from the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202053002). The subjects will be incorporated into this trial only after their signature has been obtained. The study will be performed according to the 2013 revision of the Helsinki Declaration of 1975. Personal

information of all subjects will be stored in the Department of Periodontology at Peking University School and Hospital of Stomatology. Data of the present research will be registered with the International Clinical Trials Registry Platform. Additionally, we will disseminate the results through scientific journals.

Trial status

The trial protocol received ethics approval in March 2020 and was registered at the International Clinical Trials Registry Platform (ID: ChiCTR-2000030851) on March 15, 2020. The trial will begin after the COVID-19 pandemic has been controlled; it is scheduled to be completed in October, 2021.

Acknowledgements

Not applicable

Contributors

H-DZ, K-NL, C-RZ and J-XH proposed the concept of this work. C-RZ, K-NL and J-XH designed the trial. C-RZ and H-DZ drafted the document. Z-GY, L-LM and YH revised the part on randomisation and calculation of the sample size. K-NL and J-XH reviewed and finalised the manuscript. All authors agreed to the final version.

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Competing interests

The authors declare that there is no conflict of interest

Patient and public involvement

Neither patients nor the public were associated with either the design or dissemination scheme of this work.

Patient consent for publication

Not required.

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Figure 1. Framework of the trial and CONSORT flow chart

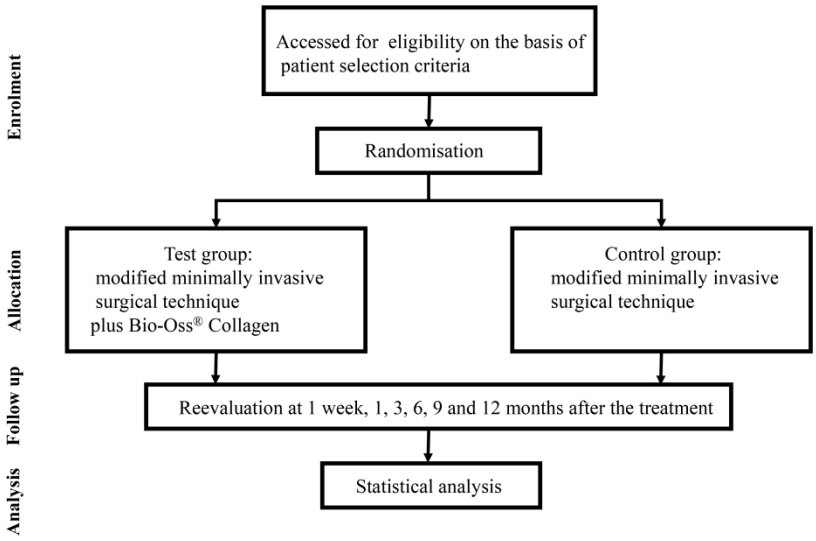

Figure 1. Framework of the trial and CONSORT flow chart

The stages of periodontitis

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TABLE 3 Periodontitis stage – Please see text and appendix A (in online *Journal of Periodontology*) for explanation

Periodontitis stage		Stage I	Stage II	Stage III	Stage IV
Severity	Interdental CAL at site of greatest loss	1 to 2 mm	3 to 4 mm	≥5 mm	≥5 mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (15% to 33%)	Extending to mid-third of root and beyond	Extending to mid-third of root and beyond
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local	Maximum probing depth ≤4 mm Mostly horizontal bone loss	Maximum probing depth ≤5 mm Mostly horizontal bone loss	In addition to stage II complexity: Probing depth ≥6 mm Vertical bone loss ≥3 mm Furcation involvement Class II or III Moderate ridge defect	In addition to stage III complexity: Need for complex rehabilitation due to: Masticatory dysfunction Secondary occlusal trauma (tooth mobility degree ≥2) Severe ridge defect Bite collapse, drifting, flaring Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	Add to stage as descriptor	For each stage, describe extent as localized (<30% of teeth involved), generalized, or molar/incisor pattern			

The initial stage should be determined using CAL; if not available then RBL should be used. Information on tooth loss that can be attributed primarily to periodontitis – if available – may modify stage definition. This is the case even in the absence of complexity factors. Complexity factors may shift the stage to a higher level, for example furcation II or III would shift to either stage III or IV irrespective of CAL. The distinction between stage III and stage IV is primarily based on complexity factors. For example, a high level of tooth mobility and/or posterior bite collapse would indicate a stage IV diagnosis. For any given case only some, not all, complexity factors may be present, however, in general it only takes one complexity factor to shift the diagnosis to a higher stage. It should be emphasized that these case definitions are guidelines that should be applied using sound clinical judgment to arrive at the most appropriate clinical diagnosis.

For post-treatment patients CAL and RBL are still the primary stage determinants. If a stage-shifting complexity factor(s) is eliminated by treatment, the stage should not retrogress to a lower stage since the original stage complexity factor should always be considered in maintenance phase management.

CAL = clinical attachment loss; RBL = radiographic bone loss.

Informed consent

Dear patients:

You will be invited to participate in a study led by Dr. Kaining Liu in the Department of Periodontology at Peking University School and Hospital of Stomatology. This study will observe the regenerative effect of modified minimally invasive surgical technique (M-MIST) combined with Bio-Oss® Collagen for isolated interdental intrabony defects. The study will last 12 months. The total number of patients is 40. Since you meet the inclusion criteria, you are invited to join this study.

This informed consent form provides you with important information to help you decide whether to participate in this study. Your participation in this study is voluntary. This study has been reviewed and approved by Ethics Committee of Peking University School and Hospital of Stomatology. If you agree to join this research, please read the following instructions:

Please read it carefully. If you have any questions, please consult the researcher in charge of the study.

Background:

A modified minimally invasive surgical technique (M-MIST) was proposed to be used in the periodontal regeneration therapy for isolated interdental intrabony defects in 2009. It has been used for more than 10 years, and there have been more than 100 cases reported in the literatures. This technique only elevates the buccal flap to ensure adequate blood supply, tighter primary wound closure and lower risk of bacterial infection. In addition, Bio-Oss® Collagen is similar to human cancellous bone, with the ability to promote bone formation. Bio-Oss® Collagen, with outstanding plasticity and spatial stability, is suitable for the small field of vision resulting from

M-MIST.

Recently, the combination of different materials and techniques has become a research hotspot. By combining the advantages of regeneration technology and materials, better regenerative results can be achieved. Therefore, we plan to research the therapeutic effect of M-MIST combined with Bio-Oss® Collagen and the use of M-MIST exclusively for periodontal tissue regeneration of isolated interdental intrabony defects.

Research plan:

You will be randomly assigned to one of the two groups. Test group: M-MIST combined with Bio-Oss® Collagen. Control group: M-MIST. All surgical operations will be performed by an experienced therapist. Before surgery, you will receive a periodontal clinical examination and have periapical radiographs and cone beam computed tomography taken. You will receive a phone-call for re-examinations at 1 week and 1, 3, 6, 9, and 12 months post-surgery. Periapical films and cone beam computed tomography of the defect associated site will be taken 6 and 12 months post-surgery. In addition, you will finish a VAS to evaluate the discomfort after 1 week of treatment.

All patients are randomly grouped according to the random sequence, and you will have an equal chance of being assigned to each group. Neither you nor the therapist can choose your treatment group.

The following criteria should be met:

(1) age 18 to 75 years;

(2) both genders will be considered for selection in the study;

(3) patients with stage III or IV periodontitis at least two months after periodontal initial

therapy;

(4) good compliance;

(5) good oral hygiene;

(6) full-mouth plaque score and full-mouth bleeding score each less than 30%;

(7) systemically healthy.

Your responsibilities:

The study will last for 12 months. You will receive a phone-call for re-examinations at 1 week and 1, 3, 6, 9, and 12 months post-surgery.

During the follow-up period, you also need to maintain good oral hygiene. The above items will not increase the number and time of your visits. Periodontal treatment has the characteristics of lasting life, so after the study, periodontal maintenance treatment will be offered for you.

The impact of participating in the study on your life:

You may feel that these visits and examinations are inconvenient. In addition, some examinations may make you feel uncomfortable. If you have any questions about the examinations and procedures in the study, you can consult the researchers.

During the entire study period, you can no longer participate in any other clinical trials related to drugs or medical devices.

Risks and adverse effects of participating in this study:

During the study, you may experience common discomfort after periodontal surgery (see the informed consent form for periodontal surgery for details). We will monitor all patients in the study for any adverse reactions. If you have any adverse reactions during the research process, please call your doctor for consultation in time, and we will perform treatment accordingly.

You need to tell your family or friends close to you that you are participating in a clinical study and they can pay attention to the events described above. If they have questions about your participation in the study, you can tell them how to contact your doctor.

Are there any other treatment options:

Although there is already evidence that the M-MIST is effective for treating isolated interdental intrabony defects, it is not guaranteed to be effective for you. The M-MIST + Bio-Oss® Collagen used in this study is not the only way to treat isolated interdental intrabony defects. You can also ask your doctor about other treatments you might get.

Expenses, compensation and remuneration for participating in this research association:

There is no compensation for this study. All examination and treatment costs are borne by the yourself.

We will arrange supportive periodontal therapy for you, as well as oral hygiene guidance and related consultations.

Confidentiality of your personal information:

If you decide to participate in this study, your participation in the experiment and your personal information in the experiment are confidential. Your name, ID number, address, phone number, or any information that can directly identify you in the research records will not be leaked outside the Peking University School and Hospital of Stomatology. We will use a unique number to represent your research information that is sent outside the Peking University School and Hospital of Stomatology. The coded information will be properly stored in Peking University School and Hospital of Stomatology.

At any time during the study, you can request access to your personal information (such as your

name and address), and modify this information if necessary.

withdraw

You can quit the research at any time. The withdrawal will not influence you further treatment in the Department of Periodontology.

Contact information

If you have any questions related to this research, or if you have any discomfort or injury during the research process, or have questions about the rights of participants in this research, you can contact Dr. Haidong Zhang, Office Tel: 010- 82195367; mobile phone: 13426305500. Or contact the Ethics Committee of Peking University School and Hospital of Stomatology, Tel: 010-82195759, Email: keyanchuethics@163.com

Competing interests

None

Subject's agreement statement:

I have read the above introduction about this research and fully understand the possible risks and benefits of participating in this research. I have voluntarily agreed to participate in the clinical research described in this article.

Name of the subject:_____

Signature date:_____ Phone number:_____

Researcher's statement:

I confirm that I have explained the details of this study to the patient, especially the possible risks and benefits of participating in this study.

Name of the researcher:_____

Signature date:_____ Phone number:_____

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions,and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,14
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5,6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7-10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7,8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	7
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	15
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	sup 2
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.