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Platelet-rich fibrin/anorganic bovine bone mineral complex as grafting materials in endodontic microsurgery with a large lesion size: study protocol for a randomised controlled trial

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ABSTRACT

Introduction: Endodontic microsurgery is a treatment of last resort for preserving natural teeth. According to radiographic evaluation, the percentage of complete healing after endodontic microsurgery is only 74.3%. The use of regenerative techniques in endodontic microsurgery for large lesions (>10 mm diameter) is therefore recommended. The most frequently used bone graft in endodontic microsurgery is anorganic bovine bone mineral (ABBM) but this only has an osteoconductive effect. Thus, when platelet-rich fibrin (PRF), a reservoir of growth factors, is used together with ABBM, it increases the regenerative effect. This study is devoted to comparing the clinical outcomes of PRF with/without ABBM as grafting biomaterials in endodontic microsurgery cases with large lesion size to provide some valuable reference data for dentists.

Methods and analysis: Sixteen patients who are in need of endodontic microsurgery will be recruited. The patients will be randomly assigned to one of two groups: an experimental group, treated with PRF/ABBM complex and collagen membrane, and a control group, treated with ABBM and collagen membrane. Clinical examination including percussion, mobility testing and presence/absence of sinus will be recorded at 7 days, and at 3, 6 and 12 months after endodontic microsurgery. A visual analogue scale (VAS) will be used by the patients to evaluate pain at 1, 3 and 7 days after endodontic microsurgery. Routine paralleling radiographs will be obtained before and at 3, 6 and 12 months follow-up after endodontic microsurgery. Cone-beam computed tomographic (CBCT) scans will be obtained at the 12-month follow-up. Bone formation will be evaluated according to CBCT and paralleling radiographs.

Ethics and dissemination: The present study received approval from the Ethics Committee of Peking University School and Hospital of Stomatology. Research data will be registered with the International Clinical Trials Registry Platform (ICTRP), ID: ChiCTR2100046684. The results will be disseminated through scientific journals.

Strengths and limitations of this study:

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

The trial will be the first clinical trial with a novel design to compare the clinical and

radiographic effects of PRF/ABBM complex and ABBM on large periapical lesions (>10 mm diameter) after endodontic microsurgery.

This study will collect longitudinal data on patients during the subsequent 12-month follow-up.

The patients with systemic disease will not be included in this research.

Keywords: Platelet-rich fibrin, Endodontic microsurgery, Large lesion size, Osteoinduction, Regeneration techniques

INTRODUCTION

With developments in equipment, instruments and biomaterials, endodontic microsurgery has become available as a treatment of last resort for cases which are no longer suitable for retreatment. [1] It is generally accepted that radiographic outcomes can be classified into complete healing, incomplete healing, uncertain healing and unsatisfactory healing. [2, 3] Complete bone repair is the ideal therapeutic outcome of endodontic microsurgery, however, the percentage of complete radiographic healing after endodontic microsurgery is only 74.3%. [4] Thus, it is imperative to promote healing of periapical bone defects more effectively. The results of endodontic microsurgery can be influenced by several factors, especially lesion size. During the healing process, epithelial cells repopulate the wound at the highest rate which results in scar formation. [5] Once complete osseous regeneration of a defect cannot occur, the defect will be filled by fibrous connective tissue. [6] It has been demonstrated that 26% of defects radiographically larger than 10 mm result in scar formation after endodontic microsurgery. [7] Thus, usage of grafting materials and membrane in cases with large lesion size (>10mm diameter) is recommended. [8-10]

There are four types of bone grafts: autogenous grafts, allogeneic grafts, xenogeneic grafts and alloplastic materials. Autogenous grafts and allogeneic grafts are osteoinductive materials, while xenogeneic grafts and alloplastic materials are osteoconductive materials due to their lack of growth factors. [11] It has been reported that a combination of osteoconductive materials (e.g. anorganic bovine bone mineral (ABBM)) and growth factors results in faster and better healing of bone in endodontic microsurgery than osteoconductive materials only. [12, 13]

PRF (platelet-rich fibrin) contains platelets, leukocytes and more than 100 types of growth factors including platelet-derived growth factor (PDGF), transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2), which promote the proliferation and differentiation of osteoblasts. [14] Moreover, the presence of leukocytes is helpful for their anti-infection and immunomodulatory effects. [15-17] Previously, PRF has been extensively used in dentistry, including the healing of extraction sockets, [18] ridge

preservation, [19] maxillary sinus augmentation and the regeneration of periodontal lesions. [20-24] It promotes the healing of soft tissue, and reduces postoperative pain, swelling, and the incidence of alveolar osteitis after the extraction of impacted mandibular third molars. [18] Compared with natural healing, horizontal and vertical dimension ridge preservation is more effective with the use of PRF. [19] The combination of PRF and deproteinized bovine bone mineral has been shown to increase bone formation in maxillary sinus augmentation compared with deproteinized bovine bone mineral alone. [20] Plenty of studies have indicated that combinations of PRF and bone grafting materials enhance periodontal regeneration in periodontal intrabony defects. [21-24] In a word, PRF has the potential for osseous regeneration and healing of soft tissue, especially when combined with various bone grafting materials. [25] It was also indicated that PRF reduces post-operative pain and infections due to the improvements in soft-tissue healing and the presence of microbial-fighting immune cells. [26] Hitherto, there have only been two case reports in which PRF was used together with osteoconductive bone grafts in periapical surgery in order to achieve a better healing outcome. [27, 28] With the exception of these two case reports, there has been no clinical research into the effects of PRF in periapical surgery.

We hypothesized that PRF combined with ABBM and collagen membrane is more effective than using only ABBM and collagen membrane in endodontic microsurgery. The hypothesis will be tested in the present double-masked randomised controlled clinical trial, the design of which is described below.

METHODS AND ANALYSIS

The study is a prospective, single-centre randomised controlled trial. It had been approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202059179). Research procedures including assessments, interventions and follow-ups will be carried out in Peking University School and Hospital of Stomatology (Beijing, China). This study has been registered with the registry of ICTRP (ID: ChiCTR2100046684). The main objective of this randomised

controlled clinical trial is to compare and evaluate the clinical effects of PRF with or without combination with ABBM in endodontic microsurgery cases with large lesion size (>10 mm diameter). The primary hypothesis is that healing of periapical lesions will be better in the PRF/ABBM complex group.

Inclusion criteria

Patients attending the Department of Cariology and Endodontology for routine planned endodontic microsurgery will be evaluated for inclusion in this clinical trial. The age of patients to be recruited will range from 18 to 65 years. Patients will have received periodontal treatment before endodontic microsurgery. Only single-rooted teeth will be included in the study, and the periapical lesions should be classified as large periapical lesions (>10mm diameter) according to cone-beam computed tomographic (CBCT) evaluation.

Exclusion criteria

1. Smokers;
2. Pregnant women;
3. Patients with systemic diseases;
4. Re-surgery;
5. Unqualified coronal restoration;
6. Teeth with deep periodontal pockets (probing depth ≥5mm).

Recruitment

Patients who are willing to participate in this study will be recruited from the Department of Cariology and Endodontology, Peking University School and Hospital of Stomatology. The necessity of endodontic microsurgery and collecting blood for PRF/ABBM complex will be explained to the participants. A signed informed consent form will be obtained and preserved confidentially in the cabinet. The procedures of this clinical trial are shown in Figure 1.

Groups, randomisation and blinding

An experienced endodontist will perform the examination, diagnosis and assessment procedures after clinical and radiographic examinations. The sequence and allocation

will be performed by a professor alone with opaque envelopes. The bone grafting material of the experimental group will be a PRF/ABBM composite, while the control group will receive ABBM only.

Interventions

All enrolled patients will be randomly divided into two groups after examination. The endodontic microsurgery will be performed by the same experienced endodontist using an endodontic microscope (F40, Leica Microsystems Ltd, Wetzlar, Germany). The procedures and principles of the endodontic microsurgeries will follow the guidelines proposed by Prof. Kim. [29] A vertical and a horizontal incision will be used to reflect the flap. Peri-radicular curettage will be performed after reaching the periapical lesion. After 3mm of the root apex is resected, retro-preparation and retro-filling with iRoot BP (Innovative BioCeramix Inc., Burnaby, BC, Canada) will be performed. In the experimental group, after peri-radicular curettage, whole blood (10 mL) will be collected and centrifuged at 960 rpm (Medical centrifuge Auto V1, JM Instrument, Beijing, China) for 2.8 minutes. The upper layer consisting of liquid PRF will be mixed with ABBM (Heal-all, ZH-Bio, Yantai, China) evenly. ABBM is a category of xenograft which has only an osteoconductive effect. The periapical defects will be filled with PRF/ABBM complex in the experimental group while the periapical defects will be filled with ABBM only in the control group. An absorbable collagen membrane (Heal-all, ZH-Bio, Yantai, China) will be applied in both groups, then the flap will be repositioned with 6-0 sutures. Amoxicillin and 0.2% chlorhexidine gluconate rinse will be prescribed to prevent postoperative infection. Sutures will be removed 5 days after the endodontic microsurgery.

Examination

Baseline examination

Before treatment, all participants will be examined carefully by a calibrated examiner. Clinical examination including percussion and mobility testing and presence/absence of sinus will be recorded simultaneously. Preoperative paralleling radiographs and CBCT scans will be obtained for each participant.

Examination during the follow-up period

Clinical examination including percussion, mobility testing and presence/absence of sinus will be performed at 7 days, 3 months, 6 months and 12 months after the endodontic microsurgery. In addition, a visual analogue scale (VAS) will be recorded at 1 day, 3 days and 7 days after the endodontic microsurgery for the evaluation of postoperative pain. Paralleling radiographs will be obtained at 3 months, 6 months and 12 months. CBCT scans will be performed at the 12-month follow-up. The grouping and the treatment plan will be confidential to the examiners. The primary parameter of the present clinical trial is radiographic bone regeneration in the area of the periapical defect. The paralleling radiographs and CBCT scans will be evaluated both before and after microsurgery. Bone regeneration in periapical osseous defects will be evaluated according to the radiopacity scoring scale of paralleling radiographs by three independent blinded and calibrated examiners at the 3-month, 6-month and 12-month follow-ups after the microsurgery and compared with the paralleling radiograph after microsurgery immediately. According to a previous study, [30] the change in the volume of the periapical defect is the evaluation index of the radiopacity scoring scale. CBCT data before and 12 months after microsurgery will be processed using medical image processing software (MIMICS, Materialise, Leuven, Belgium) to evaluate the changes of volume and density of the periapical defect. The data of the clinical trial will be input and kept in a specific computer and a locked cabinet by two designated member of staffs. The secondary parameters of this clinical trial include percussion, mobility testing and presence/absence of sinus. The VAS scores are also secondary parameters. All the data will be recorded and imputed in the computer simultaneously. Due to lack of data monitoring committee in our hospital, the data will be kept in the cabinet by two different researchers to make sure the accuracy and completeness of the data. An independent inspector will review the incoming data every 3 months. There will be no harms caused by the trial.

Sample size

The sample size of this clinical trial is determined by the following formula:

$$N1 = N2 = 2\left[\frac{\sigma(Z_{\alpha/2} + Z_{\beta})}{\delta}\right]^2$$

According to the data of a published clinical trial, which is about bone grafting in periapical osseous defects, [30] the σ/δ is around 0.49. The inspection level (α) is set to 0.05, and the power is set to 0.9. For bilateral tests, the required sample size in each group is six. Considering a missed follow-up rate of 20%, the sample size should be 7.2. Consequently 16 participants will be needed.

Statistical analysis

Data will be analysed using SPSS software (SPSS, Inc, Chicago, IL, USA). Statistical significance will be accepted for *P* values lower than 0.05. Normality and variance equality will be analysed using the Shapiro-Wilk test and the Levene variance homogeneity test, respectively. Normally-distributed data will be shown as mean ± standard deviation, while non-normally distributed data will be shown as median (lower to upper quartile). Student's *t*-test will be used to compare the difference between the two groups for the data with both normality and variance equality. Otherwise, the Mann-Whitney *U* test will be used.

Withdrawal

The patients have the right to withdraw from this clinical trial without any reason at any point during the treatment. Follow-up treatment will not be affected by the withdrawal.

Dissemination of results

The results of this clinical trial will be registered at the International Clinical Trials Registry Platform (ICTRP). In addition, the results will be published in a peer-reviewed journal.

DISCUSSION

When PRF is used together with ABBM, it increases the regenerative effect of osseous tissue, [31, 32] because ABBM only has an osteoconductive effect and osteoinduction is induced due to the growth factors in PRF. In a histological study, bone regenerative effects of PRF-TCP, rhBMP-2-coated TCP and TCP alone were compared, and the results showed that PRF-TCP resulted in more rapid bone healing compared to the other two groups. [12] The antibacterial and anti-inflammatory effects

of PRF also promoted tissue healing. [14] Although PRF has many advantages, traditional PRF is not liquid, and it is challenging to mix PRF and ABBM evenly. Thus, in previous studies, [33-35] the gelatinous PRF had to be minced and then mixed with ABBM, which is time-consuming. Exposing alveolar bone to air could result in bone resorption, and a long duration of surgery is not favourable. [36] Therefore, our group developed a new technique to obtain a type of PRF/ABBM complex and thus avoid the need to mince the PRF. [23, 24] Additionally, PRF/ABBM complex as a whole had better applicability than granular ABBM, and could significantly reduce the time required for bone grafting. [24] The complex together with a collagen membrane has been demonstrated to result in enhanced gains in attachment level compared to ABBM alone with a collagen membrane, indicating improved periodontal regeneration. [24]

To date there is still a lack of clinical trials investigating the osseous regenerative effect of PRF/bone grafting material composites in endodontic microsurgery. This randomised controlled trial is devoted to the evaluation of the clinical outcomes of the application of a PRF/ABBM complex as a grafting biomaterial in endodontic microsurgery cases with large lesion size, in the hope of providing some scientific evidence to support endodontic microsurgeries for dentists.

Ethics and dissemination

Ethical approval had been approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202059179). The data of the clinical trial will be input and kept in a specific computer and a locked cabinet by two designated member of staffs. The results will be published in a peer-reviewed journal. Research data will be registered with the International Clinical Trials Registry Platform (ICTRP), ID: ChiCTR2100046684.

Trial status

The trial has been registered at the International Clinical Trials Registry Platform (ICTRP). The identifier number is ChiCTR2100046684. Recruitment will begin in June 2021 and will end in June 2023.

Contributors

The study concept was proposed by ZW, KL, ZC and BH. The manuscript was drafted by BH and KL. CZ and ZZ revised the part on randomisation. YW and YL calculated the sample size. ZW, XW and KL revised the manuscript finally. All authors have agreed with the final version of the manuscript.

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Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer-reviewed.

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Figure Legend:

Figure 1 The flow chart of this clinical research

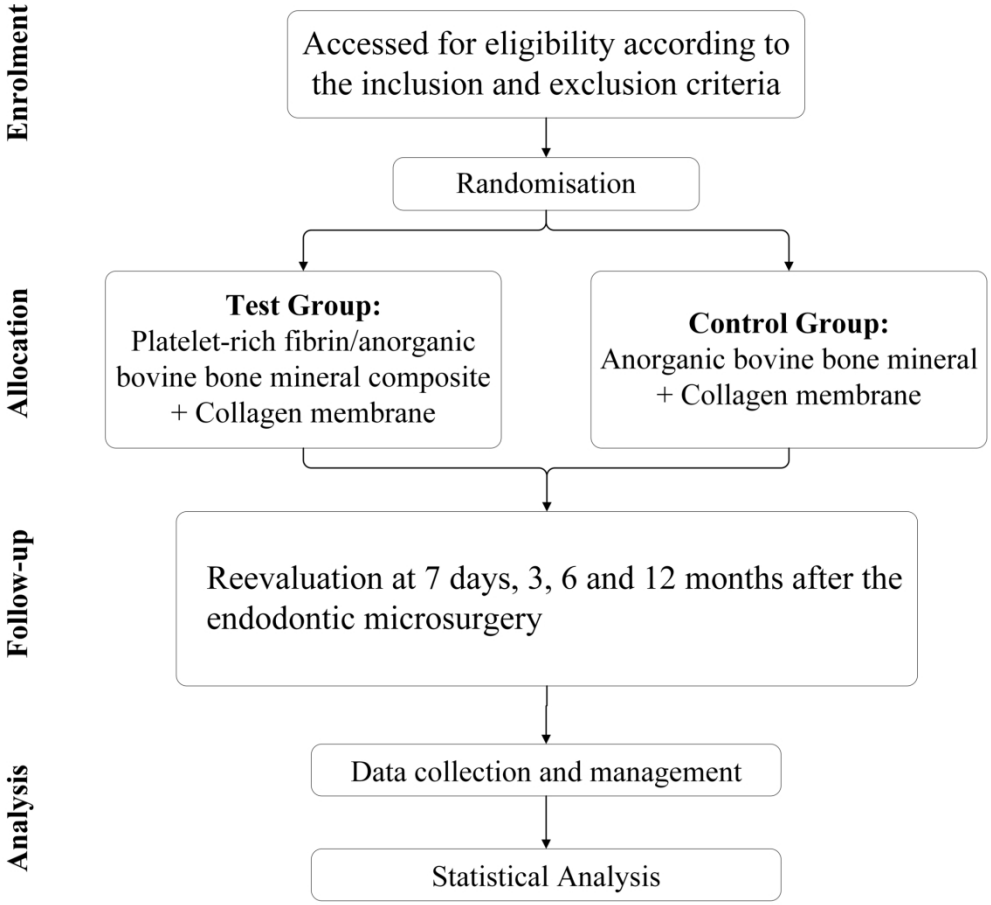

Figure 1 The flow chart of this clinical research

189x175mm (300 x 300 DPI)

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	11
Funding	4	Sources and types of financial, material, and other Support	12
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2, 11
	5b	Name and contact information for the trial sponsor	12
	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	N/A
	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)	N/A
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	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)	6
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	6, 7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, ligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	8, 9
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, 8, 9
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	9, 10
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	7, 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	7, 8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7, 8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8, 9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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	8, 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, 10
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	9
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	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A

	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)	N/A
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	9
	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	N/A
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	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	11
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)	N/A
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	N/A
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
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	11

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	N/A
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	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Additional file
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	N/A

*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

Platelet-rich fibrin/anorganic bovine bone mineral complex as grafting materials in endodontic microsurgery with a large lesion size: study protocol for a randomised controlled trial

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Platelet-rich fibrin/anorganic bovine bone mineral complex as grafting materials in endodontic microsurgery with a large lesion size: study protocol for a randomised controlled trial

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ABSTRACT

Introduction: Endodontic microsurgery is a treatment of last resort for preserving natural teeth. According to radiographic evaluation, the percentage of complete healing after endodontic microsurgery is only 74.3%. The use of regenerative techniques in endodontic microsurgery for large lesions (>10 mm diameter) is therefore recommended. The most frequently used bone graft in endodontic microsurgery is anorganic bovine bone mineral (ABBM) but this only has an osteoconductive effect. Thus, when platelet-rich fibrin (PRF), a reservoir of growth factors, is used together with ABBM, it increases the regenerative effect. This study is devoted to comparing the clinical outcomes of PRF with/without ABBM as grafting biomaterials in endodontic microsurgery cases with large lesion size to provide some valuable reference data for dentists.

Methods and analysis: Sixteen patients who are in need of endodontic microsurgery will be recruited. The patients will be randomly assigned to one of two groups: an experimental group, treated with PRF/ABBM complex and collagen membrane, and a control group, treated with ABBM and collagen membrane. Clinical examination including percussion, mobility testing and presence/absence of sinus will be recorded at 7 days, and at 3, 6 and 12 months after endodontic microsurgery. A visual analogue scale (VAS) will be used by the patients to evaluate pain at 1, 3 and 7 days after endodontic microsurgery. Routine paralleling radiographs will be obtained before and at 3, 6 and 12 months follow-up after endodontic microsurgery. Cone-beam computed tomographic (CBCT) scans will be obtained at the 12-month follow-up. Bone formation will be evaluated according to CBCT and paralleling radiographs. The study execute time including follow-ups last from June 1st 2021 to December 31st 2024.

Ethics and dissemination: The present study received approval from the Ethics Committee of Peking University School and Hospital of Stomatology. Research data will be registered with the International Clinical Trials Registry Platform (ICTRP), ID: ChiCTR2100046684. The results will be disseminated through scientific journals.

Strengths and limitations of this study:

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

The trial will be the first clinical trial with a novel design to compare the clinical and radiographic effects of PRF/ABBM complex and ABBM on large periapical lesions (>10 mm diameter) after endodontic microsurgery.

This study will collect longitudinal data on patients during the subsequent 12-month follow-up.

The patients with systemic disease will not be included in this research.

Keywords: Platelet-rich fibrin, Endodontic microsurgery, Large lesion size, Osteoinduction, Regeneration techniques

INTRODUCTION

With developments in equipment, instruments and biomaterials, endodontic microsurgery has become available as a treatment of last resort for cases which are no longer suitable for retreatment. [1] It is generally accepted that radiographic outcomes can be classified into complete healing, incomplete healing, uncertain healing and unsatisfactory healing. [2, 3] Complete bone repair is the ideal therapeutic outcome of endodontic microsurgery, however, the percentage of complete radiographic healing after endodontic microsurgery is only 74.3%. [4] Thus, it is imperative to promote healing of periapical bone defects more effectively. The results of endodontic microsurgery can be influenced by several factors, especially lesion size [5, 6]. During the healing process, epithelial cells repopulate the wound at the highest rate which results in scar formation. [7] Once complete osseous regeneration of a defect cannot occur, the defect will be filled by fibrous connective tissue. [8] It has been demonstrated that 26% of defects radiographically larger than 10 mm result in scar formation after endodontic microsurgery. [9] Thus, usage of grafting materials and membrane in cases with large lesion size (>10mm diameter) is recommended. [5, 6, 10]

There are four types of bone grafts: autogenous grafts, allogeneic grafts, xenogeneic grafts and alloplastic materials. Autogenous grafts and allogeneic grafts are osteoinductive materials, while xenogeneic grafts and alloplastic materials are osteoconductive materials due to their lack of growth factors. [11] It has been reported that a combination of osteoconductive materials (e.g. anorganic bovine bone mineral (ABBM)) and growth factors results in faster and better healing of bone in endodontic microsurgery than osteoconductive materials only. [12, 13]

PRF (platelet-rich fibrin) contains platelets, leukocytes and more than 100 types of growth factors including platelet-derived growth factor (PDGF), transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2), which promote the proliferation and differentiation of osteoblasts. [14] Moreover, the presence of leukocytes is helpful for their anti-infection and immunomodulatory effects. [15-17] Previously, PRF has been extensively used in dentistry, including the healing of extraction sockets, [18] ridge

preservation, [19] maxillary sinus augmentation and the regeneration of periodontal lesions. [20-24] It promotes the healing of soft tissue, and reduces postoperative pain, swelling, and the incidence of alveolar osteitis after the extraction of impacted mandibular third molars. [18] Compared with natural healing, horizontal and vertical dimension ridge preservation is more effective with the use of PRF. [19] The combination of PRF and deproteinized bovine bone mineral has been shown to increase bone formation in maxillary sinus augmentation compared with deproteinized bovine bone mineral alone. [20] Plenty of studies have indicated that combinations of PRF and bone grafting materials enhance periodontal regeneration in periodontal intrabony defects. [21-24] In a word, PRF has the potential for osseous regeneration and healing of soft tissue, especially when combined with various bone grafting materials. [25] It was also indicated that PRF reduces post-operative pain and infections due to the improvements in soft-tissue healing and the presence of microbial-fighting immune cells. [26] Hitherto, there have only been two case reports in which PRF was used together with osteoconductive bone grafts in periapical surgery in order to achieve a better healing outcome. [27, 28] With the exception of these two case reports, there has been no clinical research into the effects of PRF in periapical surgery.

We hypothesized that PRF combined with ABBM and collagen membrane is more effective than using only ABBM and collagen membrane in endodontic microsurgery. The hypothesis will be tested in the present double-masked randomised controlled clinical trial, the design of which is described below.

METHODS AND ANALYSIS

The study is a prospective, single-centre randomised controlled trial. It had been approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202059179). Research procedures including assessments, interventions and follow-ups will be carried out in Peking University School and Hospital of Stomatology (Beijing, China). This study has been registered with the registry of ICTRP (ID: ChiCTR2100046684). The main objective of this randomised

controlled clinical trial is to compare and evaluate the clinical effects of PRF with or without combination with ABBM in endodontic microsurgery cases with large lesion size (>10 mm diameter). The primary hypothesis is that healing of periapical lesions will be better in the PRF/ABBM complex group.

Inclusion criteria

Patients attending the Department of Cariology and Endodontology for routine planned endodontic microsurgery will be evaluated for inclusion in this clinical trial. The age of patients to be recruited will range from 18 to 65 years. Patients will have received periodontal treatment before endodontic microsurgery. Only single-rooted teeth will be included in the study, and the periapical lesions should be classified as large periapical lesions (>10mm diameter) according to cone-beam computed tomographic (CBCT) evaluation.

Exclusion criteria

1. Smokers;
2. Pregnant women;
3. Patients with systemic diseases;
4. Re-surgery;
5. Unqualified coronal restoration;
6. Teeth with deep periodontal pockets (probing depth ≥5mm).

Recruitment

Patients who are willing to participate in this study will be recruited from the Department of Cariology and Endodontology, Peking University School and Hospital of Stomatology. The necessity of endodontic microsurgery and collecting blood for PRF/ABBM complex will be explained to the participants. A signed informed consent form will be obtained and preserved confidentially in the cabinet. The procedures of this clinical trial are shown in Figure 1.

Groups, randomisation and blinding

An experienced endodontist will perform the examination, diagnosis and assessment procedures after clinical and radiographic examinations. The sequence and allocation

will be performed by a professor alone with opaque envelopes. The bone grafting material of the experimental group will be a PRF/ABBM composite, while the control group will receive ABBM only.

Interventions

All enrolled patients will be randomly divided into two groups after examination. The endodontic microsurgery will be performed by the same experienced endodontist using an endodontic microscope (F40, Leica Microsystems Ltd, Wetzlar, Germany). The procedures and principles of the endodontic microsurgeries will follow the guidelines. [29] A vertical and a horizontal incision will be used to reflect the flap. Peri-radicular curettage will be performed after reaching the periapical lesion. After 3mm of the root apex is resected, retro-preparation and retro-filling with iRoot BP (Innovative BioCeramix Inc., Burnaby, BC, Canada) will be performed. In the experimental group, after peri-radicular curettage, whole blood (10 mL) will be collected in sterile glass-coated centrifugal tube without any anticoagulant and centrifuged at 960 rpm (Medical centrifuge Auto V1, JM Instrument, Beijing, China) for 2.8 minutes. Then the buffy coat layer, the plasma layer, and 1 to 1.5 mm of the red blood cell layer below the buffy coat layer will be harvested as the liquid PRF. Finally, ABBM (Heal-all, ZH-Bio, Yantai, China) will be mixed with the liquid PRF evenly. ABBM is a category of xenograft which has only an osteoconductive effect. The periapical defects will be filled with PRF/ABBM complex in the experimental group while the periapical defects will be filled with ABBM only in the control group. An absorbable collagen membrane (Heal-all, ZH-Bio, Yantai, China) will be applied in both groups, then the flap will be repositioned with 6-0 sutures. Amoxicillin and 0.2% chlorhexidine gluconate rinse will be prescribed to prevent postoperative infection. Sutures will be removed 5 days after the endodontic microsurgery.

Examination

Baseline examination

Before treatment, all participants will be examined carefully by a calibrated examiner. Clinical examination including percussion and mobility testing and presence/absence of sinus will be recorded simultaneously. Preoperative paralleling radiographs and

CBCT scans will be obtained for each participant.

Examination during the follow-up period

Clinical examination including percussion, mobility testing and presence/absence of sinus will be performed at 7 days, 3 months, 6 months and 12 months after the endodontic microsurgery. In addition, a visual analogue scale (VAS) will be recorded at 1 day, 3 days and 7 days after the endodontic microsurgery for the evaluation of postoperative pain. Paralleling radiographs will be obtained at 3 months, 6 months and 12 months. CBCT scans will be performed at the 12-month follow-up. The grouping and the treatment plan will be confidential to the examiners. The primary parameter of the present clinical trial is radiographic bone regeneration in the area of the periapical defect. The paralleling radiographs and CBCT scans will be evaluated both before and after microsurgery. Bone regeneration in periapical osseous defects will be evaluated according to the radiopacity scoring scale of paralleling radiographs by three independent blinded and calibrated examiners at the 3-month, 6-month and 12-month follow-ups after the microsurgery and compared with the paralleling radiograph after microsurgery immediately. According to a previous study, [30] the change in the volume of the periapical defect is the evaluation index of the radiopacity scoring scale. CBCT data before and 12 months after microsurgery will be processed using medical image processing software (MIMICS, Materialise, Leuven, Belgium) to evaluate the changes of volume and density of the periapical defect. The data of the clinical trial will be input and kept in a specific computer and a locked cabinet by two designated member of staffs. The secondary parameters of this clinical trial include percussion, mobility testing and presence/absence of sinus. The VAS scores are also secondary parameters. All the data will be recorded and imputed in the computer simultaneously. Due to lack of data monitoring committee in our hospital, the data will be kept in the cabinet by two different researchers to make sure the accuracy and completeness of the data. An independent inspector will review the incoming data every 3 months. There will be no harms caused by the trial.

Sample size

The sample size of this clinical trial is determined by the following formula:

$$N1 = N2 = 2\left[\frac{\sigma(Z_{\alpha/2} + Z_{\beta})}{\delta}\right]^2$$

According to the data of a published clinical trial, which is about bone grafting in periapical osseous defects, [30] the σ/δ is around 0.49. The inspection level (α) is set to 0.05, and the power is set to 0.9. For bilateral tests, the required sample size in each group is six. Considering a missed follow-up rate of 20%, the sample size should be 7.2. Consequently 16 participants will be needed.

Statistical analysis

Data will be analysed using SPSS software (SPSS, Inc, Chicago, IL, USA). Statistical significance will be accepted for *P* values lower than 0.05. Normality and variance equality will be analysed using the Shapiro-Wilk test and the Levene variance homogeneity test, respectively. Normally-distributed data will be shown as mean ± standard deviation, while non-normally distributed data will be shown as median (lower to upper quartile). Student's *t*-test will be used to compare the difference between the two groups for the data with both normality and variance equality. Otherwise, the Mann-Whitney *U* test will be used.

Withdrawal

The patients have the right to withdraw from this clinical trial without any reason at any point during the treatment. Follow-up treatment will not be affected by the withdrawal.

Dissemination of results

The results of this clinical trial will be registered at the International Clinical Trials Registry Platform (ICTRP). In addition, the results will be published in a peer-reviewed journal.

Patient and Public Involvement

Neither patients nor the public were involved in the design, recruitment, assessment, conduct and reporting of this research. The results will be disseminated through scientific journals.

DISCUSSION

When PRF is used together with ABBM, it increases the regenerative effect of osseous tissue, [31, 32] because ABBM only has an osteoconductive effect and osteoinduction is induced due to the growth factors in PRF. In a histological study, bone regenerative effects of PRF-TCP, rhBMP-2-coated TCP and TCP alone were compared, and the results showed that PRF-TCP resulted in more rapid bone healing compared to the other two groups. [12] The antibacterial and anti-inflammatory effects of PRF also promoted tissue healing. [14] Although PRF has many advantages, traditional PRF is not liquid, and it is challenging to mix PRF and ABBM evenly. Thus, in previous studies, [33-35] the gelatinous PRF had to be minced and then mixed with ABBM, which is time-consuming. Exposing alveolar bone to air could result in bone resorption, and a long duration of surgery is not favourable. [36] Therefore, our group developed a new technique to obtain a type of PRF/ABBM complex and thus avoid the need to mince the PRF. [23, 24] Additionally, PRF/ABBM complex as a whole had better applicability than granular ABBM, and could significantly reduce the time required for bone grafting. [24] The complex together with a collagen membrane has been demonstrated to result in enhanced gains in attachment level compared to ABBM alone with a collagen membrane, indicating improved periodontal regeneration. [24]

To date there is still a lack of clinical trials investigating the osseous regenerative effect of PRF/bone grafting material composites in endodontic microsurgery. This randomised controlled trial is devoted to the evaluation of the clinical outcomes of the application of a PRF/ABBM complex as a grafting biomaterial in endodontic microsurgery cases with large lesion size, in the hope of providing some scientific evidence to support endodontic microsurgeries for dentists.

Ethics and dissemination

Ethical approval had been approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202059179). The data of the clinical trial will be input and kept in a specific computer and a locked cabinet by two designated member of staffs. The results will be published in a peer-reviewed journal. Research data will be registered with the International Clinical Trials Registry

Platform (ICTRP), ID: ChiCTR2100046684.

Trial status

The trial has been registered at the International Clinical Trials Registry Platform (ICTRP). The identifier number is ChiCTR2100046684. Recruitment will begin in June 2021 and will end in June 2023.

Contributors

The study concept was proposed by ZW, KL, ZC and BH. The manuscript was drafted by BH and KL. CZ and ZZ revised the part on randomisation. YW and YL calculated the sample size. ZW, XW and KL revised the manuscript finally. All authors have agreed with the final version of the manuscript.

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Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer-reviewed.

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Figure Legend:

Figure 1 The flow chart of this clinical research

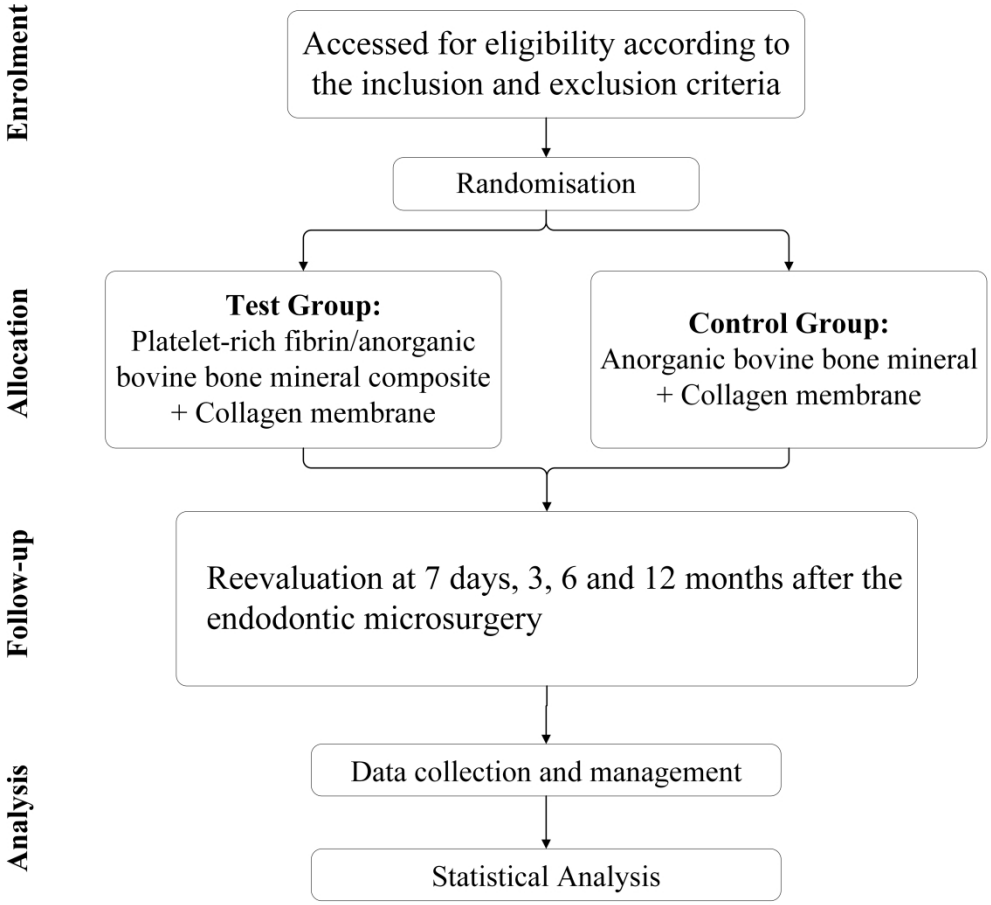

Figure 1 The flow chart of this clinical research

189x175mm (600 x 600 DPI)

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	11
Funding	4	Sources and types of financial, material, and other Support	12
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2, 11
	5b	Name and contact information for the trial sponsor	12
	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	N/A
	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)	N/A
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	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)	6
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	6, 7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, ligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	8, 9
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, 8, 9
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	9, 10
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	7, 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	7, 8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7, 8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8, 9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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	8, 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, 10
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	9
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	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A

	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)	N/A
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	9
	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	N/A
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	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	11
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)	N/A
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	N/A
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
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	11

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	N/A
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	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Additional file
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	N/A

*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

Platelet-rich fibrin/anorganic bovine bone mineral complex as grafting materials in endodontic microsurgery with a large lesion size: study protocol for a randomised controlled trial

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Platelet-rich fibrin/anorganic bovine bone mineral complex as grafting materials in endodontic microsurgery with a large lesion size: study protocol for a randomised controlled trial

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ABSTRACT

Introduction: Endodontic microsurgery is a treatment of last resort for preserving natural teeth. According to radiographic evaluation, the percentage of complete healing after endodontic microsurgery is only 74.3%. The use of regenerative techniques in endodontic microsurgery for large lesions (>10 mm diameter) is therefore recommended. The most frequently used bone graft in endodontic microsurgery is anorganic bovine bone mineral (ABBM) but this only has an osteoconductive effect. Thus, when platelet-rich fibrin (PRF), a reservoir of growth factors, is used together with ABBM, it increases the regenerative effect. This study is devoted to comparing the clinical outcomes of PRF with/without ABBM as grafting biomaterials in endodontic microsurgery cases with large lesion size to provide some valuable reference data for dentists.

Methods and analysis: Sixteen patients who are in need of endodontic microsurgery will be recruited. The patients will be randomly assigned to one of two groups: an experimental group, treated with PRF/ABBM complex and collagen membrane, and a control group, treated with ABBM and collagen membrane. Clinical examination including percussion, mobility testing and presence/absence of sinus will be recorded at 7 days, and at 3, 6 and 12 months after endodontic microsurgery. A visual analogue scale (VAS) will be used by the patients to evaluate pain at 1, 3 and 7 days after endodontic microsurgery. Routine paralleling radiographs will be obtained before and at 3, 6 and 12 months follow-up after endodontic microsurgery. Cone-beam computed tomographic (CBCT) scans will be obtained at the 12-month follow-up. Bone formation will be evaluated according to CBCT and paralleling radiographs. The study execute time including follow-ups last from June 1st 2021 to December 31st 2024.

Ethics and dissemination: The present study received approval from the Ethics Committee of Peking University School and Hospital of Stomatology. Research data will be registered with the International Clinical Trials Registry Platform (ICTRP), ID: ChiCTR2100046684. The results will be disseminated through scientific journals.

Strengths and limitations of this study:

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

The trial will be the first clinical trial with a novel design to compare the clinical and radiographic effects of PRF/ABBM complex and ABBM on large periapical lesions (>10 mm diameter) after endodontic microsurgery.

This study will collect longitudinal data on patients during the subsequent 12-month follow-up.

The patients with systemic disease will not be included in this research.

Keywords: Platelet-rich fibrin, Endodontic microsurgery, Large lesion size, Osteoinduction, Regeneration techniques

INTRODUCTION

With developments in equipment, instruments and biomaterials, endodontic microsurgery has become available as a treatment of last resort for cases which are no longer suitable for retreatment. [1] It is generally accepted that radiographic outcomes can be classified into complete healing, incomplete healing, uncertain healing and unsatisfactory healing. [2, 3] Complete bone repair is the ideal therapeutic outcome of endodontic microsurgery, however, the percentage of complete radiographic healing after endodontic microsurgery is only 74.3%. [4] Thus, it is imperative to promote healing of periapical bone defects more effectively. The results of endodontic microsurgery can be influenced by several factors, especially lesion size [5, 6]. During the healing process, epithelial cells repopulate the wound at the highest rate which results in scar formation. [7] Once complete osseous regeneration of a defect cannot occur, the defect will be filled by fibrous connective tissue. [8] It has been demonstrated that 26% of defects radiographically larger than 10 mm result in scar formation after endodontic microsurgery. [9] Thus, usage of grafting materials and membrane in cases with large lesion size (>10mm diameter) is recommended. [5, 6, 10]

There are four types of bone grafts: autogenous grafts, allogeneic grafts, xenogeneic grafts and alloplastic materials. Autogenous grafts and allogeneic grafts are osteoinductive materials, while xenogeneic grafts and alloplastic materials are osteoconductive materials due to their lack of growth factors. [11] It has been reported that a combination of osteoconductive materials (e.g. anorganic bovine bone mineral (ABBM)) and growth factors results in faster and better healing of bone in endodontic microsurgery than osteoconductive materials only. [12, 13]

PRF (platelet-rich fibrin) contains platelets, leukocytes and more than 100 types of growth factors including platelet-derived growth factor (PDGF), transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP-2), which promote the proliferation and differentiation of osteoblasts. [14] Moreover, the presence of leukocytes is helpful for their anti-infection and immunomodulatory effects. [15-17] Previously, PRF has been extensively used in dentistry, including the healing of extraction sockets, [18] ridge

preservation, [19] maxillary sinus augmentation and the regeneration of periodontal lesions. [20-24] It promotes the healing of soft tissue, and reduces postoperative pain, swelling, and the incidence of alveolar osteitis after the extraction of impacted mandibular third molars. [18] Compared with natural healing, horizontal and vertical dimension ridge preservation is more effective with the use of PRF. [19] The combination of PRF and deproteinized bovine bone mineral has been shown to increase bone formation in maxillary sinus augmentation compared with deproteinized bovine bone mineral alone. [20] Plenty of studies have indicated that combinations of PRF and bone grafting materials enhance periodontal regeneration in periodontal intrabony defects. [21-24] In a word, PRF has the potential for osseous regeneration and healing of soft tissue, especially when combined with various bone grafting materials. [25] It was also indicated that PRF reduces post-operative pain and infections due to the improvements in soft-tissue healing and the presence of microbial-fighting immune cells. [26] Hitherto, there have only been two case reports in which PRF was used together with osteoconductive bone grafts in periapical surgery in order to achieve a better healing outcome. [27, 28] With the exception of these two case reports, there has been no clinical research into the effects of PRF in periapical surgery.

We hypothesized that PRF combined with ABBM and collagen membrane is more effective than using only ABBM and collagen membrane in endodontic microsurgery. The hypothesis will be tested in the present double-masked randomised controlled clinical trial, the design of which is described below.

METHODS AND ANALYSIS

The study is a prospective, single-centre randomised controlled trial. It had been approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202059179). Research procedures including assessments, interventions and follow-ups will be carried out in Peking University School and Hospital of Stomatology (Beijing, China). This study has been registered with the registry of ICTRP (ID: ChiCTR2100046684). The main objective of this randomised

controlled clinical trial is to compare and evaluate the clinical effects of PRF with or without combination with ABBM in endodontic microsurgery cases with large lesion size (>10 mm diameter). The primary hypothesis is that healing of periapical lesions will be better in the PRF/ABBM complex group.

Inclusion criteria

Patients attending the Department of Cariology and Endodontology for routine planned endodontic microsurgery will be evaluated for inclusion in this clinical trial. The age of patients to be recruited will range from 18 to 65 years. Patients will have received periodontal treatment before endodontic microsurgery. Only single-rooted teeth will be included in the study, and the periapical lesions should be classified as large periapical lesions (>10mm diameter) according to cone-beam computed tomographic (CBCT) evaluation.

Exclusion criteria

1. Smokers;
2. Pregnant women;
3. Patients with systemic diseases;
4. Re-surgery;
5. Unqualified coronal restoration;
6. Teeth with deep periodontal pockets (probing depth ≥5mm).

Recruitment

Patients who are willing to participate in this study will be recruited from the Department of Cariology and Endodontology, Peking University School and Hospital of Stomatology. The necessity of endodontic microsurgery and collecting blood for PRF/ABBM complex will be explained to the participants. A signed informed consent form will be obtained and preserved confidentially in the cabinet. The procedures of this clinical trial are shown in Figure 1.

Groups, randomisation and blinding

An experienced endodontist will perform the examination, diagnosis and assessment procedures after clinical and radiographic examinations. The sequence and allocation

will be performed by a professor alone with opaque envelopes. The bone grafting material of the experimental group will be a PRF/ABBM composite, while the control group will receive ABBM only.

Interventions

All enrolled patients will be randomly divided into two groups after examination. The endodontic microsurgery will be performed by the same experienced endodontist using an endodontic microscope (F40, Leica Microsystems Ltd, Wetzlar, Germany). The procedures and principles of the endodontic microsurgeries will follow the guidelines. [29] A vertical and a horizontal incision will be used to reflect the flap. Peri-radicular curettage will be performed after reaching the periapical lesion. After 3mm of the root apex is resected, retro-preparation and retro-filling with iRoot BP (Innovative BioCeramix Inc., Burnaby, BC, Canada) will be performed. In the experimental group, after peri-radicular curettage, whole blood (10 mL) will be collected in sterile glass-coated centrifugal tube without any anticoagulant and centrifuged at 960 rpm (Medical centrifuge Auto V1, JM Instrument, Beijing, China) for 2.8 minutes. Then the buffy coat layer, the plasma layer, and 1 to 1.5 mm of the red blood cell layer below the buffy coat layer will be harvested as the liquid PRF. Finally, ABBM (Heal-all, ZH-Bio, Yantai, China) will be mixed with the liquid PRF evenly. ABBM is a category of xenograft which has only an osteoconductive effect. The periapical defects will be filled with PRF/ABBM complex in the experimental group while the periapical defects will be filled with ABBM only in the control group. An absorbable collagen membrane (Heal-all, ZH-Bio, Yantai, China) will be applied in both groups, then the flap will be repositioned with 6-0 sutures. Amoxicillin and 0.2% chlorhexidine gluconate rinse will be prescribed to prevent postoperative infection. Sutures will be removed 5 days after the endodontic microsurgery.

Examination

Baseline examination

Before treatment, all participants will be examined carefully by a calibrated examiner. Clinical examination including percussion and mobility testing and presence/absence of sinus will be recorded simultaneously. Preoperative paralleling radiographs and

CBCT scans will be obtained for each participant.

Examination during the follow-up period

Clinical examination including percussion, mobility testing and presence/absence of sinus will be performed at 7 days, 3 months, 6 months and 12 months after the endodontic microsurgery. In addition, a visual analogue scale (VAS) will be recorded at 1 day, 3 days and 7 days after the endodontic microsurgery for the evaluation of postoperative pain. Paralleling radiographs will be obtained at 3 months, 6 months and 12 months. CBCT scans will be performed at the 12-month follow-up. The grouping and the treatment plan will be confidential to the examiners. The primary parameter of the present clinical trial is radiographic bone regeneration in the area of the periapical defect. The paralleling radiographs and CBCT scans will be evaluated both before and after microsurgery. Bone regeneration in periapical osseous defects will be evaluated according to the radiopacity scoring scale of paralleling radiographs by three independent blinded and calibrated examiners at the 3-month, 6-month and 12-month follow-ups after the microsurgery and compared with the paralleling radiograph after microsurgery immediately. According to a previous study, [30] the change in the volume of the periapical defect is the evaluation index of the radiopacity scoring scale. CBCT data before and 12 months after microsurgery will be processed using medical image processing software (MIMICS, Materialise, Leuven, Belgium) to evaluate the changes of volume and density of the periapical defect. The data of the clinical trial will be input and kept in a specific computer and a locked cabinet by two designated member of staffs. The secondary parameters of this clinical trial include percussion, mobility testing and presence/absence of sinus. The VAS scores are also secondary parameters. All the data will be recorded and imputed in the computer simultaneously. Due to lack of data monitoring committee in our hospital, the data will be kept in the cabinet by two different researchers to make sure the accuracy and completeness of the data. An independent inspector will review the incoming data every 3 months. There will be no harms caused by the trial.

Sample size

The sample size of this clinical trial is determined by the following formula:

$$N1 = N2 = 2\left[\frac{\sigma(Z_{\alpha/2} + Z_{\beta})}{\delta}\right]^2$$

According to the data of a published clinical trial, which is about bone grafting in periapical osseous defects, [30] the σ/δ is around 0.49. The inspection level (α) is set to 0.05, and the power is set to 0.9. For bilateral tests, the required sample size in each group is six. Considering a missed follow-up rate of 20%, the sample size should be 7.2. Consequently 16 participants will be needed. Once more than 20% participants withdrawal occur, new participants will be enrolled.

Statistical analysis

Data will be analysed using SPSS software (SPSS, Inc, Chicago, IL, USA). Statistical significance will be accepted for *P* values lower than 0.05. Normality and variance equality will be analysed using the Shapiro-Wilk test and the Levene variance homogeneity test, respectively. Normally-distributed data will be shown as mean ± standard deviation, while non-normally distributed data will be shown as median (lower to upper quartile). Student's *t*-test will be used to compare the difference between the two groups for the data with both normality and variance equality. Otherwise, the Mann-Whitney *U* test will be used.

Withdrawal

The patients have the right to withdraw from this clinical trial without any reason at any point during the treatment. Follow-up treatment will not be affected by the withdrawal.

Dissemination of results

The results of this clinical trial will be registered at the International Clinical Trials Registry Platform (ICTRP). In addition, the results will be published in a peer-reviewed journal.

Patient and Public Involvement

Neither patients nor the public were involved in the design, recruitment, assessment, conduct and reporting of this research. The results will be disseminated through scientific journals.

DISCUSSION

When PRF is used together with ABBM, it increases the regenerative effect of osseous tissue, [31, 32] because ABBM only has an osteoconductive effect and osteoinduction is induced due to the growth factors in PRF. In a histological study, bone regenerative effects of PRF-TCP, rhBMP-2-coated TCP and TCP alone were compared, and the results showed that PRF-TCP resulted in more rapid bone healing compared to the other two groups. [12] The antibacterial and anti-inflammatory effects of PRF also promoted tissue healing. [14] Although PRF has many advantages, traditional PRF is not liquid, and it is challenging to mix PRF and ABBM evenly. Thus, in previous studies, [33-35] the gelatinous PRF had to be minced and then mixed with ABBM, which is time-consuming. Exposing alveolar bone to air could result in bone resorption, and a long duration of surgery is not favourable. [36] Therefore, our group developed a new technique to obtain a type of PRF/ABBM complex and thus avoid the need to mince the PRF. [23, 24] Additionally, PRF/ABBM complex as a whole had better applicability than granular ABBM, and could significantly reduce the time required for bone grafting. [24] The complex together with a collagen membrane has been demonstrated to result in enhanced gains in attachment level compared to ABBM alone with a collagen membrane, indicating improved periodontal regeneration. [24]

To date there is still a lack of clinical trials investigating the osseous regenerative effect of PRF/bone grafting material composites in endodontic microsurgery. This randomised controlled trial is devoted to the evaluation of the clinical outcomes of the application of a PRF/ABBM complex as a grafting biomaterial in endodontic microsurgery cases with large lesion size, in the hope of providing some scientific evidence to support endodontic microsurgeries for dentists.

Ethics and dissemination

Ethical approval had been approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202059179). The data of the clinical trial will be input and kept in a specific computer and a locked cabinet by two designated member of staffs. The results will be published in a peer-reviewed journal.

Research data will be registered with the International Clinical Trials Registry Platform (ICTRP), ID: ChiCTR2100046684.

Trial status

The trial has been registered at the International Clinical Trials Registry Platform (ICTRP). The identifier number is ChiCTR2100046684. Recruitment will begin in June 2021 and will end in June 2023.

Contributors

The study concept was proposed by ZW, KL, ZC and BH. The manuscript was drafted by BH and KL. CZ and ZZ revised the part on randomisation. YW and YL calculated the sample size. ZW, XW and KL revised the manuscript finally. All authors have agreed with the final version of the manuscript.

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Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer-reviewed.

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Figure Legend:

Figure 1 The flow chart of this clinical research

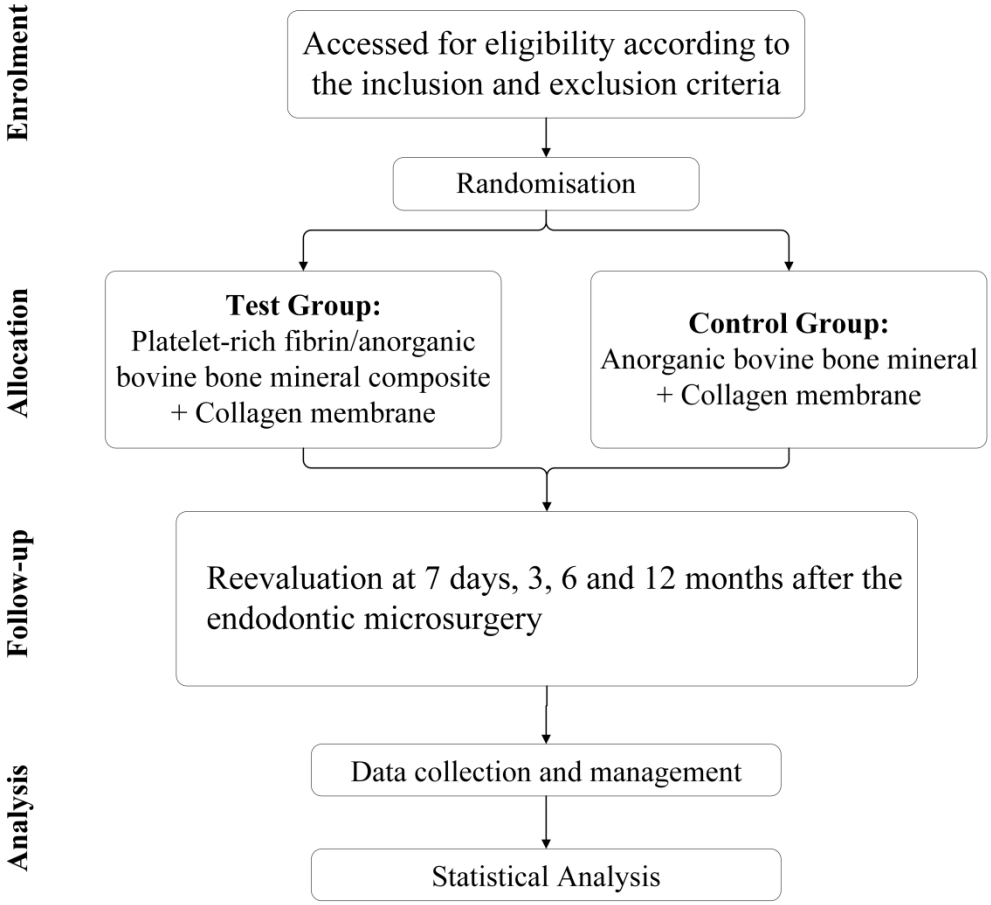

Figure 1 The flow chart of this clinical research

189x175mm (600 x 600 DPI)

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	11
Funding	4	Sources and types of financial, material, and other Support	12
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2, 11
	5b	Name and contact information for the trial sponsor	12
	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	N/A
	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)	N/A
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	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)	6
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	6, 7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, ligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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	8, 9
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, 8, 9
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	9, 10
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	7, 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	7, 8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7, 8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8, 9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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	8, 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, 10
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	9
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	10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A

	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)	N/A
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	9
	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	N/A
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	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	11
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)	N/A
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	N/A
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
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	11

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	N/A
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	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Additional file
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	N/A

*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.