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HUMAN RANDOMIZED CONTROLLED TRIAL

Treatment of periodontal intrabony defects using bovine porous bone mineral and guided tissue regeneration with/without platelet-rich fibrin: A randomized controlled clinical trial

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Abstract

Background: To investigate the regenerative effect of adjunctive use of guided tissue regeneration (GTR), bovine porous bone mineral (BPBM), and platelet-rich fibrin (PRF) in intrabony defects.

Methods: Fourteen participants were enrolled, and for each patient their left and right two sides were randomized to the test group or control group. Only the worst intrabony defect on each side was analyzed. The test group received GTR, BPBM, and PRF, whereas the control group received only GTR and BPBM. The PRF used in the trial was fluid PRF, which combined with the BPBM to form a BPBM-PRF complex. The patients were followed up by clinical and radiographic evaluation for 24 months after surgery.

Results: Probing depth (PD) in the test group was significantly less than that in the control group at 12 and 24 months after surgery, and the mean difference was ≈ 0.5 to 0.7 mm. Clinical attachment level (CAL) gain in the test group was ≈ 0.9 mm higher than that in the control group at 6 months after surgery, and the difference reached 1.0 to 1.1 mm 12 and 24 months after surgery. None of the other clinical or radiographic parameters differed significantly between the two groups at any time-point after the surgery.

Conclusion: Compared with GTR and BPBM, the combination of GTR and BPBM-PRF complex is more effective clinically, and results in better clinical outcomes.

KEYWORDS
guided tissue regeneration; periodontal; platelet-rich fibrin; randomized controlled trials

1 | INTRODUCTION

The aim of periodontal treatment is the restoration of periodontal function and esthetics by eliminating periodontal inflammation and controlling of predisposing factors.[1] If indicated, the regeneration of periodontal tissues is an important additional aim of periodontal therapy.[2] Guided tissue regeneration (GTR) is an important method for the achievement of periodontal regeneration.[2] In GTR, the space between the membrane and the root is important for

successful regeneration.[3] Thus, bone grafts are frequently used together with GTR,[4] and bovine porous bone mineral (BPBM) is a type of bone graft. In addition, using biologic agents together with GTR might be beneficial for periodontal regeneration, because biologic agents can augment the proliferation, differentiation, and chemotaxis of cells.[2–3]

Platelet-rich fibrin (PRF) contains a variety of autologous biologic agents,[5] and thus can augment the proliferation, differentiation, and migration of gingival fibroblasts, periodontal ligament cells, and osteoblasts.[6–8] The combination of PRF and open flap debridement (OFD) has advantages over OFD alone for periodontal regeneration in intrabony defects, which is reflected in the reduction of probing depth (PD), gain of clinical attachment level (CAL) and filling of bone defects.[9–11] It has also been reported that PRF enhances the regenerative effects of a variety of bone grafts in intrabony defects.[12–16] Adjunctive use of PRF and demineralized freeze-dried bone allograft (DFDBA) has clinical advantages beyond those associated with the use of DFDBA alone.[12–13] The combination of a nanocrystalline HA graft with PRF has also been shown to result in better clinical effects than nanocrystalline HA alone.[14] In another study, a greater gain of CAL was detected in the group treated with the combination of BPBM with PRF than in the BPBM alone group.[15] In a recent study, the adjunctive use of bioactive glass and PRF resulted in better clinical and radiographic results than bioactive glass alone, indicating increased periodontal regeneration.[16] In addition, a resorbable collagen membrane if used together with PRF was associated with better clinical outcomes in intrabony defects than the same membrane alone.[17]

It has been demonstrated that using BPBM together with PRF augments the regenerative effects of PRF,[18–19] which indicates that using BPBM and PRF produces better results than using either BPBM or PRF alone. Hitherto, there have been no clinical studies in which GTR, bone grafts and PRF were used together. Only in one case report,[20] GTR, BPBM, and PRF were used together in the treatment of a maxillary lateral incisor with severe intrabony defects, and both clinical and radiographic improvements were observed 15 months after treatment.

In the present study, the treatment effects in periodontal intrabony defects were compared between GTR + BPBM and GTR + BPBM-PRF complex. The parameters analyzed were PD, CAL, bleeding index (BI),[21] and radiographic bone fill. The hypothesis tested in this double-masked randomized controlled clinical trial was that adjunctive use of GTR and BPBM-PRF complex is more effective than GTR and BPBM for periodontal regeneration in intrabony defects.

2 | MATERIALS AND METHODS

2.1 | Study population

Fifteen patients (four males and 11 females) with periodontitis Stage IV Grade C diagnosed based on the 2017 consensus classification of periodontal and peri-implant diseases and conditions,[22] were recruited from the Clinic of the Department of Periodontology, Peking University School and Hospital of Stomatology between September 2016 and March 2018. Each participant had one or more intrabony defects with a depth of ≥3 mm on each side according to intraoral periapical radiographs. The PD and BI on such sites were ≥5 mm and ≥2, respectively, after initial periodontal therapy. If any patient had more than one defect on one side, only the worst defect on each side was analyzed. Smokers and any participants with systemic diseases, pregnancy, lactation or poor oral hygiene were excluded. Because subject No. 10 (female) withdrew from the study, 65 intrabony defects of 14 participants' (mean age 36.0 ± 8.6 years, range 24 to 60 years) were analyzed. This study was approved by the human subjects ethics board of Peking University School and Hospital of Stomatology and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was obtained from each subject. The procedures of this randomized clinical trial (Clinical Trials Registry-China, ChiCTR1900027581), which has been published in our previous report,[23] are explained below.

2.2 | Presurgical treatment

Each participant was subjected to initial periodontal therapy, consisting of oral hygiene instructions, scaling and root planing. Hand curettes* and an ultrasonic device† were used in the therapy, and root planing was completed under local anesthesia. PD, CAL, and BI at the intrabony defect sites were reevaluated 6 weeks after initial periodontal therapy, and were recorded as baseline clinical data. At each site, the buccal and lingual clinical parameters were recorded. One clinician (ZH), who was masked to the subsequent surgeries, performed all the clinical and radiographic measurements at baseline, as well as at all the postsurgical time points. Intra-examiner calibration was performed twice on six volunteers with severe periodontitis (three for each time-point) before and in the middle of the trial, and the results revealed that 97.4% and 98.5% of PD measurements were within 1 mm, whereas 93.6%

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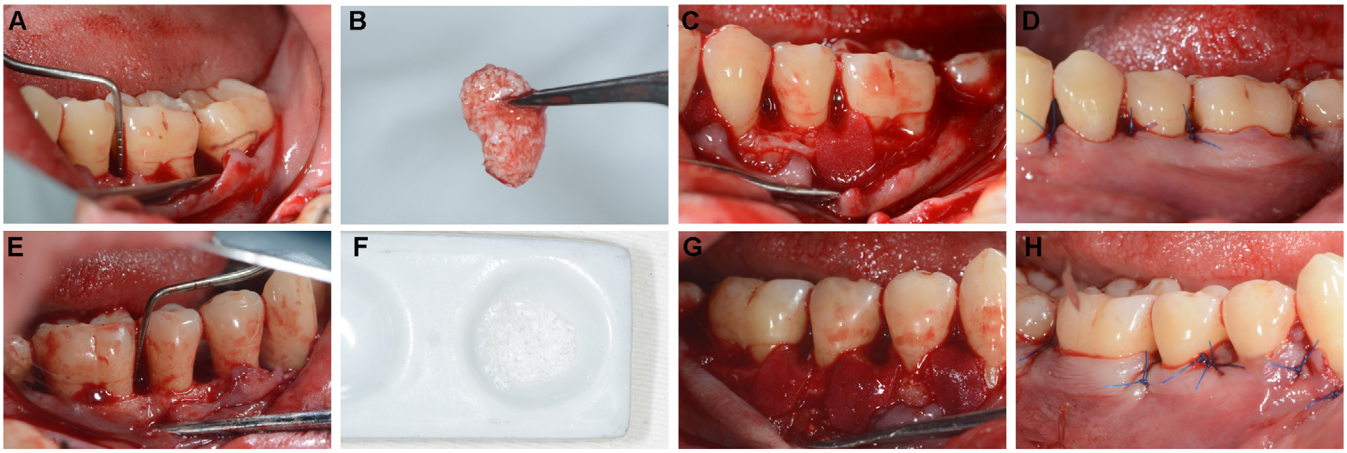

FIGURE 1 Surgical procedures in the two groups. Panel (**A**) An intrabony defect in the test group. Panel (**B**) The BPBM-PRF complex. Panel (**C**) Covering the intrabony defect with a collagen membrane after bone grafting in the test group. Panel (**D**) Tight suturing in the test group. Panel (**E**) An intrabony defect in the control group. Panel (**F**) BPBM. Panel (**G**) Covering the intrabony defect with a collagen membrane after bone grafting in the control group. Panel (**H**) Tight suturing in the control group

and 92.8% of CAL measurements showed differences of < 1 mm.

2.3 | Surgical procedure

The left and right sides of each participant were randomized to the test group or the control group by flipping a coin. In the test group, a resorbable collagen membrane was used together with BPBM-PRF complex to treat intrabony defects. In the control group, only membrane and BPBM were used in the periodontal surgery. Periodontal surgery was accomplished on the left side first, no matter whether the left side was assigned to the test group or the control group. All the randomization and surgeries were completed by the same operator (KL) who was masked to all the examinations and data analysis.

Under local anesthesia with 4% articaine containing 1:100,000 epinephrine, sulcular incisions were followed by elevation of mucoperiosteal flaps. In the test group, the subsequent steps are shown in Figure 1A-1D. After the elevation of flaps, 10 mL whole blood from the antecubital vein was collected in sterile tubes without any anticoagulant. The blood was centrifuged[‡] for 3 minutes at 700 rpm. After centrifugation, we harvested all the plasma layer, the buffy coat layer and 1 to 1.5 mm of the red blood cell layer directly below the buffy coat layer as the liquid PRF. Then, the liquid PRF was mixed with BPBM. Meanwhile, complete debridement was performed using hand curettes[§]. After debridement of the intrabony defects, the BPBM-PRF complex formed a kind of gelatinous material (Figure 1B) and was used to fill the defects. Then a resorbable collagen membrane[**] was used to cover the defects (Figure 1C), and the flaps were sutured using 5 to 0 nylon sutures (Figure 1D).

The surgery in the control group is shown in Figure 1E-1H, and the only difference was that the intrabony defects were filled with BPBM alone (Figure 1F) before being covered with membrane. So that the participants would be masked to the different treatments, when performing surgery on the control side, blood was also collected but PRF was not prepared.

The time when bone graft material was filled into each intrabony defect was recorded.

2.4 | Postsurgical treatment

Amoxicillin (0.5 g every 8 hours for 7 days) and 0.2% chlorhexidine gluconate rinses (twice a day) were prescribed.

The sutures were removed 2 weeks postoperatively. All the participants were reexamined at 6, 12, and 24 months after surgery, and PD, CAL, and BI were also recorded. In addition, the treated intrabony defects were also reexamined radiographically using intraoral periapical radiographs at each time-point. Supportive periodontal therapy was carried out after each reexamination.

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[§] Hu-Friedy, Chicago, IL
[**] B type, ZH-Bio, Yantai, China

2.5 | Radiographic analysis

The bisecting-angle technique was used to obtain radiographs. Radiographs were scanned with a scanner[††] and evaluated by the same evaluator, who was blinded to the grouping. Radiographic depth of intrabony defect and radiographic vertical bone loss were the two radiographic parameters evaluated. Because the length measured on the radiographs was not equal to the actual length, the radiographic parameters were converted into the percentage of the length of the root. The radiographic depth of the intrabony defect was calculated as the distance from the alveolar crest to the base of the intrabony defect/root length, and radiographic vertical bone loss was calculated as the distance from the cemento-enamel junction to the base of the intrabony defect/root length.

2.6 | Bias control

Two methods were used to control selection bias in the present trial.

1) The left or right side of each participant was randomized into either the test group or the control group.

2) The loss rate of participants was low, with only one participant withdrawing from the trial, and 14 participants completed the study.

In the present trial, the main method of controlling the information bias was the double-masked design. The examiner and data analyst as well as the participants were all blinded to the grouping information.

Further two methods were used to control the confounding bias in the present trial.

1) For each participant, their left and right sides were randomized to either the test group or the control group.

2) Because of the split-mouth design, no patient factors needed to be taken into account for the analysis.

2.7 | Statistical analyses

The primary outcome in the present study was CAL. The other clinical parameters, radiographic parameters and time of bone grafting were all secondary outcomes.

The calculation of sample size was as described in our previous report.[23] Briefly, the formula $N = 2[\frac{\sigma(Z_{\alpha/2}+Z_{\beta})}{\delta}]^2$ was used in the calculation. The σ/δ of CAL was ≈ 0.7 according to previous studies,[12,24] whereas α, β and the missing rate were set as 0.05, 0.1, and 20%, respectively, and 14 participants needed to be recruited.

TABLE 1 Comparison of clinical parameters before surgery

Clinical parameters	Groups	Number of intrabony defects	Before surgery
Buccal PD (mm)	Test group	14	4.6 ± 1.2
	Control group	14	4.8 ± 1.5
Lingual PD (mm)	Test group	14	6.0 ± 0.9
	Control group	14	6.0 ± 0.9
Buccal CAL (mm)	Test group	14	4.9 ± 1.4
	Control group	14	4.8 ± 1.4
Lingual CAL (mm)	Test group	14	5.4 ± 1.6
	Control group	14	5.5 ± 1.4
Buccal BI	Test group	14	2.6 ± 0.6
	Control group	14	2.6 ± 0.9
Lingual BI	Test group	14	3.5 ± 0.7
	Control group	14	3.6 ± 0.5
Depth of intrabony defects (mm)	Test group	14	4.8 ± 1.8
	Control group	14	4.4 ± 1.5
Width of intrabony defects (mm)	Test group	14	2.8 ± 0.8
	Control group	14	2.5 ± 0.7

PD, CAL gain, BI, depth and width of intrabony defects are shown as mean ± SD, and were compared using the paired-samples t test between the two groups, as was the time of bone graft filling. The radiographic depth of the intrabony defect and the radiographic vertical bone loss are shown as median (lower to upper quartile), and were compared using the Wilcoxon test between the two groups. All statistical analyses were performed using a statistical software package[‡‡]. Statistical significance was accepted when $P < 0.05$.

3 | RESULTS

The clinical parameters at baseline are presented in Table 1. PD, CAL, BI, depth, and width of intrabony defects did not differ significantly between the groups before surgery. Generally, lingual parameters were higher than the

[††] Canon, Tokyo, Japan

[‡‡] SPSS 11.5, SPSS Inc., Chicago, IL, US

TABLE 2 Comparison of clinical parameters at different time-points during the follow-up

Clinical parameters	Groups	Number of intrabony defects	6 months	12 months	24 months
Buccal PD (mm)	Test group	14	1.9 ± 1.0	1.9 ± 0.7	1.9 ± 0.5*
	Control group	14	2.4 ± 0.6	2.4 ± 0.8	2.4 ± 0.6
Lingual PD (mm)	Test group	14	2.6 ± 0.9	2.6 ± 0.8*	2.7 ± 0.6*
	Control group	14	3.0 ± 0.7	3.2 ± 0.7	3.4 ± 0.8
Buccal CAL gain (mm)	Test group	14	2.9 ± 0.4*	3.2 ± 0.6*	3.1 ± 0.5*
	Control group	14	2.0 ± 0.9	2.1 ± 1.1	2.1 ± 1.1
Lingual CAL gain (mm)	Test group	14	3.0 ± 0.9	3.1 ± 0.8*	3.1 ± 0.7*
	Control group	14	2.6 ± 0.6	2.1 ± 0.7	2.0 ± 0.8
Buccal BI	Test group	14	0.9 ± 0.6	0.9 ± 0.7	1.0 ± 0.7
	Control group	14	0.9 ± 0.6	0.9 ± 0.5	0.7 ± 0.6
Lingual BI	Test group	14	1.1 ± 0.8	1.3±0.7	1.1 ± 0.3
	Control group	14	1.3 ± 0.7	1.3 ± 0.7	1.1 ± 0.7

*$P < 0.05$.

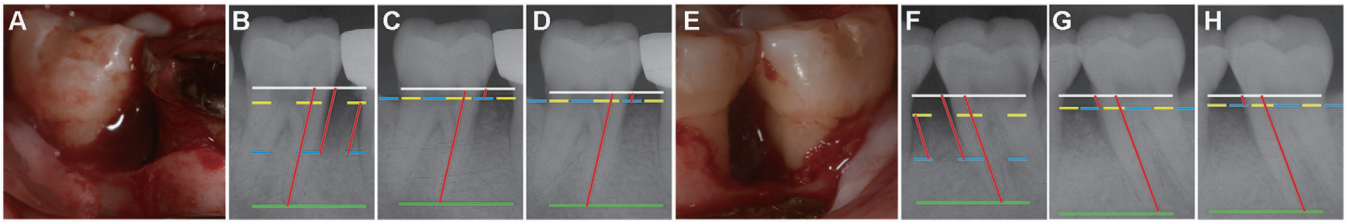

FIGURE 2 Comparison of radiographic parameters in the two groups. The white line, yellow line, blue line, and green line indicate the cemento-enamel junction, the alveolar crest, the base of the intrabony defect and the root apex, respectively. Thus, the radiographic depth of the intrabony defect was the distance between the yellow and blue lines, and radiographic vertical bone loss was the distance between the white and blue lines. The length of the root was the distance between the white and green lines. All the distances (red lines) were measured parallel to the long axis of the tooth root. Panels (**A**–**D**) Test group. Panels (**E**–**H**) Control group. Panels (A, E) Photographs of the intrabony defects. Panels (B, F) X-rays before surgery. Panels (C, G) X-rays 12 months after surgery. Panels (D, H) X-rays 24 months after surgery

corresponding buccal ones. The BI at each site with an intrabony defect was ≥2, namely bleeding on probing positive.

The time needed for bone grafting in the test group was significantly shorter than that in the control group (23.9 ± 7.4 s versus 144.0 ± 56.3 s, $P < 0.05$).

As shown in Table 2, no significant differences in buccal PD or lingual PD were observed between the two groups 6 months after surgery. As for the longer follow-up, the buccal PD in the test group was significantly less than that in the control group 24 months after surgery, as was the lingual PD. The mean difference in PD between the two groups was ≈ 0.6-0.7 mm at both 12 months and 24 months after surgery. CAL gain was found to be significantly higher in the test group than that in the control group at all the follow-up time-points except for the lingual CAL gain 6 months after surgery. The difference in the CAL gain between the two groups was 0.9 to 1.1 mm. No significant differences between the two groups were observed in buccal or lingual BI at any time-point after surgery.

Figure 2 shows the cemento-enamel junction (white line), the alveolar crest (yellow line), the base of the intrabony defect (blue line), and the root apex (green line). Thus, radiographic depth of the intrabony defect was the distance between the yellow and blue lines, and radiographic vertical bone loss was the distance between the white and blue lines. The length of the root was the distance between the white and green lines. All the distances (the red lines) were measured parallel to the long axis of the tooth root. The results demonstrated that neither the radiographic depth of the intrabony defect nor the radiographic vertical bone loss differed significantly between the two groups (Table 3).

TABLE 3 Comparison of radiographic parameters at different time-points

Radiographic parameters	Groups	Number of intra-bony defects	Before surgery	12 months	24 months
Radiographic depth of intrabony defect	Test group	14	27.3% (22.2% to 40.9%)	1.6% (0 to 5.9%)	1.4% (0 to 5.0%)
	Control group	14	26.7% (24.0% to 33.5%)	2.1% (0 to 5.4%)	1.6% (0 to 5.9%)
Radiographic vertical bone loss	Test group	14	49.2% (40.8% to 60.2%)	8.4% (6.5% to 18.5%)	9.7% (8.0% to 18.9%)
	Control group	14	44.7% (39.2% to 58.6%)	9.4% (6.9% to 13.8%)	9.9% (6.1% to 14.1%)

4 | DISCUSSION

To the best of our knowledge, this is the first clinical trial in which absorbable membrane, BPBM and PRF were used together to treat periodontal intrabony defects. The strengths of the present study comprised its randomized, double-blind design and the matching of the two groups. The results verified that PRF promoted the effects of GTR and bone grafting in reducing PD and increasing CAL. The PRF used in the present study was not traditional PRF but a type of liquid PRF which formed a BPBM-PRF complex. The liquid PRF was similar to the injectable PRF first reported in 2017,[25] although the centrifugation step was not the same. The BPBM-PRF complex provided the following advantages: 1) Because traditional PRF is gelatinous but not liquid, it is a challenge to mix PRF with bone graft materials evenly. In previous reports,[15,18,20,26] the PRF was minced into small pieces before mixing with the bone grafts. These extra steps increase the complexity and length of the whole regenerative surgery procedure, which is not favorable. Because the exposure of alveolar bone to air will lead to bone resorption,[27] elimination of the need to mince PRF reduced the duration of alveolar bone exposure. 2) The BPBM-PRF complex forms a mass but is not granular, so the applicability of the BPBM-PRF complex was better than that of granular materials such as BPBM alone or BPBM together with minced PRF. Moreover, no additional materials were needed for formation of the BPBM-PRF complex. In contrast, to make the granular complex of BPBM and minced PRF more usable, another study[26] found that additional materials needed to be added into the complex, however, the complexity of the surgery was enhanced. In the present study, the time required for the bone grafting step in the test group was significantly shorter than that in the control group because of the increased usability of the BPBM-PRF complex. As is known, contamination by saliva has adverse effects on successful periodontal regeneration by GTR and bone graft surgery. Shorter bone grafting time could reduce the possibility that bone graft materials were contaminated by saliva, which might contribute to the better clinical outcomes in the test group.

The reasons for the more effective regeneration in the test group might mainly be as follows: 1) PRF is a rich source of autologous growth factors [5] (e.g., platelet-derived growth factor, transforming growth factor β 1, epidermal growth factor) that could induce the migration, proliferation and differentiation of different periodontium-related cells, which could in turn enhance the potential for periodontal regeneration.[6–8,28] 2) PRF has antibacterial capacity against various periodontal pathogens, especially *P. gingivalis*.[29] Additionally, the injectable PRF has stronger antibacterial capacity against *P. gingivalis* than traditional PRF.[30] 3) PRF suppresses the formation of osteoclasts and osteoclastogenesis.[31] 4) Injectable PRF has anti-inflammatory activity via inhibition of the immune response of macrophages and dendric cells.[32]

It has been reported that the clinical advantages of regenerative periodontal therapy over OFD could be maintained for as long as 10 years, but the differences between different regenerative periodontal therapies have been neglectable in long-term follow-up.[33] However, there were no previous studies in which PRF was used in regenerative periodontal therapy with a follow-up of over 1 year. In the present study, PRF treatment was found to be advantageous for the control group clinically 2 years after the surgery, indicating that PRF might be a promising method in regenerative periodontal therapy. However, longer follow-up is still needed before more precise conclusion can be drawn.

There were four limitations to the present study. 1) The study was performed in one single center, and consequently the data could not be directly applied in other populations and a multicenter study will be needed in the future. 2) Although the radiographic parameters were

converted into a percentage of the length of the roots, the bisecting angle radiographs were still not as good as paralleling radiographs. 3) No histological evidence of periodontal regeneration was available because re-entry surgeries were not involved. 4) "Tossing of a coin" is not a perfect method for randomization and could result in bias. A block-randomization method should have been used.

According to recent studies, horizontal centrifugation could generate a higher yield of platelets and leukocytes in PRF.[34–35] Additionally, improvement of the centrifugation protocol for classical i-PRF could generate concentrated PRF,[34,36] which has higher yields of platelets and leukocytes, resulting in more potent induction of gingival fibroblast biological activity. Thus, such methods of enhancing the yields of platelets and leukocytes in PRF might be the focus of future work to improve clinical outcomes.

5 | CONCLUSION

In conclusion, our method of using BPBM-PRF as a complex in GTR is innovative and simple. This randomized controlled trial provided preliminary evidence that the combined application of GTR and BPBM-PRF complex might result in better clinical outcomes than GTR and BPBM in intrabony defects. However, more studies are still needed.

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AUTHOR CONTRIBUTIONS

Kaining Liu: Conceptualization, methodology, investigation, original draft writing, and funding acquisition. Zhen Huang: Investigation. Zhibin Chen: Conceptualization, methodology, investigation, project administration. Bing Han: Conceptualization, methodology, formal analysis, supervision, project administration, review writing and editing, funding acquisition. Xiangying Ouyang: Investigation and formal analysis.

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