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The effects of fulvic acids and low-level laser therapy on orthodontic retention in rats

Jianmei Zhao[1,2], Qingmei Liu[2], Caifeng Zhang[3], Kuanshou Zhang[4] and Pengfei Xin[2*]

Abstract

Background Shortening retention time and minimizing relapse rates are ongoing challenges in orthodontics. This study investigated the effects of natural fulvic acids (FAs) and low-level laser therapy (LLLT) on orthodontic retention in rats.

Methods Seventy-two male Sprague-Dawley rats underwent mesial movement of the left maxillary first molar using a 50 g force via a nickel-titanium tension spring. After three weeks of movement, the rats entered the retention phase with retainer wires and were divided into four groups: Control (no intervention), FAs (80 mg/kg orally daily), LLLT (808 nm laser twice weekly), and FAs + LLLT (both treatments). Retainers were removed on days 7, 14, and 21 for a 3-day relapse assessment. Maxillary impressions were analyzed for relapse rates using 3Shape software, alongside histological and immunohistochemical evaluations of bone morphogenetic protein-2 (BMP-2) expression in periodontal tissues, with differences among groups analyzed using an ordinary two-way analysis of variance (ANOVA).

Results The relapse rate decreased over time, particularly at 10, 17, and 24 days ($p < 0.001$). The FAs group did not significantly affect relapse rates compared to the control group ($p = 0.084$). In contrast, both the LLLT and FAs + LLLT groups significantly reduced relapse rate ($p < 0.001$), with no significant difference between these groups ($p = 0.555$). Histological examination revealed active osteoclasts on day 10, decreasing by days 17 and 24. The LLLT and FAs + LLLT groups showed less local cementum resorption and better periodontal fiber arrangement. All treatment groups significantly increased BMP-2 expression ($P < 0.05$) compared to controls. with LLLT and FAs + LLLT differing significantly from FAs ($P < 0.001$), though no difference was observed between LLLT and FAs + LLLT ($P = 0.578$).

Conclusions FAs did not significantly reduce relapse rate with retainers, while LLLT effectively reduced relapse rates, showing no additional benefit from combining FAs with LLLT. Both FAs and LLLT increased BMP-2 expression in PDL fibroblasts but with no synergistic effect.

Keywords Humic substances, Low-level light therapy, Orthodontic retainers, Osteoblasts, Osteoclasts

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Introduction

Retention is the phase of orthodontic treatment which involves using orthodontic retainer or dental splint to keep teeth in the correct position [1]. Without retention the teeth will have a tendency to return to their initial positions. This unfavorable change in the orthodontic tooth movement (OTM) from its corrected one to their initial position is known as relapse. During long-term follow-up post-orthodontic treatment, 40–90% of patients demonstrate teeth misalignment more than ten years after treatment [2]. Orthodontic retention plays a crucial role in preventing relapse following orthodontic treatment. Despite the effective use of retainers three years post-treatment, the relapse rate remains above 19% [3]. Orthodontic retention remains a challenge as relapse is a lifetime risk and retainer needed to be worn for at least 1 to 2 years, or even longer time. Just because of the long retention period, the compliance of some patients with wearing retainers decreases, resulting in less satisfactory results.

Therefore, increasing tooth stability after movement, shortening retention time, and decreasing relapse are crucial in orthodontic treatment.

The biological backgrounds of relapse are not fully understood but are felt to relate to the fibrous structures within the supporting tissues of the teeth, such as collagen turnover in the periodontal ligament (PDL), alveolar bone remodeling, and other extracellular matrix components [4–6]. The side that experienced tension in the PDL during the force-activated tooth movement can be regarded as the pressure side during relapse [5]. After tooth movement, there is still significant force remained in the periodontium. The fibers supporting teeth are stretch and displace during tooth movement, and then tend to rearrange over time. In addition, the bones are also noticed to response and rearrange to the force, and new bones are laid along the stretched fiber bundles and the complete bone realignment in some areas was only found after 232 days – more than 7 months [7, 8]. PDL plays an important role in the early relapse stage while alveolar bone and osteoclasts prefer to participate in the late [9]. Periodontal ligament fibroblasts (PDLFs), which are located in the periodontal PDL, possess large nuclei and alkaline cytoplasm. These cells are the most abundant within the PDL and play a crucial role in the repair, regeneration, and remodeling of periodontal tissues [10, 11].

Apart from the mechanical forces applied to the tooth (e.g., retainers) and surgical approaches (e.g., fiberotomy), biological interventions aimed to enhance osteoblast and/or inhibit osteoclast are some of the strategies to shorten orthodontic retention time. Photobiomodulation (PBM) and drug therapies are the most widely studied biological interventions in orthodontic treatment.

PBM, also known as low-level laser therapy (LLLT), has been applied in oral basic research and clinical practice in many terms, such as bone stimulation, etching, and surgical procedures [12–15]. LLLT has been shown promoting angiogenesis, fracture healing and osteogenic differentiation of stem cells [16]. Various signaling pathways have been reported to play an important role in LLLT-induced osteogenesis, including ROS/HIF-1α, PI3K/Akt/Bcl-2, and the Wnt and Smads 2/3-β-catenin pathway [17, 18]. Wang et al.. elaborated on the mechanisms of PBM in orthodontics via four important sections including blood vessels, inflammatory response, collagen and fibers, and mineralized tissues, and they provided valuable insights into LLLT [19]. It is worth noting that LLLT combined with a retainer may shorten the retention time, while without any retainer may increase the rate of relapse, so combining conventional retainer with LLLT can achieve the better retention effect [20, 21]. Bone morphogenetic protein-2 (BMP-2) can accelerate the regeneration of bone and cementum and the insertion of PDL fibers [22]. Multiple studies have suggested that BMP-2 induces the expression of osteogenic proteins and promotes the periodontal regeneration, which can serve as a valuable indicator in comprehending the mechanisms involved in periodontal remodeling [23–25].

Herbal, semisynthetic and synthetic drugs are used to enhance OTM and some drugs are used to prevent orthodontic relapse, such as atorvastatin, aspirin, calcitonin, raloxifene, intermittent parathyroid hormone, osteoprotegerin, reveromycin A, grape seed extract containing cyanidin, adiponectin, sulforaphane, and resveratrol [26–29]. These agents may improve the stability of tooth movement, thus enhancing orthodontic retention. However, they may also have side effects, limited indications, and unclear long-term efficacy. To date, there are no marketed formulations designed specifically to accelerate OTM or reduce orthodontic retention time. Natural fulvic acids (FAs), a fraction of humic acids (HAs) primarily derived from weathered coal, peat, and young lignite, may present a promising option for investigation as functional excipients [30]. Some studies reported that HAs may help prevent alveolar bone loss, while others believed that HAs alone did not significantly affect alveolar structure density [31–33]. Additionally, literature indicates that humic substances possess photosensitizing properties [34] as well as photothermal effects [35].

This study aimed to assess the effects of LLLT, FAs, and the combination of the two on the stability of orthodontic retention, while exploring the potential regulatory role of BMP-2 in this process. The null hypothesis was that the combination of FAs and LLLT would have a synergistic effect in shortening the orthodontic retention phase.

Materials and methods

Establishment of the rat OTM model

A total of 72 8-week-old male Sprague-Dawley (SD) rats weighing 220±20 g were obtained and reared in laboratory animal center of Shanxi medical university in this study. Male rats were selected to minimize the impact of FAs and LLLT on estrogen levels, thereby reducing their effects on bone metabolism. A sample size of 72 rats was determined through Power Analysis and Sample Size (PASS) software (Version 21, NCSS, LLC, Utah, USA), based on osteoclast data from previous literature [33], ensuring a minimum power of 0.9, $\alpha = 0.05$, and accounting for a 10% dropout rate. All procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the Laboratory Animal Ethics and Welfare Committee of Shanxi Bethune Hospital, Shanxi Academy of Medical Science (SBQDL-2022-066). The left maxillary first molars were moved mesially with a force of 50 g using a nickel-titanium tension spring (Fig. 1A). Several published studies have confirmed that applying 40–60 g of orthodontic force does not lead to incisor tilt [9, 20, 27, 33, 36–39]. The force was applied for 3 w with re-calibrated every 7 days using a force gauge.

Establishment of orthodontic retention model and grouping

After three weeks, we removed the tension springs and used twisted orthodontic wires ($\Phi = 0.2$ mm) to create retainer wires to maintain the results of tooth movement (Fig. 1B). At present, no standardized retainer specifically designed for rat models in orthodontics exists, posing challenges in obtaining consistent results. Various alternatives, such as flowable composite materials [15, 20] and orthodontic wire or wire-resin splint-type retainers [21, 27, 40], have been suggested in the literature. Consequently, the use of orthodontic wire has gained some acceptance among researchers. While these devices may seem somewhat rudimentary, they offer a viable solution at this stage and are recognized by many in the field.

Then the rats were randomly divided into four groups (18 rats per group): control group, FAs group, LLLT group, and FAs+LLLT group.

In the control group, no interventions were taken.

In the FAs group, the rats were all administered FAs (No. HFS-ZCF341, FAs content was >90%, obtained from the Humic Acid Engineering and Technology Research Center of Shanxi Province) through gavage, with a dose of 80 mg/kg body weight daily until the scheduled time (Fig. 1C).

In the LLLT group, the left maxillary alveolar bone around first molars were subjected to laser irradiation until the scheduled endpoint. A fiber-coupled laser

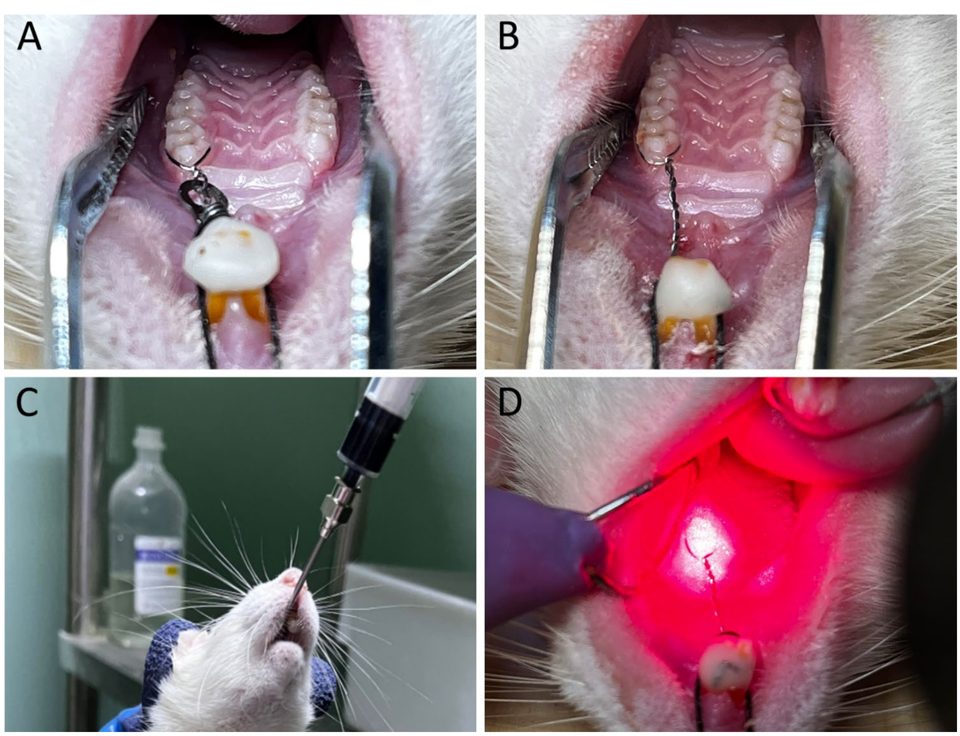

Fig. 1 Graphical representation of the models: (**A**) Orthodontic tooth movement (OTM) rat model, (**B**) the orthodontic retention model, (**C**) rats receiving fulvic acids (FAs) via gavage, and (**D**) laser irradiation applied to the left maxillary alveolar bone surrounding the first molars

system (VCL-808nmM1, Blueprint, Beijing, China) with a wavelength of 808 nm was selected. The laser was configured to continuous mode, delivering an output energy of 1 W with a parallel beam diameter of 10 mm (0.79 cm^2). Irradiation was performed on both the buccal and palatal sites of the first molar, with each site receiving an exposure of 18 s. This protocol resulted in an energy density of approximately 23 J/cm^2 per site, culminating in a cumulative irradiation time of 36 s twice a week (Fig. 1D). It is essential to emphasize the significance of energy density in achieving bio-stimulation effects. The Arndt-Schultz law provides a relevant framework for understanding the dose-dependent effects of LLLT [41], highlighting that insufficient energy may fail to induce a biological response, optimal energy can elicit photo-bio-stimulation, while excessive energy may inhibit rather than stimulate biological activity [42]. Kim et al. have indicated that energy densities in the range of 4.63 to 6.47 J/cm^2 can increase the relapse rate during the retention phase without retainers [21, 43]. Thus, the energy density employed in this study was informed by prior laser irradiation experiments on rats, which indicated that a density of 23 J/cm^2 positively influenced orthodontic retention in rat models [40, 44, 45].

In the FAs+LLLT group, the rats were given both FAs administration and laser irradiation as the same as the FAs group and LLLT group.

Measurement of relapse

Current methods for measuring OTM of the first molars include referencing the central incisors [46], second molars [36, 39, 47], third molars [33], and artificially implanted mini-screws [48]. Given the distance between the first molars and incisors, as well as the significant wear of rodent incisors, using incisors as a reference may introduce larger measurement errors. Employing mini-implants as a reference necessitates additional invasive procedures on the rats; therefore, we chose the second molars as our reference. The distance between the first and second left maxillary molars were all measured three times and the mean value was recorded before the orthodontic force was applied, and we recorded it as *d0*.

For the relapsing distance measurement and histological analysis, six rats of each group were randomly selected on scheduled days 7, 14, and 21 during orthodontic retention phase, respectively. The retainer wires were indeed used in accordance with the scheduled retention phases, specifically for durations of 7, 14, and 21 days.

The selected rats were stopped all experimental interventions and removed the retaining device, allowing the teeth to relapse for three days. The distance between the lowest point of the palatal mesial sulcus of the first and second left maxillary molars were measured and recorded as *d1* on the scheduled day (7 d, 14 d, and 21d, respectively). The distance was measured again on three days after scheduled day (10 d, 17 d, and 24 d, respectively) and we recorded it as *d2* (Fig. 2). The relapsing distance was calculated as $d2 - d1$, and the percentage of relapsing (relapse rate) was defined as $(d2 - d1) / (d0 - d1)$.

Distance measurements were conducted under general anesthesia, with all animals receiving pentobarbital sodium (3%, 1.5 ml/kg body weight, administered via intraperitoneal injection) for anesthesia induction. Initially, a silicone impression (Fig. 3A) was used to create an accurate replica of the dental arch, from which a cast (Fig. 3B) was fabricated using super-hard gypsum stone, ensuring exceptional hardness and precision. The gypsum stone cast was then scanned using a laboratory scanner (D2000, 3Shape, Copenhagen, Denmark), and the image data were saved. The Dental Manager Premium software (version 2021, 3Shape, Copenhagen, Denmark) was used to calibrate the palatal lowest point of the maxillary first molar and the corresponding point for the maxillary second molar, allowing for the measurement of the distance between these two points (Fig. 3C), which was the primary data collected for this experiment. Currently, oral scanning technologies are employed to measure tooth movements [40]; however, they facilitate the permanent storage of research data and allow for repeated measurements, making them more effective than conventional vernier calipers.

Histopathological analysis

After the distance measurement *d2* was completed, the rat was sacrificed through cervical dislocation under general anesthesia. The left maxilla of the rat was harvested (Fig. 3D), fixed in 4% paraformaldehyde solution for 48 h and demineralized in 10% ethylenediaminetetraacetic acid (EDTA) for approximately 30 d at 4 °C.

The specimens were then dehydrated, sampled, embedded in paraffin and sectioned along the molars in a mesiodistal plane for hematoxylin and eosin (HE) and immunohistochemistry (IHC) staining. Sectioning of the paraffin-embedded specimens at 4 μm thickness was performed on a microtome with a new sterile disposable blade. The specimens were then dried in an oven at 45 °C. HE staining was used to observe the PDL structures, osteoclasts, and osteoblasts on the distal side of left first upper molars in each group under a microscope.

For BMP-2 IHC analysis, rabbit anti-rat BMP-2 polyclonal primary antibody (Cohesion Biosciences, London, UK) and horseradish peroxidase (HRP) conjugated goat anti-rabbit secondary antibody (Proteintech, Wuhan, PRC) were chosen for detection. This study focuses on the expression of BMP-2 in PDLFs to assess the effects of FAs and/or LLLT on the remodeling processes of

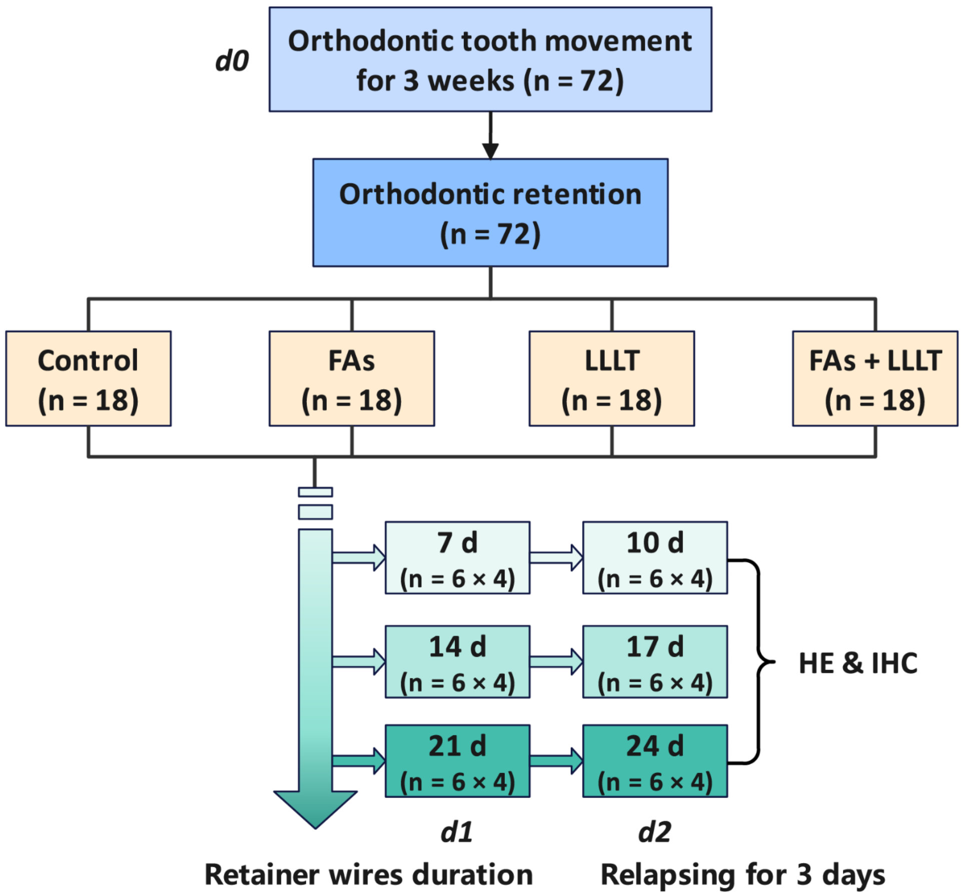

Fig. 2 Flowcharts illustrating the animal study. LLLT, low-level laser therapy

periodontal tissues in vivo. The region of interest (ROI) was defined as the area encompassing the horizontal and oblique fiber bundles of the principal PDL fibers located on the distal sides, excluding the alveolar crest, apical, and interradicular fiber groups. Five visual field were randomly chosen for the analysis of average optical density (AOD) values using ImageJ software (1.54f, NIH, Bethesda, MD, USA).

Statistical analysis

Prism software (version 10, GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis and graphing. The relapse rate was expressed as the mean value ± standard deviation (SD). Normality was tested and confirmed by the Kolmogorov–Smirnov test. Ordinary two-way analysis of variance (ANOVA) was performed to determine whether there was a difference between the groups, and the Tukey's test was used for multiple comparisons. The statistical significance level was set at 5%.

Results

All animals survived, and there was no significant decline in food consumption or body weight across the groups throughout the study.

Analysis of the relapse rate

In this study, retainers were worn for durations of 7, 14, or 21 days. Upon the removal of the retainers, all molars subjected to orthodontic movement displayed relapse, followed by a 3-day relapse observation period (Table 1; Fig. 4). A two-way ANOVA demonstrated significant effects of both time and intervention methods on the relapse rate associated with OTM during the retention phase ($p<0.001$). However, interaction analysis revealed that the interaction time and intervention methods did not significantly affect the relapse rate ($p=0.630$). The main effect analysis demonstrated that both time and intervention methods had statistically significant impacts on the relapse rate ($p<0.001$). Specifically, multiple comparisons for the main time effect showed a reduction in the relapse rate as the duration of retainer wear increased, particularly notable at 10 days, 17 days, and 24 days ($p<0.001$). In terms of intervention effects, comparisons indicated that the group receiving FAs did not produce a statistically significant difference in relapse rates compared to the control group ($p=0.084$). Conversely, both the LLLT group and the FAs + LLLT group significantly reduced relapse rates ($p<0.001$), although

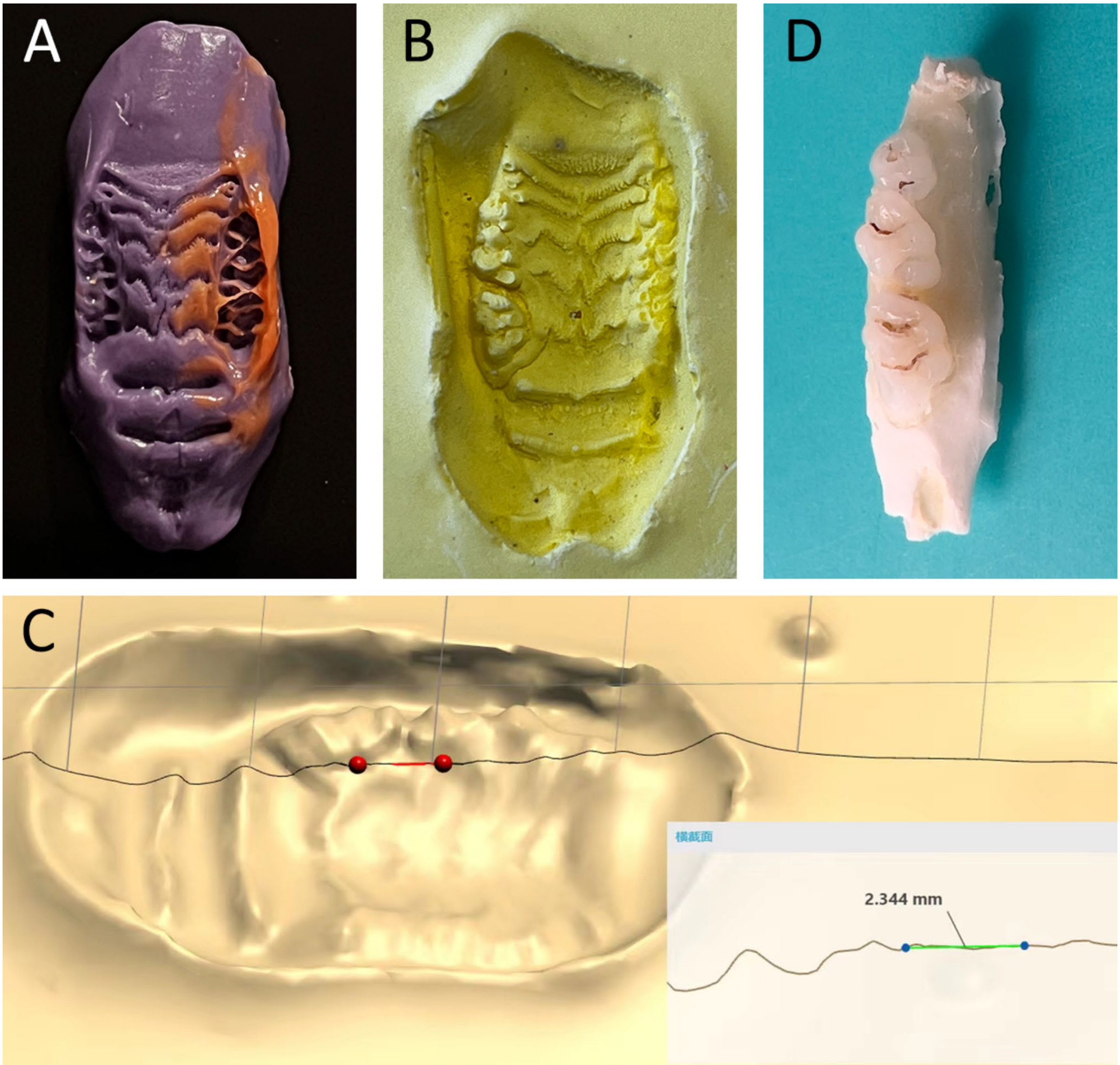

Fig. 3 Measurement of OTM: (**A**) silicone impression of the rat maxilla, (**B**) gypsum stone cast, (**C**) distance measurement using software, and (**D**) the harvested left maxilla

Table 1 Relapse rate of the upper left first molars in each group at various time points ($\bar{x} \pm s$) (total $n = 72$)

Time (d)	Relapse rate (%)			
	Control	FAs	LLLT	FAs + LLLT
10	34.99 ± 3.16^{ax}	34.48 ± 3.18^{ax}	30.75 ± 2.45^{bx}	27.65 ± 3.36^{bx}
17	31.91 ± 1.69^{ay}	28.64 ± 2.32^{ay}	25.74 ± 2.18^{by}	25.51 ± 3.07^{by}
24	24.25 ± 3.33^{az}	21.30 ± 3.29^{az}	18.52 ± 2.16^{bz}	18.19 ± 2.56^{bz}

a, b: → Different lowercase letters in the same row indicate significant differences in the main intervention effects

x, y, z: ↓ Different lowercase letters in the same column signify significant differences in the main time effects

the difference between these two groups was not statistically significant ($p = 0.555$).

Histological analysis

During the rapid relapse phase, osteoclasts were redistributed on the alveolar bone surface opposite the tooth root in response to experimental tooth movement. As the relapse phase stabilized, the number of osteoclasts gradually decreased, and osteoblasts emerged, leading to bone formation and subsequent alveolar bone remodeling.

After the removal of orthodontic force, followed by the retention of the retainer for 7 days and experiencing a

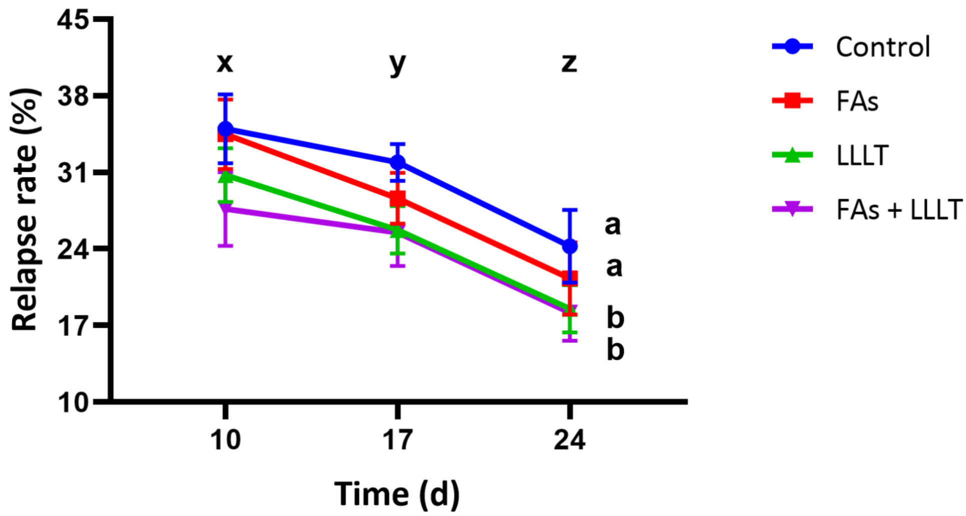

Fig. 4 Relapse rates of the upper left first molars across various time points. Identical lowercase letters denote mean values that do not demonstrate a statistically significant difference, where a and b reflect main intervention effects, while x, y, z pertain to main time effects

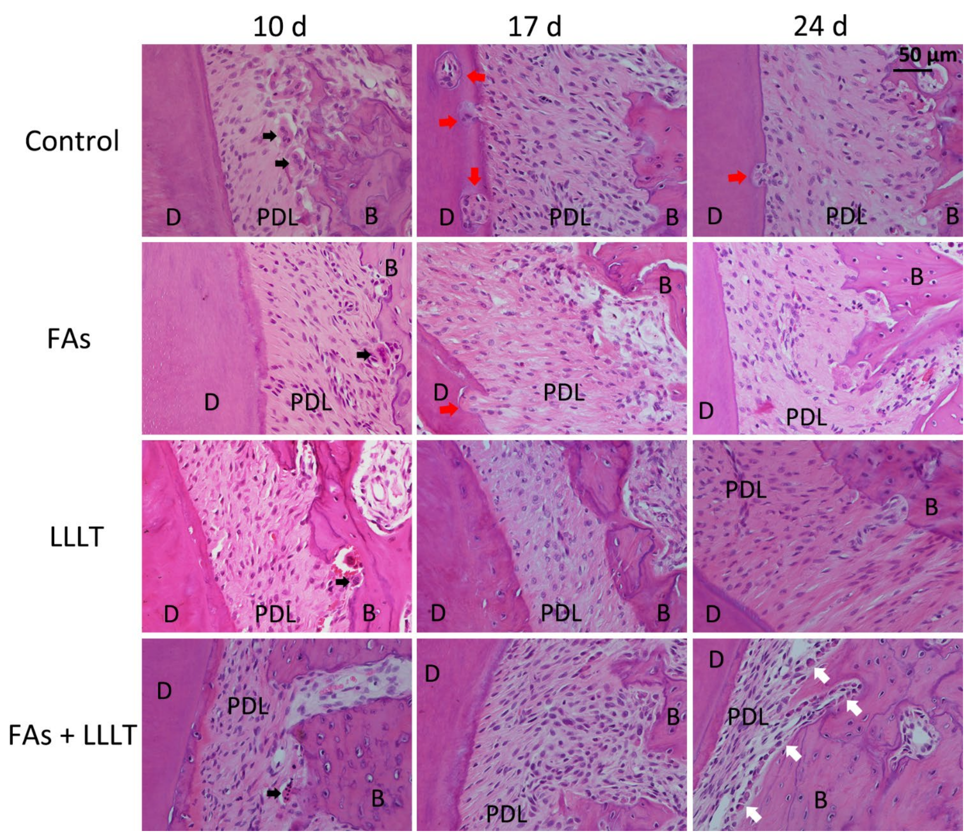

Fig. 5 Histopathological evaluation of upper first molar teeth in each group at different time points, stained with hematoxylin and eosin at a magnification of ×200. Osteoclasts are indicated by black arrows, osteoblasts by white arrows, and local cementum resorption by red arrows. B represents bone, D denotes dentin, and PDL refers to the periodontal ligament

3-day relapse, the HE staining results (Fig. 5) showed the presence of osteoclasts and local cementum resorption in the distal roots of each group, particularly in the direction of relapse. This occurrence was concomitant with disarray in the arrangement of fibroblasts and periodontal fibers in the PDL. Subsequent observations, following 14 days of retainer wear and a 3-day relapse, revealed varying degrees of cementum resorption. The LLLT group and the FAs+LLLT group exhibited lower osteoclast activity and significant reconstruction of periodontal tissues compared to the control and FAs groups. After 21 days of retainer wear and a 3-day relapse, all groups demonstrated reduced osteoclast activity and improved arrangement of periodontal fibers, leading to enhanced stability and decreased relapse rates.

IHC analysis (Fig. 6) revealed positive cytoplasmic expression of BMP-2, characterized by a brownish-yellow hue, in PDLFs across all experimental groups. Furthermore, quantitative analysis of the IHC results indicated that the interventions significantly influenced BMP-2 expression levels in the alveolar bone and PDL during the orthodontic retention phase ($p<0.001$). Nevertheless, time did not exert a significant effect on BMP-2 expression levels ($p=0.867$), which precluded the need for interaction testing to avoid false-positive results (Type I error).

Post hoc multigroup analyses comparing the main effects of the interventions revealed that all three treatment groups significantly elevated BMP-2 expression compared to the control group ($p<0.05$). Additionally, BMP-2 expression levels in the LLLT and FAs+LLLT groups showed significant differences relative to those in the FAs group ($p<0.001$). However, no statistically significant difference in BMP-2 expression emerged between the LLLT and FAs+LLLT groups ($p=0.578$).

Discussion

The findings of this study demonstrate that LLLT effectively decreases the relapse rate of OTM during the retention phase. In contrast, FAs alone do not contribute to a reduction in the relapse rate. Moreover, combining FAs with LLLT does not enhance the effects of LLLT. Thus, the null hypothesis asserting that the combination of FAs and LLLT has a synergistic effect on reducing the retention phase has been rejected.

It is important to note that the duration of orthodontic intervention can vary significantly based on individual patient needs and treatment objectives, potentially extending from one to three years or longer. Similarly, in animal studies, the duration of force application is influenced by both the experimental objectives and the magnitude of the orthodontic force used. Previous research supports retention periods of 14–60 days as acceptable in the literature [37, 39, 46, 48, 49]. Studies have shown that rats experience a rapid relapse in tooth alignment following the removal of force appliances, with relapse rates without any retainers ranging from 62.5 to 73% one day post-removal, stabilizing between 86.1% and 93% by day 21 [50, 51]. This finding informed our choice of a 21-day retention period for this study.

The physiological movement of the second molars can be understood in two perspectives. First, studies indicate that in rats, the second molars exhibit a physiological distal movement rather than a mesial movement, a phenomenon likely driven by occlusal forces [52]. Conversely, the mesial movement of the second molars—resulting in the tilting of adjacent teeth towards the gap—was found to be negligible, with minimal changes occurring within one month, especially in comparison to the movement distance of first molars subjected to external orthodontic forces. To our knowledge, existing literature does not support the practice of ligating the second and third molars to enhance accuracy, nor can it ascertain whether such ligation during the application of orthodontic traction or retention would cause unnecessary harm to experimental animals. Consequently, we adhere to the method of not ligating the second and third molars, as prescribed in previous studies.

Furthermore, the existing literature demonstrates that, even in the absence of orthodontic forces on the first molars, the positions of the second molars undergo changes [48]. Notably, when using mini-screws as reference points, the screws themselves may also shift [48]. Therefore, any physiological movement of the reference points could introduce systematic errors in measurements.

It is also important to note the distinctive dental structure of rats, which features ever-growing, rootless incisor teeth that allow for rapid regrowth as they wear down. This characteristic is crucial to consider when designing experiments. To ensure the stability of the devices and to prevent accidental displacement due to incisor wear, we used flowable resin to stabilize the retainer wires. Additionally, it was vital to apply the resin below the incisor tips during the application process to avoid unnecessary alterations in occlusion.

The remodeling of the PDL occurs over a period of three to four months [53]. Clinically, retainers (mechanical retention) are used to maintain teeth in their corrected positions until this remodeling of the supporting tissues is complete. However, biological interventions can expedite tissue recovery, enhance new bone formation, promote periodontal tissue remodeling, inhibit alveolar bone resorption, and reduce the orthodontic relapse rate while shortening the mechanical retention period [29].

Currently, there is ongoing debate regarding the impact of low-level laser therapy (LLLT) on orthodontic relapse, likely due to variations in laser wavelengths and irradiation parameters employed in different LLLT studies. The selection of LLLT parameters during the orthodontic retention phase remains an area of limited consensus in the literature. Previous studies indicate that commonly used dental lasers operate within a wavelength range of 488 to 10,600 nm, with tissue interactions occurring at energy levels of 1–1000 J/cm². Laser irradiation energy for dental alignment typically ranges from 260 to 336 J/cm² per month [6]. The total energy of laser irradiation employed in this study closely aligns with values reported

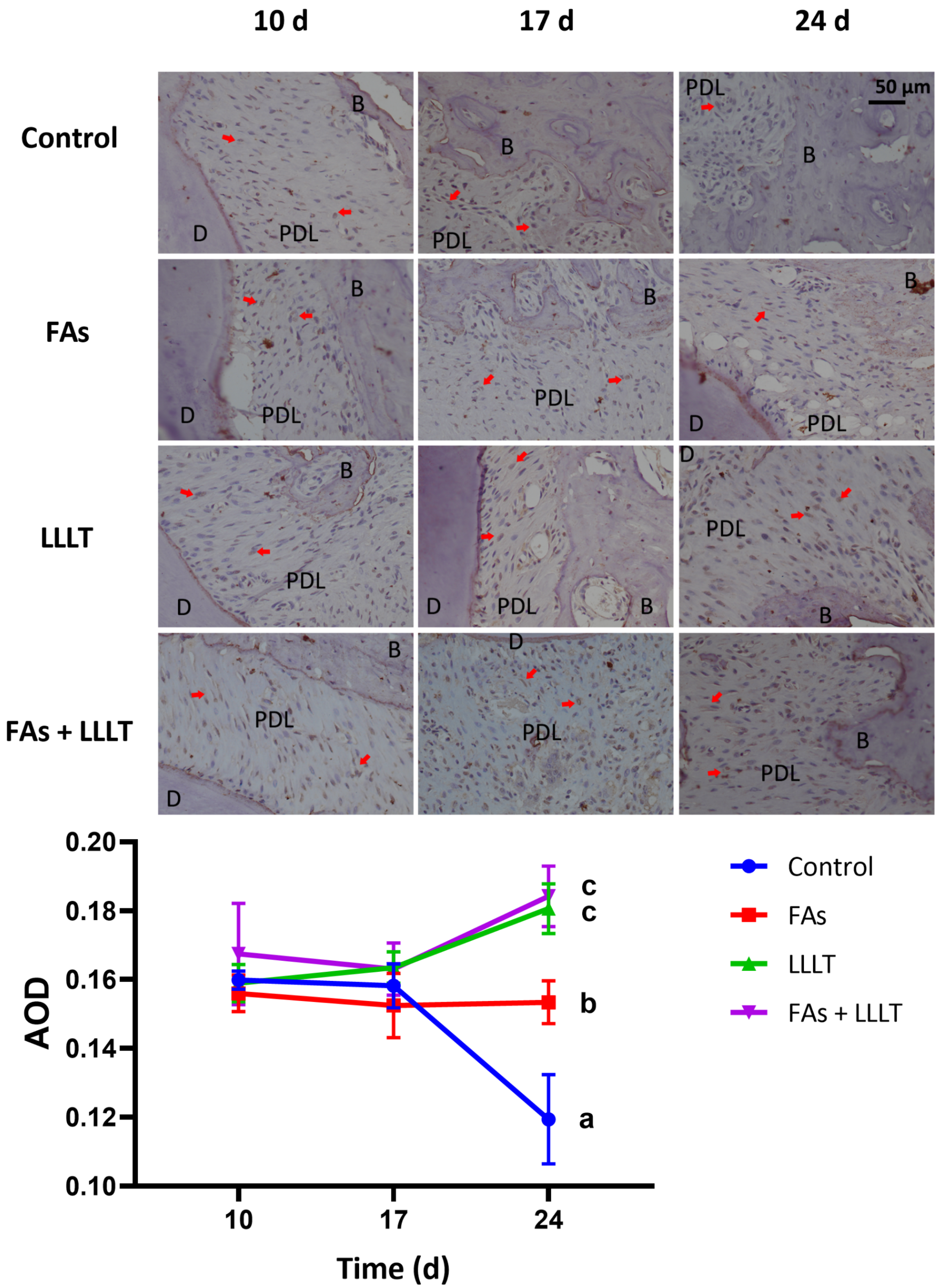

Fig. 6 Comparison of BMP-2 expression across each group at various time points, assessed through immunohistochemical staining of BMP-2 at a magnification of ×200. The same lowercase letters indicate mean values without statistically significant differences. Each subunit of the composite imagery represents a distinct region of interest (ROI), with red arrows indicating positive expression of PDL fibroblasts

in the literature. We selected an 808 nm semiconductor laser due to its cost-effectiveness and availability, as well as its favorable tissue penetration properties within the biologically transparent window (700–1100 nm) [54].

Many scholars believe that LLLT offers significant advantages in accelerating OTM, reducing pain, and decreasing orthodontic relapse [14, 20, 21, 55–57]. Research by Kim et al.. studied the effect of LLLT on the relapse of rotated teeth in dogs, indicating that LLLT (4.63–6.47 J/cm^2) may increase the relapse in rotated teeth without retainers [43]. Subsequent research further explored the effect of LLLT on OTM relapse and PDL remodeling during retention, confirming that LLLT increases the relapse rate post-treatment without retainers but can accelerate periodontal remodeling at the new tooth position when combined with retainers [21]. While Franzen et al.. suggested that LLLT may reduce the relapse tendency, partly due to bone formation in the previous tension region and redistribution of osteoclasts following orthodontic force removal [44]. Our animal experiment findings align with the conclusions of researchers regarding the efficacy of using LLLT in conjunction with wearing retainers to reduce orthodontic relapse and potentially shorten retention phase.

The results obtained from HE staining and IHC analysis provide partial insights into the issue of orthodontic tooth relapse rates. LLLT appeared to increase BMP-2 expression, facilitating the remodeling of periodontal tissue. Although the expression of BMP-2 in the FAs group was higher than that in the control group, this combination did not yield a synergistic effect compared with LLLT. Additionally, no specific correlation was identified between the relapse rates of orthodontic teeth measured at different time intervals and the corresponding histopathological findings. Such discrepancies often arise in clinical presentations and pathological assessments; for instance, certain pathological features may allow for the diagnosis of tumor type without accurately predicting tumor size or growth duration. These findings underscore the importance of mechanical retention methods, such as the use of retainers, during the overall orthodontic retention phase.

There are studies focusing on HAs combined with 808 nm laser for tumor photothermal therapy and HAs with 650 nm laser for accelerated OTM, our research specifically investigates the use of FAs combined with 808 nm laser for the orthodontic retention phase. Additionally, although FAs are a subclass of HAs, it is important to note that they possess distinct properties and mechanisms of action, which may contribute differently to orthodontic treatments compared to HAs. Çalışır et al.. found that administration of HAs at a dose of 80 mg/kg, whether given systemically (by gastric feeding) or locally (by holding small cotton pellets soaked with HAs solutions around the tooth) for 15 days until sacrifice, may have a preventive effect on alveolar bone loss and inflammation in a rat model [31, 32]. Miao et al.. have reported that sodium humate (the salt form of HAs) exhibit photothermal capabilities (808 nm, 1.1 W/cm^2) achieving a conversion efficiency of up to 76.3%, and this highlights their potential as a phototheranostic agent with promising applications in anti-tumor therapies [35]. Additionally, HAs can serve as a photothermal drug carrier (808 nm, 1.5 W/cm^2), combined with other photosensitizers (e.g., verteporfin (689 nm, 100 mW/cm^2)) to form synergistic diagnostic and therapeutic formulations with photothermal and photodynamic effects [58]. An et al.. conducted research to investigate the effects of utilizing HAs as a photosensitizer along with LLLT on OTM in rats [33]. By injecting HAs intraperitoneally into the rats (80 mg/kg, daily) and applying a semiconductor laser irradiation (650 nm, 50 mW, 50 s every other day) to the periodontal tissue surrounding the upper first molars, they observed accelerated OTM, increased number of bone absorption lacunae and osteoclasts on the alveolar bone's pressure side without any root resorption [33]. However, the available evidence regarding the using of HAs as a photosensitizer in vivo appears to be lacking as their study results did not conclusively demonstrate the production of cytotoxic reactive oxygen species (ROS) or other free radicals, cations, anions, etc., by HAs upon exposure to a 650 nm wavelength laser.

Limitations

This study acknowledges several limitations. First, the pharmacological and photochemical effects of FAs, which can be derived from coal or plant sources, may vary based on origin and extraction methods. Second, LLLT encompasses a diverse range of clinical lasers with varying wavelengths and parameters, all of which may influence experimental outcomes. Third, twisted wires used as retainers in this experiment align with extant literature; however, there remains a possibility of relapse occurring with the use of twisted wires alone or in combination with flowable composite materials. Exploring advancements in retainer designs within rat models may offer promising avenues for mitigating relapse during orthodontic retention and represents a crucial direction for future research. Subsequent studies will focus on cell-based experiments to directly assess the photothermal and photodynamic effects of FAs under low-level laser irradiation while aiming to minimize extraneous variables and investigate the upstream and downstream signaling proteins and RNAs associated with BMP-2.

Conclusions

Based on the findings of this study, the following were concluded:

1. During the retention phase following OTM in rats, the administration of FAs as an adjunctive therapy, alongside the use of retainers, does not significantly reduce the rate of orthodontic relapse.
2. LLLT effectively decreases the relapse rate, although its efficacy is not enhanced by concurrent application of FAs.
3. FAs exhibit limited effects on the remodeling of periodontal tissue during orthodontic retention, whereas LLLT significantly enhances this remodeling phase.
4. Both FAs and LLLT increase BMP-2 expression in PDLFs, yet no synergistic effect is observed.

Abbreviations

ANOVA	Analysis of variance
AOD	Average optical density
BMP-2	Bone morphogenetic protein-2
EDTA	Ethylenediaminetetraacetic acid
FAs	Fulvic acids
HAs	Humic acids
HE	Hematoxylin and eosin
HRP	Horseradish peroxidase
IHC	Immunohistochemistry
LLLT	Low-level laser therapy
OTM	Orthodontic tooth movement
PBM	Photobiomodulation
PDL	Periodontal ligament
PDLFs	Periodontal ligament fibroblasts
ROI	Region of interest
ROS	Reactive oxygen species
SD	Sprague-Dawley

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Author contributions
Each author is expected to have made substantial contributions to the conception of the work, the acquisition, analysis, and interpretation of data. J.Z. & P.X. has drafted the work or substantively revised it. All authors reviewed the manuscript.

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Data availability
The data that support the findings of this study are available from the authors but restrictions apply to the availability of these data, which were used under license from the Shanxi Bethune Hospital (Taiyuan) for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from Shanxi Bethune Hospital.

Declarations

Ethics approval and consent to participate
All procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the Laboratory Animal Ethics and Welfare Committee of Shanxi Bethune Hospital, Shanxi Academy of Medical Science.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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