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Original Article

# Effect of humic acid as a photosensitizer combined with low-energy laser on orthodontic tooth movement in rats

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## KEYWORDS

Humic acid;  
Lasers;  
Orthodontic tooth  
movement;  
Bone density;  
Photosensitizer

**Abstract** *Background/purpose:* Humic acid (HA) could promote light conversion reaction, and lasers accelerate orthodontic tooth movement. We investigated the effect of HA, as a photosensitizer, combined with low-energy laser on orthodontic tooth movement in rats.

*Materials and methods:* An orthodontic tooth movement model was established, and the upper left first molar was moved mesially by a nickel-titanium tension spring with a 50-g force. HA was injected into the rats' abdominal cavity (80 mg/kg once daily). The periodontal tissue of the upper left upper first molar on the pressure side was irradiated (50 s once every 2 days) using a semiconductor laser (wavelength, 650 nm; power, 50 mV). Distance moved by the upper left first molar was measured at different time points, and the tissue of the first molar was sectioned and scanned by micro-computed tomography to evaluate the alveolar bone density. Tartrate-resistant acidic phosphatase staining was used to observe the osteoclast number, alveolar bone, and periodontal tissue.

*Results:* HA alone did not significantly affect orthodontic tooth movement, alveolar structure density, or periodontal tissue remodeling ( $P > 0.05$ ). HA combined with a low-energy laser accelerated orthodontic tooth movement. The number of bone absorption lacunae and osteoclasts on the alveolar bone's pressure side increased significantly ( $P < 0.05$ ), while the density decreased significantly ( $P < 0.05$ ); however, no root absorption was observed.

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**Conclusion:** HA can improve the conversion rate of low-energy lasers, enhance the low-energy laser effect, and promote orthodontic tooth movement and periodontal tissue reconstruction on the pressure side in rats, without causing root resorption.

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## Introduction

In orthodontic treatment, patients and dentists aim to shorten the treatment period by accelerating orthodontic tooth movement and alveolar bone remodeling.<sup>1,2</sup> Low energy lasers promote vascular regeneration, improve blood circulation, facilitate the reconstruction of the periodontal membrane and alveolar bone, and accelerate orthodontic tooth movement.<sup>3–6</sup>

A macromolecular organic compound, humic acid (HA), is widely found in water and soil,<sup>7</sup> and has low toxicity at the cellular and animal levels.<sup>8</sup> In the 1970s and 1980s, its pharmacological, physiological, and toxicological effects were extensively studied.<sup>9</sup> Currently in dental clinics, HA is used for periodontal disease and surgery, and in cases of oral mucosal diseases, such as recurrent aphthous ulcer, herpetic stomatitis, and Sjogren's syndrome. Additionally, it is used as a periodontal plugging agent to manage apical fistula, post-extraction hemostasis, tooth replantation, and treatment of maxillofacial tumors. Çalışır et al.<sup>10</sup> found that 80 and 150 mg/kg of HA significantly reduced alveolar bone loss and inflammatory response in a rat periodontitis model.

As a natural photosensitizer, HA promotes light conversion reaction.<sup>11</sup> A previous work examined the use of HA as a photosensitizer, and proved that it has good light absorption and a high photothermal conversion rate.<sup>9</sup> Colloidal sodium humate was creatively used in diagnostic preparations with photothermal effects, and the photothermal conversion efficiency of the purified colloidal sodium humate was 76.3%, according to Cheng.<sup>12</sup>

The propagation of a laser in tissue consists of light and heat propagation.<sup>13</sup> The energy loss of the laser follows the exponential decay law when the laser penetrates the tissue.<sup>14</sup> In cases of laser energy loss when irradiating the periodontal tissue, the photosensitizer can be used to enhance the laser effect. However, to date, there is no evidence concerning the photosensitizer effect on orthodontic tooth movement combined with laser. In this study, HA as a photosensitizer, combined with local irradiation of a low-energy laser, was applied to orthodontic tooth movement in rats. The distance moved by the upper left first molar and reconstruction of the alveolar bone and periodontal tissue on the pressure side were observed at different time points. Further, the HA potential to enhance the conversion rate of low energy laser was evaluated.

## Materials and methods

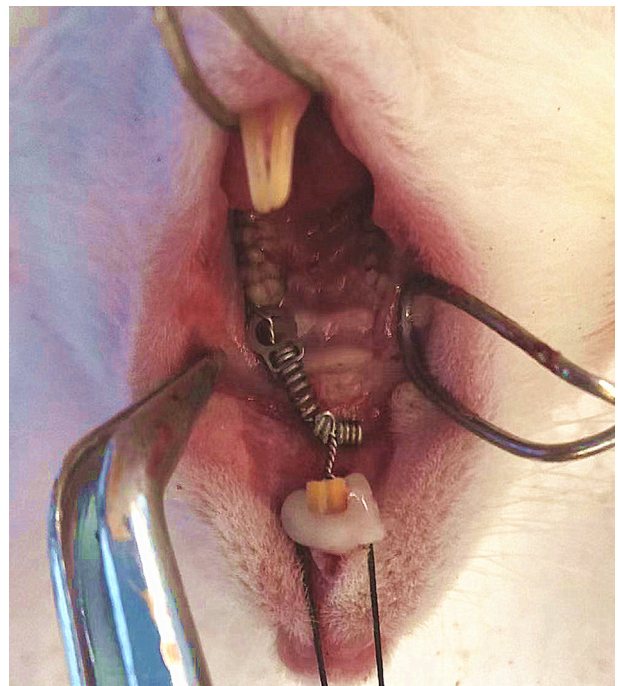
This study protocol was approved by the ethics committee of XXX, and was conducted in accordance with the regulations of XXX on the use of experimental animals.

## Establishment of the rat model

One-hundred-sixty male Wistar rats (age, 8 weeks; body weight,  $200 \pm 20$  g) were obtained from the Experimental Center of XXX. One side of a nickel-titanium tension spring was ligated to the neck of the upper left upper first molar, and the other side into the shallow groove on the necks of the two incisors (Fig. 1). The tension spring was stretched with an initial force value of 50 g, measured using an orthodontic force meter (Suhang Corp., Shenzhen, China), and was re-established every 7 days.

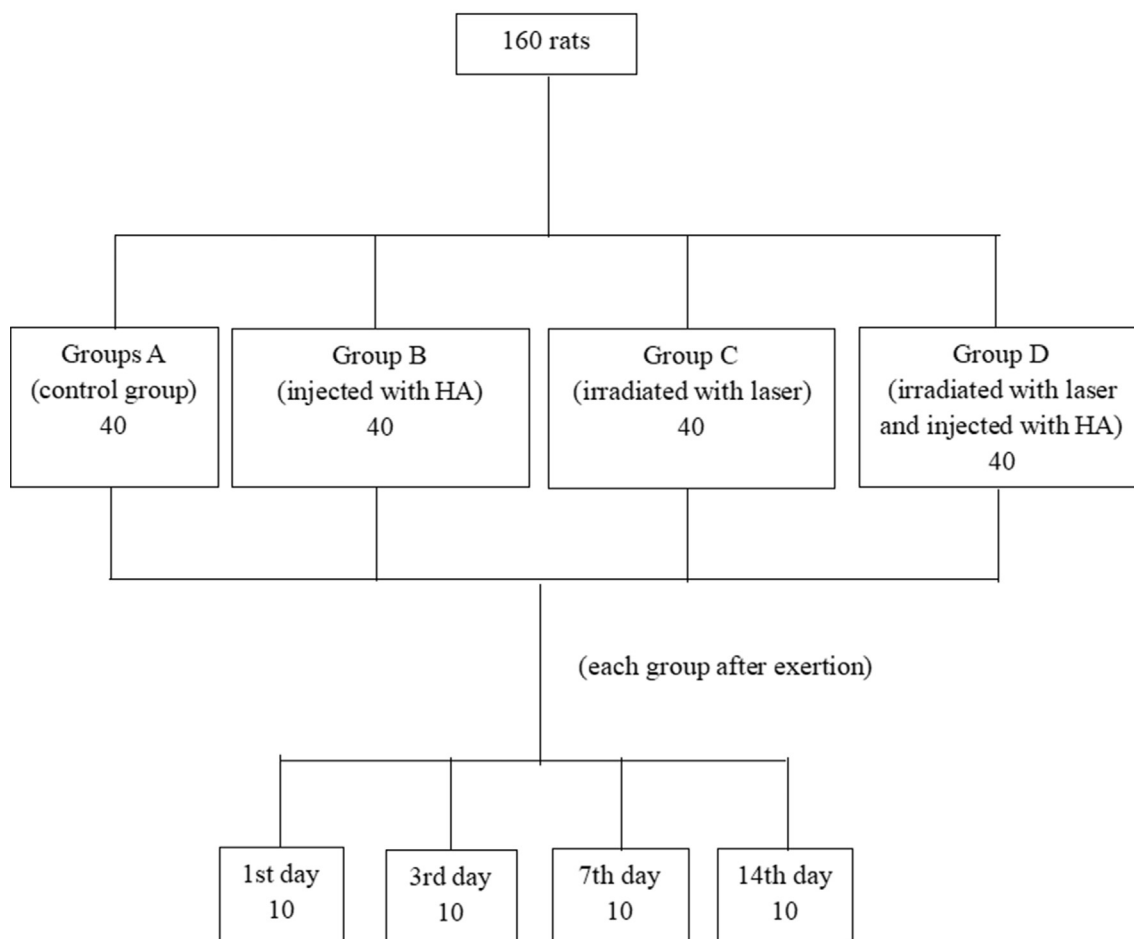
## Grouping of experimental animals

According to the time points after exertion, the 160 rats were randomly divided into four groups: the 1st, 3rd, 7th, and 14th day groups. Each group was further divided into four subgroups: Groups A (control group), B (injected with HA), C (irradiated with laser), and D (irradiated with laser and injected with HA) (Fig. 2). There were 10 rats in each subgroup.



**Figure 1** The orthodontic tooth movement rat model.

### Grouping of experimental animals



**Figure 2** The flowchart of grouping experimental animals.

#### Preparation of HA solution and evaluation of the animal safety test

HA solutions were prepared with concentrations of 2, 1, 0.5, 0.25, and 0.125 mg/mL. These were injected into the abdominal cavity of rats at a dose of 80 mg/kg,<sup>10</sup> once a day, to detect the toxic effect on rats and to determine the maximum suitable concentration.

#### Parameters of semiconductor laser and irradiation sites

A semiconductor laser with a wavelength of 650 nm and a power of 50 mV was selected (Jinxin Corp., Wuhan, China). The irradiation sites were on the mesial, mesiobuccal, and mesiopalatal surfaces of the upper left first molar; each site was irradiated for 50 s, once every 2 days.

#### Measurement of distance of tooth movement

Rats in each group were sacrificed on days 1, 3, 7, and 14 after exertion. The maxillary arches of the rats were taken,

and anhydrite models were prepared. The distance between the mesiolingual grooves of the first and third molars on both sides of the maxilla was measured with a vernier caliper. The mesial movement of the first molar was defined as the difference between the left and right sides. Measurements were performed twice, and the mean value was considered.

#### Micro-computed tomography scanning

From the maxillary specimens of the rats, the soft tissues were removed. Then, the maxilla was sectioned, retaining only the area of the first molar. The specimens were fixed in 4% paraformaldehyde for 24 h, and, then, scanned by micro-computed tomography (CT) (Skyscan-1276; Bruker Corp., Billerica, MA, USA; voltage, 70 kV; current, 90  $\mu$ A; thickness; 5.0  $\mu$ m, and pixel size, 1536  $\times$  1024). The density of the alveolar bone on the pressure side was measured.

#### Specimen preparation

Subsequently, the specimens were decalcified in 10% EDTA solution for 30 days until no resistance was observed when

**Table 1** Distance moved by the upper left upper first molar in each group at different time points (mm,  $\bar{x} \pm s$ ).

Group	1st day	3rd day	7th day	14th day
A	0.118 $\pm$ 0.013	0.290 $\pm$ 0.024 <sup>b,c</sup>	0.610 $\pm$ 0.018 <sup>b,c</sup>	0.739 $\pm$ 0.021 <sup>b,c</sup>
B	0.116 $\pm$ 0.008	0.291 $\pm$ 0.026 <sup>d,e</sup>	0.608 $\pm$ 0.013 <sup>d,e</sup>	0.742 $\pm$ 0.019 <sup>d,e</sup>
C	0.136 $\pm$ 0.010	0.440 $\pm$ 0.015 <sup>b,d</sup>	0.881 $\pm$ 0.015 <sup>b,d,f</sup>	1.272 $\pm$ 0.021 <sup>b,d,f</sup>
D	0.144 $\pm$ 0.012	0.458 $\pm$ 0.015 <sup>c,e</sup>	1.197 $\pm$ 0.013 <sup>c,e,f</sup>	1.422 $\pm$ 0.020 <sup>c,e,f</sup>

<sup>a</sup>  $P < 0.05$ :(A vs B); <sup>b</sup>  $P < 0.05$ :(A vs C); <sup>c</sup>  $P < 0.05$ :(A vs D); <sup>d</sup>  $P < 0.05$ :(B vs C); <sup>e</sup>  $P < 0.05$ :(B vs D); <sup>f</sup>  $P < 0.05$ :(C vs D).

Groups: A, control group; B, injected with humic acid; C, irradiated with laser; and D, irradiated with laser and injected with humic acid.

punctured with a pin. After conventional paraffin embedding, it was sectioned along the mesiodistal direction of the first molar (slice thickness, 5  $\mu$ m) and, then, dried in an oven.

### Tartrate-resistant acidic phosphatase staining

Tartrate-resistant acidic phosphatase (TRAP) staining was used to observe the remodeling of periodontal tissue and alveolar bone, and to count the number of osteoclasts on the pressure side in each group under a microscope.

### Statistical methods

Statistical analysis was performed using SPSS statistical software (IBM Corp., Armonk, NY, USA). All data were analyzed with one-way ANOVA, with significance set at  $P < 0.05$ .

## Results

### Animal safety test and concentration determination of HA solution

All concentrations of HA solution were injected intraperitoneally into rats (dose, 80 mg/kg), and no obvious acute or chronic toxic reactions occurred. Therefore, 2 mg/mL HA solution was used.

### Movement of the upper left first molar in rats at different time points

With time, the distance moved by the first molar increased continuously within the same group, and the intra-group comparison showed statistically significant differences in tooth movement among the groups on days 1, 3, 7 and 14 exertion ( $P < 0.05$ ) (Table 1 and Fig. 3).

At the same time point, the movement of the first molar was different among different groups, except that between Groups A and B. On day 1, there was no significant difference in movement between Groups A, B, C, and D ( $P > 0.05$ ). On day 3, the movements in Groups C and D were significantly greater than those of Groups A and B ( $P < 0.05$ ); however, there was no significant difference between Groups A and B ( $P > 0.05$ ). Although the movement in Group D was greater than that in Group C, the difference was not statistically significant ( $P > 0.05$ ). On days 7 and 14, the comparison of the movement was as follows: Group D > Group C > Group A  $\approx$  Group B ( $P < 0.05$ ).

### Density of alveolar bone on the pressure side at different time points

Micro-CT scanning showed that the density of alveolar bone decreased gradually with time in the same group and was the lowest on day 14 after exertion (Table 2 and Figs. 4 and 5). On day 1, the density of alveolar bone was similar among the four groups ( $P > 0.05$ ). On day 3, the alveolar bone densities in Groups C and D were lower than those in Groups A and B ( $P < 0.05$ ); however, there was no difference between Groups A and B or between Groups C and D ( $P > 0.05$ ). On days 7 and 14, the alveolar bone densities were as follows: Group D < Group C < Group A  $\approx$  Group B ( $P < 0.05$ ).

### Remodeling of the periodontal tissue on the pressure side

With time, TRAP staining showed that the number of absorption lacunae in the alveolar bone increased gradually in the same group, and peaked on day 14, similar to TRAP staining positive cells (osteoclasts) (Fig. 6). On day 1, alveolar bone absorption did not occur on the pressure side of the groups (Fig. 7A). On day 3, alveolar bone absorption occurred on the pressure side in all groups. The numbers of bone absorption lacunae in Groups C and D were greater than those in Groups A and B. However, there was no significant difference between Groups A and B or between Groups C and D (Fig. 7B). On days 7 and 14, periodontal fiber degeneration was obvious. The number of bone



**Figure 3** Movement of the upper left first molar in different time points.

**Table 2** Density of alveolar bone on the pressure side at different time points ( $\text{mg}/\text{cm}^3$ ,  $\bar{x} \pm s$ ).

Group	1st day	3rd day	7th day	14th day
A	2.270 $\pm$ 0.022	2.692 $\pm$ 0.018 <sup>b,c</sup>	2.510 $\pm$ 0.026 <sup>b,c</sup>	2.315 $\pm$ 0.030 <sup>b,c</sup>
B	2.785 $\pm$ 0.025	2.693 $\pm$ 0.021 <sup>d,e</sup>	2.499 $\pm$ 0.034 <sup>d,e</sup>	2.309 $\pm$ 0.031 <sup>d,e</sup>
C	2.771 $\pm$ 0.019	2.588 $\pm$ 0.046 <sup>b,d</sup>	2.410 $\pm$ 0.027 <sup>b,d,f</sup>	2.207 $\pm$ 0.016 <sup>b,d,f</sup>
D	2.772 $\pm$ 0.012	2.572 $\pm$ 0.055 <sup>c,e</sup>	2.348 $\pm$ 0.034 <sup>c,e,f</sup>	2.120 $\pm$ 0.023 <sup>c,e,f</sup>

<sup>a</sup>  $P < 0.05$ :(A vs B); <sup>b</sup>  $P < 0.05$ :(A vs C); <sup>c</sup>  $P < 0.05$ :(A vs D); <sup>d</sup>  $P < 0.05$ :(B vs C); <sup>e</sup>  $P < 0.05$ :(B vs D); <sup>f</sup>  $P < 0.05$ :(C vs D).

Groups: A, control group; B, injected with humic acid; C, irradiated with laser; and D, irradiated with laser and injected with humic acid.

absorption lacunae was as follows: Group D>Group C>Group A≈Group B (Fig. 7C, D). No significant root absorption occurred in any group, suggesting that HA alone had no effect on periodontal tissue remodeling on the pressure side during orthodontic tooth movement, and that low-energy laser can promote alveolar bone and periodontal tissue remodeling on the pressure side. However, HA combined with a low-energy laser promoted periodontal tissue remodeling on the pressure side, and the effect was greater than with low-energy laser irradiation alone.

### TRAP positive cells

TRAP staining showed that the number of TRAP positive cells (osteoclasts) in each group increased gradually with time, and peaked on day 14 after exertion; on intra-group comparison, the number of osteoclasts in the same group showed statistically significant differences at different time points ( $P < 0.05$ ) (Table 3 and Fig. 7).

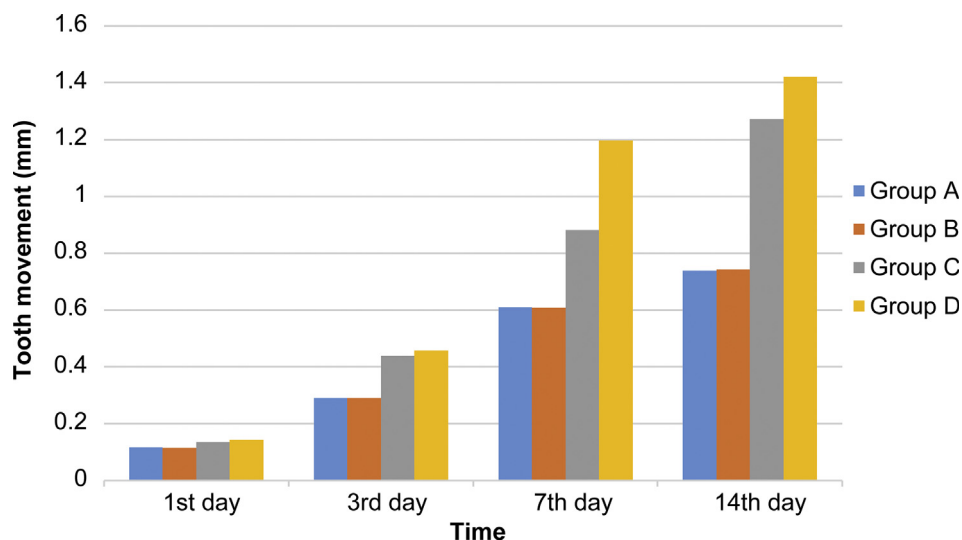
At the same time point, the number of osteoclasts in each group was different; on day 1, osteoclasts were rarely discovered on the alveolar bone surface of the pressure side in Groups A, B, C, and D ( $P > 0.05$ ). On day 3, osteoclasts appeared in the adsorption lacunae of alveolar bone in all four groups; number of osteoclasts in groups C and D was higher than that in Groups A and B ( $P < 0.05$ ). There was no significant difference between Groups A and B or between Groups C and D ( $P > 0.05$ ). On day 7, the number

of osteoclasts was as follows: Group D>Group C>Group A≈Group B ( $P < 0.05$ ). On day 14, osteoclasts in each group were similar to those on day 7, and the number of osteoclasts peaked ( $P < 0.05$ ).

### Discussion

HA has unique pharmacological properties and low toxicity and, therefore, it has long been used to treat diseases in China.<sup>12</sup> In our study, when rats were injected intraperitoneally/intravenously with HA, the lethal dose was 100–500 mg/kg, and up to 5000 mg/kg orally.<sup>8</sup> HA, at doses of 80 and 150 mg/kg, can significantly reduce alveolar bone loss and inflammatory response in rat periodontitis models, with no significant difference observed between the two doses.<sup>10</sup> Therefore, 80 mg/kg was used in this study.

Owing to photothermal, photochemical, and electromagnetic effects, lasers can be converted to biomass after being absorbed by tissue, promoting angiogenesis, improving local blood circulation, providing nutrition to the periodontal membrane, accelerating tissue repair, stimulating fibroblast and osteoblast activity, and finally accelerating osteoblast differentiation.<sup>15</sup> Yang showed that both 660-nm and 830-nm low-energy laser irradiations could accelerate the speed of orthodontic tooth movement in rats, promote the expression of bone remodeling factors related to periodontal cells on the pressure side, and advance the peak of osteoclastic activity.<sup>16</sup> At 7 days after



**Figure 4** Radiograph of micro-computed tomography scanning.

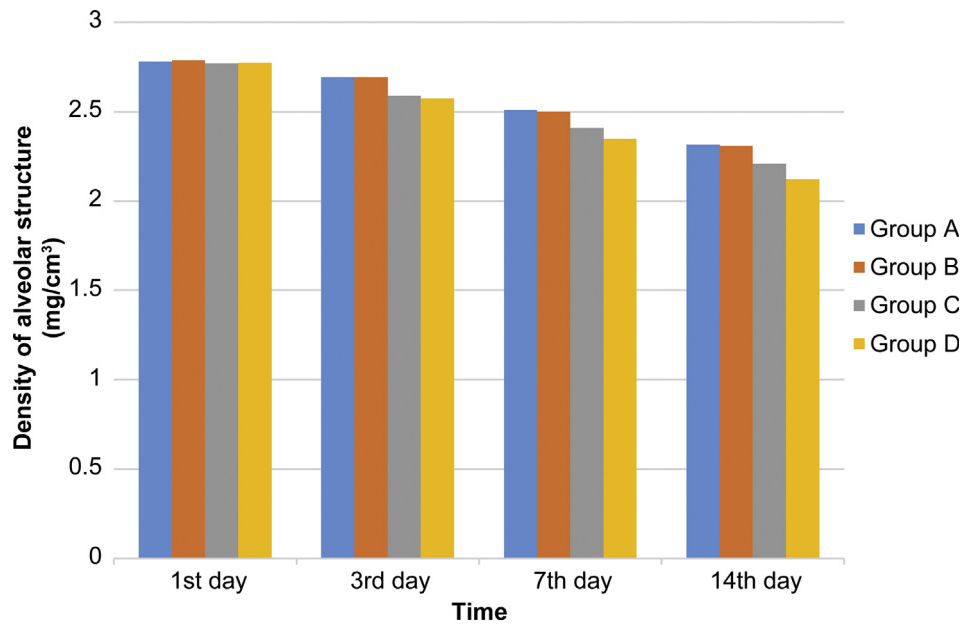


Figure 5 Density of alveolar bone in different time points.

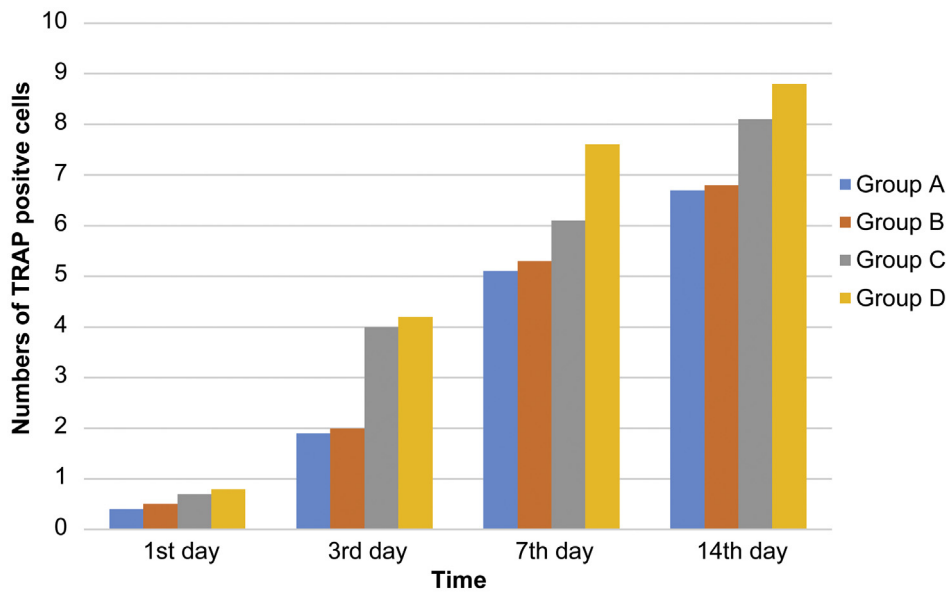


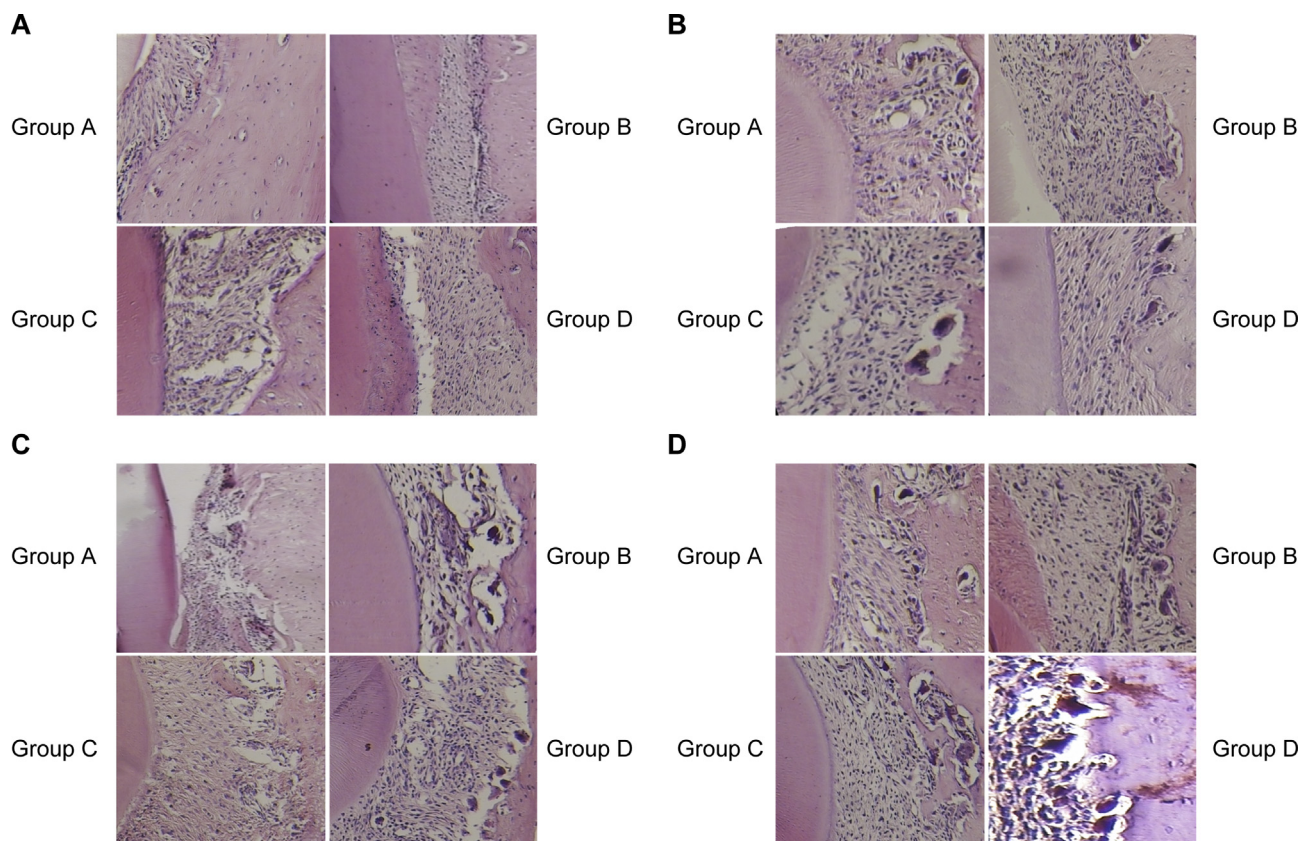
Figure 6 Number of TRAP positive cells in different time point. TRAP, tartrate-resistant acidic phosphatase.

irradiation, 660-nm low-energy laser irradiation absorbed in the periodontal tissues may be higher than that of 830 nm, indicating better acceleration of tooth movement with 660-nm laser irradiation in the early stage. However, there was no difference between the 660-nm and 830-nm low-energy laser irradiations after 14 days, which may be attributed to the "cumulative effect" of the laser; therefore, in our experiment, a 660-nm low energy laser was used.

When a low-energy laser irradiates the soft tissues around the teeth of rats, the energy is lost when penetrating the soft tissue and alveolar bone to the periodontal membrane. Moreover, lasers have a certain conversion rate in tissues. Regarding energy loss, we investigated whether

HA as a photosensitizer can improve the laser conversion rate and reduce the energy loss in this experiment. The results showed that when HA was injected intraperitoneally into rats combined with low-energy laser irradiation of the periodontal tissue of the upper first molar, it accelerated tooth movement and promoted the formation of osteoclasts on the pressure side and periodontal tissue reconstruction, without causing root resorption. Hence, HA can improve the conversion rate of low-energy laser in the periodontal tissue, reduce energy loss, and enhance the effect of laser irradiation on periodontal tissue.

Temperature affects the formation and proliferation of osteoblasts and osteoclasts. *In vitro* thermal stimulation



**Figure 7** (A) Changes of alveolar bone and periodontal membrane on the pressure side in rats on day 1 in all groups. Tartrate-resistant acidic phosphatase staining; magnification, 100 $\times$ . (B) Changes of alveolar bone and periodontal membrane on the pressure side of rats on day 3 in all groups. Tartrate-resistant acidic phosphatase staining; magnification, 100 $\times$ . (C) Changes of alveolar bone and periodontal membrane on the pressure side of rats on day 7 in all groups. Tartrate-resistant acidic phosphatase staining; magnification, 100 $\times$ . (D) Tissue changes of alveolar bone and periodontal membrane on the pressure side of rats on day 14 in all groups. Tartrate-resistant acidic phosphatase staining; magnification, 100 $\times$ .

**Table 3** Number of TRAP positive cells on the surface of alveolar bone on the pressure side in each group ( $\bar{x} \pm s$ ).

Group	1st day	3rd day	7th day	14th day
A	0.40 $\pm$ 0.52	1.90 $\pm$ 0.88 <sup>b,c</sup>	5.10 $\pm$ 0.88 <sup>b,c</sup>	6.70 $\pm$ 0.68 <sup>b,c</sup>
B	0.50 $\pm$ 0.53	2.00 $\pm$ 0.82 <sup>d,e</sup>	5.30 $\pm$ 0.95 <sup>d,e</sup>	6.80 $\pm$ 0.92 <sup>d,e</sup>
C	0.70 $\pm$ 0.48	4.00 $\pm$ 0.82 <sup>b,d</sup>	6.10 $\pm$ 0.74 <sup>b,d,f</sup>	8.10 $\pm$ 0.32 <sup>b,d,f</sup>
D	0.80 $\pm$ 0.42	4.20 $\pm$ 0.63 <sup>c,e</sup>	7.60 $\pm$ 0.52 <sup>c,e,f</sup>	8.80 $\pm$ 0.42 <sup>c,e,f</sup>

<sup>a</sup>  $P < 0.05$ : (A vs B); <sup>b</sup>  $P < 0.05$ : (A vs C); <sup>c</sup>  $P < 0.05$ : (A vs D); <sup>d</sup>  $P < 0.05$ : (B vs C); <sup>e</sup>  $P < 0.05$ : (B vs D); <sup>f</sup>  $P < 0.05$ : (C vs D).

Groups: A, control group; B, injected with humic acid; C, irradiated with laser; and D, irradiated with laser and injected with humic acid.

promotes the proliferation of MC3T3-E1 cells while promoting bone formation and inhibiting bone resorption by regulating the expression of OPG/RANKL mRNA.<sup>17</sup> Heat stimulation promotes the expression of *HSP70* in osteoblasts, promotes the formation of osteoblasts, and inhibits the proliferation of osteoclasts.<sup>18</sup> The laser has a thermal effect, and HA has photodynamic and photothermal effects. Their combined effects increase the energy of the laser. Extremely high laser energy can cause tissue damage; however, in this study, HA combined with low-energy laser irradiation did not cause damage to the gingival, palatal mucosa, periodontal membrane, or alveolar bone in rats. HA injected intraperitoneally into rats at a dose of

80 mg/kg, combined with laser (650 nm, 50 mV) irradiation of the local periodontal tissue for 50 s did not cause irreversible damage to the periodontal tissue, and did not inhibit the formation and proliferation of osteoclasts at the pressure side.

However, this experiment only showed that HA, as a photosensitizer, can improve laser conversion rate, including photothermal, photochemistry, and electromagnetism; however, this should be investigated further along with the measurement of the distribution of low-energy lasers in the soft tissue, alveolar bone, and periodontal membrane after absorption. Furthermore, the mechanism of the effect on osteoclasts should be explored *in vitro* and *in vivo*.

In conclusion, HA alone had no effect on orthodontic tooth movement, the density of alveolar structure, or periodontal tissue remodeling at the pressure side. As a photosensitizer, it can improve the conversion rate of low-energy lasers, enhance its effect, and promote orthodontic tooth movement and the reconstruction of periodontal tissue on the pressure side without causing root damage in rats. Moreover, its effect is greater than that of laser irradiation alone. The photodynamic and photothermal effects of HA did not damage the periodontal tissues of rats when combined with low-energy laser irradiation.

## Declaration of Competing Interest

There were no conflicts of interest to be declared.

## Acknowledgments

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