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Dynamics and observations of long-term orthodontic tooth movement and subsequent relapse in C57BL/6 mice

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Abstract: The risk of relapse associated with orthodontic treatment is a major problem. Despite extensive research and discussion regarding the risk of orthodontic relapse, the underlying mechanisms remain to be elucidated. This study aimed to evaluate relapse following orthodontic treatment in mice (C57BL/6) tested via the coil spring method based on tooth movement at 21 days and mechanical retention at 7 days after completion of the procedure. During the experiment, relapse was observed and evaluated over 7 days. At the end of orthodontic tooth movement, the average distance was 259.6 (± 10.9) μm, and tooth movement was observed in all mice. No significant differences in distance were observed at the end of the experimental treatment period or after 7 days of mechanical retention. The distance at the start of observation was 258.6 (± 10.4) μm, whereas that at the end was 155.4 (± 12.4) μm, indicating that the distance had decreased significantly. Relative to the total relapse distance over the 7-day period, 45.7 (± 4.3)% of the relapse was observed on Day 0–1. The mouse model established in the current study provides an effective and reproducible method for the optimal evaluation of relapse. Our findings clarified that most of the relapse occurs within 7 days during the initial observation stage.
Key words: coil spring method, mechanical retention, orthodontic tooth movement, relapse, relapse observation mouse

Introduction

Relapse after orthodontic treatment is a major problem in clinical orthodontic practice. It is a phenomenon in which the dental alignment, occlusal relationship between the upper and lower teeth, and the relative orientation of the jaws obtained through orthodontic return to the pre-treatment state [1]. While many patients experience relapse, its detailed mechanism has not yet been elucidated [2]. Basic research using rats and mice has been conducted to clarify this mechanism [3].

Since it is easier to perform surgeries that involve attaching devices in the oral cavity in rats, many basic studies in the field of orthodontics have used rats [4]. In recent years, some knockout rats have been produced following establishment of embryonic stem cells (ESCs) and advancements in genome editing technology. However, in comparison with the extent to which these techniques are used in mice, research on rats remains far behind [5]. In contrast mice have been subjected to whole-genome analysis and database maintenance and have greatly contributed to our understanding of the physiological function of numerous genes [6]. In addition, mice have been used to assess the potential utility

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of biopharmaceuticals and drugs [7].

In our previous study we used osteoprotegerin (OPG) knock-out (KO) mice (OPG KO mice) to elucidate the physiological function of OPG in orthodontic tooth movement (OTM) and to evaluate its effect on drug treatment [8]. We first utilized a method a method involving insertion of elastic between the maxillary first molar and second molar to move the tooth (Waldo method) [9], followed by a method involving method of pulling the maxillary first molar with a coil spring to move the tooth (coil spring method) [10]. When studying tooth movement in mice, using a coil spring induces greater movement than using elastic, and it is possible to observe tooth movement over a long period of time. Therefore, using that method, it is possible to conduct research in a state similar to the actual clinical environment [11]. We surmised that relapse can be easily observed and evaluated when a large distance is covered by the tooth.

Therefore, in this study we aimed to evaluate relapse following orthodontic treatment using a mouse model, subjected to the coil spring method for 21 days and mechanical retention for 7 days after completion of tooth movement. The extent of relapse over the following 7 days was observed and evaluated. Further, the oral cavity of mice is one-tenth smaller than that of rats. We endeavored to eliminate the difficulty of surgical procedures caused by the limited space in the oral cavity. To this end, we established a mouse model for observing orthodontic relapse using an effective, reproducible imaging method.

Materials and Methods

Animals

The experimental animals used in this study were 8-week-old male WT mice (C57BL/6) purchased from CLEA Japan (Tokyo, Japan) and bred in the Animal Laboratory, Faculty of Dentistry, Aichi Gakuin University. The breeding environment comprised a constant room temperature of 22 ± 2°C, humidity of 50 ± 10%, and lighting maintained at a 12-h cycle. The feed was CE-2 type powder feed (CLEA Japan), and tap water was used as drinking water, both of which were freely ingested. The animal experiments in this study followed relevant guidelines and were approved by the Animal Experiment Committee of the Faculty of Dentistry, Aichi Gakuin University (approval number AGUD465).

Experiment schedule

The experimental design for OTM, mechanical retention after OTM, and observation of relapse is presented in Fig. 1-a. Photographs of the oral cavity of the mice were taken before the start of OTM (Fig. 1-b-A), after completion of OTM, *i.e.*, at the start of retention (Fig. 1-b-B), at the start of relapse (Fig. 1-b-C), and at the end of relapse (Fig. 1-b-D).

General anesthesia was induced via intraperitoneal administration of a mixture of the following three anesthetics: medetomidine hydrochloride (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), midazolam (Astellas Pharma Inc., Tokyo, Japan), butorphanol tartrate (Meiji Seika Pharma Co., Ltd.). Under general anesthesia, a tooth movement device was placed in the oral cavity, and mesial movement of the maxillary left first molar was started (Day −28) (Fig. 1-b-A). After 21 days of tooth movement, the oral tooth movement device was removed under general anesthesia (Day −7) (Fig. 1-b-B). Impressions (Examix Fine Injection type, GC Co., Tokyo, Japan) were taken at the same time, and the distance between distal cervical region of the maxillary first molar and the mesial cervical region of the second molar was measured. A photopolymerizable composite resin (Gracefil LoFlo, GC Co., Tokyo, Japan) was built on the occlusal surfaces of the left upper molar and the second molar. The space was mechanically retained for 7 days after completion of OTM. After the end of mechanical retention, impressions of the upper jaw at the start of relapse (Day 0) (Fig. 1-b-C) and on the 1st, 2nd, 3rd, and 7th days (Day 7) (Fig. 1-b-D) after the start of relapse were taken under general anesthesia to observe the longitudinal changes in tooth position.

Creating a model mouse for relapse observation

OTM (application of corrective force): Under anesthesia, a 10 gf Ni-Ti closed coil spring (Tomy Inc., Tokyo, Japan) was attached between the maxillary incisor and the left first molar to induce mesial movement of the left first molar. In this way, an experimental OTM model mouse was created (n=5) (Day −28) (Figs. 2-a, b).

Mechanical retention after OTM: After 21 days of OTM (Day −7), the tooth movement device was removed under general anesthesia. A dental etching agent (ETCHANT, GC Co., Tokyo, Japan) was applied to the occlusal surfaces of the maxillary left first molar and the second molar, and an adhesive fixation treatment was performed. A photopolymerized composite resin was built on the occlusal surfaces of the first and second molars. The space between the two was mechanically retained for 7 days after tooth movement had been completed (Day −7). After 7 days of mechanical retention (Day 0), the photopolymerized composite resin was removed under general anesthesia.

Observation of relapse: Impressions of the maxilla were taken under general anesthesia after the end of

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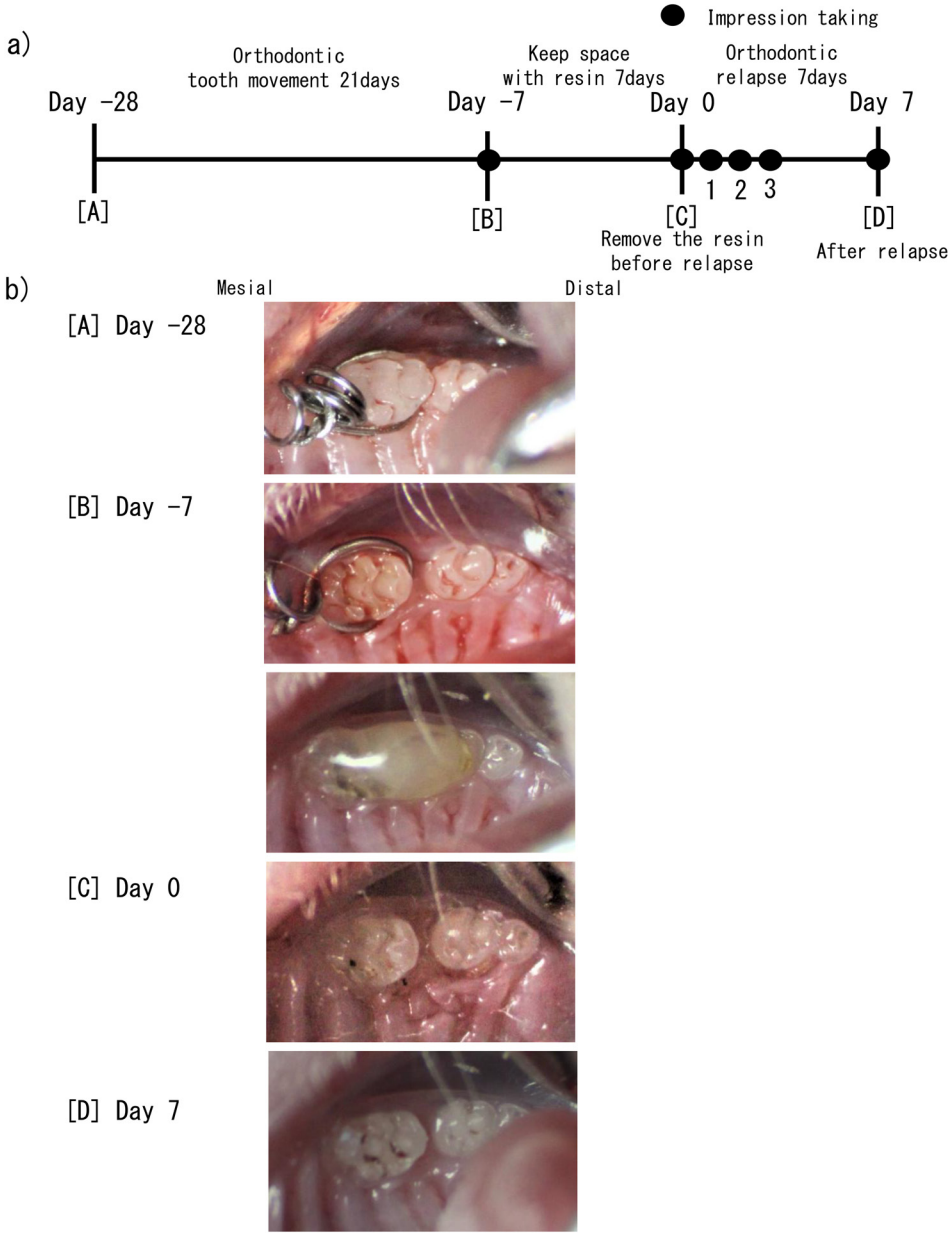

Fig. 1. a) Experimental design and schedule for the mouse model (the start of relapse is marked as Day 0); Impressions were taken at ● points; b) Oral cavity of mice [A] at the start of the 21 days of tooth movement and [B] at the end of the 21 days of tooth movement, after which the device was removed. A 7-day mechanical retention period was initiated with dental resin application. Oral cavity [C] at removal of the retention device, at the start of relapse, and [D] after a 7-day relapse period.

mechanical retention, *i.e.*, at the start of relapse (Day 0) and on the 1st, 2nd, 3rd, and 7th days (Day 7) after the start of the relapse. We observed relapse and changes in tooth positions over time.

Distance measurement between first molars and second molars: Impressions of the upper jaw were taken with a hydrophilic vinyl silicone impression material. A hydrophilic vinyl silicone impression material was placed on a metal spatula in the oral cavity of a mouse under general anesthesia. The impression was taken using a cardboard form designed for insertion into the oral cavity (Figs. 3-a and b). The obtained impression was photographed with a stereomicroscope (SMZ-10 Nikon Co., Tokyo, Japan) together with a micro ruler (KENIS LTD., Osaka, Japan) (Figs. 3-c and d). The distance between the distal cervical region of the maxillary first molar and mesial cervical region of the second molar was assessed using ImageJ software (NIH). The measurement site was the mid-point of the buccolingual dimension of the maxillary first and second molars (Figs. 3-e and f).

Relapse distance in percentage: One day after the start

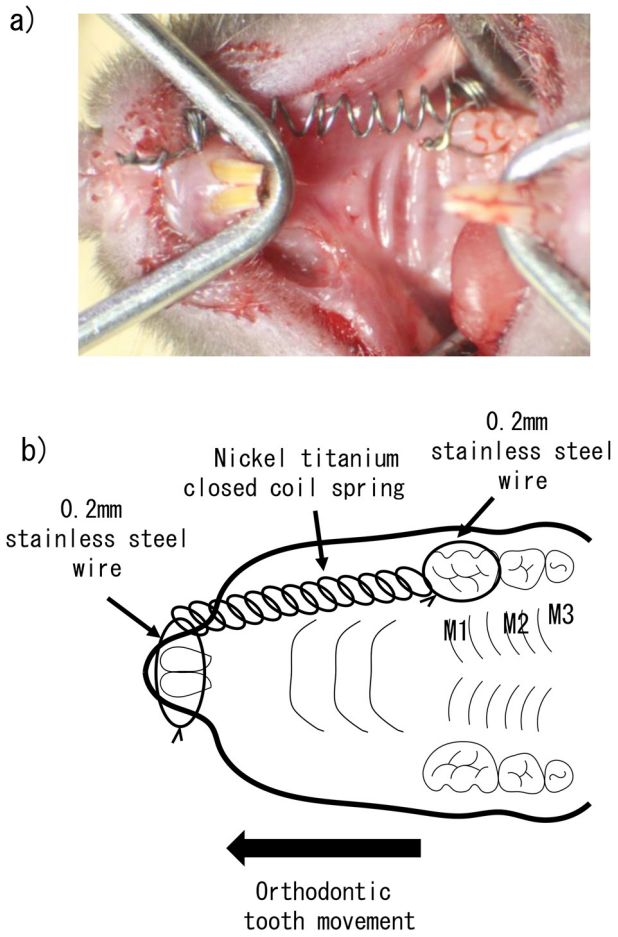

Fig. 2. a) Oral cavity of a mouse equipped with a tooth movement device; b) Intraoral schema for mice equipped with a tooth movement device (M1: maxillary left first molar, M2: maxillary left second molar, M3: maxillary left third molar).

of the relapse was defined as Day 0–1, 1–2 days after the start of the relapse were defined as Day 1–2, and 2–3 days after the start of the relapse were defined as Day 2–3. To compare the relapse distances on Days 0–1, 1–2, and 2–3 with respect to the total distance relapsed in 7 days, the ratio of each distance to the total distance was calculated as a percentage.

Histopathological observations

Maxillary bones were extracted before starting OTM (Day −28), after OTM (Day −7), and after observation of relapse (Day 7) and were fixed with 10% neutral buffered formalin solution. Next, they were decalcified with 10% EDTA (pH 7.2) at 4°C for approximately 4 weeks and then embedded in paraffin in accordance with a common method for preparing 5 μm horizontal serial tissue sections [8]. The area from the furcation to the apex was divided into three equal parts, and the tissue observation site was set to 1/3 from the furcation. Then, hematoxylin-eosin (HE) staining was performed, and the periodontal tissue around the distal palatal root of the maxillary first molar was observed under an optical microscope.

Statistical analysis

The experimental data obtained are shown as the mean and standard error. The normality of the data was confirmed using the Shapiro-Wilk test, and one-way analysis of variance (Tukey's multiple comparison test) was used to test for statistical significance. All statistical analyses were performed using Graph Pad Prism v.7 (Graph Pad Software Inc., San Diego, CA, USA). $P<0.05$ was considered to indicate statistical significance.

Results

Experimental OTM and mechanical retention

At the end of the experimental OTM (Day −7), the average distance between the maxillary first and second molars was 259.6 (± 10.9) μm. OTM was observed in all mice. The distance between the maxillary first molar and the maxillary second molar at the end of mechanical retention on the 7th day was 258.6 (± 10.4) μm. No significant difference was found in the distance between the maxillary first and second molars at the end of the experimental OTM or at the end of mechanical retention (Fig. 4).

Changes in tooth position during relapse

The distance between the maxillary first and second molars at the start of relapse observation (Day 0) was 258.6 (± 10.4) μm. It was 210.2 (± 9.6) μm on the first day, 190.8 (± 11.4) μm on the second day, and 174.4 (± 12.2) μm on the third day after the start of relapse observation. The distance between the maxillary first molar and the maxillary second molar on the 7th day after the start of the relapse (that is, at the end of the relapse observation Day 7), was 155.4 (± 12.4) μm (Fig. 5). A decrease in the distance between the maxillary first and second molars was observed in all mice. We compared the distances between Day 0 and Day 1, between Day 0 and Day 2, between Day 0 and Day 3, and between Day 0 and Day 7. In all cases, the distance between the maxillary first and second molars decreased significantly. We also compared the distances between Day 1 and Day 2 and between Day 1 and Day 3. The distance between the maxillary first and second molars decreased significantly, but the distances on Day 2 and Day 3 did not significantly differ. In addition, a significant difference was found between the distances on Day 3 and Day 7.

HE-stain findings in periodontal tissue

Before the start of OTM (Day −28), the periodontal

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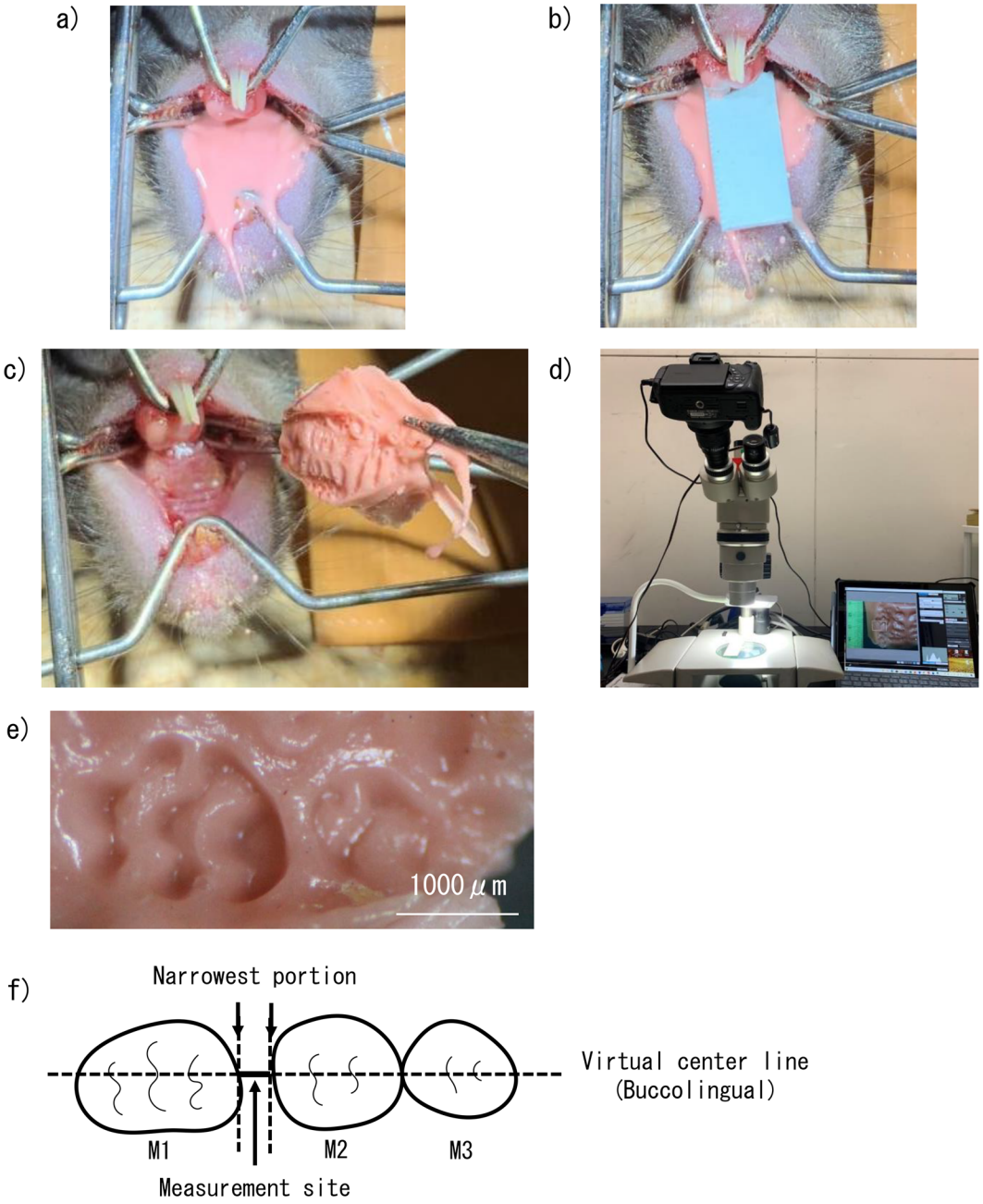

Fig. 3. Oral cavity of a mouse a) with an impression material placed on the upper jaw and b) with cardboard pressed against the impression material. c) After the impression material had cured, the impression was removed from the oral cavity. d) A stereomicroscope was used to measure the distance between M1 and M2.e) The distance between M1 and M2 was measured on the impression. f) Schema of the measurement sites on the impression (M1: maxillary left first molar, M2: maxillary left second molar, M3: maxillary left third molar).

ligament space around the distal palatal root of the maxillary first molar was uniform in width. A uniform running of the periodontal ligament fibers into the cementum was observed (Fig. 6-a). After 21 days of OTM (Day −7), significant narrowing of the periodontal ligament space was observed on the compression side. On the tension side, the width of the periodontal ligament had expanded, and tension of periodontal ligament fibers and extension of fibroblasts were observed (Fig. 6-b). After relapse observation (Day 7), the width of the periodontal ligament around the distal palatal root of the maxillary first molar recovered uniformly. Similar to before the start of OTM, we found that the periodontal ligament fibers were arranged in the direction from the alveolar bone to the cementum (Fig. 6-c).

Changes in relapse proportion

Relative to the total relapse distance over the 7-day

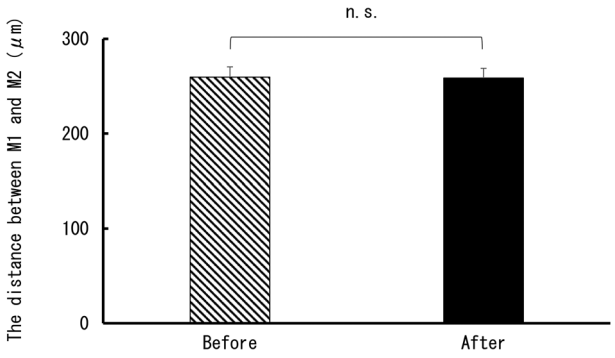

Fig. 4. Distance between M1and M2 (μm) before starting and after completing the mechanical retention phase (M1: maxillary left first molar, M2: maxillary left second molar, n.s.: not significant).

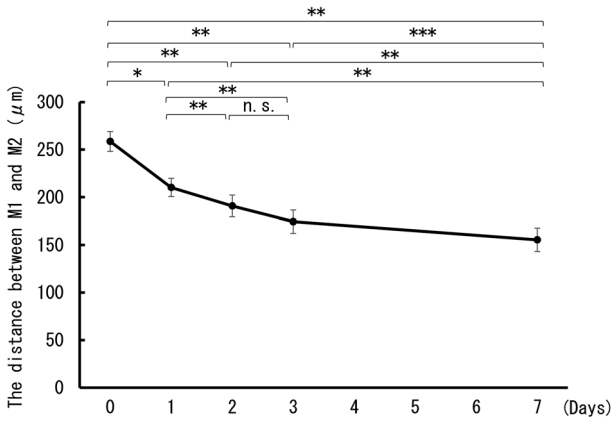

Fig. 5. Change in the distance between M1and M2 (μm) after 7 days of relapse. ***P<0.001; **P<0.01; *P<0.05 (M1: maxillary left first molar, M2: maxillary left second molar, n.s.: not significant).

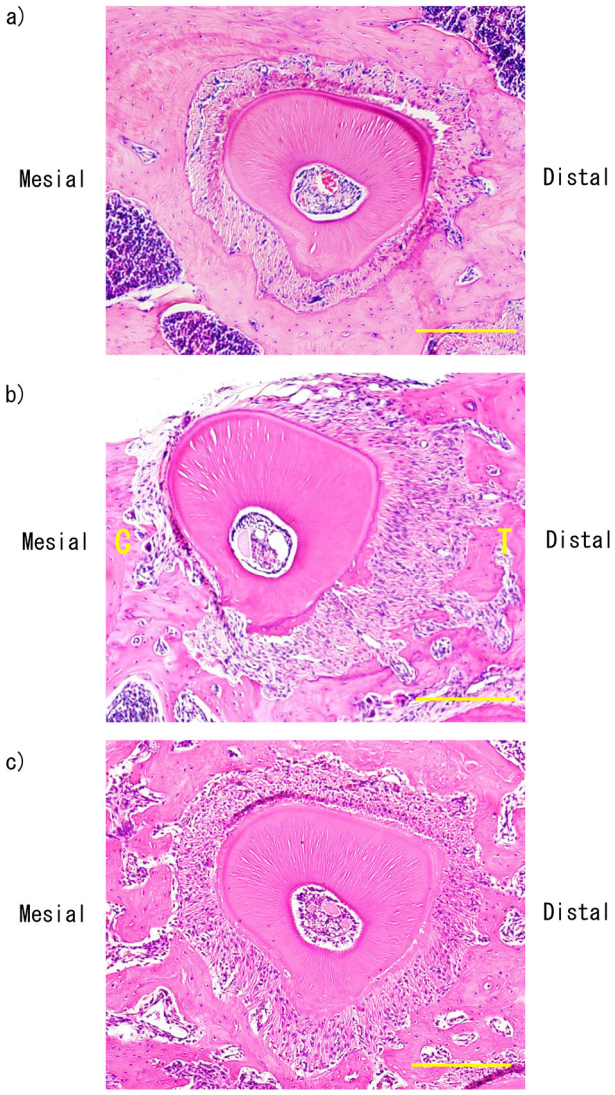

Fig. 6. a) HE stain findings around the distal palatal root of the maxillary first molar before starting OTM (Day −28). b) HE stain findings around the distal palatal root of the maxillary first molar after OTM (Day −7) (T: Tension side C: Compression side). c) HE stain findings around the distal palatal root of the maxillary first molar after relapse observation (Day 7) (Scale bar indicates 200 μm). HE, hematoxylin-eosin; OTM, orthodontic tooth movement.

period, 45.7 (± 4.3)% of the relapse was observed on Day 0–1, 20.1 (± 4.1)% on Day 1–2, and 14.9 (± 3.3)% on Day 2–3. Over the remaining 4 days, the remaining 19.3 (± 2.0)% of relapse was observed (Fig. 7). The ratio of the relapse distance on Days 0–1 was significantly larger than that on Days 2–3. The percentage of relapse distance on Days 1–2 did not significantly differ from that on Days 0–1 and 2–3.

Discussion

Currently, retention devices are used to stabilize the dentition after orthodontic treatment and prevent relapse, but poor patient compliance and certain oral environments can lead to retention failure [12]. It is difficult for dentists to predict the teeth that will and will not undergo relapse after orthodontic treatment. Teeth that were actively moving during orthodontic treatment must be actively stabilized during the retention phase. The causes of relapse include growth, perioral muscles, occlusal force, enlargement of the dental arch, archform morphology, periodontal tissue condition, and third molars. However, the extent to which each cause affects relapse has not been clarified [13]. In addition, it is well-known that dentition morphology and tooth movement occur with aging even in cases of natural dentition without orthodontic treatment. This natural tooth movement increases the difficulty of evaluating relapse after orthodontic treatment [14].

Experimental methods for achieving OTM in mice include insertion of an elastic band between the maxillary first and second molars (Waldo method) [9] and use of coil springs to pull the maxillary first molar (coil

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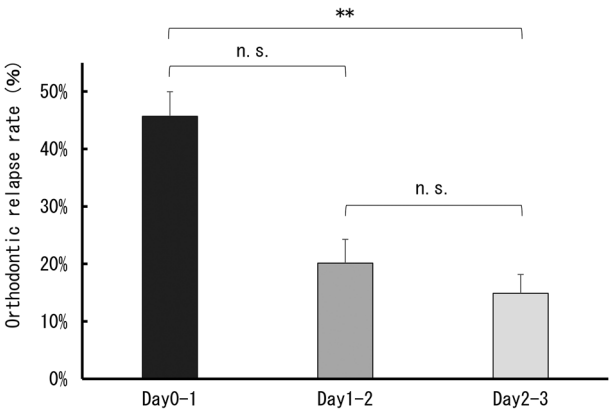

Fig. 7. Percentage of relapse distance between M1and M2. **P<0.01. (M1: maxillary left first molar, M2: maxillary left second molar, n.s.: not significant). Day 0–1: Relapse distance on Day 0–1 with respect to the total relapse distance in 7 days. Day 1–2: Relapse distance on Day 1–2 relative to the total relapse distance in 7 days. Day 2–3: Relapse distance on Day 2–3 with respect to the total relapse distance relapsed in 7 days.

spring method) [10]. In the Waldo method, the elastic falls off when tooth movement occurs. Therefore, it is only possible to observe OTM for a short period of 3–7 days. On the other hand, the device is less likely to fall off when using the coil spring method. Therefore, it is possible to observe continuous OTM over a prolonged duration and over a large movement distance, which is similar to the clinical treatment scenario in humans [11]. A large OTM distance is more advantageous for observing changes in tooth movement during the retention phase. In this study, we observed a large OTM of 259.6 (± 10.9) μm, which is approximately 15–20% of the first molar crown width. Relative to the crown width, the distance moved on application of orthodontic force for 21 days is also similar to that seen in human clinical treatment.

The factors involved in relapse, which have not yet been completely elucidated, may include remodeling of alveolar bone and periodontal ligament fibers [15]. In this experiment, changes in alveolar bone and periodontal ligament fibers could be observed over time. The roots moved beyond the width of the periodontal ligament. Therefore, it was observed that alveolar bone resorption had occurred on the compression side and that the tooth root had moved. The main factors that regulate alveolar bone remodeling in orthodontic treatment include receptor activator of nuclear factor-kappa B (RANK)/receptor activator of nuclear factor-kappa B ligand (RANK-L)/osteoprotegerin (OPG). RANK-L is expressed in osteoblasts and binds to the RANK receptor in osteoclasts to activate the master transcription factor NFATc1, leading to rapid differentiation into mature osteoclasts [16]. OPG is a soluble decoy receptor that inhibits RANK signals by binding RANK-L [17]. These factors regulate the differentiation, maturation, and function of osteoclasts as well as the destruction and resorption of calcified bone tissue. Osteoclasts are also observed in the direction of relapse when tooth relapse occurs [15]. After OTM and relapse observation, the width of the periodontal ligament fibers, which were on the tension side during OTM, decreased in our experimental mice. This suggests that tension of periodontal ligament fibers is involved in relapse. However the details are still unclear [18]. On the other hand, relapse can occur even when the periodontal ligament fibers are histologically normal [15]. Reports that periodontal ligament fibroblasts produce and secrete OPG suggest that they can also protect teeth from the osteoclasts produced during OTM and relapse and can function to suppress relapse [19]. In order to examine the dynamics of relapse, it is necessary to conduct further research on changes in the periodontal tissue on the tension side during OTM.

In this study, the maxillary molars of mice were experimentally moved in the mesial direction. A relapse in the distal direction, which is opposite to the direction of the movement, occurred in all mice. According to Fujimura *et al.*, 12 days of experimental OTM in mice led to a distance of 126.0 (± 60.0) μm. The 21-day experimental OTM in the present study led to a distance of 259.6 (± 10.9) μm. These results suggest that the distance covered by the tooth increases in proportion to the number of days of movement [20]. The following are possible reasons for stable tooth movement over a long period of 21 days. The first molar was stabilized by fixing the closed coil to the cervical region with an orthodontic ligature, and we used a special Ni-Ti closed coil spring that continued to apply a force of 10 gf even when extended from 3.0 mm to 15.0 mm. Furthermore, affixing that affixing the Ni-Ti closed coil spring through the maxilla made it possible to prevent a reaction and achieve a strong anchor point. In addition, our findings clarified that most of the relapse occurred within 7 days, at the initial stage of relapse observation. This is consistent with the findings of McManus *et al.*, who found that most of the relapse occurs early in mice [21]. In addition, the results are consistent with a clinical report according to which relapse is likely to occur immediately after the end of treatment in humans and the amount of relapse decreases with the passage of time [22]. In other words, our findings support the actual clinical phenomenon. These results emphasize importance of aggressive retention treatment immediately after the end of dynamic treatment.

In this study, we focused on investigating the dynam-

ics of the distance covered by teeth under OTM to investigate relapse. In the future, more detailed studies are necessary to elucidate the mechanism of relapse through histological observations, changes in the dynamics using knockout mice, and the effects of biopharmaceuticals and drugs on relapse.

This study had several limitations. First, the mice needed to be sedated to measure the distance; our experiments did not consider the impact of sedation on OTM and relapse. Second, the mode of OTM was limited to simple mesial inclined OTM. In clinical practice, various modes of OTM are used in addition to inclined OTM; therefore, to reproduce these modes in mice, it would be necessary to consider improving and modifying the apparatus. Third, we were unable to evaluate the extent to which the occlusal force affects tooth movement how much the occlusal force affects tooth movement. In the future, it may be necessary to develop an experimental model that eliminates bite force and conduct a comparative study.

Here, we have provided a method to successfully observe the dynamics of mechanical retention and subsequent relapse through experimental OTM of the first molars in mice. The *in vivo* orthodontic movement over 21 days resembled the application of orthodontic force on human teeth. The ability to observe tooth movement during relapse using the current mouse model may contribute to the development of novel treatment methods and plans.

In this study, we developed a method for producing a mouse model for relapse observation following OTM. After 21 days of experimental OTM, relapse was observed in all mice. Relapse was observed in all mice with experimental OTM. The distance between the maxillary first and second molars at the start of relapse observation and the end of relapse observation was compared, and a significant decrease was observed. In addition, 45.7 (± 4.3)% of the total relapse distance was observed on Day 0–1 of relapse observation. These results suggest that the ability to observe the movement of teeth at the time of relapse using a mouse model can contribute to the development of novel clinical treatment methods and plans.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Y.A.: Writing the original draft, Data curation, Formal analysis, Methodology, Visualization, S.K.: Writing the original draft, Conceptualization, Methodology, Project administration, K.M.: Writing the original draft, Conceptualization, Project administration, M.T.: Writing the original draft, Conceptualization, Methodology, Project administration, F.K.: Methodology, Revising the draft critically, K.K.: Data curation, R.K.: Data curation, T.S.: Revising the draft critically, S.G.: Conceptualization, Revising the draft critically, Supervision. All authors read and approved the final manuscript.

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