Effects of Glass Fiber-reinforced Plastic for Orthodontic Wire on Bone Remodeling during Experimental Tooth Movement: Histological Study in Dogs

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Article History
Received 28 October 2016
Accepted 22 November 2016

Abstract
Recently, we developed glass fiber-reinforced plastic (GFRP) orthodontic wire made from polycarbonate and glass fiber. The purpose of this study was to compare GFRP wire with nickel-titanium (NiTi) wire as assessed by the amount of tooth movement, the early tissue reaction of the periodontal ligament (PDL), and tartrate-resistant acid phosphatase (TRAP) expression in an experimental dog model of tooth movement in vivo.

GFRP round wire, with a diameter of 0.45 mm (0.018-in.), was prepared using 7 µm glass fibers. As a control, wires made of NiTi were also evaluated. The maxillary second and third premolars were moved buccally for 5 weeks in five beagles. The change in second and third premolar widths in NiTi and GFRP wires (P2WN, P2WG, P3WN and P3WG) were investigated. Furthermore, histological findings in the PDL and TRAP expression levels in the alveolar bone (AB)surface were measured.

The amount of tooth movement in the GFRP group was roughly equal to that in the NiTi group. The numbers of TRAP-positive cells were not significantly increased in either group at 5 weeks. No significant differences in any parameters before and after treatment were noted between the two groups.

GFRP wire showed equivalent efficiency to commercial NiTi wire in the amount of tooth movement and histological findings of periodontal tissues during experimental tooth movement. Therefore, GFRP wire can be used in place of NiTi wire at the initial leveling stage.

Keywords:
GFRP wire, tooth movement, bone remodeling, dog

Introduction
Bone modeling is the uncoupled process of activation-resorption (catabolic) or activation-formation (anabolic) on bone surfaces, resulting in changes to the shape, size, or position of the bone (1). In contrast, bone remodeling or turnover is a tightly coupled local process, which starts with bone resorption, followed by reversal and bone formation phases, resulting in the replacement of old bone with new bone (2,3). Orthodontic tooth movement is a multistep biological process characterized by sequential reactions of periodontal tissue against biomechanical forces (4). The recruitment of osteoclast- and osteoblast-progenitor cells and the balanced activation of these cells around and within the periodontal ligament (PDL) are essential for alveolar bone remodeling (5,6).

Superelasticity, as a property of nickel-titanium (NiTi) wire, is characterized by a light continuous force with a long range of activation (7–9). With this force, teeth can be moved effectively and smoothly, resulting in optimal tooth movement in humans (10,11) and rats (12). However, this wire is not esthetically pleasing and may induce allergic or toxic reactions (13). To resolve these issues, we developed glass fiber-reinforced plastic (GFRP) orthodontic wires made from polycarbonate and glass fiber (14–16). We demonstrated GFRP wire showed similar bending behavior
to NiTi wire and delivered optimal and continuous force to the teeth due to its high springback and low flexural modulus. Therefore, GFRP wires are attractive for orthodontic appliances because of their improved esthetic quality compared with commercial NiTi wire. However, little information is available regarding the behavior of clinical orthodontic tooth movement in subjects treated with GFRP wire.

The purpose of this study was to compare GFRP and NiTi wires in an experimental dog model of tooth movement. The maxillary second and third premolars were moved buccally using NiTi and GFRP wires for 5 weeks. The change in second and third premolar widths (P2WN, P2WG, P3WN and P3WG) were investigated. Furthermore, histological findings of the PDL and tartrate-resistant acid phosphatase (TRAP) expression levels in the alveolar bone (AB) surface were measured.

Materials and Methods

Materials preparation

GFRP straight wire was fabricated using a pultrusion technique described in a previous study (14,15). A polycarbonate (H4000, Mitsubishi Engineering-Plastics Corp., Tokyo, Japan) was used as the thermoplastic matrix because of its low melt viscosity. A glass fiber filament (Nitto Boseki Co., Fukushima, Japan) was used as a unidirectional reinforcement for the polycarbonate matrix. Straight prototype GFRP wire with a diameter of 0.45 mm (0.018-in
round wire) was formed in an arch shape, as described in a previous study (14) (Fig. 1a, b).

Animals

Five female beagles, periodontally healthy, each weighing 14–16 kg and between 13 and 16 months old (Sankyo Labo Service Co., Tokyo, Japan), were used for the experiment. The animals were caged individually under regulated light and temperature conditions. All dogs were fed normal soft dog chow and had access to water ad libitum. The animal experimental protocol was approved by the Ethics Committee for Animal Experiments at Nihon University School of Dentistry at Matsudo (approval no. AP15MD016). All dogs were intravenously injected with sodium propofol (Maruishi Seiyaku, Tokyo, Japan) at a dose of 25 mg/kg. The animals received dental prophylaxis to remove tartar with an ultrasonic scaler.

Orthodontic device application

A NiTi sectional wire (0.018-in round wire, Yellow Sentalloy, Tomy International, Inc., Tokyo, Japan) was placed on the right side (Fig. 2a) and a GFRP sectional wire was placed on the left side (Fig. 2b), with comparisons made to assess individual differences. The maxillary canines and fourth premolars were used as anchors, and the maxillary second and third premolars were used as experimental teeth. The mandibular second premolars were assigned as control groups. Custom bands for the maxillary canines were fabricated from band material (Tomy International, Inc.). Buccal single tubes (slot size: 0.022) (Tomy International, Inc.) were welded and then soldered to the canine bands. The second, third, and fourth premolars (micromini tube bondable, TOMY, Tokyo, Japan) were bonded with 0° offset and 0° torque.

Retentive grooves were placed in the maxillary canines and second, third, and fourth premolars using a high-speed handpiece and a #330 carbide bur. The teeth were then etched with 37% phosphoric acid gel for 30 s. A primer was applied to each tooth, after which Transbond resin (3M Unitek, Tokyo, Japan) was applied and the teeth were light-
cured. The bands were then filled with 3M Transbond XT (3M Unitek) and seated, excessive composite was removed, and the resin was light-cured. Both sectional wires were then ligated, and 0.018-in (0.045 mm) wires were placed. The ends of both wires were cinched, roughened, and bonded with composite for retention and comfort (Fig. 2c, d). The maxillary second and third premolars were moved with labial tipping with both the NiTi and GFRP wires for 5 weeks.

Measurement of force and amount of tooth movement

Orthodontic force developed by the application of NiTi and GFRP wires on the maxillary second and third premolars was measured twice using a tension gauge (OHBA SIKI, Tokyo, Japan). The condition of the appliance, teeth, gingiva, and change in body weight were evaluated. Impressions of maxillary arches were taken using silicone impression material (Genie; Sultan, Tokyo, Japan) every week for 5 weeks, and then dental casts were made with extra-hard plaster (New Fujirock; GC Corp., Tokyo, Japan). The dental casts were scanned using a contact-type three-dimensional (3D) scanner, MAESTRO (MDS400; Medic Engineering Corporation, Kyoto, Japan), which interfaced with a personal computer. Each 3D image of the dental cast combined with the image of the reference axis before and after treatment was superimposed on the medial points of the third palatal rugae and the palatal vault as the reference landmarks and area, as previously described (17), which was found to be reproducible and reliable. The second premolar width the NiTi wire (P2WN) and the second premolar width the GFRP wire (P2WG) indicates that the distance from the median palatine suture line to the cusp of the second premolars. The third premolar width the NiTi wire (P3WN) and the third premolar width the GFRP wire (P3WG) indicates that the distance from the median palatine suture line to the cusp of the third premolars. P2WN, P2WG, P3WN and P3WG were measured using a 3D form analysis software program (Body-Rugle; Medic Engineering Corporation). The measurements were performed by one examiner and repeated three times.

Tissue preparation

All 5 beagles were humanely killed after tooth movement for 5 weeks with an intravenous injection of pentobarbital (50 mg/kg). The maxilla and mandible, including the canine to the fourth premolar teeth, were dissected and fixed in G-fix (STF-01; Geno Staff, Tokyo, Japan) for 1 week. Tissue blocks (including teeth, bone, and soft tissue) were then decalcified in 1% EDTA at pH 7.2 (GCM-1; Geno Staff) for 21 weeks. Fixation and decalcification were performed at 4°C, and decalcified specimens were subsequently dehydrated using a graded series of ethanol washes and embedded in paraffin (18). Each sample was sliced continuously into 6μm sections in the bucconlingual direction (19). The sections were stained with hematoxylin and eosin (HE). Sections containing the PDL side of apical third of the root on the pressure side and cervical third of the mesial root on the tension side of maxillary second and third premolars were selected (20). Selected sections were then stained with TRAP to identify differentiated osteoclasts and osteoclast precursors. The number of TRAP-positive cells was counted on the palatal surface of the mesial root of experimental premolars (alveolar wall) and in the bone marrow cavities immediately adjacent to the alveolar wall, as previously described (21).

Histomorphometric data processing and analysis

An area measuring 872×656 μm², including the maximum tension and compressed region of the mesial root on the palatal side, was selected for measurement. The sections were selected from the apical third of the root on the pressure side and the cervical third of mesial root on the tension side, one section on each side, giving fives measurements in total. An arbitrary hot spot was observed for each area at 200× magnification under light microscopy. These selected images were manually traced at regions of interest (ROIs) to produce a binary image in Photoshop Elements 11.0 (Adobe Inc., San Jose, CA, USA). Data were cropped and transferred to ImageJ version 1.48s (http://rsbweb.nih.gov/ij). All areas of the PDL and blood vessels were measured and the blood vessel/PDL areas were calculated (Fig. 3).

Statistical analyses

Differences between groups were compared using the Mann-Whitney test with p < 0.05 considered statistically significant. The error bars in the graphs indicate 95% confidence intervals (CI).

Results

Measurements of forces

Orthodontic force developed by application of GFRP and
NiTi wires to the maxillary second premolar was approximately 50 g and that of the third premolar was approximately 75 g at initial force. There was no significant difference in orthodontic force between the two groups (data not shown).

Body weight during the experimental period

No significant differences were observed in the body weight of the dogs between the two groups (data not shown).

Amount of tooth movement

Fig. 4a-c shows color mapping for the superimpositions before (white) and after (gray) experimental tooth movement. The maxillary second and third premolars were successfully moved with labial tipping with both GFRP and NiTi wires. P2WN, P2WG, P3WN and P3WG increased by 0.6–0.7 mm with labial tipping. Additionally, P2WN, P2WG, P3WN and P3WG increased over 5 weeks in a time-dependent manner. There were no significant differences in P2W and P3W within the same group or between the NiTi and GFRP groups ($p>0.05$) (Fig. 4d, e).

Histological findings in periodontal tissues during tooth movement (HE staining)

In “tension” and “compression” areas in the maxillary second and third premolars at 5 weeks in the control group (0 g), the PDL was composed of relatively dense connective tissue fibers and fibroblasts that regularly ran in a horizontal direction from the root cementum towards the AB. Blood capillaries were mainly recognized near the AB in the PDL, and the root surfaces were relatively smooth (Fig. 5a, c).

In the tension area in the maxillary second and third premolars of the NiTi and GFRP groups, Sharpey’s fibers were extended, osteoblasts were regularly arranged, and extensive bone remodeling was observed at 5 weeks (Fig. 5e, i, m, q). The PDL areas in both the NiTi and GFRP groups were increased compared with the control group ($p<0.05$) (Fig. 6a). In addition, no significant difference in the area of the blood vessels was noted between the control and wire groups (Fig. 6b). The blood vessel/PDL areas in the wire groups were significantly decreased compared with the control group ($p<0.05$) (Fig. 6c). However, there were no significant differences in the PDL and blood vessels areas and the blood vessel/PDL areas between the wire groups.

In the compression area in the maxillary second and third premolars in the NiTi and GFRP groups, the arrangement of

Fig. 3. Images of the measurement area with hematoxylin and eosin staining and computer processing. (a) An area measuring 872 × 656 μm², including the maximum tension and compression regions (surrounded by a black line), was selected on the lingual side of the PDL. D: dentin, B: bone. (b) Extracted processing image of the PDL and vascular cross-sectional areas. The black-colored area shows the PDL cross-section.
Sharpey’s fibers and fibroblasts became coarse and irregular but did not show hyalinization and necrosis at 5 weeks (Fig. 5g, k, o, s). Resorption lacunae with few multinucleated osteoclasts were observed on the palatal root surface (Fig. 5h, l, p, t). The PDL areas in the NiTi and GFRP groups were significantly decreased compared with that in the control group ($p<0.05$) (Fig. 7c). However, no significant differences in the PDL and blood vessels areas and the blood vessels/PDL areas were noted between the wire groups.

In both the tension and compression areas, GFRP wire showed equivalent efficiency to NiTi wire according to histological changes, including the areas of the PDL, blood vessels, and blood vessels/PDL in periodontal tissues during...
Fig. 5. Histological findings in periodontal tissues during buccal tooth movement in the maxillary second and third premolars with NiTi and GFRP wires. Hematoxylin and eosin staining and TRAP staining, 200×. Arrowheads indicate osteoclasts on the alveolar bone on the compression side. B: bone, PDL: periodontal ligament, C: cementum, D: dentin. Scale bar = 50 μm. The direction of the applied force is indicated by the large arrow.
Fig. 6. Histomorphometry on the tension side in the maxillary second and third premolars. (a) The area of the PDL. (b) The area of the blood vessels. (c) The blood vessel/PDL areas. (d) The number of TRAP-positive cells were counted on the palatal surface of the mesial root and in the bone marrow cavities immediately adjacent to the alveolar wall (p<0.05). Error bars indicate 95% confidence intervals (CI).
Fig. 7. Histomorphometry on the compression side in the maxillary second and third premolars. (a) The area of the PDL. (b) The area of the blood vessels. (c) The blood vessel/PDL areas. (d) The number of TRAP-positive (TRAP⁺) cells were counted on the palatal surface of the mesial root and in the bone marrow cavities immediately adjacent to the alveolar wall (p<0.05). Error bars indicate 95% confidence intervals (CI).
experimental tooth movement.

**TRAP histochemical findings**

In the tension and compression areas in the maxillary second and third premolars at 5 weeks in the control group (0 g), no resorption lacunae with TRAP-positive multinucleated osteoclasts were observed on AB surfaces (Fig. 5b, d). Additionally, no resorption lacunae with TRAP-positive multinucleate osteoclasts were observed at 5 weeks on AB surfaces in the tension area in the wire groups (Fig. 5f, j, n, r). However, in the compression area in the maxillary second and third premolars of the NiTi and GFRP groups, few root resorption lacunae with multinucleate TRAP-positive osteoclasts were observed at 5 weeks on AB surfaces (Fig. 5h, l, p, t). A quantitative evaluation showed that TRAP expression in the compression areas in the AB and PDL was significantly increased at 5 weeks. The number of TRAP-positive osteoclasts was similar for both wire groups (Figs. 6d and 7d).

**Discussion**

In our previous studies, we developed an esthetic orthodontic wire made from GFRP composed of polycarbonate and glass fiber using pultrusion. The mechanical properties and *in vitro* biocompatibility of GFRP, such as surface characteristics, frictional properties, flexural properties, color stability, and cytotoxicity, were evaluated and we concluded that GFRP wire might be useful in orthodontic treatment (14–16). To evaluate its potential in clinical orthodontic treatment, we investigated the effects of GFRP wire on orthodontic force and changes in P2W and P3W compared with NiTi wire using an experimental dog model of tooth movement. Tao et al (18) suggested that using dogs as a research model for orthodontic tooth movement has many advantages. Given that differences in the size and anatomy of the PDL and AB between dogs and humans are rather small (22), we performed this experiment in dogs.

First, orthodontic force magnitudes of GFRP and NiTi wires were examined using a tension gauge. The force magnitudes of GFRP and NiTi wires were approximately 50 and 75 g, respectively, after ligation to the second premolars. This finding shows that the strain of GFRP wire was slightly less than that of NiTi wire after ligation (5 weeks). Considering the force magnitude during tooth movement in this study, Pilon et al (23) showed that 50 g of light force application produced significantly greater tooth movement with significantly less root resorption over a period of 120 days in relation to a heavier force application in dogs. The optimum force for the movement of the second and third premolars in dogs may be less than 50 g, as previously suggested (23), supporting the use of this model to demonstrate efficient tooth movement. The maxillary second and third premolars were moved with labial tipping with both GFRP and NiTi wires. Moreover, the amount of movement was similar between the two wires. The average distance of movement of the second and third premolars with both GFRP and NiTi wires was 0.6–0.7 mm with labial tipping in the upper arch (Fig. 4d, e). The average distance of tooth movement by an orthodontic force of 50 g was 1.0 mm in 4 weeks, which was similar to that in previous experimental dog studies (23, 24). GFRP wire showed equivalent efficiency to NiTi wire with respect to the amount of tooth movement. Therefore, GFRP wire has similar effects to NiTi wire during the initial leveling stage.

Regarding the tension area in the maxillary second and third premolars of the NiTi and GFRP groups, Sharpey’s fibers were extended, the arrangement of osteoblasts became regular, and extensive bone remodeling was observed. This may be a physiological reaction to the wire in the PDL tissue. Additionally, the PDL areas in both groups were significantly increased compared with the control group (*p*<0.05) (Fig. 6a). These findings indicate that blood circulation is maintained in the tension zone of the PDL after light force loading. In contrast, in the compression area, the arrangement of fibers and fibroblasts became coarse and irregular and did not show hyalinization and necrosis at 5 weeks (Fig. 5g, k, o, s). These findings indicate that tooth movement was achieved without causing excessive force. The PDL areas in the NiTi and GFRP groups were significantly decreased compared with that in the control group (*p*<0.05) (Fig. 7a). In addition, no significant differences in the area of the blood vessels on both sides were noted between the control and wire groups (Figs. 6b and 7b). To maintain the biological activity of a compressed PDL, compression without obstruction of blood circulation should be maintain during efficient tooth movement.

The histological findings were similar to those of conventional studies. Regarding the tension side, Tsuge et al (25) demonstrated that the total cross-sectional area of the PDL at 7 days was significant larger in experimental animals than in control animals in an experimental rat model of tooth movement. In addition, the total cross-sectional
areas of the blood vessels were not decreased, while the ratio of blood vessels to PDL was significantly decreased after orthodontic force loading. In contrast, with regard to the compression side, Noda et al (26). demonstrated that light force maintained the vascular structure in an experimental rat model of tooth movement. They concluded that a heavier force and partial or total occlusion of vessels resulted in degeneration or necrosis of the PDL.

We also examined bone resorption during tooth movement using a TRAP assay. In the tension area on AB surfaces in the NiTi and GFRP groups, no AB resorption lacunae with multinucleate TRAP-positive osteoclasts were observed at 5 weeks (Fig. 5f, j, n, r). With regard to the “compression” area in the second and third premolars of the Ni-Ti and GFRP groups, on the surface of the AB, root resorption lacunae with multinucleate TRAP-positive osteoclasts were observed at 5 weeks (Fig. 5h, l, p, t) Krishnan et al (6). suggested that depending on the magnitude of applied force, the reaction at this site differs: light pressure produces direct bone resorption while heavy forces produce hylalination. Therefore, the histological findings of this study were considered appropriate. There was no significant difference in the histological findings between the two wire types, suggesting that GFRP wire showed equivalent efficiency to NiTi wire with regard to histological changes during tooth movement.

In this study, we examined the effects of GFRP wire measuring 0.018-in in diameter. Generally, rods with narrower diameter, such as 0.014- or 0.016-in diameter, are used at the beginning of clinical orthodontic treatment. Future studies should investigate the effects of GFRP wire with narrower diameters on the amount of tooth movement and changes to periodontal tissues in vivo.

**Conclusion**

In this experimental dog model, no significant differences before or after treatment were noted with the use of GFRP and NiTi wires during labial tooth movement of the maxillary second and third premolars. The amount of tooth movement in the second and third premolars increased over 5 weeks with both wires in a time-dependent manner. Additionally, the amount of tooth movement in the GFRP group was equal to that in the NiTi group. TRAP expression in the compression areas in the AB and PDL was significantly increased at 5 weeks. GFRP wire showed equivalent efficiency to PDL tissue responses of commercial NiTi wire in labial tooth movement. The results of this study suggest that GFRP wire can be used in place of NiTi wire during the initial leveling stage.

**Acknowledgments**

This study was supported in part by a Nihon University multidisciplinary grant for (2011) and by a Grant-in-Aid for Young Scientists (B) (No. 24792155) from the Japan Society for the Promotion of Science.

**Conflict of interest**

The authors have declared that no competing interest exists.

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