Enhancement of human periodontal ligament by preapplication of orthodontic loading

Kittitat Nakdilok,[a] Sarawut Langsa-ard,[b] Suttichai Krisanaprakornkit,[b] Eduardo Yugo Suzuki,[a] and Boonsiva Suzuki[a]
Bangkok and Chiang Mai, Thailand

Introduction: The quantity of remaining periodontal ligament (PDL) on the root surface of donor teeth is essential for the success of tooth autotransplantation. Preapplication of orthodontic loading increases this quantity on rat tooth root surfaces. However, little is known about the effects of preloading on human PDL or the ease of tooth extraction. This study aimed to determine the optimal duration of preloading for enhanced PDL on the root surface of extracted human premolars and for facilitating extraction. **Methods:** Sixty patients received orthodontic preloading with a bracket connected to an archwire on one of their maxillary first premolars for 2, 4, 6, or 8 weeks, whereas the contralateral premolar was not loaded as a control. Premolar extractions were performed with a record of the duration and difficulty of extraction. The extracted premolars were fixed and stained with toluidine blue. Digitized images were recorded under a stereomicroscope, and the percentage of stained PDL was analyzed. **Results:** Orthodontic preloading for 4, 6, and 8 weeks significantly increased the percentage of stained PDL on the root surface compared with the control ($P < 0.05$). The duration and difficulty of extraction were significantly less in preloaded than that of unloaded teeth after 4, 6, and 8 weeks ($P < 0.05$). **Conclusions:** A 4-week duration of orthodontic preloading is suggested as the shortest duration to adequately enhance PDL and ease tooth extraction; both outcomes may be beneficial for tooth autotransplantation. (Am J Orthod Dentofacial Orthop 2020;157:186-93)

The use of premolars extracted because of orthodontic treatment in tooth autotransplantation to replace missing or hopeless teeth has been previously reported in the literature.[1] Compared with osseointegrated dental implants, autotransplantation of a natural tooth has several advantages, including better functional adaptation,[1] alveolar ridge preservation,[2] and esthetics,[3] because the transplanted tooth can maintain periodontal ligament (PDL) homeostasis and normal functional proprioception.[4] Thus, tooth autotransplantation is an ideal choice compared with dental implants if combined therapies between orthodontic tooth movement and tooth autotransplantation are designed for the transplanted tooth.[5,6] However, some complications after tooth autotransplantation can still occur, such as root resorption, dentoalveolar ankylosis, tooth mobility, and periodontal pocket formation.[7] In monkeys, ankylosis of the donor tooth is often due to PDL injury during extraction.[8] Therefore, the quality and quantity of remaining PDL on the root surface of the extracted donor tooth, as well as the ease of extraction (to avoid possible PDL injury) are important factors for the success of tooth autotransplantation.[8]

PDL is a highly vascular and cellular connective tissue situated between the tooth and alveolar bone that provides supportive, attachment, and sensory functions.[9] Because a principal function of the PDL is to anchor the tooth root to the jaw bone and to distribute multidirectional mechanical stresses, such as masticatory forces, it is, perhaps, not surprising that the PDL exhibits features of a shock-absorbing system.[9] Indeed, some of the most interesting features of the PDL are its adaptability to rapidly applied force levels and its remarkable

[a]Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, Bangkok, Thailand.
[b]Center of Excellence in Oral and Maxillofacial Biology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.
All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
This work was supported by Chiang Mai University grants, the Intramural Endowment Fund, Faculty of Dentistry, Bangkokthonburi University [BTU61/02]; and the Thailand Research Fund [BRG6080001], [RDG5750069], and [MRG5080347].
Address correspondence to: Boonsiva Suzuki, Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, 16/10 M.2, Taweewattana Rd, Taweewattana District, Taweewattana, Bangkok 10170, Thailand; e-mail, boonsiva.suz@bkkthon.ac.th.
Submitted, September 2018; revised and accepted, March 2019.
0889-5406/$36.00

https://doi.org/10.1016/j.ajodo.2019.03.019

capacity for repair and regeneration.[9] The PDL that remains attached to the root surface is capable of regenerating new PDL after replantation.[10] Moreover, PDL of the transplanted tooth appears to be able to induce bone production, because PDL cells can be genetically differentiated into 3 distinct types of cells,[11] particularly osteoblasts that generate bone around the transplanted tooth.[4]

A few previous studies in an animal model have suggested preapplication of orthodontic force to increase the width of PDL in order to ease tooth extraction, which may decrease damage to PDL during the extraction.[12,13] A previous case report has demonstrated increased mobility of 2 human donor teeth upon orthodontic preloading, as assessed by the Periotest.[14] The increased mobility was shown, immediately, to ease tooth extraction without PDL injury and, after a 1-year follow-up, to have aided in successful tooth autotransplantation. Therefore, it is reasonable to hypothesize that preapplication of orthodontic force to the donor tooth would be useful to increase PDL tissue on the root surface of the preloaded tooth with complete root formation and to reduce PDL injury owing to easier tooth extraction. The objective of this study was to determine an optimal duration for the stimulation of the proliferative activity of PDL tissue on the root surface and the facilitation of extraction after preapplication of orthodontic force for different periods.

MATERIAL AND METHODS

Sixty healthy patients (aged 16-37 years; 21 years, 6 months ± 4 years, 8 months [mean ± standard deviation]; 23 male and 37 female) free from any underlying medical conditions, as revealed by their medical history, were recruited from the Postgraduate Clinic, Faculty of Dentistry, Bangkokthonburi University, Thailand with written informed consent. The study protocol was approved by the human ethics committee of Bangkokthonburi University (approval number: 5/2561). The patients were planned for extractions of their maxillary first premolars as part of orthodontic treatment. The inclusion criteria were that patients had a sound first premolar in each quadrant without caries or restorations, were not taking any steroidal or nonsteroidal anti-inflammatory drugs during force application because of their inhibitory effects on PDL and bone remodeling,[15] and had good oral hygiene and healthy periodontium with pocket depths ≤3 mm. The exclusion criteria were an asymmetrical arch form or any root malformations on either side, such as root dilaceration, which might result in a complicated extraction of the maxillary first premolars, as evidenced by intraoral and extraoral radiographs.

By drawing sealed randomization envelopes prepared by a person not involved in this study, 60 patients were randomly assigned to the 4 groups (15 each), which comprised orthodontic preloading on either side of their maxillary first premolars for 2, 4, 6, or 8 weeks, respectively, whereas the contralateral premolar was not loaded as a control. The reason for choosing only maxillary premolars in this study was that local anesthesia of mandibular premolars by inferior alveolar nerve block is considered technically more challenging and less predictable than that of local infiltration[16] for the upper premolars. Hence, the duration and difficulty of extraction between different cases and quadrants could not be controlled. The preloading force was obtained from a 0.016-inch improved superelastic nickel-titanium alloy wire (Sentalloy, Tomy International, Tokyo, Japan) inserted into a slot (Roth prescription; 0.018 × 0.025-inch), attached to a preadjusted edgewise bracket (Tomy International), bonded on the maxillary first premolar using compomer (Grengloo, Ormco, Orange, Calif; Fig 1, *A*) whereas the contralateral premolar was not connected to the wire (Fig 1, *B*). The inserted wire was tied with the bracket using an elastomeric ring (DynaFlex, Emergo Group, the Hague, the Netherlands; Fig 1, *A*). According to the manufacturer's instructions (Tomy International), the wire has the shape memory effect that uses body temperature to generate continuous light force (100 g) with a deflection of 0.5-1.8 mm.

After orthodontic preloading for the indicated times, the wire was removed, followed by removal of the bracket on the preloaded tooth, using a bracket remover (Tomy International). The tooth surface was cleaned and polished with white stone (Shofu, Kyoto, Japan). Infiltration with 4% articaine with epinephrine 1:100,000 (Septanest SP, Septodont, Saint-Maur-des-Fossés, France) was conducted through the buccal vestibule on both sides. Premolar extractions were performed by the same surgeon, who was blinded to the tooth condition, with a record for the duration of the extraction procedure that began when the forceps (F150, Hu-Friedy, Chicago, Ill) was clamped on the tooth and ended when the tooth was removed out of its socket. After tooth removal, the surgeon was asked to score on a modified 4-point Likert scale (1 = very easy; 2 = easy; 3 = difficult; 4 = very difficult) based on the surgeon's perception.[17] After extraction, 500 mg of acetaminophen (paracetamol GPO, Government Pharmaceutical Organization, Bangkok, Thailand) was prescribed for the patients every 6 hours as needed for pain.

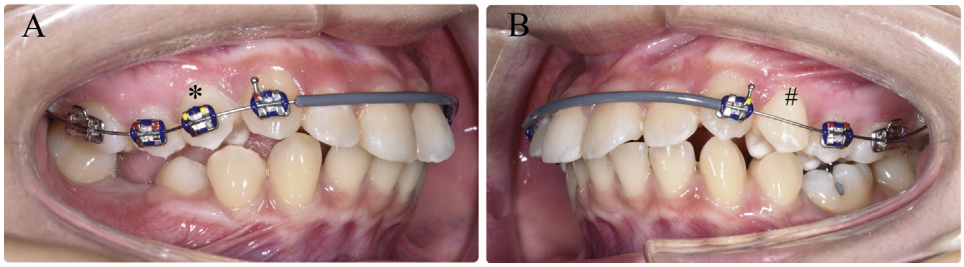

Fig 1. Intraoral photographs. **A,** Preloaded, upper right first premolar (*). **B,** Unloaded control, upper left first premolar (#).

Staining with toluidine blue was performed to determine the amount of PDL tissue with proliferative cells on the root surface using a protocol modified from Iwata et al.[18] Yee[19] reported that the number of mitotic cells representing PDL cell proliferation was increased after orthodontic loading of rats' teeth for 24 hours. In addition, cells with increased mitotic activity are usually detected by toluidine blue staining.[20] In brief, the extracted teeth were washed gently in phosphate-buffered saline (PBS), pH 7.2, and fixed within 30 minutes after extraction with 10% buffered formalin solution in 50-ml tubes (Corning, Inc., Corning, NY) for 24 hours. The teeth were then immersed in PBS for 2 days, stained with 0.04% (w/v) toluidine blue (Sigma-Aldrich, St Louis, Mo) for 10 minutes, and destained with 4 ml of PBS, which was changed daily for 14 days. After destaining, the teeth were left to air dry.

The buccal, palatal, mesial, and distal surfaces of the stained roots were observed under a stereomicroscope (Olympus SZX7; Olympus, Tokyo, Japan), and a digitized image of each root surface was recorded perpendicular to the tooth axis using a charge-coupled device (Olympus E-330; Olympus) attached to the stereomicroscope. By using ImageJ software version 1.51r (National Institute of Health, Bethesda, Md), which is commonly used in previous studies to quantify stained images,[18,21,22] the area of stained PDL in each digital image, represented by blue pixels regardless of their staining intensities, was analyzed and expressed as the percentage of the stained area against the total area, which is a combination of both blue and white pixels, representing the unstained area. The mean percentage of stained PDL in each tooth was derived from an average value of the percentages of stained PDL from all 4 surfaces of that tooth.

Statistical analysis

Differences in the percentage of stained PDL on the root surface, in the duration and ease of tooth extraction between control and preloaded teeth for the various durations of preloading, were analyzed using the paired *t*-test. Two-way analysis of variance (ANOVA) was used for the analysis of the interactions between preloading or control and the 4 periods of orthodontic preloading. Comparisons between 2 different time points within the same group were analyzed using one-way ANOVA (Duncan's multiple comparison test). Correlations between these different parameters were analyzed using either the Spearman or the Pearson correlation test. The statistical analyses were conducted using SPSS version 17.0 (IBM Corporation, Armonk, NY). P values of <0.05 were considered statistically significant.

RESULTS

Because of relocation of 2 patients in the 2-week preloading group, as well as root tip fracture of the unloaded control teeth during extraction in 5 patients in the preloading groups for 2 (3 patients) and for 8 (2 patients) weeks, both preloaded and unloaded control teeth of these 7 patients were excluded from analyses of the duration of extraction and PDL staining. Therefore, the number of remaining patients in the preloading groups for 2 and 8 weeks was 10 and 13, respectively. However, from the sample size calculation determined by G*Power software (version 3.1.9.2; Franz Faul, Universität Kiel, Kiel, Germany) with the effect size = 0.59 derived from the preliminary data, $\alpha = 0.05$ and $1\text{-}\beta = 0.95$, the calculated number of patients in each period of preloading should be ≥ 10. In addition, no difference in average ages was found among the 4 preloading groups, including 2, 4, 6, and 8 weeks (data not shown).

It was demonstrated that the duration of average extraction time in preloaded teeth determined from all 4 periods of orthodontic preloading (20.78 ± 1.94 seconds) was reduced to nearly 50% compared with that in unloaded control teeth (40.11 ± 5.29 seconds). The mean duration of

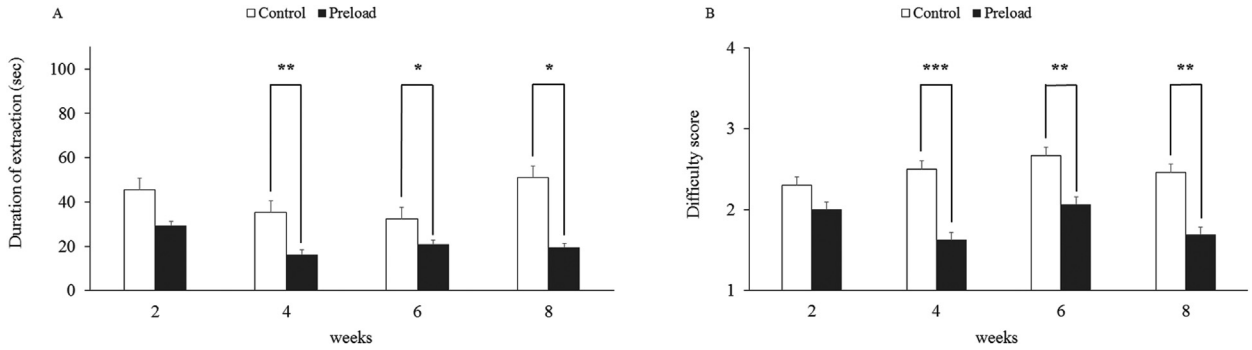

Fig 2. Facilitated tooth extraction by orthodontic preloading force. **A,** The bar graph shows a significant reduction in the duration of extraction in seconds (sec) upon preloading for 4, 6, and 8 weeks. **B,** The bar graph shows a significant decrease in the difficulty score upon preloading at 4, 6, and 8 weeks. The following indicate statistical significance: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Error bars represent standard errors.

extraction in preloaded teeth was significantly less than that of unloaded control teeth at 4, 6, and 8 weeks, but not at 2 weeks, of orthodontic preloading ($P < 0.05$; Fig 2, *A*). Similarly, the mean scores for the difficulty of extraction were significantly lower in preloaded than those of unloaded control teeth at 4, 6, and 8 weeks ($P < 0.01$; Fig 2, *B*).

Representative images of stained PDL tissue on the root surface of a preloaded tooth and an unloaded control are illustrated in Figure 3. The areas of stained PDL, irrespective of staining intensity, on all 4 root surfaces in preloaded teeth, were more significant than that of unloaded control teeth. When the average percentages of stained PDL on the 4 root surfaces from 4 different periods of orthodontic preloading were determined and compared between preloaded, and unloaded control groups, it was shown that the average percentage of stained PDL in preloaded teeth (75.67 ± 2.06) was increased by approximately 13% compared with the unloaded control teeth (63.13 ± 2.64). When the percentages of stained PDL on the combined 4 root surfaces in each period of orthodontic preloading were determined and compared, the percentages of stained PDL were significantly increased after orthodontic preloading for 4, 6, and 8 weeks, but not for 2 weeks, compared with the control ($P < 0.05$; Fig 4). When the percentages of stained PDL on each of the 4 root surfaces in each period of orthodontic preloading were compared between preloaded and unloaded groups, the percentages of stained PDL were significantly increased in at least 3 of the 4 root surfaces at 4, 6, and 8 weeks ($P < 0.05$; data not shown).

Furthermore, using a 2-way ANOVA, no interactions between either preloading or control and the 4 periods of orthodontic preloading were found ($P = 0.379$). In the preloading group, significant increases in the percentages of stained PDL were found at 4 and 8 weeks compared with that at 2 weeks ($P < 0.01$; Fig 4). However, in the control group, no differences in the percentages of stained PDL were found among the 4 periods (Fig 4), indicating that similar amounts of stained PDL remaining on the root surfaces after premolar extraction in the control group are about the same despite different individuals recruited for each period.

When correlations between each pair of the 3 parameters were determined, it was demonstrated that a significant and positive correlation was found between the duration and the difficulty of extraction ($r = 0.689$; $P < 0.001$; Fig 5, *A*), whereas a significant but negative correlation was found between the duration and the percentage of stained PDL ($r = -0.306$; $P < 0.05$; Fig 5, *B*). The correlation between the difficulty score and the percentage of stained PDL was not found to be statistically significant (Fig 5, *C*).

DISCUSSION

In this study, the preapplication of orthodontic loading facilitated tooth extraction by significantly lowering both the duration and the difficulty score of extraction at 4, 6, and 8 weeks, but not at 2 weeks (Fig 2). Consistent, significant increases in the percentages of stained PDL in preloaded premolars compared with unloaded premolars were found at 4, 6, and 8 weeks, but not at 2 weeks (Fig 4). Moreover, orthodontic preloading for 4 weeks yielded significantly higher percentages of stained PDL than that for 2 weeks (Fig 4). Consequently, a 4-week duration of orthodontic preloading is suggested by this study to be the shortest

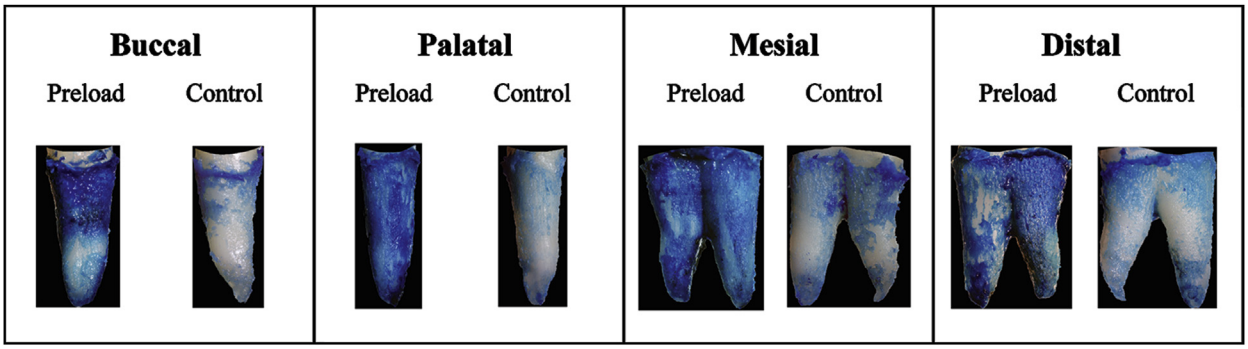

Fig 3. Representative images of PDL stained with toluidine blue on the 4 root surfaces, buccal, palatal, distal and mesial, from a preloaded tooth for 4 weeks and the unloaded control tooth.

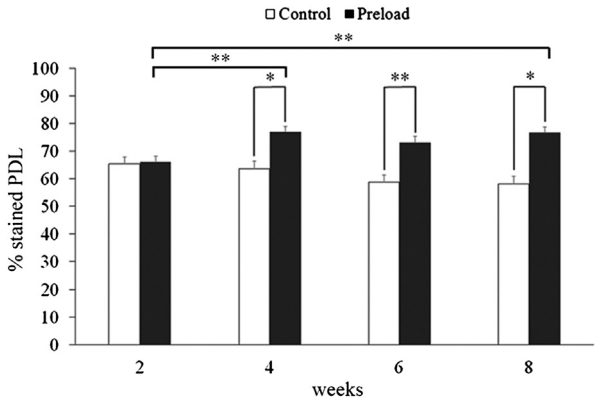

Fig 4. Significant enhancement of PDL upon orthodontic preloading force for at least 4 weeks. Note significant increases in the percentages of stained PDL on the root surfaces of preloaded teeth for 4 and 8 weeks compared with that for 2 weeks. *, ** indicate a statistically significant difference at $P < 0.05$ and $P < 0.01$, respectively. Error bars represent standard errors.

duration for both ease of tooth extraction and enhancement of PDL, both of which outcomes may increase the success rate of tooth autotransplantation. Choi et al[23] have previously reported a significant enhancement in the survival rate of intentional tooth replantation from 91.2% to 98.1% by applying extrusive force before extraction. We speculate that this increase in the survival rate, resulting from preapplication of extrusive force, is due to increased amounts of PDL, as shown in our study; however, it is not possible to replant a tooth stained with toluidine blue back into the alveolar socket.

Tooth autotransplantation is considered an accepted treatment with a 5-year survival rate of around 90% in transplanted premolars with incomplete root formation.[24,25] However, the 5-year survival rate of the transplanted premolars with complete root formation drops to 60%[24] to 84%[26] because of decreased PDL healing and increased root resorption. Moreover, PDL tissue can be damaged by conventional extraction, which results in several undesirable complications, such as root resorption and dentoalveolar ankylosis. Up to the present, a few methods for enhancing PDL tissue in extracted teeth with complete root formation have been introduced to reduce complications from damaged PDL or denuded root surface, methods such as PDL regeneration by cell culture[18] and preapplication of orthodontic force.[13,23] However, in this study a preloading force for different periods was chosen for an investigation of the remaining PDL tissue because PDL regeneration by cell culture[18] is not clinically practical because there is an increased risk of possible contamination of the cells during the transfer from the mouth to the culture plate.

In the present study, orthodontic preloading for 4, 6, and 8 weeks significantly reduced the duration of extraction. This finding differs from that shown by Choi et al,[23] in which they found no significant difference in the duration of extraction by using an atraumatic forceps technique between preloaded teeth with 50-g orthodontic extrusive force for 2-3 weeks and unloaded control teeth. This discrepancy may be explained by different periods (4-8 vs 2-3 weeks), magnitudes (100 vs 50 g) and types (leveling vs extrusion) of orthodontic preloading used in our study and theirs, respectively. However, in terms of the difficulty of extraction, our finding is consistent with that of Rai and Yadav,[17] who found a significant reduction in the difficulty of extraction in preloaded premolars with leveling orthodontic force. In another study,[13] preapplication of orthodontic force led to the widening of PDL spaces and alveolar sockets. Such widening of PDL spaces and alveolar sockets may be the reason why preapplication of orthodontic force results in facilitated extraction.

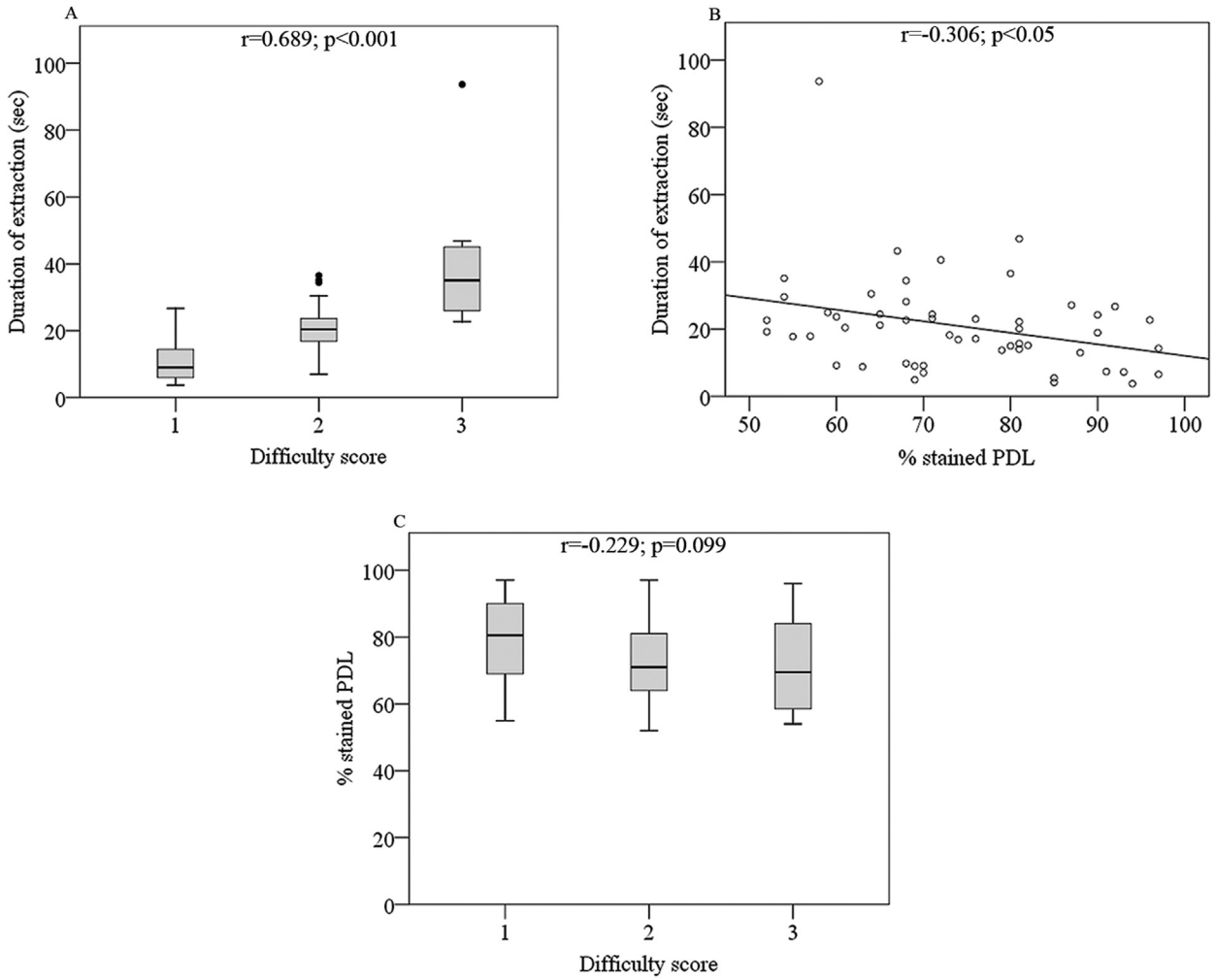

Fig 5. **A,** Significant and positive correlation between the duration in seconds and the difficulty score of extraction. **B,** Significant but inverse correlation between the duration of extraction and the percentage of stained PDL. **C,** No significant correlation between the difficulty score of extraction and the percentage of stained PDL. Small *black circles* in A represent outliers; *horizontal lines* in **A** and **C** within the boxes represent medians; a *regression line* is shown in **B**.

Staining with toluidine blue is usually recommended to detect cell proliferation.[20] Consequently, stained PDL tissue on the root surface may imply PDL cell proliferation, whereas unstained areas may represent PDL tissue without cell proliferation. In terms of the amounts of proliferative PDL tissue on the root surface, our study demonstrated significant enhancement of human proliferative PDL tissue by orthodontic preloading for at least 4 weeks. This finding corresponds with that demonstrated by Suzaki et al,[13] in which attached PDL was abundant on the root surface after force application in a rat model. Choi et al[23] suggested that PDL volume is increased on the root surface of preloaded teeth; however, there was no quantitative assessment reported in their study. Because leveling of orthodontic force was used in this study, it is appropriate to determine the overall percentages of stained PDL from the 4 root surfaces rather than to evaluate the percentage in each root surface. In addition, assessment of the combined percentage from the 4 root surfaces (Fig 4) yielded a 3-dimensional perspective of stained PDL as a whole.

When correlations between each pairing of the 3 parameters were analyzed, a significant and positive correlation was found between the duration and the difficulty of extraction. This correlation is logical because a surgeon who spends less time on the extraction of preloaded premolars would perceive the extraction as being easy. Furthermore, the inverse correlation between

the duration and the percentage of stained PDL was found to be significant. However, there was only a trend for the inverse correlation between the difficulty score and the percentage of stained PDL (Fig 5, *C*). These negative relationships imply that the facilitated tooth extraction could avoid PDL injury and then preserve more PDL tissue on the root surface.

In this study, both enhanced PDL on the root surface of extracted human premolars and facilitated tooth extraction after preloading have been demonstrated, which are clinically crucial for cases that tooth autotransplantation has been planned. It is essential to avoid any damage of the PDL on the root surface of the donor tooth that is used for autotransplantation. Moreover, fracture of root tips often complicates extraction of maxillary biradicular premolars, as evidenced in the unloaded control teeth of our preloading groups at 2 and 8 weeks, and their removal could lead to increased alveolar bone destruction, especially for thin buccal plate that would lead to a buccal bone defect and compromise the treatment outcome. Therefore, orthodontic preloading to facilitate tooth extraction would be useful for preventing PDL damage of the donor tooth and preserving the alveolar bone.

In the present study, only conventional 0.018-inch pre-adjustable edgewise brackets combined with 0.016-inch improved superelastic nickel-titanium alloy archwires were used in order to standardize the preloading procedures. The use of other different bracket systems, such as the self-ligating brackets in combination with different archwire sizes, may yield different results in terms of PDL enhancement because of the difference in friction between the bracket types. Additional studies are required to clarify the possibility of different findings. Furthermore, the findings gained from this study, based on both clinical and laboratory findings, could be fundamental for conducting future clinical research that would help determine the benefits of orthodontic preloading for an increase in the survival rate of tooth autotransplantation.

CONCLUSIONS

Orthodontic preloading force for 4 weeks is proposed to be an appropriate duration for facilitated tooth extraction and PDL tissue enhancement.

ACKNOWLEDGMENTS

The authors thank Dr Thanapat Sastraruji, Faculty of Dentistry, Chiang Mai University, Thailand, for his assistance in statistical analysis; Somyot Limpanaputtajak, Faculty of Dentistry, Bangkokthonburi University, Thailand, for his role as an oral surgeon; and Dr M. Kevin O. Carroll, Professor Emeritus of the University of Mississippi School of Dentistry, and Professor at the Bangkokthonburi University, Faculty of Dentistry, for his critical reading of this manuscript.

REFERENCES

1. Slagsvold O, Bjercke B. Indications for autotransplantation in cases of missing premolars. Am J Orthod 1978;74:241-57.
2. Czochrowska EM, Stenvik A, Album B, Zachrisson BU. Autotransplantation of premolars to replace maxillary incisors: a comparison with natural incisors. Am J Orthod Dentofacial Orthop 2000;118:592-600.
3. Zachrisson BU, Stenvik A, Haanaes HR. Management of missing maxillary anterior teeth with emphasis on autotransplantation. Am J Orthod Dentofacial Orthop 2004;126:284-8.
4. Tsukiboshi M. Autotransplantation of teeth: requirements for predictable success. Dent Traumatol 2002;18:157-80.
5. Choi YJ, Han S, Park JW, Lee DW, Kim KH, Chung CJ. Autotransplantation combined with orthodontic treatment to restore an adult's posttraumatic dentition. Am J Orthod Dentofacial Orthop 2013;144:268-77.
6. Fujita K, Kanno Z, Otsubo K, Soma K. Autotransplantation combined with orthodontic treatment in adult patients. Orthod Waves 2008;67:128-34.
7. Kokai S, Kanno Z, Koike S, Uesugi S, Takahashi Y, Ono T, et al. Retrospective study of 100 autotransplanted teeth with complete root formation and subsequent orthodontic treatment. Am J Orthod Dentofacial Orthop 2015;148:982-9.
8. Andreasen JO, Kristerson L. The effect of limited drying or removal of the periodontal ligament. Periodontal healing after replantation of mature permanent incisors in monkeys. Acta Odontol Scand 1981;39:1-13.
9. Lekic P, McCulloch CA. Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. Anat Rec 1996;245:327-41.
10. Andreasen JO. Periodontal healing after replantation and autotransplantation of incisors in monkeys. Int J Oral Surg 1981;10:54-61.
11. Yamamura T, Shimono M, Koike H, Terao M, Tanaka Y, Sakai Y, et al. Differentiation and induction of undifferentiated mesenchymal cells in tooth and periodontal tissue during wound healing and regeneration. Bull Tokyo Dent Coll 1980;21:181-221.
12. Qu H, Saito S, Morohashi T, Ohmae M, Seki K, Yamada S, et al. Effects of pre-transplantable jiggling force on root resorption of the experimental autotransplanted of teeth in vivo. Orthod Waves 2001;60:213-25.
13. Suzaki Y, Matsumoto Y, Kanno Z, Soma K. Preapplication of orthodontic forces to the donor teeth affects periodontal healing of transplanted teeth. Angle Orthod 2008;78:495-501.
14. Cho JH, Hwang HS, Chang HS, Hwang YC. Application of orthodontic forces prior to autotransplantation—case reports. Int Endod J 2013;46:187-94.
15. Bartzela T, Türp JC, Motschall E, Maltha JC. Medication effects on the rate of orthodontic tooth movement: a systematic literature review. Am J Orthod Dentofacial Orthop 2009;135:16-26.
16. Hargreaves KM, Keiser K. Local anesthetic failure in endodontics: mechanisms and management. Endod Top 2002;1:26-39.
17. Rai AK, Yadav BK. Facilitating orthodontic teeth extraction–a technique suggestion. Saudi J Dent Res 2016;7:96-100.

18. Iwata T, Mino C, Kawata T. In vitro proliferation of periodontal ligament-like tissue on extracted teeth. Arch Oral Biol 2017;75: 31-6.
19. Yee JA. Response of periodontal ligament cells to orthodontic force: ultrastructural identification of proliferating fibroblasts. Anat Rec 1979;194:603-14.
20. Chieco P, Pagnoni M, Romagnoli E, Melchiorri C. A rapid and simple staining method, using toluidine blue, for analysing mitotic figures in tissue sections. Histochem J 1993;25:569-77.
21. Jensen EC. Quantitative analysis of histological staining and fluorescence using ImageJ. Anat Rec 2013;296:378-81.
22. Grove C, Jerram DA. jPOR: an ImageJ macro to quantify total optical porosity from blue-stained thin sections. Comput Geosci 2011;37:1850-9.
23. Choi YH, Bae JH, Kim YK, Kim HY, Kim SK, Cho BH. Clinical outcome of intentional replantation with preoperative orthodontic extrusion: a retrospective study. Int Endod J 2014;47: 1168-76.
24. Andreasen JO, Paulsen HU, Yu Z, Schwartz O. A long-term study of 370 autotransplanted premolars. Part III. Periodontal healing subsequent to transplantation. Eur J Orthod 1990;12:25-37.
25. Schwartz O, Bergmann P, Klausen B. Resorption of autotransplanted human teeth: a retrospective study of 291 transplantations over a period of 25 years. Int Endod J 1985;18:119-31.
26. Sugai T, Yoshizawa M, Kobayashi T, Ono K, Takagi R, Kitamura N, et al. Clinical study on prognostic factors for autotransplantation of teeth with complete root formation. Int J Oral Maxillofac Surg 2010;39:1193-203.