



OPEN

Low-intensity pulsed ultrasound attenuates replacement root resorption of avulsed teeth stored in dry condition in dogs

Saemi Seong^{1,3}, Dohyun Kim^{1,3}, Dasun Lee¹, Hyung-Ryong Kim^{2✉} & Yooseok Shin^{1✉}

This study aimed to investigate the effects of low-intensity pulsed ultrasound (LIPUS) on replacement root resorption after replantation of avulsed teeth stored in a dry condition in dogs. A total of 73 premolar roots from four male mongrel dogs were intentionally avulsed with forceps and divided into four groups—HN, HL, DN, and DL—according to storage conditions and whether or not they received LIPUS treatment. Thirty-eight roots were kept in Hanks' Balanced Salt Solution for 30 min (HN and HL groups), whereas the remaining 35 roots were left to dry in the air for an hour (DN and DL groups) prior to replantation. Following replantation, the roots in the HL and DL groups (21 and 18 roots, respectively) received a 20-min daily LIPUS treatment for 2 weeks. The animals were euthanized 4 weeks after the operation. Micro-computed tomography images were acquired for each root and the amount of replacement root resorption was measured three-dimensionally. Histological assessments were also carried out. There was significantly less replacement root resorption for the roots in the DL group compared to the DN group ($p < 0.01$). Histological findings in the DN group demonstrated evident replacement root resorption, whereas the DL group revealed less severe resorption compared to the DN group. Within the limitations, these results suggest that LIPUS could attenuate the replacement resorption of avulsed teeth stored in a dry condition, thereby improving their prognosis.

Tooth avulsion affects 0.5–16% of traumatic dental injuries in permanent dentitions and is considered to be the most serious type of them¹. Both pulp and periodontal tissues suffer from extensive damage during an extra-alveolar period, with healing reactions dependent upon various factors such as storage media, length of the extra-alveolar period, root development, and contamination of the root surface^{2–4}. While tooth replantation is the indicated treatment for some of the avulsions, external root resorption is seen as a major complication encountered after replantation. A meta-analysis on the incidence of root resorption after the replantation of avulsed teeth showed that the incidence of surface, inflammatory, and replacement root resorption was 13.3%, 23.2%, and 51.0%, respectively⁵. Surface root resorption can be seen in a normal healing process and considered as a favorable type of periodontal healing. Inflammatory root resorption is associated with necrosis and infection of the pulp. Replacement root resorption appears as a fusion between the tooth and the alveolar bone, and is related to the condition of periodontal ligaments cells from the injured root surface. The periodontal healing after replantation of avulsed teeth have been the subject of numerous experimental studies. A series of histological analyses of avulsed human teeth showed that almost half of the periodontal ligament was lost after replantation⁶. This indicates serious healing complications that may result in replacement root resorption being commonly observed in avulsed teeth after replantation. This has been confirmed by experimental replantation in dogs⁷, monkeys^{8,9}, and humans¹⁰. Many studies were conducted to improve regenerative responses and healing of periodontal tissues to minimize replacement root resorption after replantation of avulsed teeth. Preventive methods to reduce root resorption susceptibility were also introduced¹¹. However, a reliable therapeutic protocol has not yet been universally established.

The use of ultrasound for medical applications has been investigated for decades¹². Low-intensity pulsed ultrasound (LIPUS) is a specific type of ultrasound that delivers impulses at a low-intensity and outputs pulsed waves. The effectiveness of LIPUS treatments is mostly ensured by non-thermal effects, including acoustic cavitation

¹Department of Conservative Dentistry and Oral Science Research Center, Yonsei University College of Dentistry, Seoul 03722, Republic of Korea. ²College of Dentistry, Dankook University, Cheonan 31116, Republic of Korea. ³These authors contributed equally: Saemi Seong and Dohyun Kim. ✉email: hrkimdp@gmail.com; densys@yuhs.ac

	LIPUS treatment	No LIPUS treatment	Total
HBSS storage (30 min)	21 (group HL)	17 (group HN)	38
Dry storage (60 min)	18 (group DL)	17 (group DN)	35
Total	39	34	73

Table 1. Allocation of roots for each group.

and biological signaling¹³. LIPUS treatment has been mostly used in the field of orthopedics. It has been shown that LIPUS enhances and accelerates fracture-healing through increased bone formation in cases of delayed and/or impaired bone healing, and significantly decreases period of bone healing^{14,15}. Its applications for orthopedic treatment have been approved by the US Food and Drug Administration (FDA) in 1994 and 2000¹⁶. Although the impact of LIPUS in the osteogenic responses of the bone has been well documented, elucidating the clinical effectiveness of LIPUS in orofacial regions warrants further study. Soft tissue healing and significant decrease in areas of root resorption lacunae have been reported to this date^{17,18}. It was found that the application of LIPUS for sinus augmentation promotes new bone formation in rabbits¹⁹. A pilot study demonstrated enhanced alveolar bone healing of extraction sockets²⁰, while another showed the prevention of bisphosphonate-related osteonecrosis of the jaw, both of which were carried out in a rat model²¹. Recently, it was found that LIPUS treatment not only enhances the migration of periodontal ligament stem cells in vitro²², but also accelerates 2-wall alveolar bone defects in canines, in addition to inhibiting root resorption after luxation and immediate replantation in rats^{18,23}.

A few more studies have been carried out in relation to orthodontically-induced root resorption. It has been reported that LIPUS treatment minimized orthodontically-induced inflammatory root resorption caused by torque in rats²⁴ as well as in healthy human patients²⁵. It has been shown that LIPUS treatment increases the thickness of predentin and cementum in rats²⁶ and beagles²⁷. It is considered that LIPUS regulates osteoclast differentiation and bone remodeling²⁸. However, no study to date has evaluated the effects of LIPUS on root replantation after avulsion solely dependent on periodontal healing, excluding the inflammatory effects due to infected pulp tissues, which would more accurately reflect the effects of LIPUS on replacement root resorption of avulsed teeth. Thus, this study aimed to investigate the effects of LIPUS on replacement root resorption after replantation of avulsed dog teeth stored with different conditions and periods, excluding the adverse effects from necrotic pulp. It was hypothesized that there would be no difference in replacement root resorption between the roots exposed to LIPUS treatment and those receiving no supplementary treatment after replantation.

Methods

Animals. Four healthy male mongrel dogs, 10–14 months of age and weighing approximately 25–30 kg, were included in this study. The animals were housed and monitored daily for the duration of the study in the Department of Laboratory Animal Resources, Yonsei Biomedical Research Institute, Seoul, Republic of Korea. They were allowed a week for acclimation prior to the experiment. They were kept in individual cages at 22 °C with relative humidity of 50%, and a 12-h light/dark cycle. Approximately 500 g of solid food (Purina; Nestle SA, Vevey, Switzerland) was provided to each animal every day, and water was available ad libitum during the experimental period. Experiments were carried out in compliance with the ARRIVE guidelines, approved by the Yonsei University Health System Institutional Animal Care and Use Committee, Seoul, Republic of Korea (Approval No. 2019-0068). Informed consent was obtained from the owners, and all experiments were performed in accordance with relevant guidelines and regulations.

Surgical procedures. Premolar teeth of each animal were included for the experiment. The roots were divided into four groups according to storage conditions before replantation and whether or not they received a LIPUS treatment after replantation. The roots in each group were evenly distributed among the four dogs, such that each group equally included the right and left, maxillary and mandibular premolar roots. Congenitally missing, impacted, or root-fractured teeth were excluded from the study. Thirty-eight roots were kept in Hanks' Balanced Salt Solution (HBSS) for 30 min (HN and HL groups), whereas the remaining 35 roots were left to dry in the air for an hour (DN and DL groups) before the replantation. The roots in the HL and DL groups received a daily LIPUS treatment after the replantation (Table 1).

Three experienced dental clinicians performed all surgical procedures under general and local anesthesia under aseptic routines. General anesthesia was induced by alfaxalone (Alfaxan; Jurox Pty Ltd., Rutherford, NSW, Australia), medetomidine hydrochloride (Tomidine; Provet Ltd., Istanbul, Turkey) intravenously and was maintained by 2% isoflurane (Ifiran Liq; Hana Pharm Co Ltd., Republic of Korea) in conjunction with pure oxygen by inhalation.

Preoperative root canal treatments were performed aseptically on all experimental teeth to minimize the effects of pulpal infection as a stimulus for external root resorption. After occlusal reduction and access opening with sterile diamond bur, coronal flaring was carried out using Gates-Glidden drills (Dentsply Maillefer, Baillagues, Switzerland). The working length was determined using size #20 stainless steel k-type hand instruments (Dentsply Maillefer, Baillagues, Switzerland) and a periapical radiograph. Nickel-titanium rotary instruments (Profile; Dentsply Maillefer, Baillagues, Switzerland) and k-type hand instruments were used for pulp extirpation and root canal preparation to apical delta. The apical enlargements of root canals were accomplished up to size #50–70 with k-type hand instruments. After shaping of the root canal was completed, final irrigation was performed using 5 mL of 2.5% NaOCl solution using a syringe with a 30-gauge needle. Lastly, the root canals

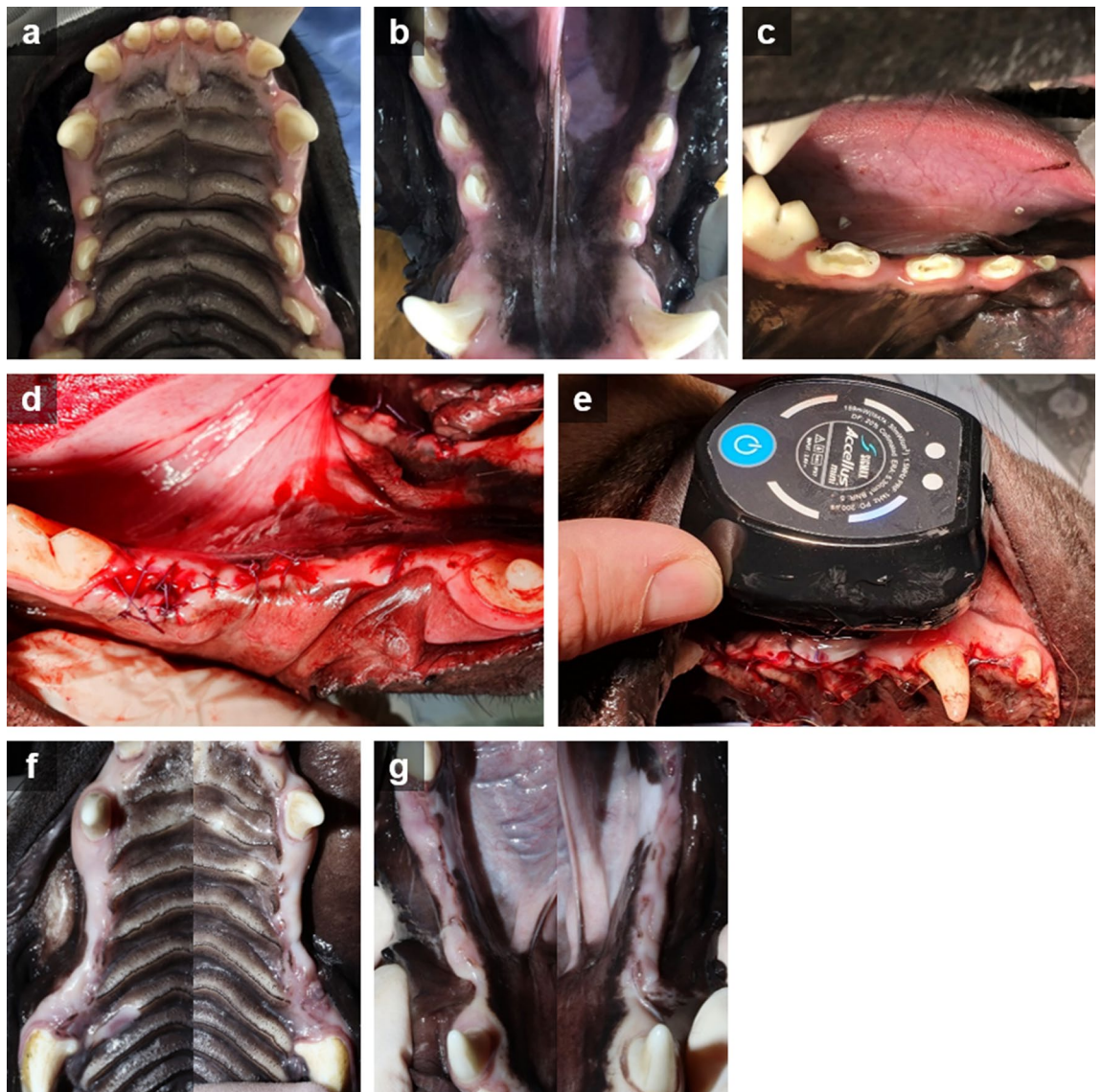


Figure 1. Clinical photographs during experimental periods. (a, b) Pre-operation. (c) After root canal treatment. (d) Post-operation. Replanted roots were covered by gingiva and sutured. (e) LIPUS treatment (f, g) 4 weeks after the operation.

were dried with sterile paper points and obturated with Vitapex paste (J Morita, Tokyo, Japan). Occlusal access holes were filled with resin-modified glass ionomer (Fuji II LC; GC, Tokyo, Japan) (Fig. 1a–c).

An intentional avulsion was performed as atraumatically as possible with forceps (Physics Forceps; GoldenDent, Detroit, MI, USA) and gentle bucco-lingual luxation forces. After extraction, two-rooted premolars were hemisectioned into two single roots. The crown portion of the tooth was removed at the cemento-enamel junction with a high-speed diamond bur to avoid occlusal forces during healing periods. Each root was placed in its prepared designated space in a 16-well plate. For the HN and HL groups, each well was filled with 2.5 mL of HBSS, ensuring that the root was completely submerged in the solution for 30 min. For the DN and DL groups, the root was swiftly air dried and then placed in an empty well to dry for an hour. After the given time, the roots were replaced in the socket in their own position and sutured with 4-0 Coated Vicryl (Ethicon Inc, Sommerville, NJ, USA) such that each root was adequately covered with gingiva (Fig. 1d).

Periapical radiographs were taken before the operation, after the root canal treatment, and after the replantation to confirm each procedure, respectively (Figs. 2a–c and 3a–c). After the surgical procedures, the animals were administered ketorolac tromethamine (Keromin inj; Hana Pharma Co Ltd., Republic of Korea), Cefazolin sodium (30 mg/kg, Chonkundang, Republic of Korea), Meloxicam (0.2 mg/kg, Boehringer Ingelheim, Greece) for a week.

LIPUS exposure. After replantation, the roots in the HL and DL groups received LIPUS treatment starting from the day of surgical procedure for 2 weeks (Fig. 1e). The LIPUS instrument used in this study (Accellus Mini; Nippon Sigmax Co, Tokyo, Japan) consisted of a circular transducer with a diameter of 26 mm and an effective

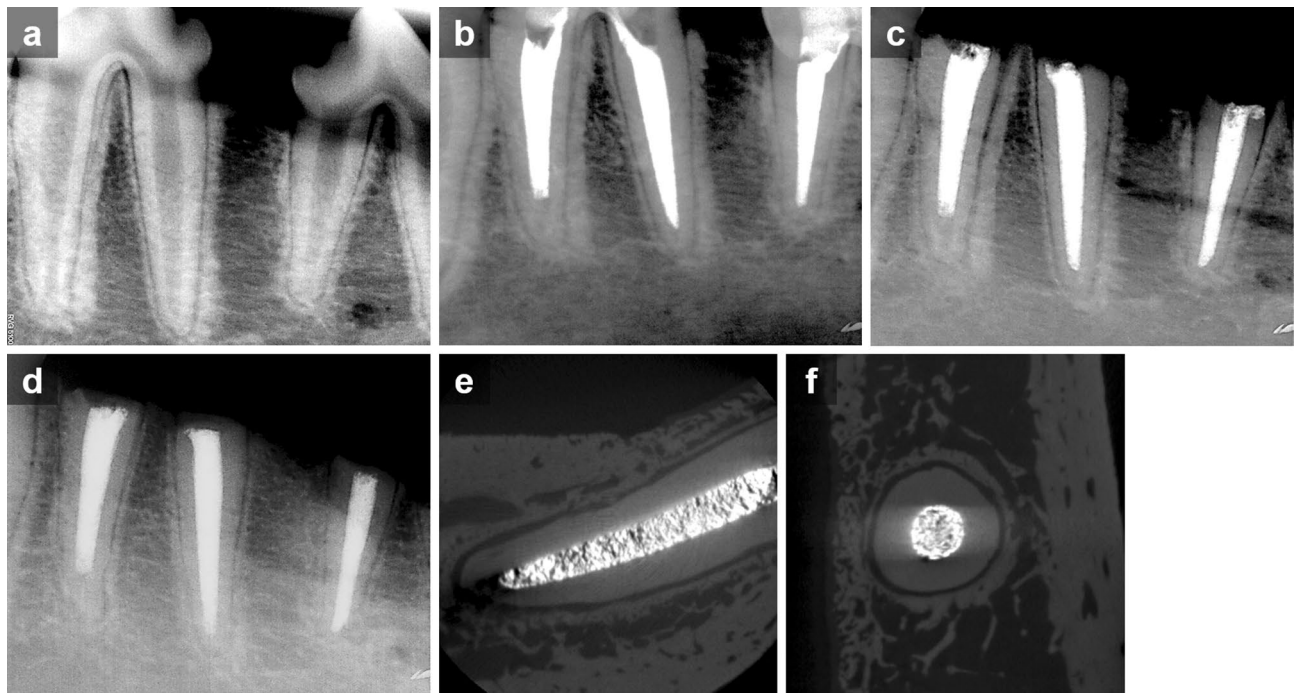


Figure 2. Representative serial radiographs and micro-CT images of roots in the HL group. (a) Pre-operation. (b) After root canal treatment. (c) After replantation. (d–f) 4 weeks after the operation. Note that the roots were surrounded by intact lamina dura.

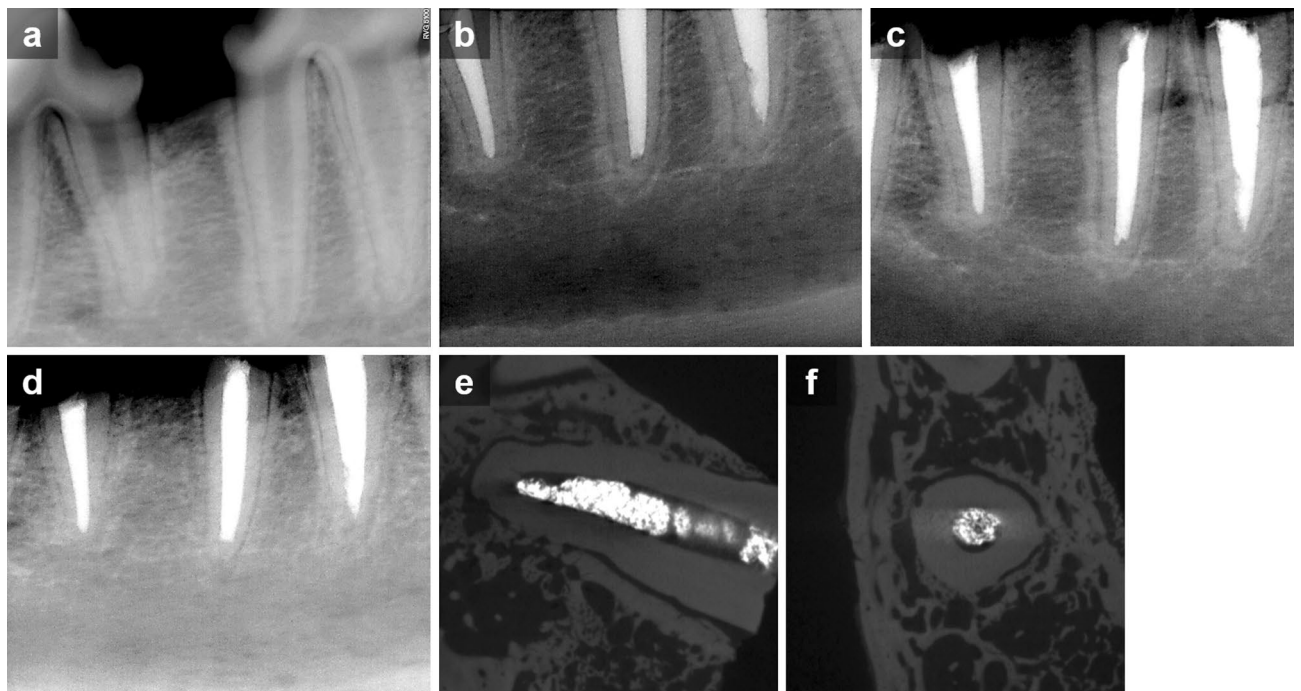


Figure 3. Representative serial radiographs and micro-CT images of roots in the DN group. (a) Pre-operation. (b) After root canal treatment. (c) After replantation. (d–f) 4 weeks after the operation. Note that the lamina dura became indistinct and the evidence of replacement root resorption was seen in the micro-CT images.

radiating area of 5.3 cm². The pulsed ultrasound signal had a 1.5 MHz frequency, 30 mW/cm² ultrasound intensity, and 1 kHz pulse repetition rate. For daily ultrasonic exposure, intravenous general anesthesia was delivered to each animal. The transducer was placed in contact with the buccal gingiva, in the region corresponding to the designated quadrant. A single practitioner located the device on the buccal mucosa to cover the targeted area. A coupling gel was kept constantly in place to optimize penetration of the ultrasound energy into the tissues. The ultrasound was used for 20 min daily for 14 consecutive days.

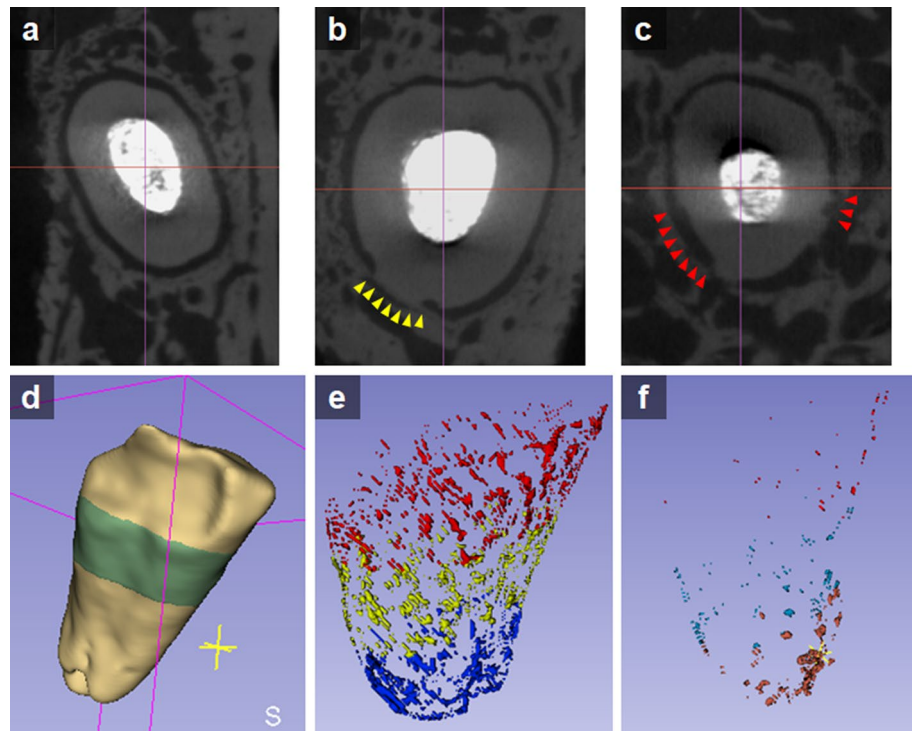


Figure 4. Representative micro-CT images and 3D reconstruction for quantitative measurement of the amount of root resorption. The root embedded in the alveolar bone alone was included. (a) Normal root surface without any root resorption. Regular and continuous radiolucent areas between the root surface and the alveolar bone, indicating PDL space, can be seen. (b) Replacement root resorption presenting direct connection between the root surface and the alveolar bone without interposition of a radiolucent area (yellow arrowheads). (c) Surface root resorption presenting craters or holes in the root surface (red arrowheads) (d) Visualization of the root by 3D reconstruction. (e) Distribution of replacement root resorption. (f) Distribution of surface root resorption.

Three-dimensional (3D) analysis. Four weeks following the operation (Fig. 1f,g), the dogs were euthanized under general anesthesia using an overdose of potassium. After taking periapical radiographs for all roots (Figs. 2d and 3d), both maxillary and mandibular premolar areas were removed en bloc with the surrounding hard tissues and fixed in 10% buffered paraformaldehyde.

The blocks were attached in a custom attachment and scanned by a micro-computed tomography (micro-CT) imaging system (Quantum GX; PerkinElmer, Hopkinton, MA, USA) at an isotropic resolution of 40 μm . The images of each sample were quantitatively assessed with regard to both replacement root resorption and surface root resorption. In each coronal cross-section image (Fig. 4a), absence of radiolucent area between the alveolar bone and the root surface was measured for replacement root resorption (Fig. 4b) and distribution of craters or holes in the root surface were measured for surface root resorption (Fig. 4c) using MeshLab²⁹ 2020 (<https://www.meshlab.net/>). Images of each specimen were reconstructed with 3D Slicer³⁰ 4.11.0 (<https://www.slicer.org/>) that provided transaxial, coronal, and sagittal cross sections and visualization, including volume rendering of the samples (Fig. 4d). The areas of replacement and surface root resorption were reconstructed three-dimensionally (Fig. 4e,f), and the percentage of replacement and surface root resorption to whole root surface were calculated respectively.

Histologic evaluation. The specimen blocks were decalcified in 10% ethylenediaminetetraacetic acid solution (pH 7.4) at 4 °C for 4 weeks embedded in paraffin. Serial, 3 μm -thick sagittal sections of selected roots were cut including the surrounding tissue. For histological analyses, the sections were stained with hematoxylin and eosin (H & E) or tartrate-resistant acid phosphatase staining of osteoclasts (TRAP) and observed under light microscopy.

Statistical analysis. The percentages of surface and replacement root resorption were compared among the four groups using a one-way analysis of variance followed by a Tukey's post-hoc test. All statistical analyses were performed under a 95% confidence level using the SPSS 25 (IBM Corp, Somers, NY, USA) software program.

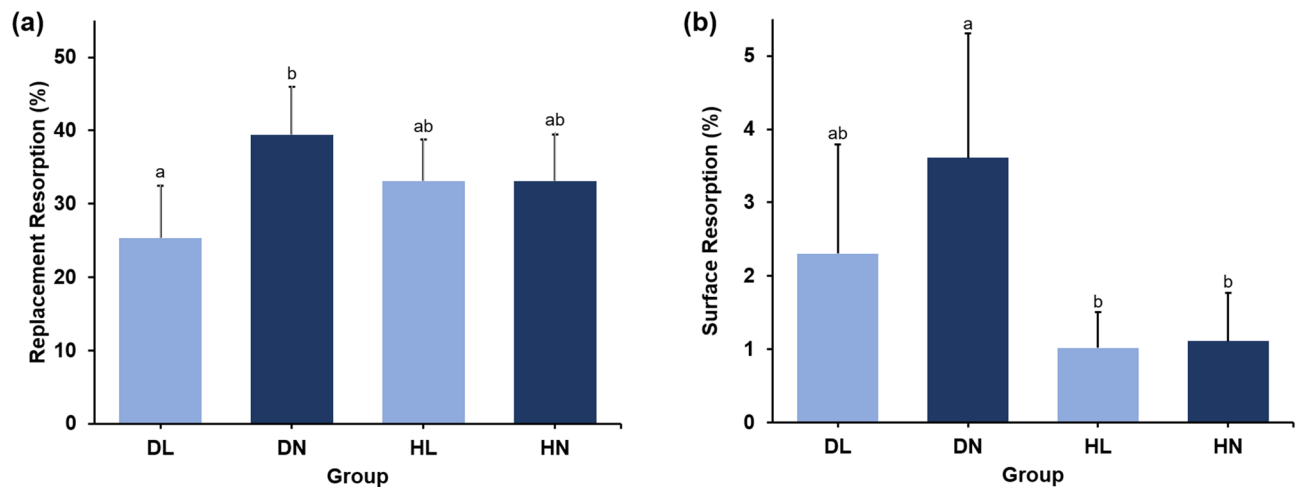


Figure 5. Percentage of root resorption area in each group. (a) Replacement resorption. (b) Surface resorption. Different letters denote significant different among the groups ($p < 0.05$).

Results

Postoperative clinical observations. Postoperative clinical healing was uneventful at all sites. Pus discharge from the gingiva was noticed in some experimental teeth with buccal bone fracture at 2 weeks, which were excluded. No visible adverse reactions, including suppuration, abscess formation or increased tooth mobility, were observed in all roots after 4 weeks.

Amounts of root resorptions. In dry storage conditions, there was significant less replacement root resorption in the LIPUS-treated group (DL) compared to the non-treated group (DN) ($p < 0.01$). Neither the DN nor the DL group was significantly different from the HL or HN group. However, the roots in the DN group tended to have the largest percent of replacement resorption, while those in the DL group had the least (Fig. 5a). The representative radiographs and micro-CT images of roots in the HL and DN groups are shown in Figs. 2 and 3, respectively.

Both HBSS storage groups (HL and HN) had significantly reduced surface resorption compared to the DN group, regardless of LIPUS exposure ($p < 0.01$ and $p < 0.05$, respectively). There were no significant differences between the HBSS treated groups. Within the dry storage groups (DL and DN), the roots in the DL group showed decreased surface resorption compared to those in the DN group. However, there was no statistically significant difference. In addition, the DL group demonstrated no significant differences in surface root resorption compared to the HL or HN groups (Fig. 5b).

Descriptive histological results. Cross sections were evaluated for healing according to the criteria of Andreasen³¹. For most roots in the HL and HN groups, the cementum integrity could be observed with periodontal ligament (PDL) space preserved with relatively uniform thickness, where the root was clearly separated from the alveolar bone (Fig. 6a,b). In contrast, histological findings in the DN group demonstrated evident replacement root resorption, where the cementum of the root and alveolar bone was connected, with no clear border in between (Fig. 6c,d). Osteoclastic cells were observed through TRAP staining as well (Fig. 6e,f). Meanwhile, the DL group revealed a substantially inhibited development of severe root resorption lacunae compared to the DN group. Similar degree of slight surface resorption was shown in all groups, corresponding to the micro-CT analysis results.

Discussion

This study focused on the effects of LIPUS treatment on replacement root resorption of avulsed teeth after replantation with different storage conditions and periods in dog models. 3D quantitative measurements of replacement root resorption and surface root resorption were carried out by micro-CT images to assess the effects of LIPUS on the healing of cementum and periodontal ligaments after a given trauma. In most previous studies, the amounts of root resorption were histologically measured³¹. Histological measurements help estimate the proportion of the root resorption area, but one should keep in mind that root resorption is not evenly distributed on the root surface and may preferentially affect corner surfaces^{32,33}. If a single axial section is used as a reference of resorption, one section direction might show only little root resorption, whereas another section may demonstrate much more resorption.

Recently, 3D images of the microarchitecture of bone specimens from different species and body parts have been obtained by using micro-CT. This produces high resolution images by micro-CT and digitally reconstructs the tooth and surrounding alveolar bone, allowing for the quantitative assessment of root surface defects or bony attachments^{34,35}. Nevins et al. showed that micro-CT can be used for the 3D quantitative assessment of alveolar bone³⁶. De Paula Reis et al. showed, when compared to histologic analyses, that micro-CT in *in vivo* studies can be a promising method for the identification of the different stages of tooth resorption and repair, in addition

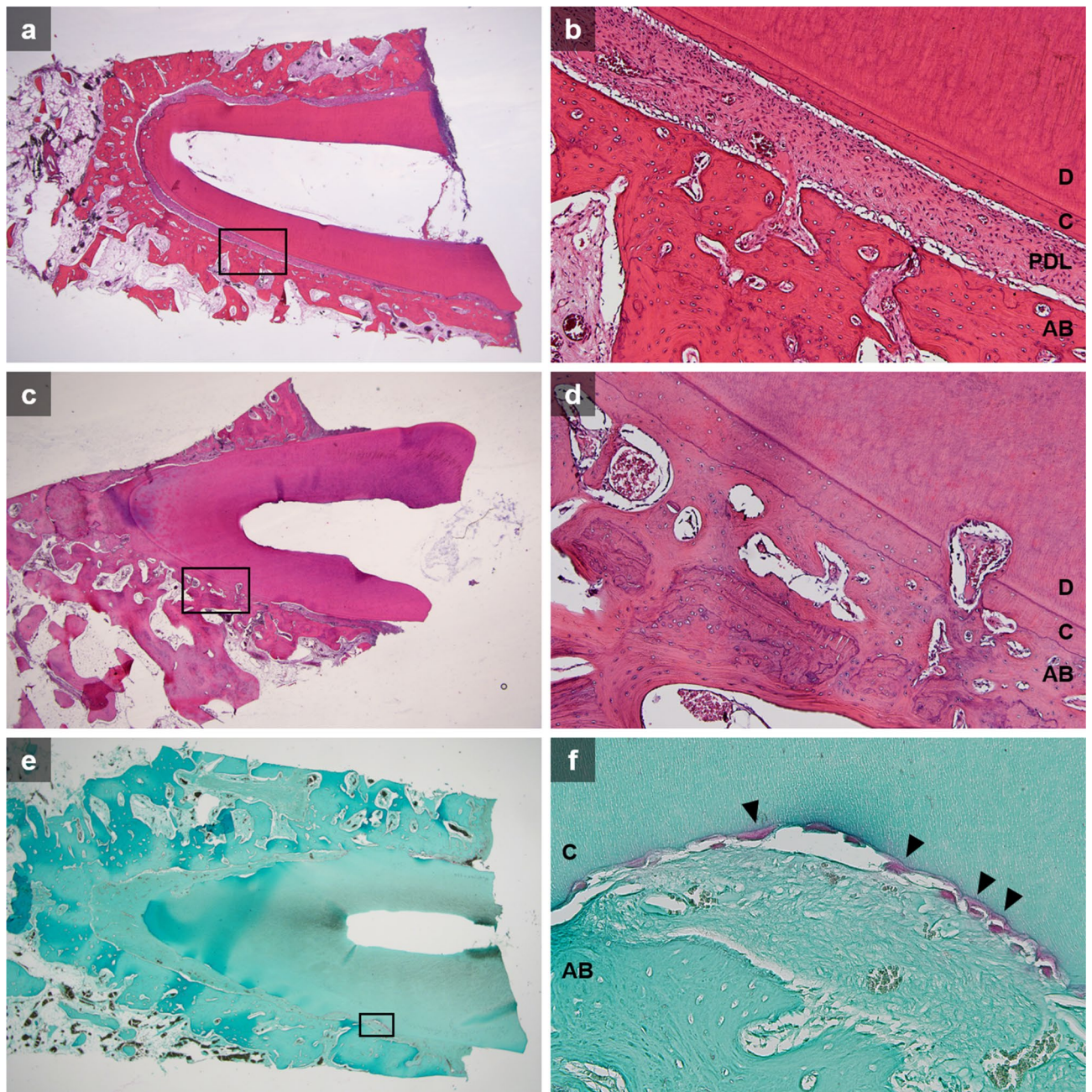


Figure 6. Histologic overview of sections of the replanted roots. **(a, b)** A root in the HL group, pointing to minimal root resorption. Note that normal periodontium organized PDL with collagen fibers and cementum layer. **(c–f)** A section of root in the DN group, showing replacement root resorption. **(e, f)** TRAP staining showed osteoclastic cells (black arrows) confined to the cementum surface. (*D* dentin, *C* cementum, *PDL* periodontal ligament, *AB* alveolar bone).

to the evaluation of the total extension of the periodontium³⁷. In this study, replacement root resorption, fusion between the alveolar bone and the root surface, and surface root resorption, craters or holes in the root surface, were measured in each coronal and cross-sectional image. The root resorption areas were reconstructed three-dimensionally and the percentages of resorption area to whole root surface were calculated. To the best of our knowledge, this is the first published report utilizing micro-CT to calculate the total surface area of a root to identify specific pathological parameters.

Dry storage for 60 min was used as a factor to increase damage to the periodontal ligament covering the root surface, according to the International Association of Dental Traumatology (IADT) guidelines for the management of avulsed permanent teeth where 60 min of extra-oral drying time is suggested as a criterion in making treatment plans and prognosis³⁸. The length of dry extra-alveolar storage period is regarded as one of the strongest factors for PDL healing after tooth avulsion, affecting the survival of the PDL cells along the root surface. The guidelines indicate that after an extra-alveolar dry time of 30 min, most PDL cells are non-viable^{39,40}.

Andreasen et al. reported an increasing rate of ankylosis after delayed replantation over 5 min⁴¹. Maslamani et al. said that when the extra-oral dry time exceeds 60 min, it is considered that most PDL cells do not survive, and surface root resorption or dentoalveolar ankylosis of the tooth can progress further⁴². To exclude any potential factors that may influence or aggravate root resorption other than storage conditions, preoperative root canal treatment was performed and calcium hydroxide paste was added to minimize the progression of inflammatory root resorption due to infection of necrotic pulp⁴³. In addition, decoronized roots were covered with gingiva as excessive initial force might cause severe root and bone resorption^{44,45}. The lack of a negative control was based on the fact that an ideal storage medium would preserve the teeth in an environment that is similar to that of an immediate replantation (i.e. the positive control) and based on the extensive literature showing higher levels of replacement resorption for teeth that have been kept dry^{8,46}. HBSS has been shown to be the best storage medium during the extra-oral phase⁴⁷.

This study's results showed that the surface resorption of the DN group was significantly higher than that of the HN and HL group ($p < 0.05$ and $p < 0.01$, respectively), possibly because of the low survival rates of PDL cells on the root surfaces, similar to those of previous studies^{41,48}. In contrast, the DL group did not show any significant differences, whether with DN or with HN or HL. These results imply that LIPUS may positively affect regeneration of the periodontal tissue after intense avulsion conditions. However, since preventive root canal treatment measures were taken, the overall degree of surface resorption was minimal because of its self-limiting characteristics demonstrating repair with new cementum^{49,50}. Most resorption lacunae are superficial and confined to the cementum, which is consistent with previous studies.

On the other hand, replacement resorption yielded more definite results. A significant reduction in replacement root resorption was found in the DL group compared to the DN group ($p < 0.01$). The HN and HL yielded similar outcomes. These results also implied that LIPUS may improve the recovery of cementum and periodontal tissue while necrotic PDL has undergone inflammatory change. These results are consistent with those of previous studies which showed that LIPUS accelerates the healing of resorption areas with the reparative cementum of orthodontic patients⁵¹ and stimulates the cementum regeneration of periodontal defects in canine models³⁵. Furthermore, several in vitro studies demonstrate that LIPUS has an anabolic effect on human periodontal ligament cells by promoting mature cementoblasts⁵² and accelerating the differentiation of immature cementoblasts⁵³. It has also been shown that LIPUS has the potential to accelerate endogenous periodontal mesenchymal stem cell recruitment for periodontal tissue regeneration on a molecular level²². It has been shown that ultrasound, if applied correctly, can stimulate tissue repair and wound healing^{54,55}. Ultrasound during the inflammatory phase of tissue repair can lead to an acceleration of this phase, which eventually leads to an anti-inflammatory effect by LIPUS exposure⁵⁶. Rego et al. suggested that LIPUS may contribute to reducing the inflammatory reaction by impairing the TNA- α signaling pathway^{18,57}. They also demonstrated the inhibitory effect of a 21-day LIPUS application on root resorption using an experimental model of tooth replantation involving luxation and the immediate replacement of maxillary first molars in rats¹⁸.

Within the limitations of this animal study, both the 3D quantitative measurements of micro-CT and the histopathological examination demonstrated that LIPUS stimulation helped prevent the replacement resorption of avulsed teeth after delayed replantation. These results highlight the therapeutic implications of ultrasound stimulation in periodontal regeneration, suggesting the potential of LIPUS to inhibit dentoalveolar ankylosis. Additional studies are warranted to evaluate and capitalize on its full potential in the orofacial area. This animal study demonstrates one aspect of the application between therapeutic ultrasound and dental research. Considering that the ultrasound is actively used in medical fields, it could certainly contribute to the advancement of dental research.

Received: 22 October 2020; Accepted: 3 June 2021

Published online: 18 June 2021

References

- Andreasen, J. O., Andreasen, F. M. & Andersson, L. *Textbook and Color Atlas of Traumatic Injuries to the Teeth* (Wiley, 2018).
- Petrovic, B., Markovic, D., Peric, T. & Blagojevic, D. Factors related to treatment and outcomes of avulsed teeth. *Dent. Traumatol.* **26**, 52–59 (2010).
- Andreasen, J. O., Borum, M. K., Jacobsen, H. L. & Andreasen, F. M. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. *Endod. Dent. Traumatol.* **11**, 76–89 (1995).
- Andreasen, J. O., Borum, M. K., Jacobsen, H. L. & Andreasen, F. M. Replantation of 400 avulsed permanent incisors. 2. Factors related to pulpal healing. *Endod. Dent. Traumatol.* **11**, 59–68 (1995).
- Souza, B. D. M. *et al.* Incidence of root resorption after the replantation of avulsed teeth: A meta-analysis. *J. Endod.* **44**, 1216–1227 (2018).
- Haas, M., Kenny, D. J., Casas, M. J. & Barrett, E. J. Characterization of root surface periodontal ligament following avulsion, severe intrusion or extraction: Preliminary observations. *Dent. Traumatol.* **24**, 404–409 (2008).
- Yamada, H., Maeda, T., Hanada, K. & Takano, Y. Reinnervation in the canine periodontal ligament of replanted teeth using an antibody to protein gene product 9.5: An immunohistochemical study. *Dent. Traumatol.* **15**, 221–234 (1999).
- Andreasen, J. O. A time-related study of periodontal healing and root resorption activity after replantation of mature permanent incisors in monkeys. *Swed. Dent. J.* **4**, 101–110 (1980).
- Nasjleti, C. E., Caffesse, R. G., Castelli, W. A. & Hoke, J. A. Healing after tooth reimplantation in monkeys. A radioautographic study. *Oral Surg. Oral Med. Oral Pathol.* **39**, 361–375 (1975).
- Breivik, M. & Kvam, E. Histometric study of root resorption on human premolars following experimental replantation. *Scand. J. Dent. Res.* **95**, 273–280 (1987).
- Abbott, P. V. Prevention and management of external inflammatory resorption following trauma to teeth. *Aust. Dent. J.* **61**(Suppl 1), 82–94 (2016).
- Ensminger, D. & Bond, L. J. *Ultrasonics: Fundamentals, Technologies, and Applications* (CRC Press, 2011).
- Lin, G., Reed-Maldonado, A. B., Lin, M., Xin, Z. & Lue, T. F. Effects and mechanisms of low-intensity pulsed ultrasound for chronic prostatitis and chronic pelvic pain syndrome. *Int. J. Mol. Sci.* **17**, 1057 (2016).

14. Schandelmaier, S. *et al.* Low intensity pulsed ultrasound for bone healing: Systematic review of randomized controlled trials. *BMJ* **356**, j656 (2017).
15. Rutten, S., van den Bekerom, M. P., Sierevelt, I. N. & Nolte, P. A. Enhancement of bone-healing by low-intensity pulsed ultrasound: A systematic review. *JBJS Rev* **4**, 01874474-201603000-00006. <https://doi.org/10.2106/JBJS.RVW.O.00027> (2016).
16. Rubin, C., Bolander, M., Ryaby, J. P. & Hadjiargyrou, M. The use of low-intensity ultrasound to accelerate the healing of fractures. *J. Bone Joint Surg. Am.* **83**, 259 (2001).
17. Khanna, A., Nelmes, R. T., Gougoulias, N., Maffulli, N. & Gray, J. The effects of LIPUS on soft-tissue healing: A review of literature. *Br. Med. Bull.* **89**, 169–182 (2009).
18. Rego, E. B. *et al.* Ultrasound stimulation attenuates root resorption of rat replanted molars and impairs tumor necrosis factor- α signaling in vitro. *J. Periodontol. Res.* **46**, 648–654 (2011).
19. Takebe, H., Nakanishi, Y., Hirose, Y. & Ochi, M. Effect of low intensity pulsed ultrasound stimulation on sinus augmentation in rabbits. *Clin. Oral Implants Res.* **25**, 735–741 (2014).
20. Kang, K. L., Kim, E. C., Park, J. B., Heo, J. S. & Choi, Y. High-frequency, low-intensity pulsed ultrasound enhances alveolar bone healing of extraction sockets in rats: A pilot study. *Ultrasound Med. Biol.* **42**, 493–502 (2016).
21. Hidaka, K. *et al.* Low-intensity pulsed ultrasound prevents development of bisphosphonate-related osteonecrosis of the jaw-like pathophysiology in a rat model. *Ultrasound Med. Biol.* **45**, 1721–1732 (2019).
22. Wang, Y. *et al.* Low-intensity pulsed ultrasound promotes periodontal ligament stem cell migration through TWIST1-mediated SDF-1 expression. *Int. J. Mol. Med.* **42**, 322–330 (2018).
23. Shirakata, Y. *et al.* Periodontal tissue regeneration after low-intensity pulsed ultrasound stimulation with or without intra-marrow perforation in two-wall intra-bony defects—A pilot study in dogs. *J. Clin. Periodontol.* **47**, 54–63 (2020).
24. Dahhas, F. Y., El-Bialy, T., Afify, A. R. & Hassan, A. H. Effects of low-intensity pulsed ultrasound on orthodontic tooth movement and orthodontically induced inflammatory root resorption in ovariectomized osteoporotic rats. *Ultrasound Med. Biol.* **42**, 808–814 (2016).
25. Raza, H., Major, P., Dederich, D. & El-Bialy, T. Effect of low-intensity pulsed ultrasound on orthodontically induced root resorption caused by torque: A prospective, double-blind, controlled clinical trial. *Angle Orthod.* **86**, 550–557 (2016).
26. Alshihah, N., Alhadlaq, A., El-Bialy, T., Aldahmash, A. & Bello, I. O. The effect of low intensity pulsed ultrasound on dentoalveolar structures during orthodontic force application in diabetic ex-vivo model. *Arch. Oral Biol.* **119**, 104883 (2020).
27. Al-Daghreer, S. *et al.* Effect of low-intensity pulsed ultrasound on orthodontically induced root resorption in beagle dogs. *Ultrasound Med. Biol.* **40**, 1187–1196 (2014).
28. Liu, Z., Xu, J., E, L. & Wang, D. Ultrasound enhances the healing of orthodontically induced root resorption in rats. *Angle Orthod.* **82**, 48–55 (2012).
29. Cignoni, P. *et al.* Meshlab: An open-source mesh processing tool. *Eurogr. Ital. Chapter Conf.* **2008**, 129–136 (2008).
30. Fedorov, A. *et al.* 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn. Reson. Imaging* **30**, 1323–1341 (2012).
31. Andreasen, J. O. & Andersson, L. Critical considerations when planning experimental in vivo studies in dental traumatology. *Dent. Traumatol.* **27**, 275–280 (2011).
32. Andreasen, J. O. Experimental dental traumatology: Development of a model for external root resorption. *Endod. Dent. Traumatol.* **3**, 269–287 (1987).
33. Andersson, L. *et al.* Evaluation of statistics and desirable experimental design of a histomorphometrical method for studies of root resorption. *Endod. Dent. Traumatol.* **3**, 288–295 (1987).
34. Sasai, H., Iwai, H., Fujita, D., Seto, E. & Izumi, Y. The use of micro-computed tomography in the diagnosis of dental and oral disease in rabbits. *BMC Vet. Res.* **10**, 209 (2014).
35. Wang, Y., Qiu, Y., Li, J., Zhao, C. & Song, J. Low-intensity pulsed ultrasound promotes alveolar bone regeneration in a periodontal injury model. *Ultrasonics* **90**, 166–172 (2018).
36. Nevins, M. L., Camelo, M., Rebaudi, A., Lynch, S. E. & Nevins, M. Three-dimensional micro-computed tomographic evaluation of periodontal regeneration: A human report of intrabony defects treated with Bio-Oss collagen. *Int. J. Periodont. Restorative Dent.* **25**, 365–373 (2005).
37. de Paula Reis, M. V. *et al.* Histologic and micro-computed tomographic analyses of replanted teeth stored in different kind of media. *J. Endod.* **40**, 665–669 (2014).
38. Fouad, A. F. *et al.* International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Dent. Traumatol.* **36**, 331–342 (2020).
39. Barbizam, J. V. *et al.* Histopathological evaluation of the effects of variable extraoral dry times and enamel matrix proteins (enamel matrix derivatives) application on replanted dogs' teeth. *Dent. Traumatol.* **31**, 29–34 (2015).
40. Andreasen, J. O. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. *Int. J. Oral Surg.* **10**, 43–53 (1981).
41. Andreasen, J. O., Borum, M. K. & Andreasen, F. M. Replantation of 400 avulsed permanent incisors. 3. Factors related to root growth. *Endod. Dent. Traumatol.* **11**, 69–75 (1995).
42. Maslamani, M., Almusawi, A., Joseph, B., Gabato, S. & Andersson, L. An experimental model for studies on delayed tooth replantation and ankylosis in rabbits. *Dent. Traumatol.* **32**, 443–449 (2016).
43. Andreasen, J. O. Relationship between surface and inflammatory root resorption and pathological changes in the pulp after replantation of mature permanent incisors in monkeys. *J. Endod.* **7**, 294–301 (1981).
44. Kiyokawa, T. *et al.* A preliminary study of effects of low-intensity pulsed ultrasound (LIPUS) irradiation on dentoalveolar ankylosis. *J. Oral Sci.* **59**, 447–451 (2017).
45. Mine, K., Kanno, Z., Muramoto, T. & Soma, K. Occlusal forces promote periodontal healing of transplanted teeth and prevent dentoalveolar ankylosis: An experimental study in rats. *Angle Orthod.* **75**, 637–644 (2005).
46. Panzarini, S. R. *et al.* Histological and immunohistochemical analyses of the chronology of healing process after immediate tooth replantation in incisor rat teeth. *Dent. Traumatol.* **29**, 15–22 (2013).
47. Sigalas, E., Regan, J. D., Kramer, P. R., Witherspoon, D. E. & Opperman, L. A. Survival of human periodontal ligament cells in media proposed for transport of avulsed teeth. *Dent. Traumatol.* **20**, 21–28 (2004).
48. Ehnevid, H., Lindskog, S., Jansson, L. & Blomlof, L. Tissue formation on cementum surfaces in vivo. *Swed. Dent. J.* **17**, 1–8 (1993).
49. Andreasen, J. O. & Hjorting-Hansen, E. Replantation of teeth. II. Histological study of 22 replanted anterior teeth in humans. *Acta Odontol. Scand.* **24**, 287–306 (1966).
50. Andreasen, J. O. & Andreasen, F. M. Root resorption following traumatic dental injuries. *Proc. Finn. Dent. Soc.* **88**(Suppl 1), 95–114 (1992).
51. El-Bialy, T., El-Shamy, I. & Graber, T. M. Repair of orthodontically induced root resorption by ultrasound in humans. *Am. J. Orthod. Dentofacial Orthop.* **126**, 186–193 (2004).
52. Inubushi, T. *et al.* Effects of ultrasound on the proliferation and differentiation of cementoblast lineage cells. *J. Periodontol.* **79**, 1984–1990 (2008).
53. Dalla-Bona, D. A. *et al.* Effects of ultrasound on cementoblast metabolism in vitro. *Ultrasound Med. Biol.* **32**, 943–948 (2006).
54. Dyson, M., Pond, J. B., Joseph, J. & Warwick, R. The stimulation of tissue regeneration by means of ultrasound. *Clin. Sci.* **35**, 273–285 (1968).

55. ter Haar, G. Therapeutic applications of ultrasound. *Prog. Biophys. Mol. Biol.* **93**, 111–129 (2007).
56. Mukai, S. *et al.* Transforming growth factor-beta1 mediates the effects of low-intensity pulsed ultrasound in chondrocytes. *Ultrasound Med. Biol.* **31**, 1713–1721 (2005).
57. Rego, E. B. *et al.* Ultrasound stimulation induces PGE2 synthesis promoting cementoblastic differentiation through EP2/EP4 receptor pathway. *Ultrasound Med. Biol.* **36**, 907–915 (2010).

Author contributions

Conceptualization, D.K. and H.K.; methodology, D.K. and Y.S.; formal analysis, S.S. and D.K.; investigation, S.S., D.K., D.L., and Y.S.; writing—original draft preparation, S.S. and D.K.; writing—review and editing, H.K. and Y.S.; visualization, S.S. and D.K.; supervision, H.K. and Y.S.; project administration, D.K. and Y.S.; funding acquisition, H.K. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Bio & Medical Technology Development Program (NRF-2017M3A9E4047243) funded by the Ministry of Science, ICT and Future Planning, Republic of Korea.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to H.-R.K. or Y.S.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021