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Chinese Journal of Dental Research

Editorial

It has been 25 years since the first issue of the Chinese Journal of Dental Research was published in 1998. Over the past decades, the journal has developed continuously under the great support and cooperation from the authors, reviewers, editors and readers. We have also received long-term, kind support from Quintessence Publishing. We thank all our excellent collaborators and colleagues for their great contributions to the development of the journal.

On 23 February 2023, the Chinese Journal of Dental Research held its new editorial committee meeting to enrol many new members after around a year of preparation. They are international scholars with a great academic reputation or young national scholars with outstanding achievements. The new list of members appears in this issue. We hope their membership will help the journal to develop further, and we thank them in advance for their support and contribution.

In this issue, I would like to recommend several articles to our readers. The review entitled “Interactions between orthodontic treatment and gingival tissue” by Dr Yuan Liu et al at Xinjiang Medical University, School and Hospital of Stomatology, China, summarises the current evidence and solid knowledge of periodontal soft tissue problems in orthodontic treatment and prevention strategies, which I think will prove interesting to all clinicians concerned. The original research article “Hydrogen sulphide alleviates senescence of human periodontal ligament stem cells by TRPV4 channel mediated calcium flux” by Dr Yi Kun Zhou et al at Peking University School and Hospital of Stomatology, China, seeks to explore whether hydrogen sulphide can protect human periodontal ligament stem cells from senescence and the possible underlying mechanisms. It is a more basic investigation in oral biology that may attract researchers’ attention.

The articles “Evaluation of extension type of canalis sinuosus in the maxillary anterior region: a CBCT study” by Dr Leila Khojastepour et al at the School of Dentistry, Shiraz University of Medical Sciences, Iran and “Effect of different obturation techniques on sealer penetration in teeth with artificial internal root resorption: a confocal laser microscope analysis” by Dr Zeliha Uğur Aydin et al at the Faculty of Dentistry, Bolu Abant Izzet Baysal University, Turkey present offerings from other countries.

A system analysis from Prof Wei Ran Li’s team at Peking University School and Hospital of Stomatology, China, entitled “Effect of premolar extraction on the upper airway in adult and adolescent orthodontic patients: a meta-analysis” also features in this issue, and analyses the effects of premolar extraction on the upper airway in adult and adolescent orthodontic patients using CBCT. The case report entitled “Management of crown-root fracture with 180-degree rotation replantation: a report of two cases” contributed by Prof Bin Peng’s team at the School and Hospital of Stomatology, Wuhan University, China, shares an available treatment method and successful experiences in managing intentional replantation for teeth with complicated crown-root fracture.

Whether for basic research purposes or clinical dental practitioners, these articles provide much inspiration and valuable experience. We hope our readers will find them interesting and helpful.

Finally, I wish you all a happy, healthy and prosperous 2023 and hope all your wishes come true.

Prof Chuan-bin Guo
Editor-in-Chief, Chinese Journal of Dental Research
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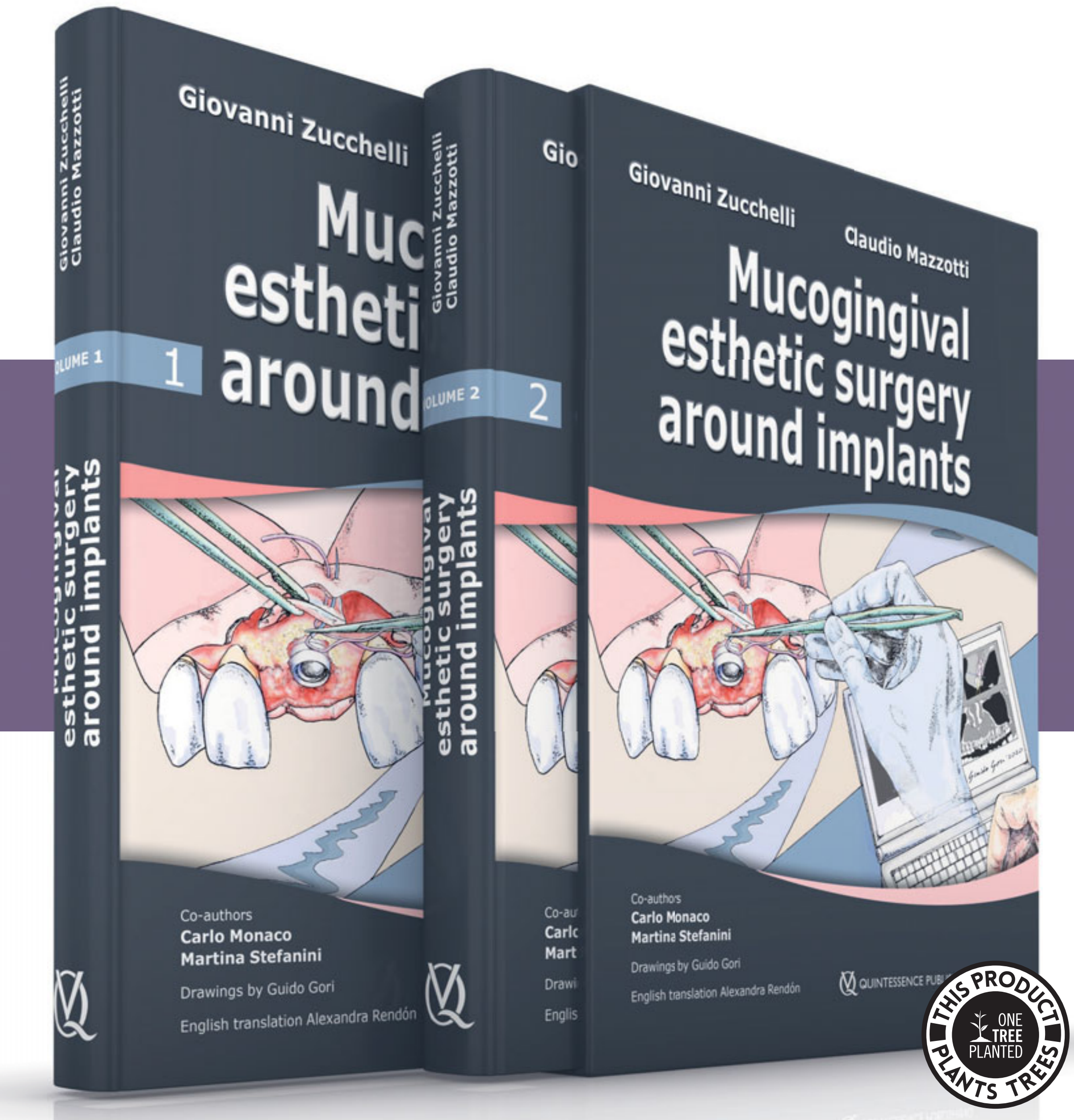

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Interactions between Orthodontic Treatment and Gingival Tissue

Yuan LIU[1#], Chen Xi LI[2,3#], Juan NIE[4], Cong Bo MI[5], Yi Ming LI[6]

In recent years, as the number of adults seeking orthodontic treatment has increased, so too has the number of periodontal tissue problems, particularly regarding the impact on periodontal tissue of receiving orthodontic treatment. Orthodontic treatment improves the occlusion and appearance of teeth by moving the teeth appropriately. These movements have a significant impact on the interactions between the teeth and periodontal tissues. Orthodontic treatment can also recover tooth alignment for patients with tooth displacement caused by periodontitis; however, orthodontic treatment also often has adverse effects on periodontal soft tissue, such as gingivitis, gingival enlargement and gingival recession. The purpose of this review is to summarise the current evidence and solid knowledge of periodontal soft tissue problems in orthodontic treatment and outline some prevention strategies.

Key words: *orthodontic treatment, periodontal tissue health, principle of treatment*
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Orthodontic treatment can help patients with malocclusion to obtain regular tooth alignment, normal oral physiological function, a coordinated face profile, healthy dentomaxillofacial development, and even improved quality of life[1,2]. The arrangement of teeth achieved is also conducive to oral hygiene and reduces the risk of caries lesions, gingivitis and periodontitis. Moreover, normal occlusion can improve the function of the stomatognathic system, increase masticatory efficiency and swallowing ability, improve pronunciation and prevent further trauma of the temporomandibular joint (TMJ)[3,4]. Since orthodontic treatment can change the facial features and appearance directly, evidence indicates that it could enhance patients' confidence and reduce psychological disorders[5,6]. Furthermore, if performed at a specific time, orthodontic treatment can promote the development of soft and hard dentomaxillofacial tissues[7,8]; however, in addition to the therapeutic benefits of orthodontic treatment, the risks it can pose to the dental pulp, TMJ, occlusion and periodontal tissue must not be overlooked, The principle of orthodontic movement is to move the teeth by applying directional external force followed by altering the periodontal tissues, which results in two requirements[9,10]: firstly, the orthodontic process should not result in destruction of an excessive amount of periodontal tissue or any irreversible damage, and secondly, the periodontal tissues should remain

Fig 1 Gingivitis in the anterior region during orthodontic treatment.

stable after the teeth have been moved into their new position. Orthodontic movements are achieved through the reconstruction of periodontal tissue[11]; however, the conditions of the jaws and alveolar bone, the tissue structure characteristics and the response to external stimuli remain variable, and changes in the periodontal soft and hard tissues are not detected or corrected in a timely manner, thus continuing the problem[12]. Periodontal soft tissue abnormalities during orthodontic treatment mainly include gingivitis, gingival enlargement, gingival recession and gingival invagination[13,14]. These problems may affect patients' oral hygiene, cause tooth sensitivity, impact gum aesthetics and even affect the results and success of orthodontic treatment[15,16]. This article reviews the causes, prevention and treatment of periodontal soft tissue abnormalities during orthodontic treatment, aiming to draw attention and consideration to the colleagues.

Orthodontic treatment and gingivitis

The main cause of periodontal inflammation in orthodontic patients is chronic gingivitis (Fig 1), which is caused by the accumulation of dental plaque near the gingival margin. Wearing orthodontic devices affects patients' self-cleaning and increases the difficulty of local plaque control[17], which affects patients' personal oral hygiene maintenance and oral flora composition[18]. A previous study found that the detection rate of *Actinobacillus actinomycetemcomitans* in children with orthodontic devices is 85%, which is significantly higher than that in children without such devices (15%)[19]. It was reported that after 3 months, the detection rate of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* increased significantly, with the Plaque Index (PI), Gingival Index (GI) and bleeding on probing (BOP) being significantly higher in individuals having received orthodontic treatment[20]. A systematic evaluation conducted by Verrusio et al[21] noted an increase in periodontal parameters after orthodontic treatment, indicating that it influenced the accumulation and composition of the subgingival microbiota and subsequently induced more inflammation and higher BOP; however, orthodontic treatment is not the direct cause of gingivitis, and good self-administered plaque control can reduce the probability of gingivitis during orthodontic treatment significantly. Thus, oral hygiene should be administered before orthodontic treatment and repeated during every visit. In addition, regular tooth brushing is the primary way of controlling dental plaque.

Prevention and treatment of gingivitis

The most effective measure for preventing gingivitis is to offer chairside oral health education and manage existing periodontal disease before orthodontic treatment[22]. At present, it is generally believed that it is not sufficient for patients with periodontal disease to only carry out self-administered plaque control during the maintenance period. Instead, professional mechanical plaque control should be performed regularly during the orthodontic process[23], and interventions should be carried out in areas of the dentition that are often neglected and difficult to clean. In addition, the design and manufacture of orthodontic devices should follow the principles of facilitating oral plaque control and avoiding gingival irritation[24].

If orthodontic patients have gingivitis, they should be transferred immediately to the periodontal department for systematic periodontal examination and treatment. The treatment should remove plaque and calculus thoroughly and eliminate factors that may cause local plaque retention and gingival irritation[25]. For patients with severe gingivitis, local medication can be used appropriately. During this period, orthodontic force should be stopped until the condition of the gingiva has been recovered entirely.

For orthodontic patients with excessive plaque deposition and severe gingival inflammation, it is necessary to remove the orthodontic device completely for periodontal treatment until the inflammation is under control[26]. This process may lead to a relapse of the orthodontic effect and longer treatment time. Thus, some patients and orthodontists are reluctant to remove the orthodontic device for periodontal treatment. Sometimes, airborne-particle abrasion under the guidance of a stain can remove plaque more effectively, improve the efficiency of inflammation control, and reduce the removal rate of fixed orthodontic devices to a certain extent[27].

Orthodontic treatment and gingival hypertrophy

Gingival enlargement is another problem that is common in orthodontic patients (Fig 2), especially adolescents[28]. In adolescents with poor oral hygiene, local stimulation of orthodontic devices and changes in sexual hormone levels often lead to inflammatory gingival swelling, accompanied by compensatory proliferation of cells and collagen fibres. The manifestations are globular or nodular enlargement of the gingival papilla, pink colour of the gingival margin, thickening and difficulty in bleeding on probing. Histologically, the basal layer and mesothelium showed excessive growth and a lack of chronic inflammatory exudation. In severe cases, gingival inflammatory hyperplasia may cover the tooth surface or orthodontic appliances, which can affect the progress of orthodontic treatment[29].

Analysis of related elements between orthodontic treatment and gingival hypertrophy

Gingival hypertrophy usually occurs 1 to 2 months after orthodontic treatment[30]. Many factors can aggravate gingival inflammation and cause gingival fibrosis and hypertrophy, such as reduced plaque control, chemical and physical stimulation of adhesives, mechanical band stimulation and food impaction.

Although plaque is often believed to be the leading cause of gingival inflammation and hypertrophy, it has been reported that gingival hypertrophy also occurs in patients with good oral hygiene, suggesting that orthodontic force and periodontal remodelling may also be associated with gingival hypertrophy[31]. For example, Surlin et al[32] found that out of 22 fixed orthodontic patients, 15 developed gingival hypertrophy. The level of matrix metalloproteinase (MMP)-8 in these patients was significantly higher than that in the standard orthodontic treatment group (no periodontal lesions in the latter group)[32]. The authors also found a positive correlation between the degree of gingival hypertrophy and the expression of MMP-9/IV collagen in gingival tissue in orthodontic patients without inflammation[29]. Based on these results, they believe that the increase in MMP level caused by orthodontic force may be one of the causes of gingival hypertrophy[29,32], but whether pure orthodontic power is a direct factor in gingival hypertrophy still needs to be explored further.

In addition, it was shown that a continuous low concentration of nickel ion stimulation in some orthodontic devices is an essential cause of gingival hypertrophy in orthodontic treatment[33]. Nickel ions may stimulate the growth of epithelial cells and the proliferation of keratinocytes by inducing T-lymphocytes to produce interferon and interleukin (IL)-2, IL-5, and IL-10, which may lead to gingival hypertrophy. Nickel ion release may be a time-dependent type IV allergic reaction[33]. Thus, it is necessary to know whether the patient has a history of nickel allergy to avoid the occurrence of gingival hypertrophy during orthodontic treatment.

Fig 2 Gingival hypertrophy in the site of the mandibular canines during orthodontic treatment.

Prevention and treatment of gingival hypertrophy

To prevent gingival hypertrophy, besides reasonable plaque control, careful diagnosis and regular periodontal maintenance during orthodontic treatment are also needed[22]. Prior to orthodontic treatment, detailed inquiries about patients' allergies, chairside oral hygiene guidance, rational selection of orthodontic instruments and careful removal of bonding materials can effectively avoid gingival hypertrophy. Orthodontic patients with gingival hypertrophy should undergo standard periodontal treatment[10]. In some patients, the problem can be eliminated partially or entirely. If the gingival hypertrophy has not completely subsided, gingivoplasty is required to restore the gingival shape to normal. Some patients who have gingivoplasty with periodontitis will require periodontal flap surgery[26].

Traditional scalpels, electric knives or lasers can remove hypertrophic gingival tissue. Traditional plastic surgery is the classical option, but bleeding is obvious with this approach, and a periodontal plug is needed to protect the wound[34]. Patients' pain and discomfort are obvious after the operation. Use of an electrotome is simple and can stop bleeding at the same time, but it may cause thermal damage to adjacent tissues,

Fig 3 Gingival recession in the site of the mandibular canines and right first premolar during orthodontic treatment.

leading to delayed wound healing. Use of lasers has many advantages, such as analgesia, rapid haemostasis, antibacterial and anti-inflammatory effects, and promotion of tissue healing, which greatly improves patient comfort during and after the operation. A previous study used Nd:YAG and CO_2 lasers to remove hypertrophic gingiva in orthodontic patients, and the length of the clinical crown and depth of the gingival sulcus improved significantly afterwards[34]. There was no pain, and the operative site healed quickly[35]. Thus, the use of oral soft-tissue laser gingivectomy and gingivoplasty is a possible choice.

Orthodontic treatment and gingival recession

Gingival recession refers to root exposure caused by the movement of the gingival margin to the root of the enamel–cementum boundary. Gingival recession may lead to poor aesthetic effects, increased root sensitivity and susceptibility to caries lesions[10,36]. In addition to anatomical factors, gingival recession is related to age, periodontal disease, improper brushing, occlusal trauma and invasion of biological width[37]. Whether orthodontic treatment causes gingival recession remains controversial (Fig 3).

Studies on the factors of gingival recession

An epidemiological investigation showed that the incidence of gingival recession during orthodontic treatment was around 1.3% to 12.0%[38]. The mandibular central incisors are most prone to gingival recession, which is linked to the thinner buccal bone plate[39] and insufficient keratinised gingiva[37]. It is now generally accepted that gingival recession during orthodontic treatment is mainly due to the movement of teeth, which is beyond the physiological range of alveolar processes, such as excessive arch expansion and immoderate inclination of teeth[40]. Orthodontic force causes the root to squeeze the thin bone plate, resulting in rapid absorption and secondary gingival recession[41]; however, other studies have not found such associations between tooth movement and gingival recession[16,18,42]. Ruf et al[43] investigated 392 mandibular incisors of adolescents treated with a Herbst appliance. The appliance resulted in a mean incisor movement of 8.9 degrees (0.5 to 19.5 degrees)[43]. Only 12 patients had gingival recession or aggravation, and there was no correlation between the anterior edge of the mandibular incisor and gingival degeneration[43]. A systematic review of labial movement and gingival recession of mandibular incisors also found no association[44]. These studies suggested that insufficient free gingival thickness, a narrow mandibular-chin junction, poor plaque control and excessive brushing are potential risk factors for gingival recession[41-43]; however, Renkema et al[45] compared the incidence of gingival recession between 100 orthodontic patients after orthodontic treatment and 120 non-orthodontic patients in a retrospective study and found that the incidence of gingival recession in the orthodontic group was always higher than that in the non-orthodontic group, and there was more gingival recession in the orthodontic group. The odds ratio (OR) of gingival recession in the orthodontic group was 4.48, indicating that orthodontic treatment was a risk factor for gingival recession[45]. A systematic review found that orthodontic movement is one of the factors affecting gingival recession, and tooth movement beyond the physiological range of the alveolar process may lead to more gingival recession[46]. However, due to an absence of an optimal evaluation mechanism and a limited research sample size, strong evidence is lacking for both sides and further investigation is required.

Prevention and treatment of gingival recession

Before orthodontic treatment, the width and thickness of the compressed lateral keratinised gingiva should be evaluated and the range of alveolar process and bone plate thickness should be analysed using CBCT, whether there is bone dehiscence or fenestration, and the potential risks should be discussed; this is necessary to prevent orthodontic-related gingival recession[37,47,48]. If the periodontal soft tissue or bone is insufficient, early intervention, such as soft tissue transplantation or incremental bone surgery, is needed to ensure that the teeth being moved always move within the physiological range of the alveolar process[49]. To prevent gingival recession during orthodontic treatment, dental practitioners or orthodontists should avoid exerting force on single teeth and moving teeth back and forth, and recommend use of segmented arch technology. In addition, it is always essential for orthodontic patients to maintain good oral hygiene.

For mild and asymptomatic gingival recession, there is only a need to maintain good local plaque control without surgical periodontal therapy. If gingival recession continues to progress, aetiological treatment is required, such as removing local risk factors, correcting improper brushing or flossing, and adjusting the orthodontic or occlusal force[50]. Once the gingival recession exceeds 2 mm, it is necessary to stop the movement of the teeth to the loading side immediately and to consider shortening the orthodontic treatment time[51]. If necessary, periodontal surgery should be performed after routine scaling and root planing[51].

Periodontal surgery includes mucogingival surgery and guided bone regeneration[52]. Mucogingival surgery involves free gingival transplantation, pedicled gingival flap transplantation and subepithelial connective tissue transplantation. It is used for a single tooth or a few teeth with gingival recession, with a mean root coverage rate of around 65% to 98%[53]. For patients with a thin alveolar bone plate or bone dehiscence, the mean root coverage rate of guided bone regeneration is around 48% to 92%[54]. Systematic reviews suggest that autogenous connective tissue transplantation or enamel matrix derivatives combined with use of a coronally advanced flap increase the possibility of root coverage in Miller type I and II single gingival recession[55]. A meta-analysis also showed that laser-assisted gingival surgery has clinical advantages in terms of improving keratotic tissue width, depth of the probe and attachment level[56]. The complete root coverage rate was 70% to 90%, which was significantly different to that of the non-laser group[56].

At present, type I and II gingival recession can be covered completely as a result of surgical treatment, while type III recession can be partially covered. Patients with type IV gingival recession are advised to undergo prosthetic treatment for coverage.

Orthodontic treatment and gingival invagination

Gingival invagination is a type of gingival cleft that occurs during orthodontic space closure with at least 1 mm depth or a vertical and horizontal probe depth of at least 2 mm in patients undergoing extraction[57]. The incidence of gingival invagination ranges from 30% to 100%[58]. It can affect plaque control, increase the risk

Fig 4 Gingival invagination in the extraction site of the maxillary left first premolar during orthodontic treatment.

of periodontal tissue injury, prolong closure time and disturb the orthodontic effect and stability[57,58]. Thus, sufficient attention should be paid to gingival invagination (Fig 4).

Factors relating to gingival invagination

The relapse factor after closure of the orthodontic space may be the cause of gingival recession[59]. When the extraction space is closed, the gingival epithelium on the tension side becomes loose and 'red triangular plaque' forms[60]. At the same time, because the teeth do not move along with the gingiva, they squeeze the gingival tissue and alveolar bone on the pressure side, making the gingiva roll in, thus causing gingival invagination. Changes in bone tissue may also be a cause of gingival invagination[60]. The results showed that the immediate closure group had higher bone mineral density, less alveolar bone resorption and lower incidence of gingival invagination than the 12-week closure group. In addition to the interval time of closing the gap, the speed also has an impact on alveolar bone absorption, thus affecting the occurrence of gingival invagination[59,60]. Some studies have found that the severity of gingival invagination increased significantly in patients whose closure speed was less than 6.0 mm per month compared with those for whom it was > 1.2 mm per month[59,60]. The difference in gingival invagination between the maxilla and mandible also indirectly explains the effect of the closure space. A retrospective study showed that the incidence and severity of maxillary gingival invagination was lower than that of the mandible (maxilla 30%, mandible 70%)[58]. The closure speed was faster and bone resorption was reduced because the maxilla was less dense than the mandible[58]. These studies indicate that gingival tissue lacks the ability to rapidly remodel, and changes occur to its morphology along with bone resorption[58-60]. There is no doubt that the more serious the bone resorption, the more obvious the gingival invagination[61].

Prevention and treatment of gingival invagination

Animal experiments and retrospective studies have found that a long interval between orthodontic closure and extraction is a potential risk factor for gingival invagination[58,61]. One of the most effective ways of preventing gingival invagination is to move teeth 2 to 4 weeks after extraction[58]. Reichert et al[62] found that early closure of the gap can only shorten the treatment time and fails to prevent or reduce the incidence and severity of gingival invagination; however, there are fewer samples in relevant studies[58,59,61], so the effect of time factors on gingival invagination requires a further design of randomized controlled trials with a larger sample size for in-depth analysis.

Multiple studies have reported that the application of different bone substitute materials at the tooth extraction site for site preservation can prevent gingival recession effectively[60,62,63]. The results indicated that the incidence of gingival invagination was 83.3% in the group in which the extraction socket underwent natural healing after complete closure of the extraction space, whereas no gingival invagination occurred in the site preservation group[61,62]. In addition, there was no gingival invagination in the guided tissue regeneration (GTR) for the group that received Gore-Tex film (Gore, Newark, DE, USA), whereas gingival invagination occurred at extraction sites in the control group[64]. Moreover, compared with the Gore-Tex group, the control group showed significant horizontal absorption of the alveolar crest, indicating that GTR combined with a barrier membrane could reduce the occurrence of gingival invagination[61]. These results suggest that guaranteeing adequate local bone mass for space maintenance may play a role in preventing gingival invagination[61,63].

Patients with gingival invagination should be treated actively to avoid it impacting their periodontal health and the outcome of orthodontic treatment at a later stage. Simple gingival resection and gingivoplasty, GTR and bone grafting can be selected based on the severity of gingival invagination. In patients with poor oral hygiene maintenance, gingival invagination is limited to the soft tissue, and patients with poor gingival morphology can undergo gingivectomy and gingivoplasty[65]. Guided periodontal tissue regeneration and bone grafting should be performed if gingival invagination penetrates the interdental papilla or accompanies alveolar bone defects[60].

Conclusion

Orthodontic treatment is closely related to gingival tissue changes and the two interact with each other. While paying attention to patients' orthodontic situation, orthodontists should cooperate closely with periodontal doctors to monitor any periodontal changes. Prior to orthodontic treatment, periodontal risk factors should be assessed and prognostic judgements should be made to predict possible risks and provide effective prevention in addition to effective chairside oral health instruction. Periodontal examination and maintenance should be performed regularly during orthodontic treatment, and periodontal problems should be addressed actively. There is still a need to increase patients' awareness of oral hygiene maintenance after treatment to ensure they maintain their periodontal health. Orthodontists should strengthen interdisciplinary cooperation with periodontal practitioners to achieve the best therapeutic effect.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Chen Xi LI drafted the manuscript, conceptualised and designed the study, and reviewed the manuscript; Dr Yuan LIU summarised the literature and drafted the manuscript; Prof Cong Bo MI conceptualised and designed the study, and critically revised the manuscript; Drs Juan NIE and Yi ming LI took charge of bibliography retrieval and collected and integrated the literature material. All authors approved the final manuscript.

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Hydrogen Sulphide Alleviates Senescence of Human Periodontal Ligament Stem Cells by TRPV4 Channel Mediated Calcium Flux

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Objective: *To explore whether hydrogen sulphide (H_2S) could protect human periodontal ligament stem cells (PDLSCs) from senescence and the possible underlying mechanisms.*
Methods: *Cell cycle assay and Ki-67 assay were used to measure proliferation of PDLSCs. Real-time polymerase chain reaction (PCR) was used to measure cellular senescence–related p16 and p21. Calcium influx was detected by measurement of Ca^{2+} imaging. In addition, we analysed the possible mechanisms underlying H_2S acting on PDLSCs by microarray.*
Results: *The cell proliferation rate of aging PDLSCs decreased significantly. The expression of cellular senescence–related p16 and p21 significantly increased in aging PDLSCs. H_2S donor (GYY4137) treatment increased the proliferation rate of senescence PDLSCs. Furthermore, the donor of H_2S treatment effectively prevented cell cycle arrest of PDLSCs during the aging process and inhibited the expression of cellular senescence–related markers. Mechanically, H_2S donor treatment could activate the calcium influx in PDLSCs. Moreover, pretreatment with TRPV4 inhibitors significantly attenuated the calcium influx induced by H_2S donor treatment in PDLSCs. It also alleviated the protective effect of H_2S on the senescence of PDLSCs.*
Conclusion: *H2S alleviated the senescence of human PDLSCs by TRPV4 channel mediated calcium flux. These results provide a potential strategy to deal with cell aging and may facilitate cell therapy for oral diseases.*
Key words: *calcium flux, cell senescence, hydrogen sulphide, periodontal ligament stem cells, transient receptor potential cation channel subfamily V member 4*
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Mesenchymal stem cells (MSCs) are pluripotent non-hematopoietic progenitor cells with self-renewal and multilineage differentiation, which play a key role in tissue regeneration and homeostasis[1]. Periodontal ligament stem cells (PDLSCs), one type of MSCs isolated from periodontal tissues, have self-renewal and multi-directional differentiation potential, which can provide a reliable source of cells for clinical applications such as periodontal regenerative therapy[2]; however, as MSCs increase in number during in vitro culture, their proliferation ability decreases, and they will eventually enter a state of replication stagnation[3]. This replication stagnation, called cell senescence, could be caused by various stressors, which are characterised by cell cycle arrest[4]. To provide references for cell therapy and tissue regeneration, it is urgent to explore new strategies to inhibit or control cell senescence.

Hydrogen sulphide (H_2S) is now considered the “third gasotransmitter” alongside nitric oxide and carbon monoxide. In recent years, it has been reported that H_2S mediates various biological processes through multiple

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signalling pathways, and abnormal H_2S metabolism is related to the dysfunction of MSCs[5]. For example, H_2S epigenetically mitigates bone loss through OPG/RANKL regulation during hyperhomocysteinemia[6]. An H_2S deficiency in bone marrow mesenchymal stem cells (BMMSCs) will weaken osteogenesis and proliferation. Interestingly, the serum and intracellular H_2O_2 levels in cystathionine β-synthase (CBS) deficient mice was decreased, leading to severe osteoporosis phenotypes[7]. As observed in BMMSCs and dental pulp stem cells (DPSCs), our studies have reported the new function of H_2S in these dental stem cells[8]. H_2S is necessary for periodontal tissue homeostasis, and studies have confirmed that cystathionine-γ-lyase (CSE) is essential for mechanical load–induced bone remodelling[9]. The study also showed that H_2S treatment increased the tooth movement rate in vivo by promoting osteogenesis and osteoclast formation in alveolar bone[10]. Perridon et al[11] showed that H_2S has a direct or indirect protective effect on aging characteristics except telomere wear. NaHS protects human umbilical vein endothelial cells from cell aging, possibly by regulating SIRT1 activity and improving the function of aging cells[12,13]. H_2S also induces S-vulcanisation of MEK1, which leads to PARP-1 activation and DNA damage repair and protects cells from aging[14]. In addition, the lack of CSE in mouse embryonic fibroblasts leads to the early development of cell senescence[15]. Whether H_2S might be able to regulate the senescence of PDLSCs is still unclear.

Ca^{2+} signalling is central to driving morphological changes that are the hallmarks of senescence. A senescence-associated increase in cell volume is normally countered by regulatory volume decrease (RVD), which preserves the structural integrity of the cell[16]. Transient receptor potential cation channel V4 (TRPV4) is a member of the transient receptor potential (TRP) superfamily, which is characterised by a weak voltage-dependent nonselective cation channel. This channel has been proven to regulate the homeostasis of intracellular calcium concentration Ca^{2+} and participate in the integrity of osmotic adjustment, endothelial barrier, nociception and bone homeostasis[17-21]. Our previous study reported that the Ca^{2+} influx triggered by H_2S maintained the bone and MSC homeostasis, and TRPV1 played an important role in maintaining MSC capacity[22,23]. Whether H_2S could regulate the calcium influx in PDLSCs and detailed molecular mechanism needs to be elucidated.

Here, we demonstrated that H_2S alleviates senescence of PDLSCs shown as increased cell proliferation and inhibited cell cycle arrest, which was mediated by TRPV4 channel–mediated calcium flux.

Materials and methods

Reagents and cell culture

Cell viability was confirmed by toluidine blue staining (Sigma-Aldrich, St Louis, MO, USA). Chemicals were purchased from Sigma-Aldrich, and TRIzol and RevertAid Reverse Transcriptase were purchased from Thermo Fisher Scientific (Carlsbad, CA, USA). TRPV4 siRNA was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Transfection reagent was purchased from Invitrogen (Waltham, MA, USA). PDLSCs were provided by the Oral Stem Cell Bank (Beijing, China) and isolated as previously reported. PDLSCs were cultured in α-MEM supplemented with 15% foetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. This study was approved by the Animal Care and Use Committee of the Health Science Centre, Peking University (no. 2015-186).

Real-time PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and LightCycler 96 (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instruction. The qPCR primers were designed as follows: p21 Forward:5`-AGGTGGACCTGGAGACTCTCAG-3`,Reverse:5`-TCCTCTTGGAGAAGATCAGCCG-3`;p16Forward:5`-CTCGTGCTGA TGCTACTGAGGA-3`,Reserve:5`-GGTCGGCGCAGTTGGGCTCC-3`; IL-6 Forward:5`-TTCTGCGCAGCTTTAAGGAG-3`,Reverse:5`-AGGTGCCCATGCTACATTTG-3`;IL-8 Forward:5`- ATGACTTCCAAGCTGGCCGTG -3`,Reserve:5`- TGTGTTGGCGCAGTGTGGTC-3`;MCP1 Forward:5`- AGGGAACTTGAGCGTGAATC -3`, Reserve: 5`- TCACTTGTCTGTTGCACACG -3`;GAPDH Forward:5`-AGCCGCATCTTCTTTTGCGTC-3`,Reserve: 5`-TCATATTTGGCAGGTTTTTCT-3`.

Senescence-associated β-galactosidase (SA-β-gal) staining

Senescence-associated β-galactosidase (SA-β-gal) expression was visualised using an SA-β-gal staining kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's protocol. Cells were washed with phosphate buffer solution (PBS) twice and fixed using a fixative solution at room temperature for 15 minutes, and then stained with X-gal solution for 24 hours at 37°C (without CO_2). Cells were observed using a light microscope (Leica, Wetzlar, Germany) with a magnification of ×100, and the percentage of SA-β-gal-positive cells in 10 random fields was calculated.

Western blotting

Western blotting was performed as previously described[22]. The antibodies used included p21, p16 and Actin (Thermo Fisher Scientific).

Senescence-associated heterochromatin foci (SAHF) analysis

To determine that SAHF formation had occurred, cells were cultured directly on glass cover slips and then fixed with 4% paraformaldehyde. After washing with PBS, cells were permeabilised with 0.2% Triton X-100/PBS for 10 minutes. DNA was visualised by 4′-6′-diamidino-2-phenylindole (DAPI) staining (1 mg/ml) for 1 minute and then washed with PBS twice. Cover slips were mounted in a 90% glycerol PBS solution and examined under a laser confocal microscope.

Flow cytometric analysis

To detect the levels of Ki-67, PDLSCs were washed twice and incubated with Ki-67 antibodies at 4°C for 40 minutes. Isotype antibodies served as controls. Cells were analysed by flow cytometry using a BD FACSVia flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Each analysis included 20,000 events.

Cell cycle assay

To determine the effect of H_2S on cell cycle progression, PDLSCs from the log phase were grown in 6-well plates and treated with 50 µM GYY4137 for 12 hours. Cells were collected using trypsinisation and centrifugation for 5 minutes at 300 × g centrifuge force and fixed with 70% ethanol at 4°C overnight. They were stained using a Cell Meter Fluorimetric Fixed Cell Cycle Assay Kit (AAT Bioquest, Pleasanton, CA, USA) following the manufacturer's instructions, then subjected to flow cytometric analyses with FACSCalibur and CellQuest software (both BD Biosciences). Cell cycles were analysed and the proportion of cells in the G0/G1, S and G2/M phases was recorded.

Microarray

Total RNAs were extracted from the control and 50 µM GYY4137 treated PDLSCs with an RNeasy kit (QIAGEN, Hilden, Germany). Microarray assays were performed at the Genome Centre at Children's Hospital Los Angeles using 1.0 ST arrays (Affymetrix, Santa Clara, CA, USA). The gene expression difference was analysed using Partek Genomics Suite (Partek, St Louis, MA, USA) and Ingenuity Pathway Analysis (IPA) software (QIAGEN). Global gene expression profiles rank ordered by relative fold-change values were analysed using Gene Set Enrichment Analysis software (Broad Institute, MIT, Cambridge, MA, USA).

Measurement of Ca^{2+} imaging

PDLSCs (1×10^5) were seeded onto 60-mm culture dishes and cultured for 24 hours at 37 °C in 5% CO_2. Next, PDLSCs were stimulated by GYY4137 with a concentration of 50 µm for 24 hours. Then, the cells were loaded with fura-2 AM (Invitrogen) and incubated for 1 hour at 37°C in 5% CO_2 in the dark. Ca^{2+} levels were measured by the ratio of emission in response to excitation at 340 and 380 nm on an Olympus Optical IX71 microscope (Olympus Life Science, Tokyo, Japan).

Statistical analysis

P values were analysed using a two-tailed Student *t* test for the difference between two groups or one-way analysis of variance to compare the difference from more than two groups using SPSS 18.0 software (IBM, Armonk, NY, USA). $P < 0.05$ was considered significant.

Results

Hydrogen sulphide alleviated senescence of PDLSCs

Firstly, we compared the cell viability of PDLSCs from passage 5 (P5) and passage 15 (P15) and found that when the cells were passaged to P15, the PDLSCs became flat and the number of cells was significantly reduced, which may be because the cells differentiated. GYY4137, a slow-release agent of H_2S, treatment could increase cell density, especially in the PDLSCs in the P15 group (Figs 1a and b). The expression of p21 and p16, which are cyclin-dependent kinase inhibitors and play a vital role in cell senescence, increased significantly when PDLSCs expanded to passage 15, while the addition of H_2S donor could partially decrease the expression of p16 and p21 (Figs 1c, d and g). Besides, the staining results showed that the expression of β-galactosidase in senescent cells was obviously upregulated compared with PDLSCs at P5 (Figs 1e and f). As shown in Fig 1h, senescence also led to pronounced DNA SAHF formation, which was visualised by DAPI staining (Figs 1h and i). Compared with the control, GYY4137 significantly suppressed the senescence-induced SA-β-gal activity and SAHF forma-

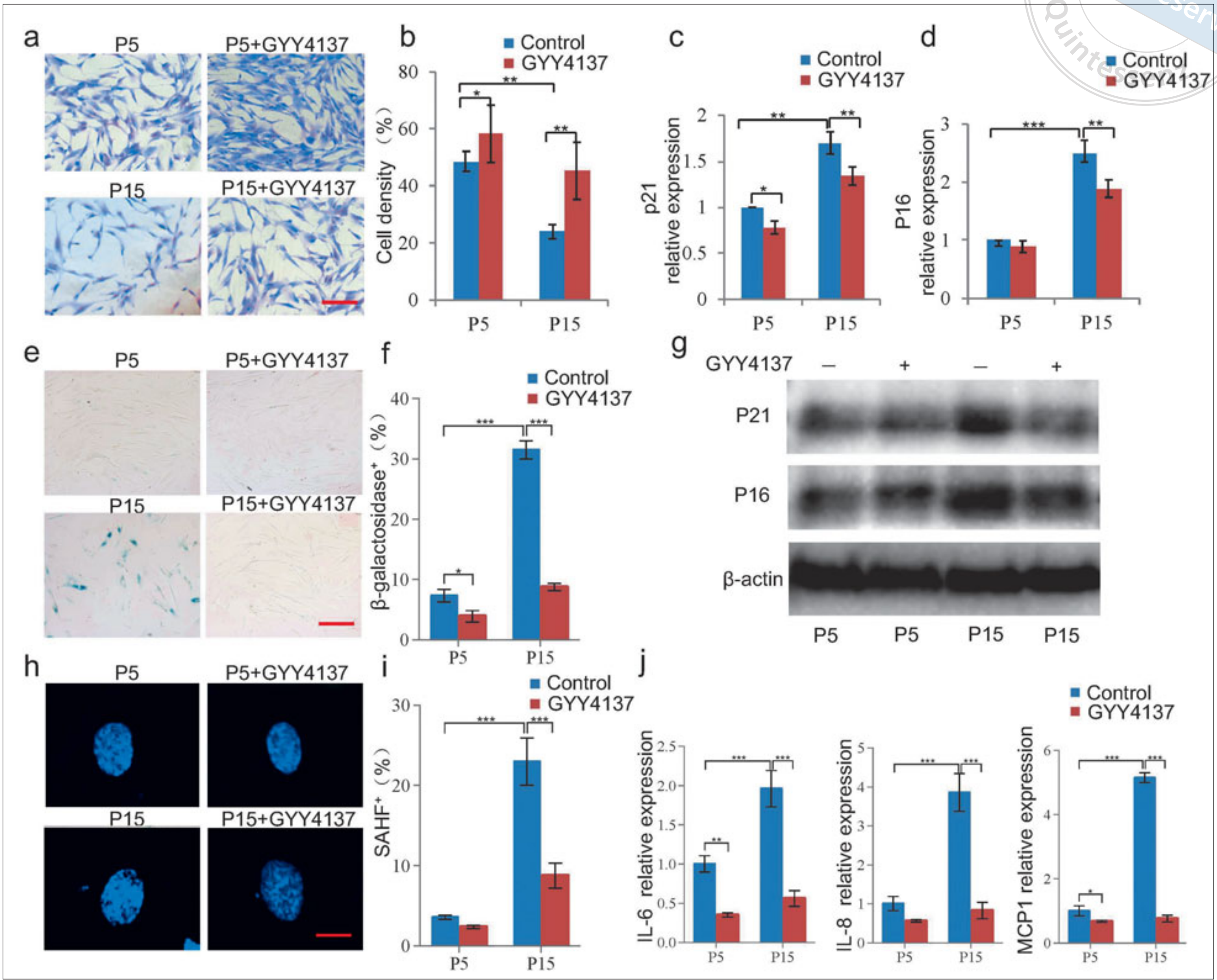

Fig 1 Hydrogen sulphide alleviates senescence of PDLSCs. **(a)** Toluidine blue staining of PDLSCs at P5 and P15 treated with or without 50 µm GYY4137. Scale bar: 50 µm. **(b)** Semi-quantitative analysis of cell density of PDLSCs at P5 and P15 treated with or without 50 µm GYY4137. **(c-d)** Expression of p21 **(c)** and p16 **(d)** in PDLSCs at P5 and P15 treated with or without 50 µm GYY4137, as assessed by qPCR. **(e)** β-galactosidase staining of PDLSCs at P5 and P15 treated with or without 50µM GYY4137. Scale bar: 50 µm. **(f)** Semi-quantitative analysis of β-galactosidase positive of PDLSCs at P5 and P15 treated with or without 50 µm GYY4137. **(g)** Expression of p21 and p16 in PDLSCs at P5 and P15 treated with or without 50 µm GYY4137, as assessed by western blot. **(h)** Cells were stained for DAPI to visualise SAHF formation. Scale bar: 10 µm. **(i)** Semi-quantitative analysis of SAHF positive of PDLSCs at P5 and P15 treated with or without 50 µm GYY4137. **(j)** Expression of SASP (IL-6, IL-8 and MCP1) in PDLSCs at P5 and P15 treated with or without 50 µm GYY4137, as assessed by qPCR. Data are presented as mean ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$), n = 3.

tion in PDLSCs at P15. Senescence associated secretory phenotype (SASP) is a phenomenon whereby senescent cells increase the expression and secretion of certain cytokines, chemokines and other proteins. Notably, the SASP-related gene expression (IL-6, IL-8, MCP1) was significantly higher in the PDLSCs in the P15 group compared to those in the P5 group. Moreover, the expression of SASP-related cytokine genes was also inhibited by the H_2S donor treatment (Fig 1j).

To further characterise the effects of H_2S on the senescence of PDLSCs, we analysed the proliferation of PDLSCs and found that it was markedly decreased when passaged to P15 compared with P5, while GYY4137 treatment could increase the proliferation of PDLSCs at P15, which was assessed by the expression level of Ki67 using flow cytometry analysis (Figs 2a and b). Cell cycle arrest during the G1 phase is a characteristic exhibited by senescent cells. Our results demonstrated that

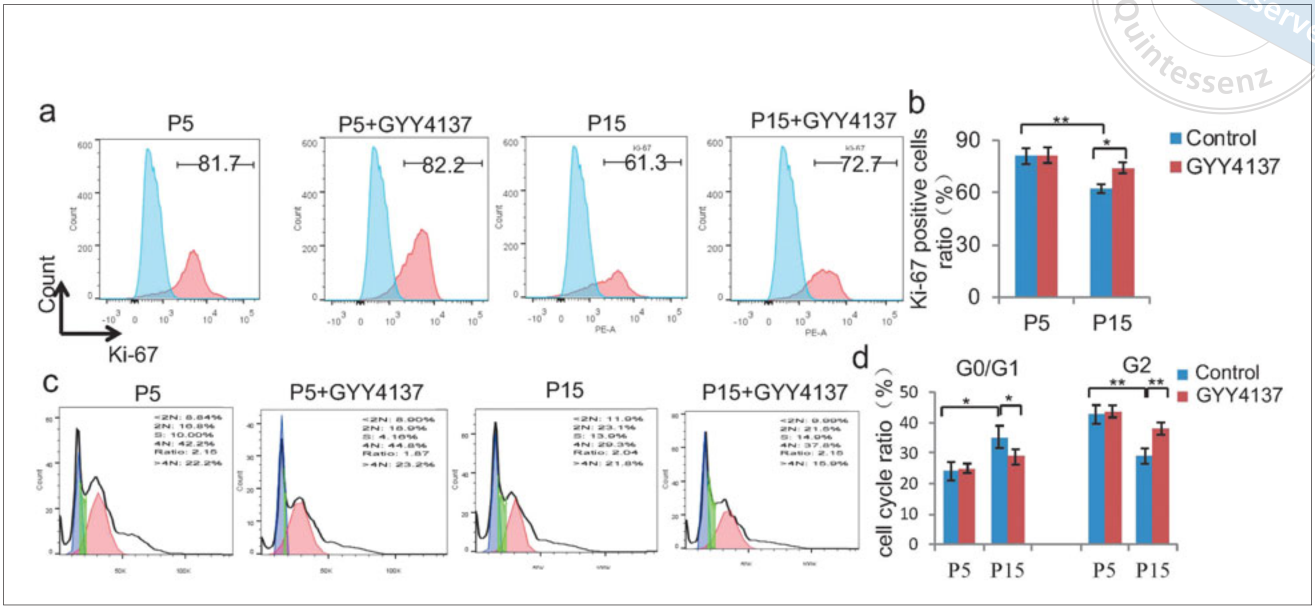

Fig 2 Hydrogen sulphide treatment increased the proliferation of PDLSCs **(a-b).** The proliferation rate of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by flow cytometry. **(c-d)** Cell cycle of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by flow cytometry. Data are presented as mean ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$), n = 3.

PDLSCs at P15 stagnated in the G0/G1 phase as the proportion of cells in the G0/G1 phase increased compared to those at P5. Moreover, the proportion of PDLSCs in the G2 phase decreased at P15 compared with the ones at P5. H_2S treatment reduced the proportion of PDLSCs during the G0/G1 phase and increased the ratio of cells during the G2 phase in the P15 group (Figs 2c and d).

Hydrogen sulphide treatment increased the calcium pathway in PDLSCs

To investigate the stem cell biology regulated by H_2S, we performed a microarray using PDLSCs with or without 50 μm GYY4137 treatment. The heatmap depicting the significant upregulated and downregulated genes (fold change > 2, $P < 0.05$) is shown in Fig 2a. The calcium pathway was one of the top 10 significantly enriched signalling clusters after H_2S treatment, analysed by Kyoto Encyclopaedia of Genes and Genomes (KEGG) (Figs 3a and b). Most of the genes related to the calcium pathway were significantly upregulated after H_2S treatment in PDLSCs (Fig 3c). Furthermore, H_2S donor treatment induced Ca^{2+} influx in PDLSCs (Fig 3d). Sequencing results showed that the calcium-related signalling pathway increased significantly after H_2S stimulation. Next, we analysed the differential genes using Gene Set Enrichment Analysis and the results showed that the TRPV channel was one of the top enriched clusters (Fig 3e).

Hydrogen sulphide activated TRPV4-channel mediated Ca^{2+} influx

Calcium entry could be activated through TRPV4 channels[19,24]. We found that the Ca^{2+} influx induced by H_2S was partially blocked by TRPV4 siRNA treatment (Figs 4a and b). In addition, compared with the control group, treatment with TRPV4 inhibitor GSK2193874A also blocked the calcium influx induced by H_2S (Fig 4c). Moreover, the H_2S donor treatment induced significantly higher Ca^{2+} in PDLSCs from P5 compared with the ones from P15 (Fig 4d). To confirm the role of H_2S treatment, we analysed the expression level of TRPV4. The results showed that TRPV4 mRNA were increased after H_2S treatment (Fig 4e). These results indicated that H_2S activated Ca^{2+} influx via the TRPV4 channel in PDLSCs.

Hydrogen sulphide alleviated senescence of PDLSCs by TRPV4 channel-mediated calcium flux

To further verify the role of TRPV4 mediated by calcium on senescence of PDLSCs, we pretreated PDLSCs with TRPV4 siRNA and found that the effects of GYY4137 against senescence of PDLSCs were attenuated by TRPV4 siRNA treatment. For instance, the cell density and viability of PDLSCs at P15 reversed by H_2S treatment were decreased after TRPV4 siRNA pretreatment (Figs 5a and b). The expression of p16 and p21 in PDLSCs at P15 decreased by H_2S donor treatment was partially

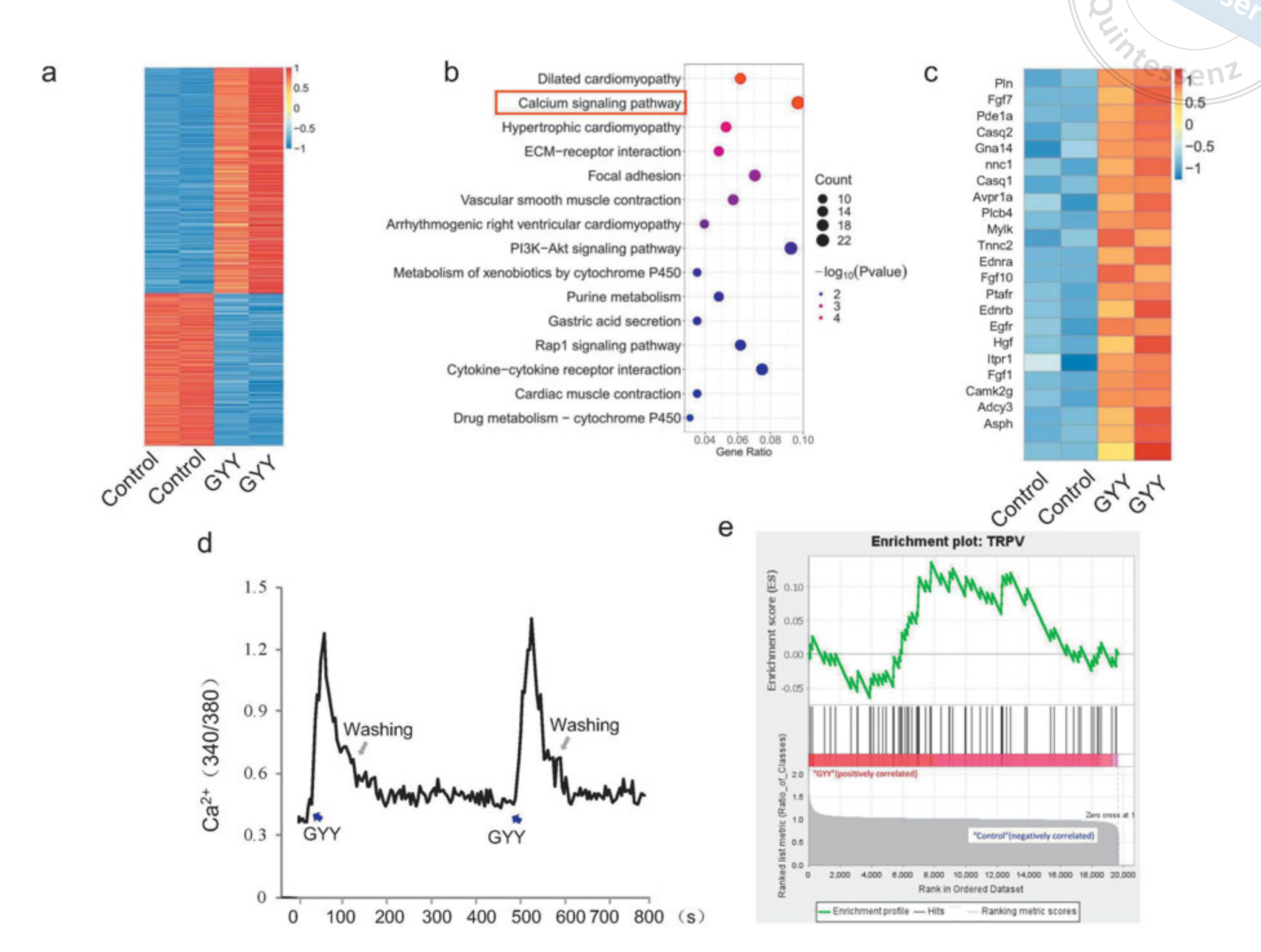

Fig 3 Hydrogen sulphide treatment increased the calcium pathway in PDLSCs. **(a)** Heatmap of genes differentially expressed in control and GYY4137 treated PDLSCs, as analysed by microarray. **(b)** The calcium signalling pathway is one of the top ten enriched clusters between control and GYY4137 treated PDLSCs as assessed by KEGG pathway analysis. **(c)** The different expression genes related with the calcium signalling pathway between control and GYY4137 treated PDLSCs. **(d)** The Ca^{2+} influx induced by GYY4137 treatment in PDLSCs, as assessed by Ca^{2+} levels using the ratio of emission in response to excitation at 340 and 380 nm. **(e)** The TRPV channels were significantly enriched based on the differential genes, as assessed by GSEA.

attenuated after TRPV4 siRNA pretreatment (Figs 5c and d). The proliferation rate of PDLSCs at P15 increased by GYY4137 stimulation was alleviated after TRPV4 siRNA pretreatment (Fig 5e). Furthermore, the cell cycle arrest reversed by GYY4137 was partially attenuated when PDLSCs received TRPV4 siRNA pretreatment (Fig 5f). These results demonstrated that inhibition of TRPV4 attenuated the protective effect of H_2S against senescence in PDLSCs.

Discussion

Cell senescence is a process whereby cells irreversibly withdraw from the cell cycle and stop dividing in response to various stresses[25]. In this study, we obtained senescent cells through continuous passaging of stem cells and found that when PDLSCs were passaged to P15, the cell viability and proliferation were decreased significantly compared with PDLSCs at P5. The ratio of the β-galactosidase positive cells increased significantly when passaged to P15. In addition, the senescence-related markers SAHF and SASP also increased remarkably at P15 compared to P5, and the PDLSCs at P15 showed cell cycle arrest with the higher expression of cyclin-dependent kinase inhibitor p16 and p21. All these results demonstrated that long-term in vitro culturing and expansion caused cell senescence in PDLSCs.

Our previous study showed that H_2S could promote

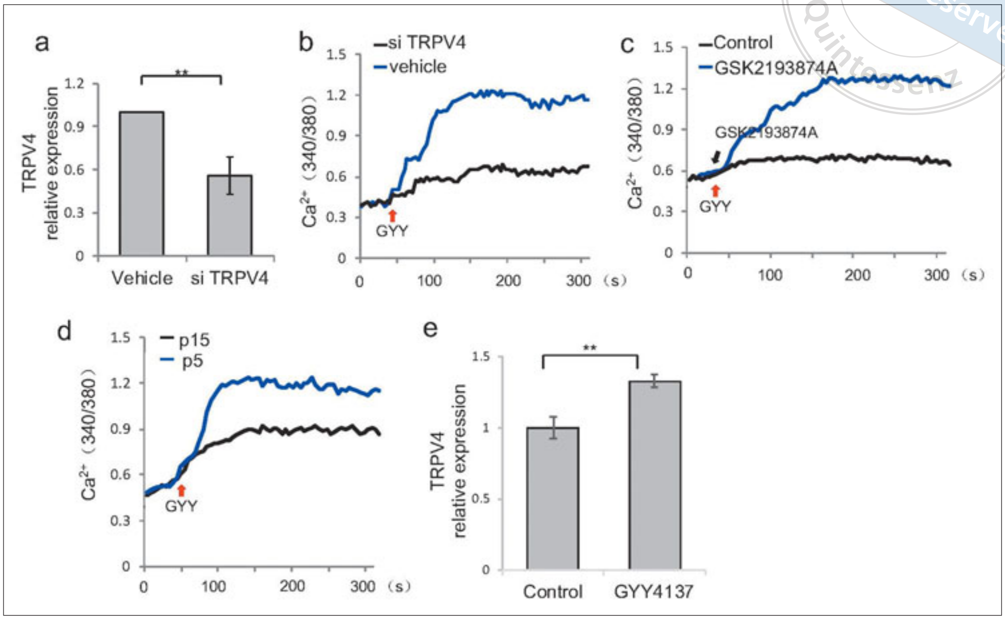

Fig 4 Hydrogen sulphide activated TRPV4 channel–mediated Ca^{2+} influx. **(a)** Efficacy of TRPV4 siRNA, as assessed by qPCR. **(b)** Ca^{2+} influx induced by NaHS treatment in PDLSCs with or without TRPV4 siRNA treatment. **(c)** The Ca^{2+} influx induced by NaHS treatment in PDLSCs was inhibited with TRPV4 inhibitor GSK2193874A treatment. **(d)** Ca^{2+} influx induced by NaHS treatment in PDLSCs of P5 and P15. **(e)** Expression of TRPV4 in control and GYY4137 treated PDLSCs, as analysed by qPCR. Data are presented as mean ± SD (***P* < 0.01).

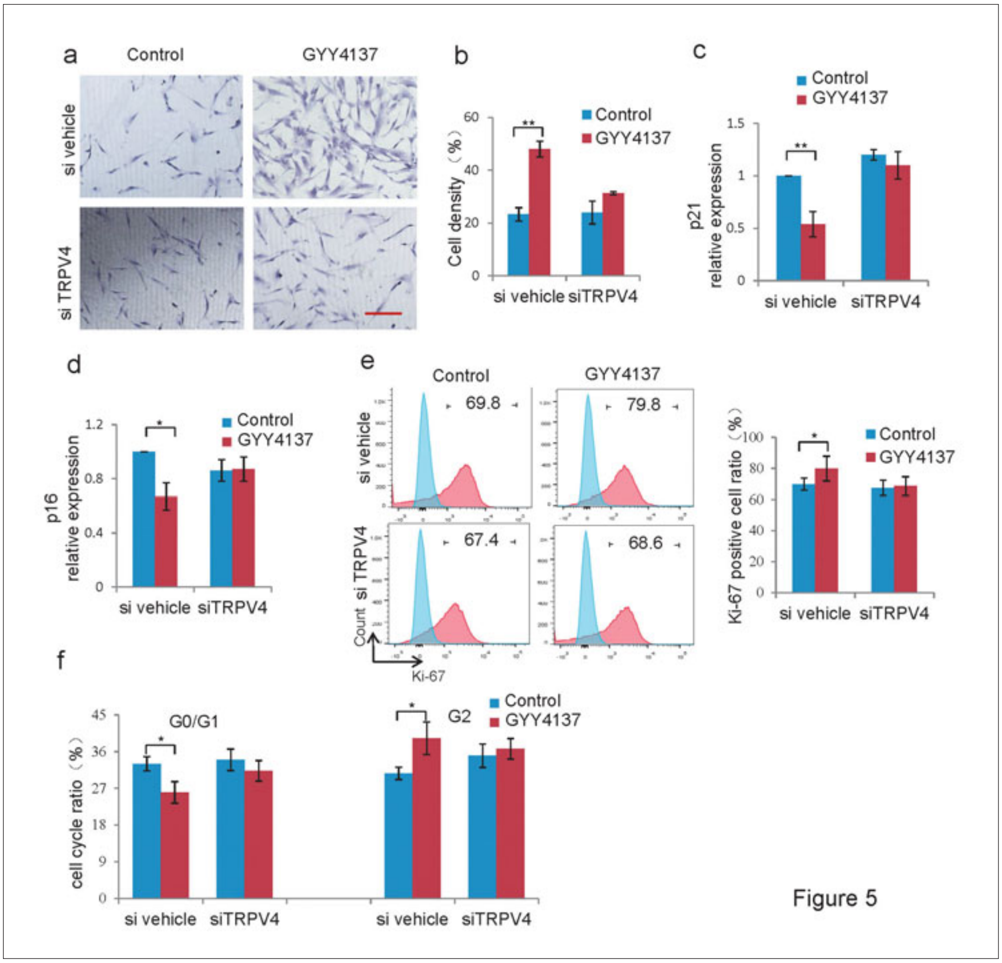

Fig 5 The capacity of H_2S to alleviate senescence of PDLSCs was inhibited by TRPV4 siRNA treatment. **(a)** Toluidine blue staining of control and TRPV4 siRNA treated PDLSCs with or without 50 µm GYY4137 stimulation. **(b)** Semi-quantitative analysis of cell density of control and TRPV4 siRNA treated PDLSCs with or without 50 µm GYY4137 stimulation. **(c-d)** Expression of p21 **(c)** and p16 **(d)** in control and TRPV4 siRNA treated PDLSCs with or without 50µM GYY4137 stimulation, as assessed by qPCR. **(e)** Proliferation rate of control and TRPV4 siRNA treated PDLSCs with or without 50 µm GYY4137 stimulation, as assessed by flow cytometry. **(f)** Cell cycle of control and TRPV4 siRNA treated PDLSCs with or without 50 µm GYY4137 stimulation, as assessed by flow cytometry. Data are presented as mean ± SD (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

bone regeneration by increasing osteogenic differentiation of MSCs[22]. Here we showed that H_2S donor GYY4137 treatment could protect PDLSCs from cell senescence with continuous passage. It has been reported that endogenous H_2S signalling maintains the proliferation ability of PDLSCs and neural stem cells[26-28]. The aging of MSCs is a complex process with comprehensive mechanisms. There is an urgent need for a strategy to produce a large number of MSCs that retains stemness and pedigree plasticity. Thus, many alternative methods have been tried to prevent or reverse aging and improve the clinical application of MSCs. Antioxidants such as ascorbic acid, cirsium setidens, lactoferrin and N-acetyl-l-cysteine can slow the aging of MSCs by inhibiting the production of reactive oxygen stress (ROS) in MSCs[29-31]. Some genetic engineering methods can also effectively slow the aging of MSCs. It was reported that knocking out migration inhibitory factor (MIF) could induce the aging of young MSCs, while overexpression of MIF led to regeneration of old MSCs[32]. In addition, some molecular compounds that activate endogenous telomerase, such as aspirin, vitamin C and FGF-2, were also used to save the proliferation potential of aged MSCs and restore their osteogenic ability[33-35]. Our results suggested that H_2S might be responsible for retarding the aging process, but the mechanisms for such properties need further investigation.

Furthermore, we investigated the possible underlying mechanism for the protective role of H_2S in PDLSCs senescence. First, we found that the expression of calcium influx–related pathway proteins in cells treated with H_2S was upregulated significantly by sequencing. Up to now, only a few studies have clarified the effect of calcium on cell senescence. One study revealed that calcium chelation could protect cells from premature senescence by reducing the DNA damage response (DDR) activation with a subsequent decrease in the p53/p21/Rb pathway[36]. It was reported that the basal calcium level of replicative aging fibroblasts was higher compared with non-aging cells[36]. Calcium protease is activated in the aging cells and leads to the enhancement of IL-1α processing. Chelating Ca^{2+} can inhibit the activation of calpain and the processing of IL-1α and alleviate cell senescence[37]. Our studies showed that Ca^{2+} influx was necessary to maintain the proliferation activity and steady state of MSCs, which was consistent with previous results[22,23]. Activation of TRPV4 channels appears to cause calcium events that result in the opening of eBK channels, endothelial hyperpolarisation and subsequent vasodilation[38]. Our results showed that H_2S protected cells against senescence through TRPV4-mediated calcium influx. To further confirm the role of TRPV4-mediated calcium influx on stem cell senescence, we used TRPV4 inhibitor or SiRNA to reduce the expression of TRPV4. We found that TRPV4 inhibition attenuated the protective effect of H_2S on cell senescence. It was reported that the transient receptor potential TRP) family mediated Ca^{2+} channels were sulfhydrylated by H_2S[22].The decrease in H_2S levels leads to cascade reactions in BMMSCs, including altered Ca^{2+} channel (at least including TRPV6, TRPV3 and TRPM4) sulfyhydration, and osteogenic differentiation[22]. H_2S-mediated vasodilation involves the activation of TRPV4-dependent Ca^{2+} influx and eBK channel activation in vascular endothelial cells[38]. H_2S also promotes the activation of T cells and differentiation of Th-cells (Treg cells) to control the homeostasis of the immune system. The specific molecular mechanisms that show H_2S regulated calcium influx in PDLSCs need to be investigated further.

Conclusion

The current research illustrates that H_2S could inhibit the senescence of PDLSCs and determines the intermediary role of TRPV4-mediated calcium ion influx in the protective effect of H_2S on senescence. It provides a potential strategy to prevent cell aging and may facilitate cell therapy for oral diseases.

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Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Yi Kun ZHOU and Rui Li YANG contributed to the collection and assembly of data, data analysis and interpretation, and manuscript drafting; Drs Rui Li YANG and Xiao Mo LIU contributed to the overall design of the study, critical editing of the manuscript, and financial support. All authors read and approved the final manuscript.

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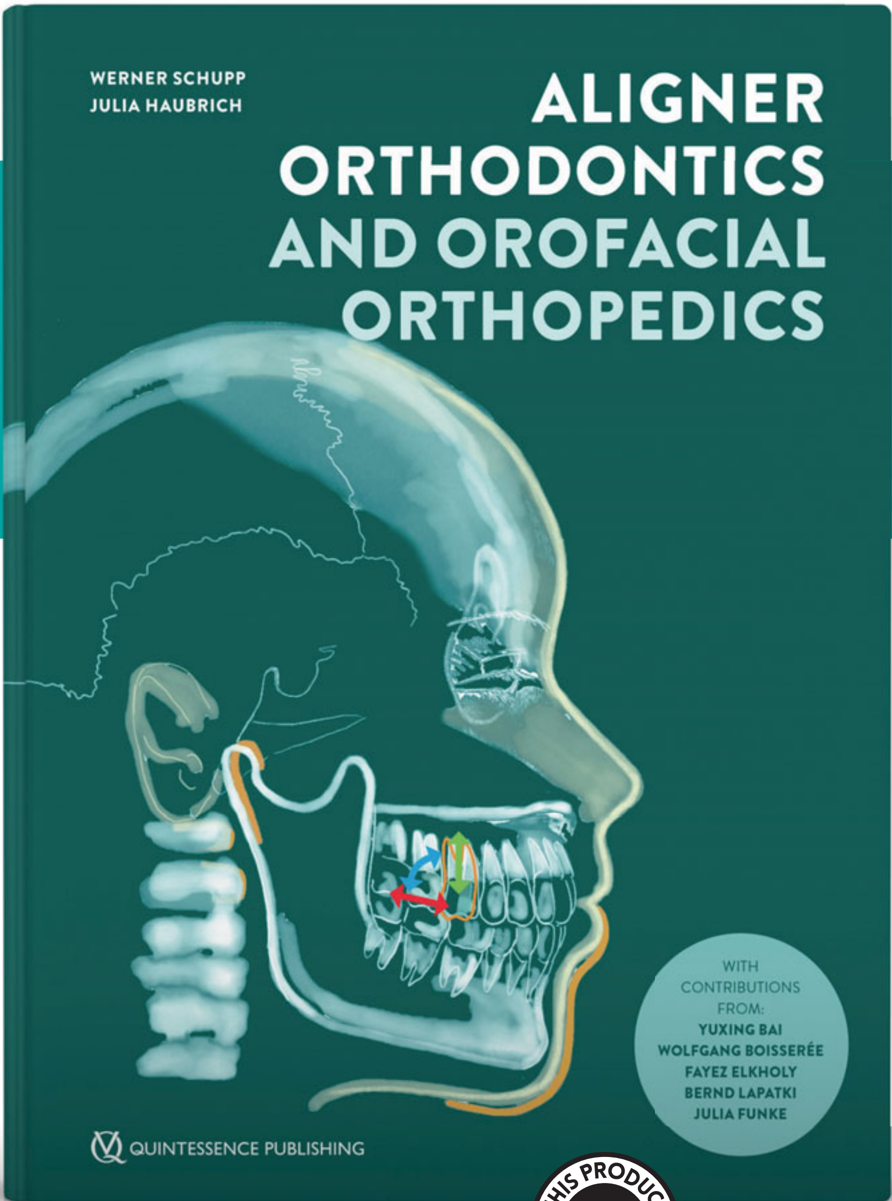

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Evaluation of Extension Type of Canalis Sinuosus in the Maxillary Anterior Region: a CBCT Study

Leila KHOJASTEPOUR[1], Fatemeh AKBARIZADEH[1]

Objective: *To evaluate the extension of canalis sinuosus (CS) into the alveolar crest for surgical reference in the anterior maxilla.*

Methods: *In this cross-sectional study, 485 CBCT images were evaluated in three orthogonal planes (axial, coronal and sagittal). The type of extension of CS into the alveolar ridge in the anterior maxilla was evaluated. The alveolar ridge was divided into four equal parts in a vertical and horizontal direction. In a vertical direction from apical to incisal and in a horizontal direction from labial to palatal, the four parts were designated as types 0, I, II and III, respectively. The extension of CS into the alveolar ridge was then traced.*

Results: *CS was present in 380 subjects (78.35%), and the extension type was unilateral in 217 of them (57.11%) and bilateral in 163 of them (42.89%). There was no significant relationship between incidence of CS and sex. Regarding the distribution of vertical and horizontal types, type II (the third quadrant of the ridge from apical to incisal and from labial to palatal, respectively) was significantly more prevalent than other types.*

Conclusion: *The most common location of CS into the alveolar ridge in both horizontal and vertical directions was type II (which is not close to the cortex). Awareness about the presence and possible locations of CS helps to reduce the risk of unjustifiable postoperative complications.*

Key words: *alveolar process, CBCT, incidence, maxilla*
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The canalis sinuosus (CS) is a neurovascular structure which was first introduced by Jones[1] in 1939. It is a branch of the anterior superior alveolar nerve and vessels, and originates from the infraorbital foramen, extending laterally towards the nasal cavity, ending in the anterior alveolar maxillary region[2]. This region has thin cortical bone, which makes it susceptible to invasion during surgical procedures[3]. The increasing amount of surgical manipulation that takes place in this region requires surgeons to have thorough knowledge about the anatomical structures prior to surgery[4-7].

Several studies have evaluated the presence and frequency of CS in the last decade. In 2012, Neves et al[6] described CS a rare anatomical structure, but in further articles it was reported as being common[8-10]. Recently, Brücker et al[4] and Lello et al[11] reported CS to be present in 97% and 100% of subjects, respectively. In fact, CS is now considered to be a normal variation rather than a rare anatomical landmark.

The main aspects of this topic that have been evaluated to date include the distance between CS and the surrounding structures, and the mesiodistal location of CS regarding the teeth in that area. Manhães Júnior et al[12] and Tomrukçu et al[13] measured the distance between CS and three surrounding structures (nasal cavity floor, buccal cortical edge and alveolar ridge crest). They reported these distances numerically, and both assumed that CS shows significant variation in its location relative to these structures. The horizontal and vertical distance of CS from the orbit and nasal cavity was measured by Lello et al[11], and they detected minor

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variations in the distance of CS from surrounding structures between various individuals.

The mesiodistal location of CS was classified first by de Oliveira-Santos et al[5]. They represented the distribution of CS relative to the teeth/incisive foramen, and found the canine region to be the predominant location. Shan et al[14] classified the location and opening of CS regarding the teeth and reported that the predominant location was between the central and lateral incisors.

In all the studies that have been published thus far, there has been profound variation in alveolar ridge dimensions in different people. Accordingly, most accurate knowledge regarding the extension of CS into the alveolar ridge can be acquired by evaluating the ratio of extension of CS into the ridge proportionally, instead of addressing the extension using numbers. There has not been any such assessment regarding CS up to now.

The use of CBCT enables a low dose of radiation exposure but detailed bone evaluation for diagnostic aims such as assessment of neurovascular structures, as it has the highest spatial resolution for bone evaluation[15,16].

The aim of this study is to assess the course of extension of CS into the alveolar ridge proportionally. Obtaining this information by means of CBCT would be beneficial in minimising the risk of injury to CS during surgical procedures in the anterior maxilla.

Materials and methods

The protocol of current study was approved by the Ethical committee of Shiraz University of Medical Sciences (protocol no. IR.SUMS.DENTAL.REC.1398.124).

Study design

CBCT images captured from June 2016 to June 2020 from the Oral & Maxillofacial Radiology Department were evaluated. The inclusion criterion was images with the field of view including the whole maxilla. The exclusion criteria were poor-quality radiographs, artefacts due to metallic restorations which led to false interpretation of the image, and subjects with a history of trauma/manipulation in the anterior maxilla. Systematic random sampling was employed. Finally, we selected 485 high-quality CBCT scans with a desirable field of exposure from the archive for further assessment. Informed consent was provided by the subjects when the images were captured, giving permission for their radiographs to be used without their names for further research.

CBCT imaging

The whole radiographs were captured under standard conditions using one device (NewTom VGI EVO CBCT unit; Bologna, Italy) with 75-110 Kvp tube voltage and 1-32 mA tube current, and the field of view size for all scans was the whole maxilla. The most recent CBCT innovation, the automatic exposure control mechanism, was used. As a result, the exposure parameters differed from patient to patient, based on their anatomy and size. The mean voxel size was 0.3 mm.

All radiographs were evaluated simultaneously by two oral and maxillofacial radiologists. The evaluation was done in three orthogonal planes (axial, coronal and sagittal) using NNT Viewer Software (NNT 9.21, NewTom).

Imaging analysis

In the first step, to detect the presence of CS, we evaluated continuous coronal cross-sectional cuts. The slice thickness and distance between the slices were both selected as 0.5 mm for more detailed examination of the area (Fig 1).

After that, in all subjects in whom CS was found to be present in coronal sections, we explored the maxilla by scrolling the axial view to confirm this finding (Fig 2).

In subjects in whom CS was confirmed to be present in both the coronal and axial views, we evaluated its extension into the alveolar ridge in sagittal view (Fig 3). We divided the alveolar ridge into four equal parts (0, I, II and III), in both an apico-incisal (vertical) and labio-palatal (horizontal) direction.

In the vertical aspect, we divided the ridge from the nasal floor to the alveolar ridge crest into four equal parts. The most apical compartment of the ridge (which was nearest to the nasal floor) to the most incisal part (which was nearest to the alveolar ridge crest) were designated as type 0 to III, respectively (from an apical to an incisal direction) (Fig 4).

In the horizontal aspect, the ridge was divided into four equal parts too. The most labial part to the most palatal part were labelled as type 0 to III, respectively (from a labial to a palatal direction) (Fig 5).

We traced the extension of CS and its terminal portion into the alveolar ridge in sagittal view. Based on the termination of CS in one of the mentioned vertical and horizontal quadrants, the subjects were categorised into four vertical and four horizontal types individually.

Additionally, the most anterior extension of CS regarding the teeth was specified. To this end, the extensions to the central incisor, lateral incisor, canine

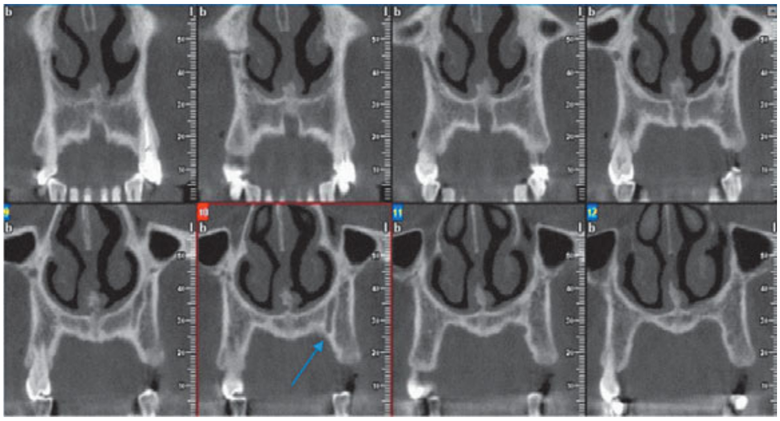

Fig 1 Detection of CS in coronal view of CBCT.

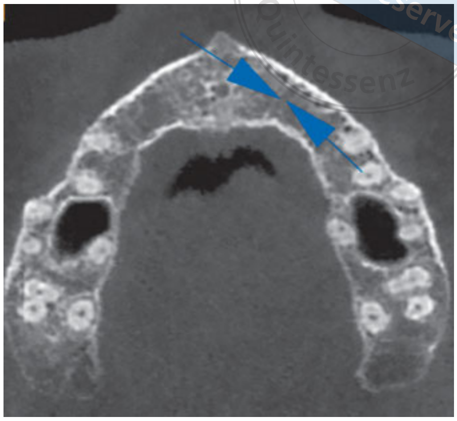

Fig 2 Confirmation of CS in axial view of CBCT.

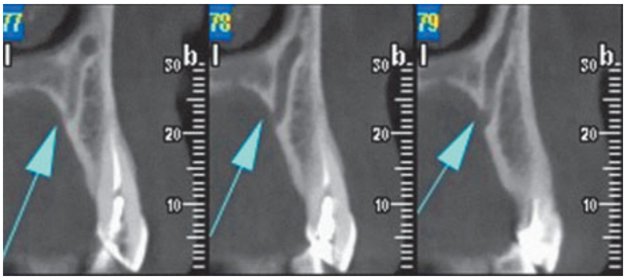

Fig 3 Evaluation of extension of CS in sagittal view of CBCT.

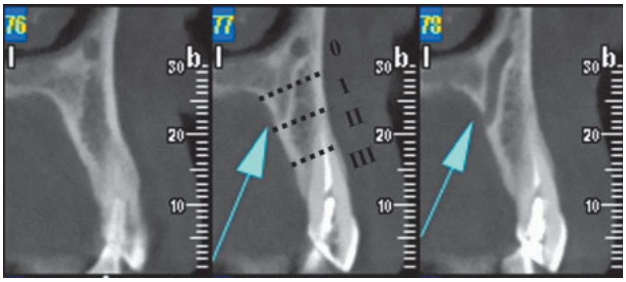

Fig 4 Division of the alveolar ridge into four equal parts (0, I, II and III) in an apicoincisal (vertical) direction. The most apical compartment of the ridge to the most incisal part was designated as type 0 to III, respectively (from an apical to an incisal direction).

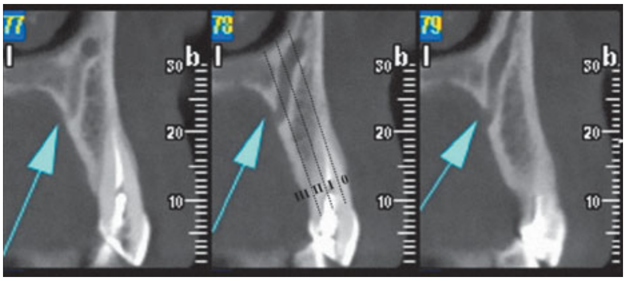

Fig 5 Division of the alveolar ridge into four equal parts (0, I, II and III) in a labiopalatal (horizontal) direction. The most labial compartment of the ridge to the most palatal part was designated as type 0 to III, respectively (from a labial to a palatal direction).

and first premolar were categorised as type 0, I, II and III, respectively.

We also evaluated the mean diameter of CS in the middle part of this structure in all subjects, and the participants were classified into two groups based on this diameter: larger than 1 mm or smaller than 1 mm.

Statistical analysis

SPSS software version 18.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. A chi-square test was used to assess the correlation between sex and frequency of CS, between sex and unilateral/bilaterality of CS, and also between sex and diameter type of CS. A Fisher exact test was applied to evaluate the frequency of horizontal and vertical extension types of CS into the alveolar ridge. *P* values < 0.05 were considered statistically significant.

Results

Among the 485 participants in the present study, 228 (47.01%) were male and 257 (52.99%) were female. The mean age of the subjects was 38.50 years. CS was present in 380 subjects (78.35%). Of these, it was unilateral in

Table 1 Frequency, unilaterality/bilaterality and diameter of CS and their relation with sex.

Variable		Sex		OR	95% CI	*P* value*
		Female	Male			
CS	Present	201 (78.21%)	179 (78.50%)	0.969	0.62–1.49	0.888
	Not present	56 (21.78%)	49 (21.49%)			
	Unilateral	113 (56.21%)	104 (58.10%)	1.07	0.49–2.32	0.866
	Bilateral	88 (43.78%)	75 (41.89%)			
	Diameter ≥ 1 mm	23 (11.44%)	35 (19.55%)	0.523	0.21–1.30	0.158
	Diameter < 1 mm	178 (88.55%)	144 (80.44%)			

**P* < 0.05. CI, confidence interval; OR, odds ratio.

Table 2 Comparison of extension of CS in different planes (vertical, horizontal and most anterior extension).

Plane	Type				
	0	I	II	III	*P* value
Vertical	15 (3.94%)	145 (38.15%)	200 (52.63%)	20 (5.26%)	0.040
Horizontal	11 (2.89%)	33 (8.68%)	206 (54.21%)	130 (34.21%)	0.000
Most anterior extension	22 (5.78%)	246 (64.73%)	83 (21.84%)	29 (7.63%)	0.078

217 individuals (57.11%, 113 women and 104 men), and bilateral in 163 cases (42.89%, 88 women and 75 men). There was no significant relationship between sex and unilaterality/bilaterality of this structure (Table 1).

The prevalence of CS among men and women was 179 (78.50%) and 201 (78.21%), respectively. There was no significant relationship between the incidence of CS and sex ($P = 0.888$, CI 0.63–1.49 odds ratio [OR] 0.97) (Table 1).

Among the subjects in whom CS was present, 80.44% and 88.55% of men and women, respectively, had a diameter smaller than 1 mm. There was no association between the diameter of CS and sex ($P = 0.158$, CI 0.21–1.30, OR 0.523) (Table 1).

Table 2 presents the vertical and horizontal distribution and most anterior extension of all 380 subjects with CS in both sexes. There was no significant difference regarding the most anterior extension of CS among subjects, although type II (lateral incisor tooth) was the most prevalent type ($P = 0.078$).

There was a significant difference between the frequencies of different vertical types. Type II was significantly more prevalent than other types, followed by types I, III and 0 ($P = 0.040$) (Table 2).

Regarding the horizontal direction, the prevalence of the four types differed significantly. The most common type was again type II, followed by types III, I and 0 (Table 2).

Discussion

CS is a common but often overlooked anatomical landmark extending from the infraorbital foramen to the anterior maxilla[2]. Precise evaluation of CS and its extension into the alveolar ridge is essential for avoiding encroachment to this neurovascular structure.

We found the frequency of CS to be 78.6%. The prevalence of CS was found to vary in previous articles due to a combination of factors. As there is no consensus about the diameter for detection of CS, Oliveira-Santos et al[5], Shan et al[14] and von Arx et al[17] determined that the diameter was just over 1 mm and reported a frequency of 15.7%, 36.9% and 27.8%, respectively; however, in other studies by Orhan et al[10], Brücker et al[4], Wanzeler et al[18] and Gurler et al[19], like as in this study, structures with a diameter lower than 1 mm were also included and higher frequencies were reported, namely 70.8% ,97.4%, 88.0% and 100.0%, respectively. The frequency of CS with a diameter smaller than 1 mm was found to be 84.73% in the present study, and there is an accepted notion that encroachment into even minute canals under 1 mm may lead to neurovascular symptoms[5]. Based on this view, evaluation of canals < 1 mm also seems essential. Ethnic discrepancies also cause differences in the frequency of anatomical variations[4,5,14,20]. The other plausible justification would be due to differences in the quality of CBCT devices and the selected pixel size for images, which differs in each article.

In the present study, the diameter of CS was smaller than 1 mm in most cases. There was an insignificant difference between diameters in both sexes. This was in accordance with the findings of Khabadze et al[8]. In contrast, Shan et al[14] reported the diameter of CS to be larger in men. We also found no significant difference in the prevalence of CS between the sexes; this was in line with the findings of Shan et al[14], Orhan et al[10] and de Oliveira-Santos et al[5]. Conversely, Khabadze et al[8], Machado et al[21], Tomrukçu et al[13] and Aoki et al[22] reported a higher frequency of CS in men. Although this research suggests that CS is mostly unilateral, Aoki et al[22] found a significantly higher percentage of bilateral cases. The reason for all these differences could be ethnic diversity.

The notion of evaluating the extension of CS into the maxillary alveolar process was proposed by de Oliveira-Santos et al[5], who classified the mesiodistal location of CS. According to their classification, there are seven regions for extension of CS: the central incisor region, between the central and lateral incisors, the lateral incisor region, canine region, first premolar region, lateral to the incisive foramen and posterior to the incisive foramen. They determined that the most common location was near the incisors or canines[5]. We found the lateral incisor to be the most common location for extension of CS, which is consistent with the findings of some other studies[8,9,12]. This result aligns with those of Aoki et al[22], Shan et al[14] and von Arx et al[17], who established the incisors as the most common location.

We evaluated the ratio of extension of CS into the alveolar ridge by dividing the ridge into four parts both horizontally and vertically. In the vertical aspect, we designated the four parts of the ridge from coronal to apical as 0, I, II and III, respectively. In the horizontal aspect, we named the four parts of the ridge from labial to palatal 0, I, II and III, respectively.

The prevalence of extension of CS into the alveolar ridge in a labiopalatal direction for the mentioned types was II > III > I > 0. This result approximately agrees with the findings obtained in previous studies. Several studies reported the location of CS opening in the palatal aspect of the alveolar ridge[8,13,17,21].

The present results illustrate that although CS is most commonly extended in the third quadrant of the ridge (from labial to palatal), other parts, even the most labial quadrant, are possible regions for CS expansion. This highlights the importance of thorough examination of the alveolar ridge before any surgical manipulation that cannot be achieved other than through CBCT examination. Intraoral and panoramic views are insufficient for presurgical assessment of the anterior maxilla, which was previously considered a safe region, because the field of view is so limited in them and there are superimpositions.

The present evaluation of CS into the alveolar ridge in a vertical direction revealed that the spread order of CS extension for the mentioned types was II > I > III > 0. The apico-incisal location of CS has only been evaluated previously in two studies, to the best of the present authors' knowledge[12,13]. Júnior et al[12] and Tomrukçu et al[13] assessed the distance between CS and the alveolar crest and reported a statistically significant difference between subjects. As for the horizontal direction, the most common location for CS in a vertical direction was type II (the third quadrant of the ridge that is not closest to the alveolar ridge). As mentioned previously, detailed examination of the entire alveolar ridge in both horizontal and vertical directions for detection of CS is essential.

Clinical knowledge about the frequency and possible locations of CS would be beneficial in different fields and may be especially helpful for surgeons. Even subtle manipulation in the anterior maxilla require detailed evaluation of the entire ridge to prevent encroachment onto the neurovascular structures, and this needs to be done using advanced modalities as it may be overlooked in conventional imaging. This reduces the risk of unjustifiable postoperative complications.

In the present study, we evaluated CS exclusively in dentate subjects. A potential limitation of the study is its lack of evaluation of CS extension type in edentulous subjects compared to dentate patients. Also, considering the diverse reports about the frequency of CS among populations, extension type of CS may vary in different ethnic groups; thus, evaluation of extension type of CS in other populations is recommended.

Conclusion

There is diversity in the frequency of CS among different populations. In present study, the frequency was reported to be 78% and there was no significant difference between the sexes. In most cases, CS was unilateral and the diameter was smaller than 1 mm, with no significant difference between the sexes. The most common mesiodistal location of CS was the lateral teeth. The most common opening location of CS into the alveolar ridge in both horizontal and vertical directions was type II, which is not closest to the alveolar ridge, the third quadrant of the ridge from labial to palatal and from apical to incisal, respectively.

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Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Both authors participated in the data collection process, statistical analysis and writing of this article.

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Effect of Premolar Extraction on the Upper Airway in Adult and Adolescent Orthodontic Patients: a Meta-analysis

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Objective: *To analyse the effects of premolar extraction on the upper airway in adult and adolescent orthodontic patients using CBCT.*

Methods: *The Embase, Web of Science, Cochrane Library and Medline (via PubMed) databases were searched with no language restrictions. Longitudinal studies in which CBCT was applied to assess the effects of tooth extraction on the upper airway were included in the analysis. Two authors performed the study selection, methodological quality assessment, data extraction and data synthesis independently.*

Results: *A total of 12 studies were included, six of which were eligible for quantitative synthesis. In the adult group, the nasopharynx and oropharynx volume showed no significant change, and the minimum cross-sectional area of the upper airway demonstrated a non-significant decrease compared to the non-extraction group. In the adolescent group, the nasopharynx volume, oropharynx volume and minimum cross-sectional area of the upper airway increased in a non-significant manner.*

Conclusion: *The currently available evidence indicates that tooth extraction does not increase the risk of airway collapse in adult and adolescent patients. The present findings should be interpreted with caution and evaluated in further high-quality studies.*

Key words: *airway, extraction, meta-analysis, orthodontic*

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The goals of orthodontic treatment are aesthetics, stability and function. An important concern in any orthodontic procedure is respiratory function, particularly in the upper airway, which includes the nasopharynx, oropharynx and hypopharynx. The oropharynx is surrounded by soft tissue (the soft palate, tongue and pharyngeal wall) and lacks skeletal support, and thus could be easily affected by orthodontic procedures. Changes in the upper airway dimensions have been reported following rapid maxillary expansion, the use of mandibular advancement appliances and orthognathic surgery[1-3].

Premolar extraction is performed to alleviate crowding, reduce facial convexity and correct anteroposterior discrepancies for orthodontic patients. The effect of premolar extraction on the upper airway has been investigated in previous studies. Some believe that it may predispose patients to oropharyngeal collapse, which is associated with a decreased oral cavity volume and posterior displacement of the tongue, especially for patients with protruding anterior teeth[4-6]. As such, an important concern is that the narrow airway caused by extraction may lead to obstructive sleep apnoea (OSA). The upper airway is a 3D structure, but the airway analyses in these studies were performed using a lateral cephalometric radiograph[4-6]. Because of the limitations of two-dimensional (2D) radiographs, lateral cephalometric radiographs yield only anteroposterior data and cannot be used to evaluate the upper airway volume.

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Recently, CBCT has been used widely to evaluate the upper airway change, which enables 3D imaging of the upper airway and analysis of its morphology and volume[7]. Several studies reported the change in upper airway volume after premolar extraction treatment using CBCT[8-10]. Their results supported that extraction-induced reduction of the dental arch perimeter did not affect the upper airway volume and respiratory function, and extraction treatment was not an aetiology factor in the development of OSA[11]. At present, there is still no strong evidence for the effect of premolar extraction on the upper airway.

On the other hand, ageing has been found to influence the upper airway morphology[12,13]. As the upper airway undergoes growth during adolescence, the effects of extraction on the upper airway in adolescent and adult patients should be analysed separately, but are less reported. In this meta-analysis, we evaluated the effects of premolar extraction on this area in adult and adolescent orthodontic patients using CBCT.

Materials and methods

Protocol and registration

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[14] and was registered in the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) with registration number INPLASY202040175.

Eligibility criteria

The eligibility criteria were based on the participants, intervention, comparison, outcome and study (PICOS) criteria:

- Participants: Orthodontic patients with premolars extracted were included without age or sex restriction.
- Intervention: Orthodontic treatment with premolar extractions aiming to retract anterior teeth (moderate to maximum anchorage). The type of appliance, technique and treatment duration were not restricted.
- Comparison: Untreated patients or orthodontic patients without premolar extraction.
- Outcomes: The upper airway volume and minimum cross-sectional area analysed by CBCT were selected as the primary outcomes. To ensure comparability, the included patients should bite in centric occlusion and breathe normally without swallowing during CBCT scanning.
- Study: Randomised controlled trials (RCTs), controlled clinical trials (CCTs) and cohort studies were included. Considering the untreated patients were limited and the airway volume was relatively stable in adult patients, self-controlled studies, which could provide clinically beneficial information, were also included. Cross-sectional studies, case reports, animal studies and reviews were excluded.

Literature search strategy

An electronic search was performed of the Embase, Web of Science, Cochrane Library and Medline (via PubMed) databases from their inception up to 10 March 2022, with no language restrictions. The detailed search strategy for PubMed is illustrated in Table 1. Similar searches using a revised strategy were performed of the other databases with the assistance of a librarian, and the reference lists of the included studies were searched manually to identify relevant articles.

Study selection

The study selection was performed by two independent authors (Guo RZ and Li LW). After eliminating duplicate studies, the titles and abstracts of all the included studies were examined based on the eligibility criteria. The full texts were obtained and evaluated when the titles and abstracts provided insufficient information. Any conflicts regarding article selection were resolved by consultation and discussion with a third author (Li WR).

Data extraction

Two authors (Guo RZ and Li LW) independently extracted the following study characteristics: study design, sample size, patient age, diagnosis and treatment plan, radiographic method, airway measurements and outcomes. The upper airway volume and minimum cross-sectional area determined by CBCT were extracted for quantitative analysis. The reference line of airway volume and space in the individual studies was recorded due to the lack of a uniform definition of the upper airway. The treatment plan, including extraction sites, anchorage status and extent of incisor retraction, was recorded. Any disagreement between the two authors was resolved through discussion with a third author (Li WR).

Assessment of methodological quality

The quality of the included non-randomised studies was assessed using the Methodological Index for Non-

Table 1 Search strategy for Medline via PubMed.

Search terms		PubMed results
# 1	orthodontics [Mesh] OR orthodontic* OR tooth movement OR teeth movement	92229
# 2	airway OR oropharynx [Mesh] OR pharynx OR pharyngeal OR oropharyngeal	286313
# 3	Tooth extraction [Mesh] OR extract*	994460
# 4	computed tomography OR cone-beam computed tomography OR cbct	609996
# 5	#1 AND #2 AND #3 AND #4	24

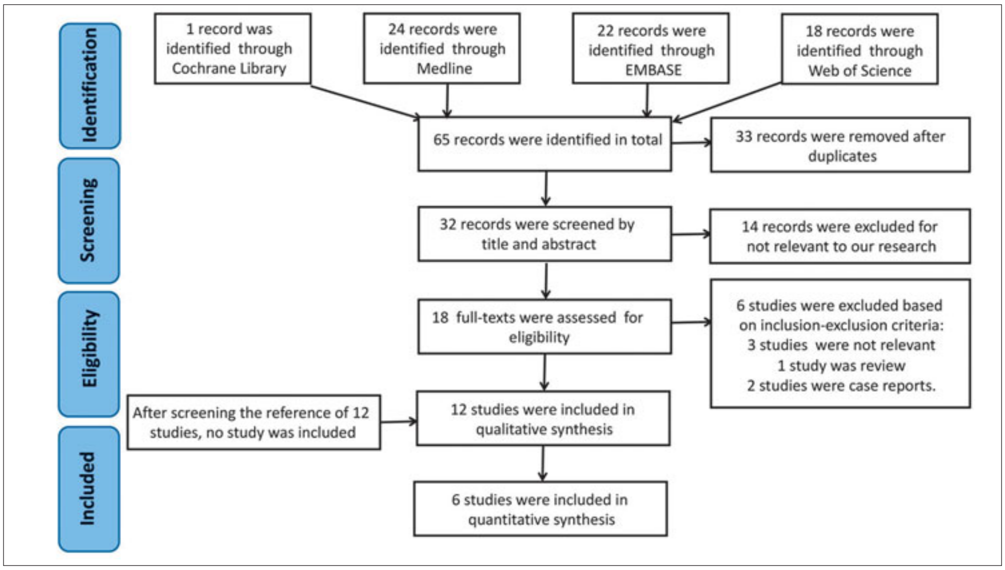

Fig 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

randomized Studies (MINORS). Only the first 8 of the 12 MINORS criteria were used to assess the quality of self-controlled studies. The overall quality of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach[15]. Two authors (Zhang LW and Yu QY) independently evaluated the included studies, and any conflicts were resolved through consultation and discussion with a third author (Li WR).

Data synthesis

Review Manager 5.3 (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark) was used for the meta-analysis. Heterogeneity was assessed using chi-square and I^2 tests. A random-effects model was used in cases of high heterogeneity ($I^2 > 50\%$); otherwise, the fixed-effects model was used. The level of statistical significance was set at $P \leq 0.05$. Studies with a high level of clinical heterogeneity such that data synthesis was not possible were described qualitatively.

Results

Study selection

Initially, 32 studies were identified using the search strategy. After title and abstract review, 18 were considered potentially eligible for full-text evaluation. Based on the eligibility criteria, 12 studies were included in the review, and six of these were eligible for quantitative synthesis. A flowchart of the study selection process is shown in Fig 1.

Study characteristics

The 12 studies comprised 8 cohort studies[8-10,16-20] and 4 self-controlled studies[21-24]. As for cohort studies, six studies included a non-extraction group[8-10,16-18] and two included an untreated control group[19,20]. In all studies, the premolars were extracted to retract the anterior teeth. Of these, five studies reported that maximum anchorage (miniscrew) was used to retract the anterior teeth[8,17,19,21,22], and the remaining studies did not report the anchorage characteristics. The characteristics of the included studies are summarised in Table 2.

Assessment of methodological quality

The risk of bias for the included studies is shown in Table 3. The MINORS score for the eight cohort studies ranged from 14 to 19, and for the four self-controlled studies it ranged from 8 to 11. All the included studies were retrospective. Among the MINORS criteria, the inclusion of consecutive patients, prospective collection of data, loss to follow-up less than 5% and prospective calculation of the study size were the main items that posed a potentially high risk of bias. The high clinical heterogeneity of the included studies was a methodological limitation.

3D airway analyses

The nasopharynx (from the top of the airway to the palatal plane level) and oropharynx (from the palatal plane to the uvula level) volumes and the minimum cross-sectional area analysed by CBCT were extracted for data synthesis. Based on age, the included patients were divided further into the adult group (aged > 18 years) and adolescent group (aged < 18 years) for assessment. There were seven studies in the adult group and five in the adolescent group. The adult and adolescent subjects were analysed separately, both quantitatively and qualitatively.

In the adult group, three studies quantitatively evaluated the nasopharynx and oropharynx volume and the minimum cross-sectional area of the upper airway[8,9,16]. After extraction, the nasopharynx (mean difference $0.07cm^3$; 95% confidence interval [CI] −0.58 to $0.73cm^3$; $P = 0.82$) and oropharynx (mean difference $0.21cm^3$; 95% CI −0.76 to $1.19cm^3$; $P = 0.67$) volumes showed no significant change (Fig 2). A quantitative synthesis of three studies[8,9,16] indicated that there were no significant differences in nasopharynx and oropharynx volume between extraction and nonextraction (Fig 3). The nonsignificant decrease in the minimum cross-sectional area of the upper airway (mean difference $0.37cm^2$; 95% CI 0.06 to $0.68cm^2$; $P = 0.11$) also did not differ significantly compared to the nonextraction group. Considering the extraction site and anchorage type among these three studies, two studies reported that at least two premolars were extracted, but the anchorage types were not stated[9,16]. One study included patients with extraction of four premolars and retracted the anterior teeth with miniscrews[8]. Zhang et al[19] found that extraction caused mainly morphological changes rather than a decrease in size in the upper airway. In contrast to our findings, Sun et al[21] and Zheng et al[22] reported a high risk of oropharyngeal collapse after maximum extraction of anterior teeth in adult patients.

In the adolescent group, three studies quantitatively evaluated the nasopharynx[10,17,20] and five quantitatively evaluated oropharynx volumes and the minimum cross-sectional area of the upper airway[10,17,18,20,21]. Unlike in the adult group, the nasopharynx volume (mean difference −0.10 cm^3; 95% CI −0.38 to 0.18 m^3; $P = 0.48$), oropharynx volume (mean difference −1.01 cm^3; 95% CI −2.48 to 0.47 cm^3; $P = 0.18$) and minimum cross-sectional area of the upper airway (mean difference −0.17 cm^3; 95% CI −0.49 to 0.14 cm^3; $P = 0.29$) increased in a non-significant manner (Fig 4). These changes were not significantly different from those in the nonextraction group (Fig 5). Considering the extraction site and anchorage type, all patients in the five included studies had their four first premolars extracted. Among these, one study reported that miniscrews were used[17]; the others did not report the anchorage type[10,18,20,21].

Risk of bias across studies and additional analyses

Due to the limited number of included studies, it was not possible to assess publication bias. The quality of evidence of the outcome was low in the adult group and very low in the adolescent group. The overall quality of evidence for each outcome assessed by GRADE is shown in Table 4.

Discussion

Effects on the airway are a concern in orthodontics. Rapid maxillary expansion, mandibular advancement appliances and orthognathic surgery increase the dimensions of the upper airway and alleviate the symptoms of OSA. At present, there is no strong evidence for the influence of extraction treatment on the upper airway. In this meta-analysis, we analysed the effects of premolar extraction on the upper airway in adult and adolescent orthodontic patients using CBCT.

Our meta-analysis quantitatively analysed the upper airway change in adult and adolescent patients after tooth extraction. The upper airway was not affected in adult or adolescent extraction compared to nonextraction patients, which was consistent with the findings of Alswairki et al[25]. Hu et al[26]performed a systematic review to analyse the effect of extraction treatment on the upper airway and reported that the retraction of the anterior teeth might lead to a narrowing of the oropharynx. On the other hand, the present systematic review combined the results of studies using lateral cephalometric radiographs and CBCT, which could cause bias. The

Table 2 Characteristics of the included studies.

Author/Year	Study design	Sample size	Average age	Diagnosis	Treatment Plan	Airway measurements	Outcomes
Park et al[8]	Cohort study	Extraction group 16, non-extraction group 17	22.18±3.99 y for both groups	Dental Class II	Distalization with and without extraction (mini-screw anchorage)	Volume of oropharynx (velopharynx and glossopharynx); Minimum cross-section area (MCA)	There were no significant changes in the airway volume or the MCA of the oropharynx among two groups
Joy et al[9]	Cohort study	Extraction group 41, non-extraction Group 42	Extraction group 26.1±7.1 y, non-extraction Group 26.0±8.0 y	Dental Class I and Class II	Extraction group: at least two premolars extracted	Volume of nasopharynx, retropalatal and retroglossal; MCA	Extractions in nongrowing patients have no negative consequences on airway measurements
Stefanovic et al[10]	Cohort study	Extraction group 31, non-extraction group 31	Extraction group 12.97±1.15 y, nonextraction group: 12.86±0.74 y	Dental Class I and Class II	Extraction of four first premolars	Volume of nasopharynx and oropharynx	An extraction or non-extraction choice for orthodontic treatment would not affect the pharyngeal airway
Pliska et al[16]	Cohort study	Extraction group 26, non-extraction group 48	Extraction group 27.4±9.7 y, non-extraction group 31.9±12.0 y	Dental Class I and Class II	Extraction group: at least two premolars extracted	Volume of nasopharynx, retropalatal and retroglossal; MCA	Dental extractions in conjunction with orthodontic treatment have a negligible effect on the upper airway in adults
Chen et al[17]	Cohort study	Extraction group 25, non-extraction group 25	Extraction group 12.2±1.2 y, non-extraction group 12.4±1.5 y	Dental Class I	Extraction of four premolar (mini-screw anchorage)	Volume of nasopharynx, oropharynx and hypopharynx; MCA	The airway volume and MCA were significantly decreased after treatment
Valiathan et al[18]	Cohort study	Extraction group 20, non-extraction group 20	Extraction group 13.8±1.3 y, non-extraction group 13.8±1.2 y	Dental Class I	Extraction of four first premolars	Volume of oropharynx; MCA	Extraction of four premolars with retraction of incisors does not affect oropharynx airway volume
Zhang et al[19]	Cohort study	Extraction group 18, untreated group 18	Extraction group 24.1 ± 3.8 y	Dental Class II	Extraction of four premolar (mini-screw anchorage)	Volume of nasopharynx, velopharynx and hypopharynx	The airway changes after extraction in adults are mainly morphological changes, rather than a decrease in size
Lei et al[20]	Cohort study	Extraction group 27, untreated group 30	Extraction group 13.62±1.49 y, nontreatment group 13.21±1.73 y	Dental Class II	Extraction of four premolar	Volume of nasopharynx, oropharynx, and hypopharynx MCA	Extraction treatment can widen the airway in adolescent patients.
Sun et al[21]	Self-controlled study	30	31.6±3.9 y	Dental Class I with bimaxillary protrusion	Extraction of four premolar (mini-screw anchorage)	Volume of nasopharynx, oropharynx and hypopharynx	The oropharynx was constricted and the pharyngeal resistance was increased after incisor retraction in adult patients.
Zheng et al[22]	Self-controlled study	30	25.87±0.78 y	Dental Class I with bimaxillary protrusion	Extraction of four premolar (mini-screw anchorage)	Volume of nasopharynx, oropharynx and hypopharynx; MCA	The risk of pharyngeal collapsing become higher after extraction with maximum anchorage in adult patients with bimaxillary protrusion.
Shi et al[23]	Self-controlled study	18	21.2±2.9 y	Dental Class II	Extraction of maxillary first premolars and mandibular second premolars (maxilliary molar intrusion with mini-screw)	Volume of nasopharynx, oropharynx and hypopharynx; MCA	By using mini-screw to intrude maxillary molars, orthodontic premolar extraction treatment could increase the upper airway dimensions
Sun et al[24]	Self-controlled study	30	13.7±1. 5 y	Dental Class I	Extraction of four first premolars	Volume of nasopharynx, oropharynx and hypopharynx; MCA	The impact of orthodontic extraction treatment on oropharyngeal airway was generally small in skeletal class I adolescents.

Table 3 Methodological index for non-randomised studies (MINORS).

Study	MINORS score												
	1	2	3	4	5	6	7	8	9	10	11	12	Total
Park et al[8]	2	2	1	2	2	2	0	0	2	2	2	2	19
Joy et al[9]	2	0	1	2	2	2	0	0	2	2	2	2	17
Stefanovic et al[10]	2	0	0	2	2	2	0	0	2	2	2	2	16
Pliska et al[16]	2	0	0	2	2	2	0	0	2	2	2	2	16
Chen et al[17]	2	0	0	2	2	2	0	0	2	2	2	2	16
Valiathan et al[18]	2	0	2	2	2	2	0	0	2	2	2	2	16
Zhang et al[19]	2	0	0	2	2	2	0	0	1	2	2	1	14
Lei et al[20]	2	0	1	2	2	2	0	0	2	2	2	2	17
Sun et al[21]	2	1	2	2	2	2	0	0					11
Zheng et al[22]	2	0	0	2	2	2	0	0					8
Shi et al[23]	2	0	0	2	2	2	0	0					8
Sun et al[24]	2	0	0	2	2	2	0	0					8

Items 1–12 represent: 1, a clearly stated aim; 2, inclusion of consecutive patients; 3, prospective collection of data; 4, endpoints appropriate to the aim of the study; 5, unbiased assessment of the study endpoint; 6, follow-up period appropriate to the aim of the study; 7, loss to follow-up less than 5%; 8, prospective calculation of the study size; 9, an adequate control group; 10, contemporary groups; 11, baseline equivalence of groups; and 12, adequate statistical analysis.

*Items scored 0 means not mentioned, 1 means reported but inadequate and 2 means reported and adequate. The total score was 24 for cohort studies and 16 for self-controlled studies.

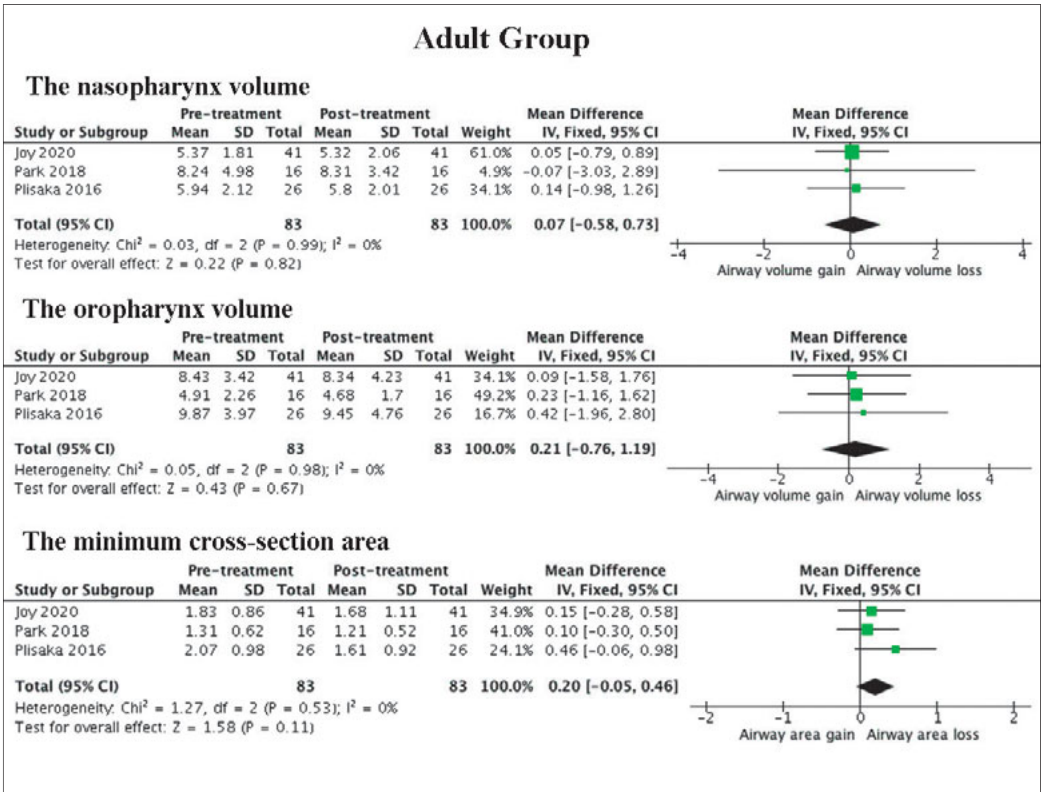

Fig 2 Forest plots of upper airway changes after extraction in the adult group.

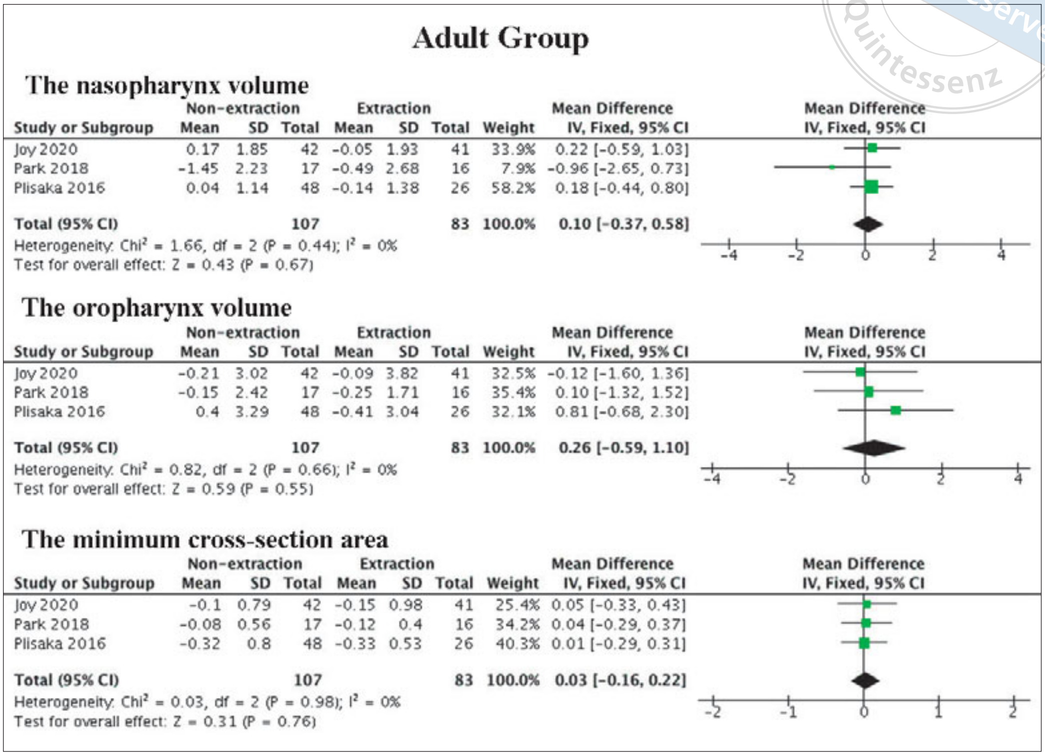

Fig 3 Forest plots of upper airway changes compared for extraction and nonextraction in the adult group.

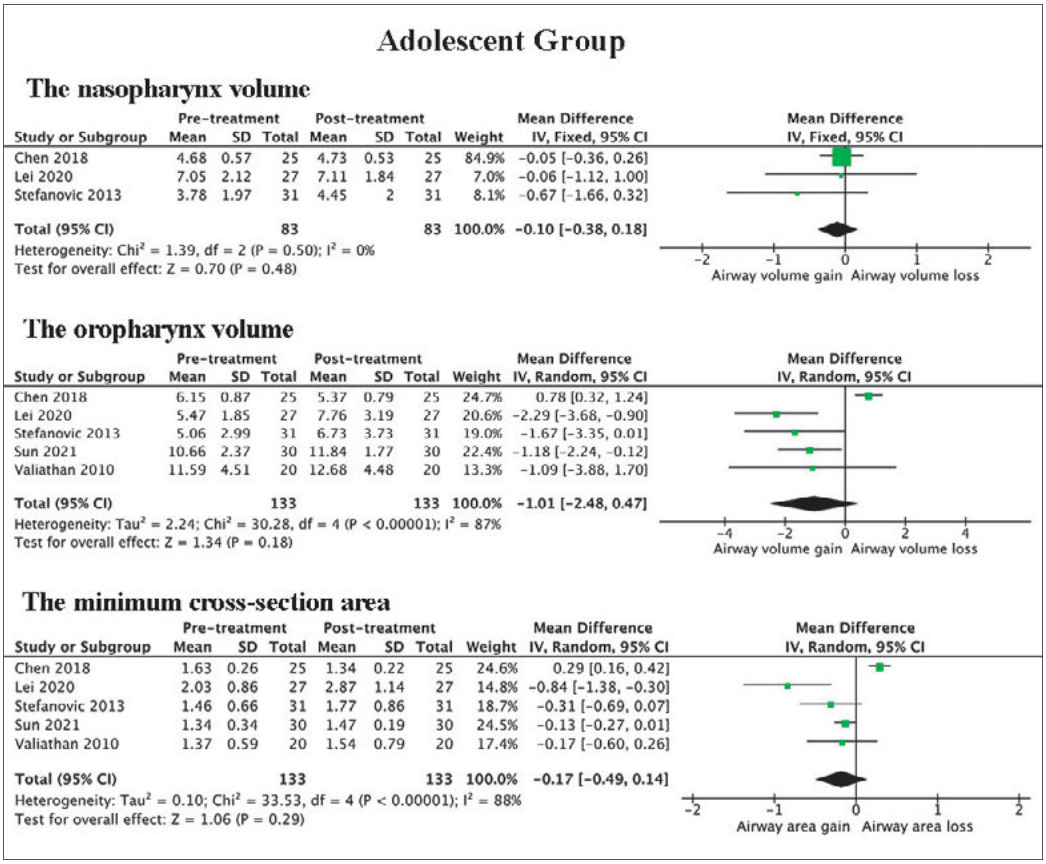

Fig 4 Forest plots of upper airway changes after extraction in the adolescent group.

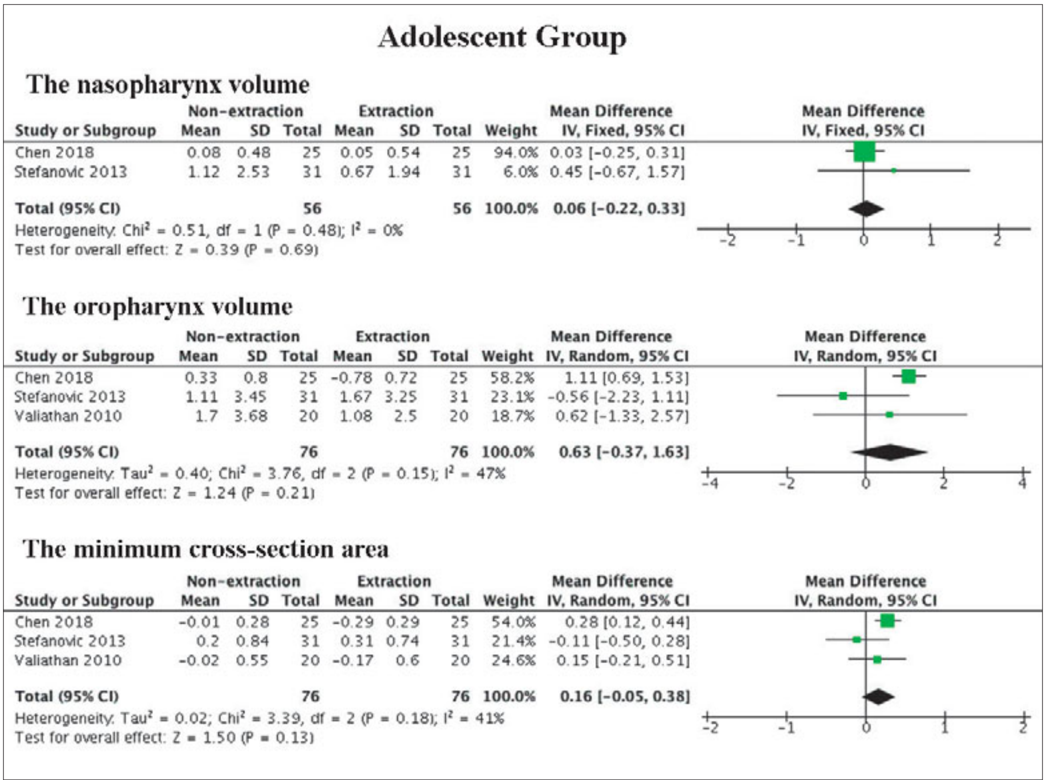

Fig 5 Forest plots of upper airway changes compared for extraction and nonextraction in the adolescent group.

Table 4 Quality of available evidence using GRADE.

Outcome	Downgrade					Upgrade	Overall quality
	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
Airway volume analysis in adult group	Serious[a]	Not serious	Not serious	Serious[c]	None	None	Low
Airway volume analysis in adolescent group	Serious[a]	Serious[b]	Not serious	Serious[c]	None	None	Very low

[a]All studies were retrospective with a high risk of bias.

[b]Higher statistical heterogeneity was involved.

[c]Participants included in the meta-analysis were limited, and 95% CIs were wide.

nasopharynx volume was stable, likely because it is supported by bone and cartilage. Indeed, although it is surrounded by soft tissue rather than bone, the oropharynx volume was also not significantly changed after extraction. The minimum cross-sectional area of the airway decreased in a non-significant manner in the adult group. Changes in the minimum cross-sectional area detected by CBCT are less reliable and more easily influenced by head position, tongue position and mode of breathing[27]. Other airway measurements reported in the included studies were analysed qualitatively. There was a close relationship between the upper airway and hyoid bone[6,28]. The latter was displaced posteriorly and inferiorly after retraction of the anterior teeth. Bhatia et al[4] suggested that posterior and inferior movement of the hyoid bone prevents encroachment of the tongue into the oropharynx. Zheng et al[22] assessed variation in airflow characteristics in the upper airway using computational fluid dynamics (CFD) and reported increased oropharynx resistance. One limitation of the CFD model is that the upper airway is assumed to have an inflexible wall; the adaptive ability of soft tissue is not considered.

In the adult group, the airway volume analysed using CBCT scans was not affected by extraction. Extraction treatment did not increase the risk of airway collapse in adult patients, while the anteroposterior airway space analysed using lateral cephalometric radiographs was mostly reported to decrease[4-6]. Lateral cephalometric radiographs typically show that after extraction, the upper airway narrows in the two-dimensional view, but the 3D airway volume is indeed not affected. We assumed that airway morphology might adapt to anteroposterior compression and transverse broadening; thus, the airway volume was maintained, which was consistent with Zhang et al[19]. Compared to lateral cephalometric radiographs, CBCT enables two- and 3D airway analyses, which could be more useful in airway morphology evaluation.

In some previous studies, the negative effects of extraction on the upper airway were thought to be a result of retraction of the anterior teeth, causing posterior displacement of the tongue and compression of the soft tissue and leading to upper airway narrowing[4,17,21]. This theory fails to consider the adaptive ability of the upper airway to maintain the airflow. Besides, healthy individuals with a narrow airway reportedly maintain patency by dynamically dilating the airway during inspiration[29]. Among the three included studies, two reported the lower incisor was retracted approximately 3 mm[8,16]. The remaining study did not state the extent of incisor retraction but reported that there was no significant relationship between initial crowding and changes in airway volume in the extraction group[9]. Park et al[8] used a modified C-palatal plate to further retract the anterior teeth after extraction space closure; the airway volume was not significantly changed. Thus, retraction of the anterior teeth seems to have no negative effect on airway volume. Interestingly, Shi et al[23] extracted four premolars of Class II high-angle patients and used miniscrews to further intrude the maxillary molars and found that mandibular counterclockwise rotation could increase the upper airway dimension in extraction patients.

There was a tendency towards increased airway volume in the adolescent group, possibly because of growth. The growth of the skeletal structure and the shrinking of soft tissue (tonsils and adenoids) contribute to the increase in the upper airway volume from infants (0 to 5 years) to children (6 to 9 years) and adolescents (12 to 16 years)[30,31]. The patients in our adolescent group ranged from 12 to 16 years of age, and the use of age-matched controls precluded elimination of the influence of growth. The airway changes were not significantly different between the extraction and nonextraction group. In this study, we distinguished the effects of extraction and growth factors and found that the airway changes caused by growth exceeded the effects of extraction.

The relationship between airway dimensions and OSA is a concern of orthodontists. OSA is common in both adult and adolescent patients; the prevalence is 5% to 14% and 1% to 4%, respectively[11]. The role of the orthodontist in the management of OSA, as suggested by the American Association of Orthodontists, is to screen and refer at-risk patients to a physician[11]. At present, there are no cutoff airway volumes and cross-sectional areas that indicate a high risk of OSA. Lowe et al[32] reported that the mean airway volume in OSA patients was 13.9 cm^3. Although airway narrowing is important in the pathogenesis of OSA, other risk factors, such as craniofacial morphology, obesity, menopause, increasing age and male sex, are also involved[33,34]. As breathing is a dynamic process, CBCT scans provide information only on the static anatomy of the upper airway and do not reflect breathing. There is also no direct link between airway volume analyses and polysomnography (PSG) results[11]. To diagnose and monitor OSA, the radiographic measurement of the airway should be interpreted in combination with other clinical symptoms and PSG results. This meta-analysis evaluated the effects of extraction only on airway morphology; airway function should be assessed in further studies. At present, there is no evidence that extraction treatment will result in the development of OSA[11].

Compared with lateral cephalometric radiographs, CBCT scans enable 3D measurement of the upper airway, including morphology and volume. Although CBCT is superior to 2D measurements to analyse the upper airway, it still has some limitations. Firstly, the cost and radiation dose are relatively high. Secondly, the upper airway is easily affected by head position and respiratory status during the CBCT scanning; thus, the reliability of upper airway assessment using CBCT, especially nasopharynx and hypopharynx assessments, has been reported to be generally low[35]. Finally, CBCT images can only reflect the static images of a dynamic breathing process.

Limitations

The main limitation of this meta-analysis was the absence of high-quality studies. All the included studies were retrospective and so had a relatively high risk of bias. As a result of the varied landmarks and reference lines selected for measurements, relatively few studies could be analysed quantitatively. Moreover, there was a high level of clinical heterogeneity among the included studies. Several factors, such as extraction site, anchorage type, skeletal patterns and body mass index varied and were generally not well reported. Because of the lack of sufficient high-quality studies, the findings should be interpreted with caution and further evaluation is needed. Finally, more attention should be paid to the effects of extraction on airway function and long-term changes.

Conclusion

Within the limitations of this meta-analysis, the evidence indicates that premolar extraction does not elevate the risk of airway collapse in adult or adolescent orthodontic patients. The findings of this meta-analysis apply only to healthy patients without OSA. Further studies are required to evaluate the effect of extraction on the upper airway in patients with OSA.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Run Zhi GUO carried out the review and drafted the manuscript; Dr Lin Wei LI performed the study selection and data extraction; Drs Li Wen ZHANG and Qian Yao YU participated in the data synthesis and quality assessment; Drs Wei Ran LI and Yi Ping HUANG participated in the research design and revised the manuscript.

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Effect of Different Obturation Techniques on Sealer Penetration in Teeth with Artificial Internal Root Resorption: a Confocal Laser Microscope Analysis

Zeliha UĞUR AYDIN[1], İrem Cansu KARA[2], Gamze ER KARAOĞLU[2], Tülin DOĞAN ÇANKAYA[3]

Objective: *To investigate the efficacy of different obturation techniques on sealer penetration in teeth with internal root resorption using confocal laser microscopy.*

Methods: *An artificial internal resorption cavity (3 mm deep and 1.2 mm in diameter) was formed in the round-shaped root canals of 45 single-rooted teeth at a distance of 7 mm from the apex, then roots were instrumented (size 40/.06). The samples were divided into three groups (n = 15) according to the obturation technique: lateral compaction (LC), warm vertical compaction (WVC) and carrier-based (CB).*

Results: *In the resorption regions, the sealer penetration depth in the CB and LC groups was significantly higher than that in the WVC group (P < 0.05).*

Conclusion: *Within the limitations of this study, the penetration depth of the sealer in the resorption region was higher in the CB and LC groups as compared to that in the WVC group.*

Key words: *carrier-based technique, internal root resorption, sealer penetration, warm vertical compaction*

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Internal root resorption (IRR), which is the result of various factors, such as chronic infection and trauma, is characterised by the gradual destruction of the dentine along the root canal walls[1]. IRR is not always clinically symptomatic and is thus often detected during routine radiographic examinations. In teeth with IRR, the root canal space or pulp chamber is enlarged on radiographs[2]. Endodontic treatment can be performed in teeth with IRR considering restoration and prognosis[1]; however, 3D chemomechanical shaping and obturation of the resorption area can be difficult for endodontic treatment in teeth affected by it[3]. According to the literature, insufficient root canal obturation in teeth with IRR causes bacterial leakage and consequent apical periodontitis[4]. Thus, the prognosis of endodontic treatment in teeth with IRR depends on the selection of an appropriate obturation technique[4,5]. The current available obturation techniques include lateral compaction (LC), warm vertical compaction (WVC) and carrier-based (CB) techniques.

The LC obturation technique allows gutta-percha to be placed in the root canal in a controlled manner, but also has disadvantages, such as a long application time, lack of homogeneity of obturation due to the formation of gaps between the gutta-percha, inadequate adaptation to root canal walls, and an increased risk of vertical root fracture[6,7]. Previous studies reported that LC, which is widely used in clinical practice, is insufficient to provide 3D obturation in the IRR cavity[8]. Thus, the use of alternative obturation methods for LC in teeth with IRR has been proposed[9].

WVC is a hybrid root canal obturation technique that involves the combined use of downpacking and back-fill procedures. Using this technique, the apical portion of the root canal is filled using a down-packing unit. The remaining root canal cavity is obstructed by the back fill unit, which allows the gutta-percha to be applied in a controlled manner[10,11]. Using this tech-

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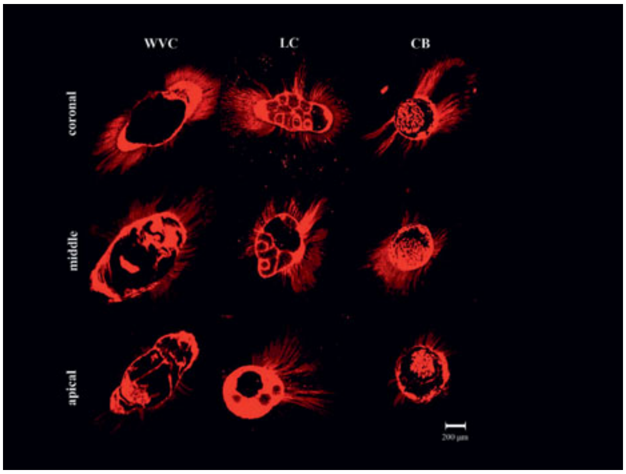

Fig 1 CLSM images representative of sealer penetration in the apical, middle and coronal region for each group.

nique, gutta-percha, which becomes fluent with the application of heat, adapts to root canal irregularities and the root canal walls[12,13].

The CB obturation technique involves the use of thermoplasticised gutta-percha as root obturation material. This material consists of a plastic or crosslinked gutta-percha carrier coated with α-phase gutta-percha[14]. In this technique, root canals are obturated using a homogeneous gutta-percha mass[15]. Selection of an appropriate obturation method to ensure hermetic obturation of the resorption area in teeth with IRR, which is difficult to manage clinically, is important with regard to the treatment prognosis[16,17]. In the present study, we investigated the effect of different obturation techniques on root canal sealer penetration in teeth with IRR.

The aim of this study was to investigate the influence of different obturation techniques on sealer penetration in teeth with artificial internal root resorption through confocal laser microscopy (CLSM).

Materials and methods

The study design was approved by the local ethics committee (ethics committee no. 2019/191). Forty-five teeth with round-shaped canals and without root caries, resorption, fractures, cracks or root canal treatment were included in this study. After removing the crowns under water cooling, the root length was standardised to 15 ± 1 mm. Using a diamond disc, the roots were divided into two parts at a distance of 7 mm from the apex. To simulate the resorption defect on the surfaces facing each other of both parts, a resorption cavity (1.5 mm deep and 1.2 mm in diameter) was prepared using a low-speed diamond cylindrical bur[9]. Two sections of each sample were joined from the outer root surface using flowable composite resins without using a dental bonding agent so that they were in the initial position, then the LED light was used for polymerisation of the composite. The working length was determined using a #10 K-type file (Dentsply Maillefer, Ballaigues, Switzerland) that was 0.5 mm shorter than the apex. After the teeth were embedded in the alginate (Blueprint, Denstply Sirona, Charlotte, NC, USA) to simulate the resistance of periodontal tissues, the root canals were prepared using ProTaper Next Rotary X1, X2, X3 and X4 files (Dentsply Maillefer)[18,19]. The root canals were irrigated with 2.5 ml 2.5% NaOCl (Wizard; Rehber Kimya, Istanbul, Turkey) after each file change, then 5 ml 2.5% NaOCl, 5 ml 17% ethylenediaminetetraacetic acid (Wizard; Rehber Kimya) and 5 ml distilled water were used for the final irrigation procedure. The procedure was carried out using a sideport irrigating needle (NaviTip; Ultradent, South Jordan, UT, USA). The root canals were dried with paper points (Diadent, Chongju, South Korea). The samples were randomly divided into three groups (n = 15) according to the root canal obturation technique used.

For the LC group, gutta-percha (ISO 40, 0.2 mm; Diadent) was coated with a sealer (AH Plus; Dentsply DeTrey, Konstanz, Germany) labelled with 0.1% rhodamine B dye (Sigma Aldrich, St Louis, MO, USA) and placed in the canal. Using 25, 20 and 15 finger spreaders (Dentsply Maillefer), gaps were created in the root canal and accessory cones were then placed. The canal was deemed to be sufficiently obturated when no more spreaders could be placed in the canal orifice[10].

For the WVC group, ProTaper X4 gutta-percha (Dentsply Maillefer) was coated with AH Plus sealer, labelled with 0.1% rhodamine B dye (Sigma Aldrich Co.) and placed in the canal. A Buchanan Heat Plugger Fine 0.04 taper (Kerr Dental, Orange, CA, USA) connected to the down-pack unit of an elements Free Obturation System (Kerr Dental) was adjusted to be 4 mm shorter than the working length. The down-packing was carried out by advancing the Buchanan Heat Plugger Fine 0.04 taper in an apical direction. The elements Gutta Percha Cartridge (Kerr Dental) was heated to 200°C. The remaining root canal space was gradually obturated with gutta-percha using the back-fill unit (Kerr Dental). A size 9 posterior Schilder plugger (Dentsply Maillefer) was used to condense the gutta-percha in an apical direction.

For the CB group, using a ISO 40, 0.2 mm paper point (Diadent), the canal walls were covered with AH Plus sealer and labelled with 0.1% rhodamine B dye. A size 40 Thermafil Plus obturator (Dentsply Sirona) was prepared in a ThermaPrep 2 Oven (Dentsply Sirona)

Table 1 Mean values and standard deviations (SD) of the sealer penetration depth (mm) into the dentinal tubules.

Region	WVC	LC	CB
Apical	0.232 ± 0.188^{Aa}	0.508 ± 0.400^{Aa}	1.096 ± 0.606^{Ab}
Middle	1.279 ± 0.743^{Ba}	2.225 ± 0.636^{Ab}	1.616 ± 0.650^{ABab}
Coronal	1.330 ± 0.538^{Ba}	2.454 ± 0.479^{Bbc}	2.003 ± 0.474^{Bb}

Different superscript letters indicate statistically significant differences between groups (*P* < 0.05) (upper case columns, lower case rows).

Table 2 Mean ± standard deviation (SD) of the sealer penetration area (mm2) into the dentinal tubules.

Region	WVC	LC	CB
Apical	4112.62 ± 607.91^{Aa}	5213.22 ± 141.08^{Aa}	7875.91 ± 351.59^{Ab}
Middle	60878.06 ± 1065.35^{Ba}	103969.48 ± 3001.13^{Bb}	75702.06 ± 1820.45^{Bb}
Coronal	102339.44 ± 2782.70^{Ca}	109832.59 ± 4073.16^{Ba}	123552.64 ± 3586.06^{Ca}

Different superscript letters indicate statistically significant differences between groups (*P* < 0.05) (upper case columns, lower case rows).

according to the manufacturer's instructions and then placed in the root canal with gentle pressure for about 5 seconds until it reached the working length. The handles of the obturators were cut at the level of the canal orifice, and the gutta- percha was then condensed using a size 9 posterior Schilder plugger.

In all groups, the gutta-percha was cut 1 mm below the canal level and the cavity was closed with Cavit (ESPE, Seefeld, Germany). After the obturation procedures, periapical radiographs of each tooth (buccolingual and mesiodistal angles) were taken to confirm that there were no voids in the obturation. The samples were then incubated at 37°C in a 100% humidified medium for 2 weeks. Using a low-speed diamond disc (Buehler, Lake Bluff, IL, USA) at 200 rpm under cooling, 1.0 ± 0.1 mm sections were obtained from each sample from the apical (3 mm), middle (resorption area, 7 mm) and coronal (10 mm) regions[20,21]. The sections were polished under water using silicon carbide sandpaper.

Each sample was mounted on a glass slide and examined using CLSM (Nikon Eclipse C1; Nikon Canada, Mississauga, ON, Canada) with ×10 magnification. In cases where the entire canal could not be viewed in an image, images of sections were taken and then combined into a single image using Photoshop (Adobe Systems, San Jose, CA, USA). The maximum penetration depth (mm) and penetration area (mm^2) of the root canal paste in the images obtained were determined using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were performed using SPSS Statistics version 21.0 (IBM, Armonk, NY, USA). A Shapiro-Wilk test was performed to determine the normal distribution of the data. The data were analysed using a one-way analysis of variance and post-hoc Tukey test. The level of statistical significance was set at $P < 0.05$.

Results

In the CB group, the sealer penetration area was significantly larger in the apical region compared with that in the other groups ($P < 0.05$). In the resorption region, the sealer penetration area was significantly larger in the CB and LC groups compared with that in the WVC group ($P < 0.05$). In the coronal region, there was no significant between-group difference in sealer penetration area ($P > 0.05$).

In all groups, the sealer penetration depth in the apical region was significantly lower than that in the coronal region ($P < 0.05$) and the sealer penetration area in the apical region was significantly lower than that in the coronal region ($P < 0.05$). The sealer penetration depth in the apical region was significantly higher in the CB group as compared with that in the other groups ($P < 0.05$). The sealer penetration depth in the resorption region in the CB and LC groups was significantly higher than that in the WVC group ($P < 0.05$), as shown in Table 1. As illustrated in Table 2, the sealer penetration area increased significantly from the apical to the coronal region in the CB and WVC groups but not in the LC group ($P < 0.05$).

Discussion

In the present study, the effect of different obturation techniques on root canal sealer penetration was evaluated in teeth with IRR. The null hypothesis was rejected as there was a difference between the obturation techniques in terms of penetration of the root canal sealer. In clinical practice, the steam lock phenomenon occurs in the root canal system due to the difference in pressure between the root canal and the periodontal ligament during irrigation and preparation procedures. For this reason, various materials such as alginate, gelatine, saline solution, sponge, agar and polyvinyl siloxane have been used to mimic the resistance of periapical tissues in many in vitro studies[19]. In this study, considering the advantages such as ease of application and low cost, a closed-ended root canal model was created by embedding the teeth in polyvinyl siloxane impression material and simulating in vivo environmental conditions.

In the literature, different imaging methods have been used to evaluate root canal sealer penetration (light microscopy, scanning electron microscopy [SEM], stereomicroscopy and CLSM)[22,23]. A previous study reported that it was difficult to distinguish between sealer and dentine levels when evaluating root canal sealer penetration by light microscopy[24]. In SEM imaging, sample preparation requires multiple processing steps and procedures, such as dehydration, which can damage the sample[22]. Furthermore, the images obtained by SEM are only two-dimensional and only allow evaluation of limited areas of the root canal wall due to high magnification[25,26]. In this study, we used CLSM to evaluate tubular penetration of the root canal seal. CLSM permits histotomographic imaging and examination of the dentine at the subsurface level[25]. An important advantage of CLSM is that there is no need to dehydrate the samples and no artefact formation[27,28]. In addition, using CLSM, the distribution of sealer in dentinal tubules can be visualised at a high magnification and sections can be reconstructed[26,29,30]. To visualise root canal sealer with CLSM, a fluorescent dye is required. In a previous study, rhodamine B dye had no effect on the penetration of resin-based root canal sealer into dentin tubules[25]. Thus, in this study, AH Plus root canal sealer was labelled with rhodamine B dye.

In all the obturation techniques tested in this study, the penetration area and depth of the root canal sealers were greater in the coronal regions than the apical regions. In line with these findings, other studies that used CLSM to investigate root canal sealer penetration reported that it decreased from the coronal to the apical region[31,32]. This decrease in sealer penetration may be due to the decrease in the diameter and number of dentinal tubules in the apical region and increased sclerosis in dentinal tubules[14]. Another explanation may be that more of the smear layer obstructing tubules in root canals is removed in the coronal region than the apical region and that some areas of the latter are covered with cementum-like tissue[33].

In a literature review, we found no previous studies on the effect of different obturation techniques on sealer penetration of dentinal tubules based on a CLSM analysis of teeth with IRR. According to the results of the present study, the root canal sealer penetration area and depth in the apical region were greater in the CB group than the other groups. There was no difference in these parameters in the WVC group versus the LC group. Contrary to these findings, Kok et al[25] found no difference in sealer penetration of dentinal tubules in a CLSM analysis of the apical region in the CB and LC groups. In a CLSM analysis, Marciano et al[34] reported that sealer penetration in both CB and WVC groups in the apical region of mesial canals of mandibular molar teeth with moderate curvature was similar to sealer penetration in an LC group. Keles et al[5] reported no difference between volume percentages and voids of root canal obturation between the LC and WWC groups in the apical region in a microcomputed tomography analysis. Several studies found that the high pressure produced in the apical region increased the penetration of this thin layer of sealer into the root canal walls[33-35]. In the literature, a thinner layer of sealer was needed for CB-based obturation techniques[35]. Based on this idea, the CB obturation technique results in higher sealer penetration in the apical region as compared with that of other obturation techniques.

The present results revealed no difference between the CB and LC groups in terms of sealer penetration in the middle (resorption area) and coronal regions, and the penetration area and sealer depth were smaller in the WVC group as compared with that in the other groups. Similarly, Goldberg et al[35] detected no difference between the LC and CB techniques in terms of obturation efficacy based on stereomicroscopy and radiographic imaging of teeth with IRR. Contrary to the findings of our study, Gençoğlu et al[9] reported that WVC obturation performed in teeth with experimental IRR yielded better results than CB or LC. Marciano et al[34] reported that sealer penetration in the middle and coronal regions of mesial canals of mandibular molar teeth with moderate curvature was higher in an LC group than in a CB group using CLSM analysis. The divergence between the study results may be due to differences in tooth selection, resorption size, sealer selection, preparation size and evaluation methods.

Conclusion

Within the limitations of the present study, tubular penetration of root canal sealer in both the apical and resorption regions in teeth with IRR was higher using the CB technique than the WVC technique.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Zeliha UĞUR AYDIN contributed to the study design, supervision, statistical analysis and literature search; Drs İrem Cansu KARA, Gamze ER KARAOĞLU and Tülin DOĞAN CANKAYA contributed to the experiment, literature search and writing of the manuscript.

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Management of Crown-root Fracture with 180-degree Rotation Replantation: a Report of 2 Cases

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Intentional replantation involves a combination of periodontics, endodontics, prosthodontics and oral surgery. Crown-root fracture management is still complicated nowadays. A fracture line extending longitudinally to the subgingival area and intruding biogical width could affect infection control, gingival health and crown restoration. In the present study, we present two cases. Case 1 involved a 23-year-old man who presented at our hospital with crown-root fracture of the maxillary left central incisor. A radiographic image of the tooth revealed a fracture line under the alveolar crest. The fractured tooth was treated with intentional replantation with 180-degree rotation, root canal treatment and veneer restoration. The patient was followed up for 60 months. The replanted tooth functioned well, and no symptoms of resorption or ankylosis were observed by radiographic examination. Case 2 involved a 20-year-old woman who was referred to our hospital for crown-root fracture of her maxillary teeth. A radiographic examination revealed complicated crown-root fracture of the maxillary right lateral incisor and both maxillary central incisors. The central incisors were treated with intentional replantation with 180-degree rotation. At the 48-month follow-up, the fractured teeth were found to have regained normal function based on clinical and radiographic examination. Limited case reports are available on a long-term follow-up of intentional replantation with 180-degree rotation. These two cases, particularly case 2, presented optimal healing after 4 years with unideal crown–root ratios. This case report suggests that this old method of preserving teeth with crown-root fractures can be used as a last resort to save teeth owing to its timesaving and microinvasive procedure.

Key words: *180-degree rotation, crown-root fracture, intentional replantation*
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Maxillary anterior teeth are prone to dental trauma[1]. Crown-root fractures account for 5% of dental injuries. The fracture lines always appear from the crown on the labial side to the subgingival palatal region, travelling longitudinally across the pulp chamber. In most patients with crown-root fracture, tooth extraction is advised due to invasion of the biological width; however, in some cases with a considerable crown–root ratio, the fractured tooth can be saved using methods such as crown lengthening, orthodontic extrusion and intentional replantation.

Crown lengthening surgery with an apically positioned flap and alveolar bone removal is a minimally invasive procedure; however, it involves the adjacent teeth and potential gingival recession, which may give unfavourable results in the aesthetic zone. Orthodontic extrusion is a relatively safe procedure, but it is time-consuming and orthodontic relapse is possibly inescapable due to stretching of the periodontal fibres. Intentional replantation is also known as intra-alveolar transplantation. Currently, the survival rate of intentional replantation is 88% to 95%[2]. In general, intentional replantation is indicated when periapical surgery requires extensive bone removal or in cases with odontogenic maxillary sinusitis and suspected root fracture. In recent years, a modification of intentional replantation with 180-degree rotation has been proven feasible to preserve teeth with deep subgingival fractures. Several authors have reported many cases of suc-

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cessful intentional replantation with 180-degree rotation with a follow-up period of 3 months to 3 years[3-6]. Kim et al reported a case of successful intentional replantation with a 90-month follow-up[7]. Thus, this old technique has been re-evaluated. Limited data are available regarding the success rate of this procedure, with the major concerns being root resorption, ankylosis and inflammatory response.

This case report presents two cases with promising prognosis at a 4- and 5-year follow-up, providing new insights into this longstanding technique.

Case 1

A 21-year-old man was referred to the present authors' hospital for pain after having injured his maxillary teeth in an accident while playing basketball the day before. He claimed that he did not experience nausea, tiredness or dizziness after the trauma. The patient's dental, medical and family histories were non-contributory. An extraoral examination revealed no abnormality concerning the temporomandibular joint. An intraoral examination revealed that the maxillary right central incisor had an enamel defect with mild percussion tenderness and mild mobility. The maxillary left central incisor had a fracture line 3 mm above the labial gingival margin, extending to the palatal subgingival side. The fractured part was mobile and attached with gingiva. After removal of the fractured fragment, the palatal tooth margin extended apically 2 mm under the alveolar crest (Figs 1a to d). Radiographic examination revealed a complicated crown-root fracture of the maxillary left central incisor and crown fracture of the maxillary right central incisor (Fig 2a). The maxillary right central incisor was treated with a composite resin restoration. Multiple treatment options were available for the maxillary left central incisor, including crown lengthening, orthodontic extrusion and intentional replantation. After being informed of the risks and benefits of each of the available treatment options, the patient chose intentional replantation by repositioning the root against the socket with 180-degree rotation for the maxillary left central incisor (buccolingual reverse).

Under local anaesthesia, the maxillary left central incisor received pulp extirpation with NiTi instruments, irrigation with 1% sodium hypochlorite and intracanal medication with iodoform calcium hydroxide, then the access cavity was filled with glass ionomer (Fig 1e). The maxillary left central incisor was extracted carefully using forceps, avoiding injury to the alveolar crest and pericementum. The root surface was kept wet with saline and examined under a microscope to rule out suspected root fracture. The blood-filled socket was well protected from saliva contamination using a cotton roll. The prospective crown–root ratio of the maxillary left central incisor after the operation was 0.8. After repositioning the tooth in its socket with 180-degree rotation with slight extrusion, the initial degree of mobility of tooth 21 was 3 degrees, and the labial fracture margin was parallel to the gingival level. The maxillary left central incisor was fixed using steel wire and resin (Figs 1f to h). The patient was advised to avoid biting with his maxillary anterior teeth until the next visit and was prescribed chlorhexidine mouthrinse and antibiotics for 3 days. Instant radiography images showed a wedge-shaped shadow around the apex (Fig 2b). The ligature was removed 10 days after replantation and the degree of mobility of the maxillary left central incisor was 1 degree. After 1 month, the maxillary left central incisor exhibited physiological mobility and was passive to palpation and percussion. It underwent root canal obturation and temporary resin veneer restoration was performed 3 months later. The maxillary right central incisor was subjected to aesthetic resin repair. The wedge-shaped shadow was repaired radiographically at the 3-month follow-up (Fig 2c). After 7 months, porcelain laminate veneer was fabricated for the maxillary left central incisor (Figs 1i to j). The pericementum was continuous and complete at the 7-month follow-up (Fig 2d). At the 12-, 18-, 24- and 60-month follow-ups, the patient was asymptomatic and no symptoms of root resorption or ankylosis were observed radiographically, and the restoration functioned well (Figs 1k to l and 2e to h). Additionally, the supernumerary tooth in the maxillary anterior region without clinical symptoms was suggested to be followed up.

Case 2

A 20-year-old woman presented to our hospital with an injury to her maxillary teeth due to a car accident 2 hours before. The patient reported no other discomfort such as nausea, tiredness or dizziness after the trauma. Her dental, medical and family histories were non-contributory. Clinical and radiographic examinations revealed crown-root fractures of the maxillary right lateral incisor and the maxillary central incisors. Fracture lines were parallel to the gingival margin on the labial side and the subgingival margin on the palatal side (Figs 3a and b and Fig 4a). The risks and benefits of various treatment options were explained to the patient, and she chose intentional replantation with root repositioning against the socket with 180-degree rotation (buccolingual reverse) for the maxillary central incisors. All

Fig 1 **(a)** Pretreatment labial view of the maxillary central incisors. **(b)** Pretreatment palatal view of the maxillary central incisors. **(c)** Occlusal view after crown extraction of the maxillary left central incisor. **(d)** Clinical photograph of removed crown. **(e)** Clinical photograph after pulp extirpation and temporary filling with glass ionomer. **(f)** Tooth extraction using forceps. **(g)** Evaluation of the extracted root. **(h)** Splinting after intentional replantation with 180-degree rotation and slight extrusion of the maxillary left central incisor. **(i)** Labial view after veneer restoration at the 7-month follow-up. **(j)** Palatal view after veneer restoration at the 7-month follow-up. **(k)** Labial view of the replanted tooth at the 60-month follow-up. **(l)** Palatal view of the replanted tooth at the 60-month follow-up.

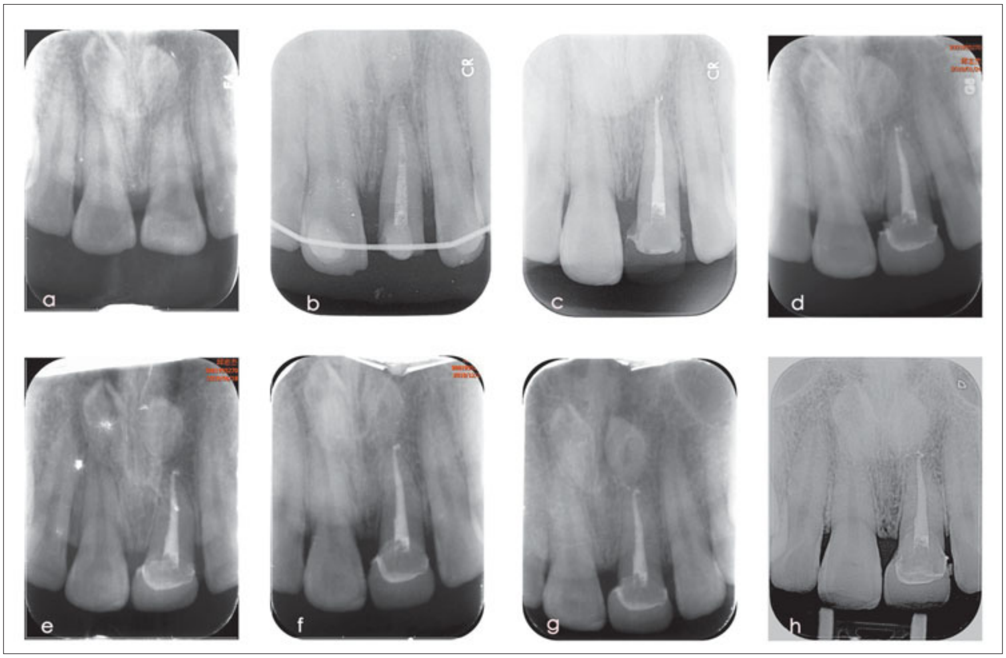

Fig 2 **(a)** Radiograph taken at the time of injury. **(b)** Radiograph taken after replantation and splinting showing a wedge-shaped shade around the apex. **(c)** Radiograph taken after root canal therapy at the 3-month follow up. **(d)** Radiograph taken after veneer restoration at the 7-month follow up. **(e)** Radiograph taken at the 12-month follow up. **(f)** Radiograph taken at the 18-month follow up. **(g)** Radiograph taken at the 24-month follow up. **(h)** Radiograph taken at the 60-month follow up.

three teeth would undergo root canal therapy followed by crown restoration.

Under local anaesthesia, pulpectomy was performed on the three teeth. Root canals were prepared using NiTi instruments and irrigated with 1% sodium hypochlorite and saline, then sealed with glass ionomer. The maxillary central incisors were removed carefully using forceps and examined closely under a

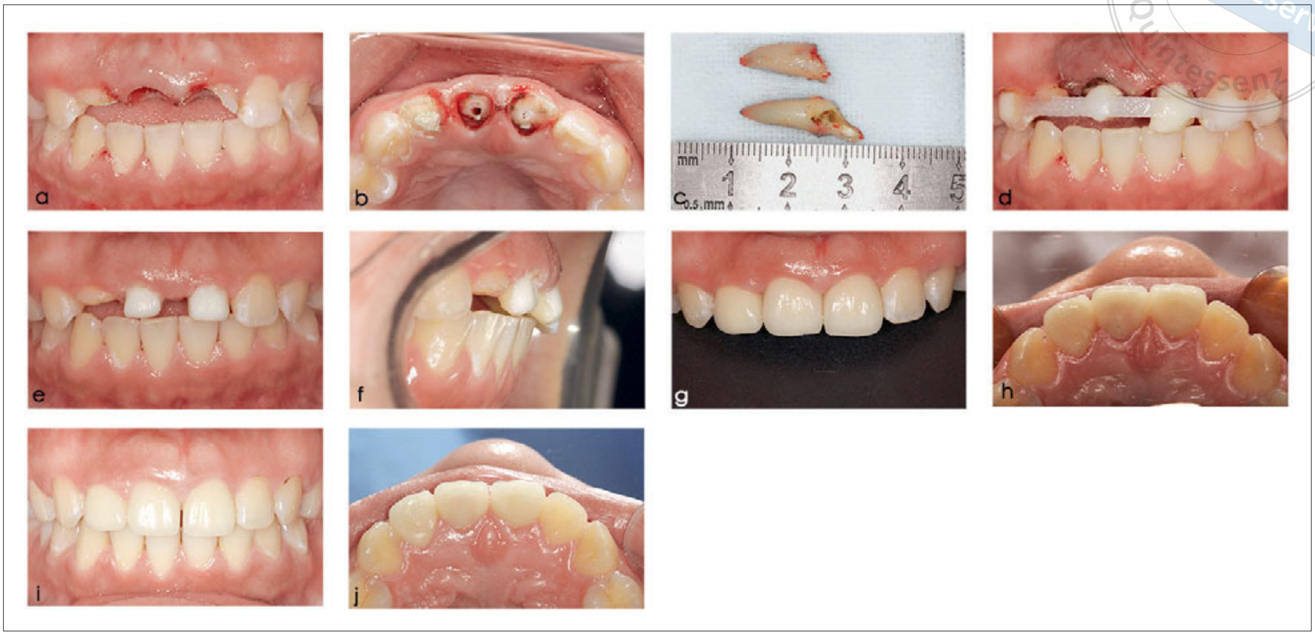

Fig 3 **(a)** Pretreatment labial view of the maxillary right lateral incisor and central incisors. **(b)** Pretreatment occlusal view: the maxillary right lateral incisor had a palatal fracture line parallel to the palatal gingival margin, the maxillary right central incisor had a palatal fracture line 7 mm under the palatal gingival margin and the maxillary left central incisor had a fracture line 5 mm under the palatal gingival margin. **(c)** Evaluation of the extracted roots of the maxillary central incisors: the prospective crown–root ratios after the operation were 1.25 and 1.00 for the maxillary right and left central incisors, respectively. **(d)** Splinting after intentional replantation with 180-degree rotation and slight extrusion of the maxillary central incisors. **(e)** Clinical photograph after splinting removal. **(f)** No occlusal contacts after splinting removal of replanted teeth. **(g)** Labial view after crown restoration at the 4-month follow up. **(h)** Palatal view after crown restoration at the 4-month follow up. **(i)** Labial view at the 48-month follow-up. **(j)** Palatal view at the 48-month follow-up.

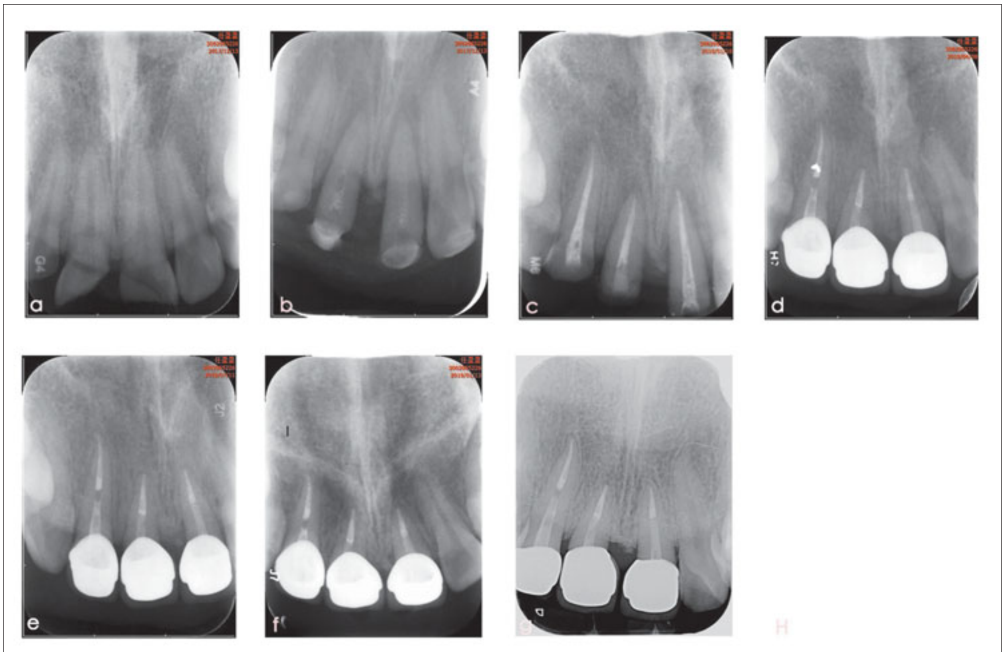

Fig 4 **(a)** Radiograph taken at the time of injury. **(b)** Radiograph taken after replantation showing a wedge-shaped shade around the apex. **(c)** Radiograph taken at the 1-month follow-up. **(d)** Radiograph taken after crown restoration at the 4-month follow-up. **(e)** Radiograph taken at the 7-month follow-up. **(f)** Radiograph taken at the 13-month follow-up. **(g)** Radiograph taken at the 48-month follow-up.

microscope to rule out suspected root fractures. The blood-filled socket was well protected from saliva contamination with a cotton roll. After repositioning the teeth in their respective sockets with 180-degree rotation and slight extrusion, the initial degree of mobility of the maxillary central incisors was 3 degrees and the labial fracture margin was parallel to the gingival level. The replanted teeth were stabilised with fibreglass splints (Figs 3c and d). The patient was advised to follow a soft diet until the next visit and was prescribed chlorhexidine mouthrinse and antibiotics for 3 days. Instant radiographic images showed an obvious trilateral radiolucent area around the apex (Fig 4b). After 10 days, the splints were removed. Mild mobility was detected for the maxillary central incisors (Figs 3e and f). One month after replantation, both teeth exhibited physiological mobility and root canal treatment was completed (Fig 4c). Post-core crown restoration was performed 4 months after replantation (Figs 3g and h and 4d). At the 7- and 13-month follow-ups, the patient was asymptomatic. Radiographic images showed complete apical healing of the replanted teeth and slightly cervical external root resorption on the distal surface of the maxillary right central incisor (Figs 4e and f). External root resorption appeared to have stopped by the 48-month follow-up (Fig 4g). Intraoral examination at 48 months revealed a gap between the maxillary central incisors (Figs 3i and j), which may have been related to the suboptimal crown–root ratio and occlusal trauma.

Discussion

In general, intentional replantation is deemed successful if radiographic observation indicates a complete continuous periodontal membrane with no signs of resorption or ankylosis; if there is no discomfort, palpation or percussion; and if there are no signs of infection or inflammation such as a sinus tract, swelling or deep periodontal pocket[8].

Typically, trauma to the periodontal ligament is the main reason for root resorption and root ankylosis[9]. External surface resorption is defined as pressure-related resorption, and it is noninfective and self-limiting[10]. Nonprogressive root resorption is a common occurrence after replantation, which is observed in 30% of cases (95% confidence interval 25%–37%) according to a recent literature review[11]. The extraction procedure causes injury to the root surface as well as the periodontal cells. After the initial inflammatory reaction, recolonised surrounding periodontal cells could repair this new surface; thus, root resorption is also described as a repair-related process[12,13]. Root ankylosis can be seen as a subsequent replacement of tooth tissue by bone after resorption. The bone tissue laid down by osteoblasts as part of the repair process can replace the resorption area, which results in ankylosis. Ankylosis is more likely to occur when the periodontal ligament defect is > 4 mm^2, where the bone cells can attach to the root surface faster than periodontal cells[14]. Progressive root resorption and ankylosis are minor events. Appropriate medication could arrest the resorptive process. Calcium hydroxide retards resorptive cells and promotes healing[15]. In Case 2, root resorption of the maxillary right central incisor was seen at the 7- and 13-month follow-ups, presenting an irregular-shaped area around the distal root borders. Radiographic observation at the 48-month follow-up revealed that the distal root surface of the maxillary right central incisor had been repaired, and the initial resorption process was proven to be nonprogressive. The intrusion or prolonged extraoral time can cause an irreversible defect that increases the possibility of ankylosis and root resorption. The common ground on this issue is to minimise extraoral time as much as possible. Some authors have suggested that extraoral time > 15 minute has a negative impact on the re-establishment of periodontal cells, which is consistent with previous studies[16,17].

Traditionally, passive and flexible splinting is recommended to promote periodontal healing for a replanted tooth. Research has verified that slight mobility and function after replantation can promote periodontal healing[18]. Physiological splinting, rigid splinting and non-splinting are applicable for the replanted tooth. In these two cases, wire with resin for rigid fixation and fibreglass for physiological fixation both seemed to provide a promising prognosis. When non-traumatised inflammation of the periodontal ligament is minimal, the periodontal ligament cells can differentiate properly and reform a normal structure of the pericementum. The time required for reattachment of the epithelium at the cementoenamel junction is approximately 1 week. The recommended splinting time varies among studies from 2 to 6 weeks according to stability after replantation[19]. Recent studies have demonstrated that successful periodontal healing is probably unaffected by splinting time[20].

With the increased promotion of dental implants nowadays, crown-root fractured teeth are likely to be extracted. In the present two cases, which were followed up for a short time, intentional replantation with 180-degree rotation proved to be an effective option. It reduced clinical time, complications and expenses compared with other options. Additionally, in these two cases, slight resorption was observed on the alveolar crest radiographically. In case 2 specifically, an interval was observed between the maxillary central incisors, and this may be related to the suboptimal crown–root ratio and alveolar crest resorption.

Intentional replantation is not recommended in conditions such as with nonrestorable dental caries or defects, severe periodontal disease, curved roots with the possibility of fracture during extraction and ankylosed teeth[8]. Good case selection and appropriate handing could confer good results. Long-term follow-up of replanted teeth is recommended.

Conclusion

Intentional replantation with 180-degree rotation is an optional treatment for complicated tooth fracture and could provide a good long-term prognosis under appropriate management.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Lin YANG and Qiang LIU contributed to the conceptualisation, data collection, writing, review and editing of the study; Drs Ming Wen LIU, Fan GU and Zi Jun WANG contributed to the data collection; Drs Yan ZUO and Yao LI contributed to the review and editing; Prof Bin PENG contributed to the supervision of the complete study.

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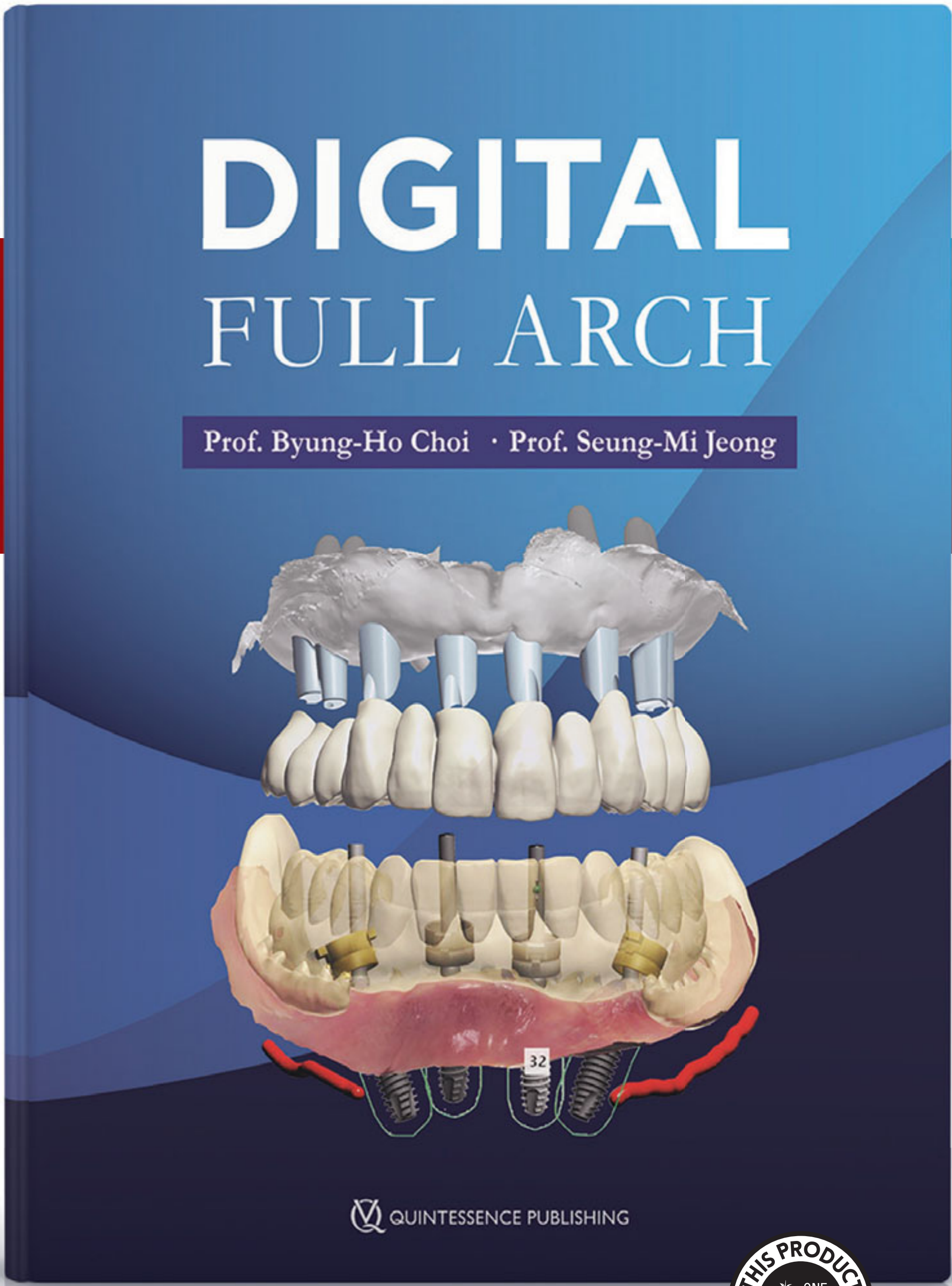

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