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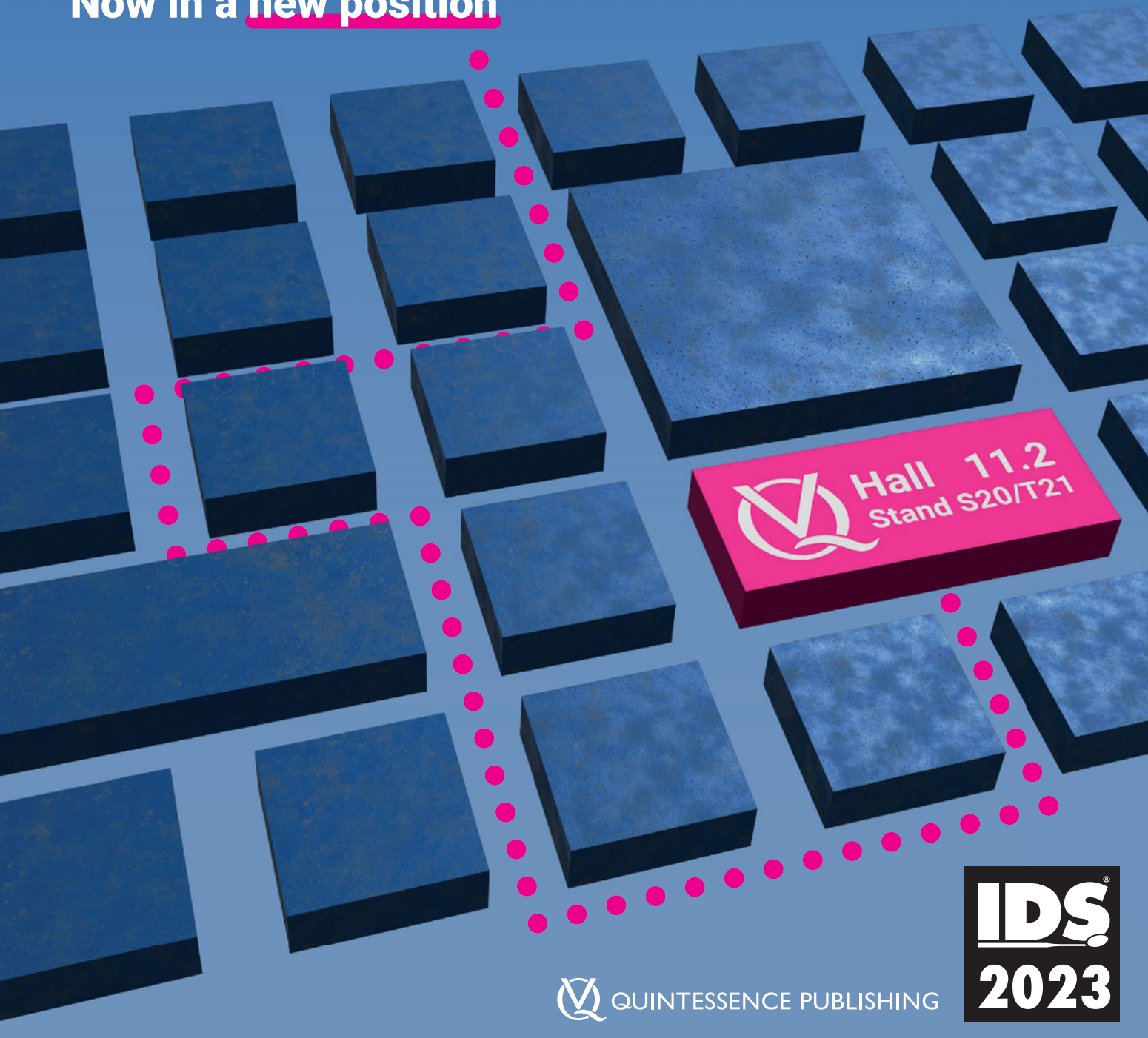

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Status Quo and Advanced Progress in Oral Health Care and Treatment of Children with Autism Spectrum Disorder: A Literature Review

Lu GAO[1], Xue Nan LIU[2]

Autism spectrum disorder (ASD) has become one of the fastest growing diseases in the world, causing a great burden to ASD children's families and society. Children with ASD face more disadvantages relating to their oral health than those without ASD. There is a positive correlation between prevalence of caries lesions and severity of ASD. Poorer oral hygiene, higher detection rates of dental calculus and far more frequent cases of gingivitis occur in children with ASD. Traumatic injuries and various types of malocclusions are more frequent in children with ASD. Poorer oral health care and treatment status are caused by multiple adverse factors. Ways of promoting effective oral health care and treatment include pretreatment counselling; improvement of the individualised treatment environment; routine behaviour guidance techniques (BGTs) including tell-show-do, distraction, role model presentation, voice control, visual education and social stories, encouragement and reinforcement; targeted BGTs including visual education, behaviour modelling, applied behaviour analysis (ABA) and systematic desensitisation; passive BGTs including protective restraint, pharmaceutically administrated sedation and general anaesthesia; oral health education for guardians; and interdisciplinary collaboration and professional dental care/treatment. Dentists, families with children with ASD and schools should cooperate to improve family-centred oral health care and treatment for ASD children not only in China, but also the whole world.

Key words: *autism spectrum disorder, behaviour management, children, oral health care, promotion*
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Autism spectrum disorder (ASD) was first reported by Professor Kanner in 1943[1]. Researchers from various countries studied similar cases and continuously adjusted the diagnostic boundary criteria of ASD spectrum[2,3]. In the diagnostic and statistical manual of disorders[4], ASD was defined as a single category, whereas subcategories such as Asperger's syndrome and pervasive developmental disorder not otherwise specified (PDDNOS) were removed. ASD has become one of the fastest growing disorders in the world. In 2011, the prevalence rate in the United Kingdom was 1.6%[5], then in 2018, it was reported to have exceeded 1/50 in the United States[6]. In 2019, the prevalence rate exceeded 1/100 in China, and it continues to rise[2,7,8-12]. The pathogenesis of ASD is still not entirely clear. It causes a great burden to families of children with ASD and society.

Children with ASD face greater disadvantages concerning their oral health than those without. Multiple adverse factors lead to poorer oral health care and treatment status, including perioral muscle characteristics and eating habits, perioral paraesthesia, oral microbial environment and drugs, bad oral habits, self-injurious behaviours, communication skills and behavioural characteristics, and unbalanced medical service resource allocation[1,2,8,13,14]. Various generalised and specialised methods of effective oral health care in children with

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ASD have been explored and need to be optimised further in larger populations, such as pretreatment counselling, individualised treatment environments, routine behaviour guidance techniques (BGTs), targeted BGTs, passive BGTs, oral health education for guardians, interdisciplinary collaboration, and professional dental care/treatment[15-19]; however, the current situation and advanced progress on oral health care and treatment for children with ASD have not been updated, summarised or analysed.

This review sought to provide an overview of the current situation in oral health care and treatment of children with ASD, influencing factors of oral health status in children with ASD, and ways to promote effective treatment and daily oral health care in children with ASD.

Oral health status of children with ASD

Dental caries

There is a positive correlation between the prevalence of caries and the severity of ASD[2,13]. Compared with children without ASD, those with ASD have a higher incidence of caries[20]. A meta-analysis conducted by Da Silva et al[21] showed that the caries prevalence rate in children with ASD is around 60.6%. The caries prevalence of children aged 3 to 5 years and with ASD is 50% to 60% in some areas of China, which is close to the national average (the fourth Chinese Oral Health Epidemiological Survey found that the prevalence of primary teeth in 3-, 4- and 5-year-old groups was 50.8%, 63.6% and 71.9%, respectively[22]). Insufficient frequency of tooth brushing and frequent consumption of sugary foods and drinks contribute most to this[2,8].

Periodontal status

Children with ASD have poor oral hygiene. The detection rates of gingival bleeding and dental calculus are significantly higher than those for children without ASD[14], and almost all children with ASD suffer from gingivitis[22,23]. The latter are also more likely to have bad breath and food impaction[8-10,12]. Insufficient daily oral health care, such as tooth brushing, and use of psychotropic drugs or antiseizure drugs (e.g., phenytoin sodium) are associated with the status[2,8,24].

Dental trauma

There is a significant difference in the prevalence of dental trauma between children with ASD and those without. Marra et al[25] found that girls with ASD (57%) suffer more traumatic injuries than boys with ASD (43%). The comparison of different dental trauma types (enamel fracture, enamel/dentine/pulp fracture, root fracture, avulsions) and different soft tissue lip injury locations (upper and lower lip) for the ASD group and non-ASD group showed a statistically significant difference ($P < 0.05$)[25]. The lower lip was found to be injured more often than the upper lip ($P < 0.005$). Furthermore, the long-term extraction rate of traumatic teeth in children with ASD is higher than that in those without ASD[25]. Oral self-injurious behaviours in children with ASD often lead to traumatic ulcers and tooth loss[26]. In addition, children with ASD are prone to tooth grinding, tongue thrusting and chewing/swallowing foreign bodies, resulting in an increased risk of damage to the oral soft and hard tissues[27,28].

Malocclusions

Self-injurious behaviours and unhealthy oral habits (bruxism, tongue thrusting, lip biting, thumb sucking and object chewing) contribute to the incidence of relevant malocclusions in children with ASD[29,30]. Skeletal and dentoalveolar deformation may occur due to the frequency and severity of the behaviours and habits[31]. There is no specific type of malocclusion for children with ASD, though a tendency towards increased horizontal overlap, increased anterior open bite, increased posterior reverse articulation and a deeper palatal vault has been observed[31-33].

Influencing factors of oral health status in children with ASD

Influencing factors of oral health

Perioral muscle characteristics and eating habits

Dietary habits, oral hygiene habits and oral environmental factors of children with ASD affect their oral health care and promote caries lesions and gingivitis. Children with ASD have greater perioral muscular tension and poorer tongue flexibility, resulting in insufficient coordination of chewing and swallowing movements, which leads to food being contained in the mouth. They also prefer soft and sticky foods, which are likely to become attached to the teeth. In addition, desserts are often used as a reinforcement for behavioural rehabilitation training, leading to increased sugar consumption and greater risk of caries lesions[1,2,8-10].

Perioral paraesthesia

Perioral paraesthesia occurs in more than 90% of children with ASD and is mainly manifested as hypersensitivity. The physical stimulation of tooth brushing or taste stimulation of toothpaste can cause discomfort and activate the gag reflex, increasing the risk of toothpaste being swallowed by accident. Children with ASD with poorer hand mobility and comprehension skills find it difficult to complete daily tooth brushing[2,8,9,12].

Oral microbial environment and drugs

Studies have found that compared to children without ASD, the oral flora of children with ASD consist of more periodontal pathogens, leading to a stronger gingival immune inflammatory response. In addition, children with ASD children often also experience epilepsy, depression and other symptoms and thus require long-term use of psychotropic drugs that affect the amount of saliva produced and flow rate, impairing oral self-cleaning and increasing susceptibility to caries; however, more evidence is required to establish the effect of psychotropic drugs on caries risk[34-36].

Bad oral habits

The higher prevalence of malocclusions in children with ASD is due to bad oral habits such as tongue thrusting and mouth breathing. The main types of malocclusion are maxillary transverse deficiency, open bite or deep vertical overlap, mandibular retrusion and dental crowding. These malocclusions affect children's facial aesthetics, as well as their physical and mental health development. In addition, low awareness of how to protect themselves leads to an increased risk of anterior dental trauma in children with ASD with protruded anterior teeth. Furthermore, malocclusion can be accompanied by airway obstruction and sleep apnoea symptoms, increasing the adverse effect on growth and development[1,8,13,25].

Self-injurious behaviours

Children with ASD, especially those with impaired linguistic ability, have a significantly higher incidence of self-injurious behaviours. This may be due to their inability to convey physical pain. More than 75% of the injury sites consist of the head and neck regions, and the behaviours are manifested as stabbing of the gingival soft tissues with the fingers or foreign bodies, lip and cheek biting, and even extraction of teeth by children themselves[37-39].

Influencing factors of treatment

Communication skills and behavioural characteristics

Children with ASD have poor communication skills, and it is difficult for them to express their feelings and describe their oral symptoms in a timely manner. Guardians often focus on the children's behavioural correction and rehabilitation training while ignoring their oral health status and appropriate timing of treatment.

Behavioural characteristics of children with ASD are the main factors affecting the treatment of oral diseases. These children are highly sensitive to sound, light, smell, touch and unfamiliar environments. Their dental fear is more severe than that of children without ASD. They exhibit multiple self-stimulating behaviours, including twisting hands, flapping arms, crying and other repetitive movements, and trying to escape from the treatment environment. They may even damage objects or hurt others by scratching, biting, kicking or head hitting. If there is no effective way to comfort them, these children are prone to injuring themselves and others, and indeed compromising the diagnosis and treatment process[13,14,39,40].

Unbalanced medical service resources

In China, the number of professional paediatric dentists is inadequate, medical service resources are unbalanced[2,9,10] and paediatric stomatologists often lack behaviour management training for children with ASD. Families of children with ASD devote most of their energy and financial resources to behavioural correction and rehabilitation training, meaning they have limited energy and financial resources to spend on oral health care and treatment. In addition, ignorance and implicit discrimination still exist towards children with ASD and their families. All these factors increase the difficulties medical treatment poses for children with ASD[2,8-13].

Promotion methods of effective treatment in children with ASD

Pretreatment counselling

Prior to treatment, guardians of children with ASD should communicate with stomatologists to prepare for the treatment process and be able to cooperate with doctors about the necessary behaviour management. Guardians should provide information about their children's unique preferences and specific behaviours; this is of great value for doctors in developing personalised be-

haviour guidance methods. Professionals should learn about the children's previous diagnoses and treatment history for oral diseases and concomitant diseases, developmental age and status, life skills, language expression level and reading ability[2,41].

Treatment environment

The treatment environment should be changed based on the needs of children with ASD to relieve anxiety and reduce resistance behaviours so they can gradually become familiar with their new environment and adapt to it. An independent waiting area and single treatment room should be reserved to ensure the same environment for each visit. Relaxing and slow music or white noise can be added, and lighting conditions should be improved[2,42].

Routine BGTs

BGTs are crucial to ensure a smooth treatment process and are used widely in paediatric dental treatment, including tell-show-do (TSD), distraction, role model presentation, voice control, encouragement and reinforcement[17,18,43-45]. Children with ASD often experience attention and comprehension deficit; thus, the effect of single BGT methods is limited, and it is often necessary to combine multiple methods in real situations[19,45].

Encouragement and reinforcement is an important and necessary BGT (especially for children aged from 3 to 6 years) to improve cooperation of in children with ASD[46]. Forms of reinforcement include doctors' pronunciation and intonation, language content, facial expressions, body movements and material objects. For children with ASD with understanding and attention deficit, simple linguistic encouragement (e.g. "you're great!") is not sufficient. Doctors can choose tangible rewards based on children's preferences. The frequency and timing of rewarding also influence the reinforcement effect. Too high a frequency will reduce the effect, whereas too low a frequency will fail to counter the effect of negative stimuli. Specific methods should be used according to the specific situation[45].

Targeted BGTs

Targeted BGTs can be used to improve the quality and efficiency of treatment. Individualised BGTs should be adopted based on the specific situation, and a combination of techniques can be applied. Persistence of guardians and oral health professionals is key.

Visual education

Children with ASD are often visual thinkers. As such, visual education methods are effective for them. By conveying information through video and images, visual education helps children with ASD to learn relevant skills[45]. They and their guardians can learn from pictures and videos of dental clinical processes to repeatedly exchange information with the outside world and become familiar with the treatment environment[19].

Narzisi et al[47] used MyDentist software (Bilbao, Spain) to provide rich and personalised image and video material for children with ASD. With its social story models and relaxing games relating to oral disease treatment, this software helps children with ASD become familiar with the treatment process[47].

In addition, with the development of information technology, the application of virtual reality (VR) technology is increasing; however, VR technology is not used widely in the treatment of oral diseases. It serves primarily to create a safe and progressive virtual environment using music, videos and game scenes so as to distract attention, make ASD children more relaxed and better adapt to the treatment environment. At present, the equipment requirement threshold and technology sensitivity of VR limit its wide application. In addition, its ethical significance should be considered fully[18].

Behaviour modelling

Behaviour modelling is based on the rigid repetitive behaviour characteristics of children with ASD. By using certain ingrained behaviours or habits, this method can help control the dietary habits and daily oral health behaviours of children with ASD. The effect of this method largely depends on long-term training implanted by guardians; however, once a good behaviour pattern is formed, daily oral health care will be simplified greatly[45].

Applied behaviour analysis

Applied behaviour analysis (ABA) is a successful psychological treatment approach for patients with ASD and has been validated for its effect of improving various behaviours. By dividing complicated target tasks into simpler ones and training patients with ASD step by step using the appropriate reinforcements, this method helps such patients finally complete complicated target tasks. ABA has been used successfully to improve the performance of children with ASD when sitting still and during intravenous injection, physical examination, oral

examination, pit and fissure sealing and periapical radiograph examination[46].

The following steps can be used for ABA training: repeatedly shining a light into children's mouth with a flashlight or mobile phone torch and helping children learn to open and close their mouth appropriately using demonstration if necessary; gradually illuminating children's teeth with oral mirrors and counting their teeth with probes; and helping children to sit in dental chairs, encourage them to contact treatment tools. Attention should be paid to any changes in children's facial expression and reinforcers should be used in a timely manner[19]. It should be noted that when unexpected behaviours occur, the best approach is to ignore them and wait for children to stop these behaviours[19,48].

Systematic desensitisation

The basic principle of systematic desensitisation is reciprocal inhibition, helping patients weaken and eventually cut the connection between stimuli and their anxiety responses[49]. Step-by-step clinical activities help children with ASD to overcome their fear of the clinical environment and treatment process. Systematic desensitisation can also be combined with other BGT methods.

Researchers at the Affiliated Stomatology Hospital of Tongji University used systematic desensitisation and found it to have an obvious effect on the treatment of children with ASD[45]. Most of the children trained made significant improvements in their cooperation[45]. Xia[50] focused on oral tactile disorders in children with ASD (sensory input disorders relating to the oral cavity, vestibule and skin), helping them to reduce oral sensory sensitivity and improve oral sensation through systematic desensitisation.

Passive behaviour control techniques

Protective restraint

When facing emergencies or prior to procedural sedation, children with ASD may need to be placed in protective restraints to prevent uncontrolled body movements that could endanger their own safety and that of others.

Use of protective restraints for children with ASD is now controversial. A study showed that pressure on the body can help children with ASD remain emotionally stable (deep pressure theory), and appropriate physical pressure can provide a calm and comforting effect[51]. Medical professionals should take the time to explain the method and process to guardians and receive informed consent prior to using protective restraints. During the treatment process, close attention should be paid to the tightness of restraints to avoid unforeseeable damage due to movements children may make while struggling[2]. For children with ASD who are older and stronger, it is difficult to use protective restraints[52,53].

Pharmaceutical administrated sedation

Comfortable treatment techniques including local anaesthesia, nitrous gas sedation and treatment of oral disease under general anaesthesia are a developing trend in dental treatment, especially in paediatric dentistry, providing new opportunities for children with difficulties to cooperate with routine treatment procedures[2,45].

Nitrous oxide (NO) sedation is the most convenient sedation method that can be controlled by dentists with a fast effect, rapid metabolism and no burden on the child's body[54]. A study showed that the success rate of treatment with 50% NO in children with ASD reaches 87.5%[55]. However, other studies have shown that children with ASD with 5,10-methylenetetra hydrofolate reductase (MTHFR) gene mutation suffer from folic acid metabolism disorder, and thus can die from long-term exposure to high-concentration NO under general anaesthesia, indicating that MTHFR deficiency is a contraindication for NO sedation[56,57]. Currently, the feasibility or effect of NO sedation in oral disease treatment of children with ASD has not been validated fully, but there is no evidence of it having a fatal impact.

It has been reported that the combination of oral benzodiazepines (e.g., diazepam and midazolam) and NO sedation has a success rate of 77% to 100%[51,58,59]. Careful planning and evaluation should be carried out by qualified physicians[59]. Doctors should discuss the benefits and risks of NO sedation with guardians before making decisions[2,45].

General anaesthesia

ASD is one of the most common indications for oral disease treatment under general anaesthesia. When children with ASD cannot cooperate with sedation, general anaesthesia can help reduce treatment time and relieve the psychological burden on guardians[60]. Informed consent must be obtained and indications and contraindications must be controlled strictly[60]. Adverse events related to general anaesthesia in children with ASD include disruptive behaviour, postoperative vomiting, postoperative bleeding and postoperative seizures. The latter two are relatively rare[61]. Dentists should explain the ASD diagnosis, its complications and any potentially risky preoperative behaviours to anaesthesiologists to make a

perioperative presurgical plan[62]. Guardians and medical professionals should help desensitise children with ASD so they can adapt to the treatment process and hospital environment in advance[2,45].

Methods for promoting daily oral health care in ASD children

Visual education and social stories

Visual education and social stories have been used widely to help children with ASD learn various skills. Visual education has been described in the previous section.

Social stories are typically short stories that describe common social experiences and clarify what is happening in a particular situation and what the appropriate behaviours are[63,64]. They play an effective role in teaching children with ASD oral health care skills[65,66]. Du et al[15] combined visual education and social stories to help children with ASD learn how to master tooth brushing skills. The 119 preschool ASD children they studied showed significantly improved tooth brushing skills and oral health status after 3 to 6 months of training[15,44].

Visual education in various forms (pictures, video, software, etc.) can help preschool children and adolescents with ASD improve their oral health care skills. Effective observation and continuous training are critical to children's performance[43,44,67,68]. Children with ASD of higher intelligence will benefit more from the social story method because social stories require comprehension skills[64,65].

ABA

When faced with resistance from children with ASD, daily tooth brushing can be split into the following smaller tasks: massage and touch the child's head, neck, face, nose and lip; gently sweep the child's hard palate, buccal mucosa, gingiva and tongue with the toothbrush; and add water to the toothpaste, and gradually transfer to tooth brushing[19,48].

Oral health education for guardians

Guardians play a central role in the long-term oral health care of children with ASD. Oral health education for guardians can compensate for the information imbalance between families and oral professionals and help guardians adopt individualised methods to better deal with possible emergencies[69-71].

To overcome multiple difficulties, guardians explore a variety of methods with great patience, love and creativity, including:

- Making plans: ritualising the tooth brushing process and recording it on a calendar as a reminder, and providing a clear time frame by counting, singing or using a timer[16];
- Acting as a role model: the whole family complete oral health care at the same time or demonstrate for children with ASD, and use visual education materials[16];
- Incorporating amusement: creating a game situation when completing oral health care, and rewarding children with individualised reinforcers after oral health care[16].

In addition, ASD children may swallow toothpaste which can lead to risks. Thus, fluoride toothpaste with a tolerable taste and texture should be tried and selected. Fluoridated milk, mouthrinse and other fluoride oral care methods can also be considered[2].

Interdisciplinary collaboration and professional level improvement

Interdisciplinary cooperation will play an effective role in improving children with ASD's oral health care status. If children have fixed psychiatrists and rehabilitation therapists, oral professionals should discuss with them to evaluate the children's characteristics, habits and skill learning status in order to offer personalised treatment[66,72-74]. Although this method is time-consuming, as the number of psychiatrists and rehabilitation therapists grows, interdisciplinary collaboration will become more popular. The families of children with ASD hope for and require professional oral health care education and treatment[75,76]; thus, oral health care professionals around the world should work harder to boost further cooperation.

The promotion methods for effective treatment and daily oral health care for children with ASD have been developed by clinicians and parents in the last few decades, especially in more developed cities such as Shanghai and Hong Kong[15,44,45]. Nevertheless, the general status for children with ASD in China is still behind that of those in developed countries with more experience and greater exploration of different methods.

Further improvements should focus on family-centred oral health care education. Dentists, families of children with ASD and schools should cooperate more effectively to improve oral health care and help children with ASD of appropriate age receive timely oral

health care management, such as caries risk assessment, pit and fissure sealing and fluoride application. Furthermore, doctors' professional skills in treating children with ASD should be improved. Training in generalised and specialised BGTs and comfortable treatment techniques should be strengthened[2,8].

Conclusion

There is a positive correlation between prevalence of caries lesions and severity of ASD. Traumatic injuries and long-term extraction of traumatic teeth are more frequent in children with ASD, and various adverse factors cause them to have poorer oral health care and a poorer treatment status. Various generalised and specialised oral health care methods have been explored for children with ASD and need to be optimised further in the wider population. Dentists, families of children with ASD and schools should cooperate to enhance family-centred oral health care and oral health treatment services for children with ASD not only in China, but also in the whole world.

Conflicts of interest

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

Author contribution

Dr Lu GAO contributed to the literature collection and drafting the manuscript; Dr Xue Nan LIU supervised the study design and reviewed and revised the manuscript.

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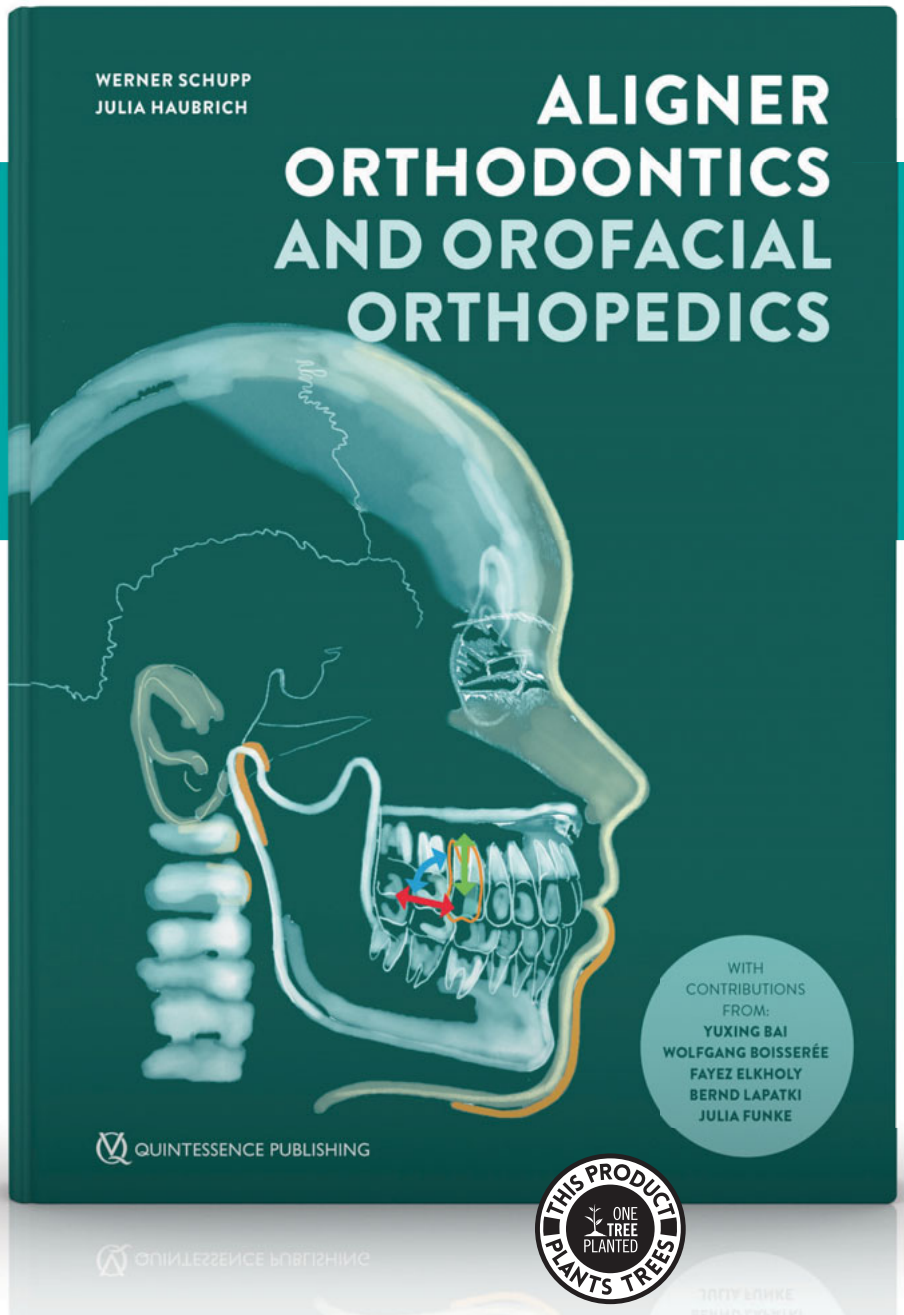

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Sappanone A Aggrandises Ionising Radiation–induced Damage in Oral Epithelial Cells

Xue Feng ZHAO[1#], Zhi Yong WANG[1#], Xue Mei QIU[1], Hang ZHAO[1], Rui LIU[1], Qian Ming CHEN[1]

Objective: *To analyse the role played by Sappanone A, a bioactive ingredient isolated from the heartwood of Caesalpinia sappan, in the regulation of oral epithelial cell viability under radiation.*

Methods: *Cell viability of human oral keratinocytes (HOKs) and mouse salivary gland cells under ionising radiation was analysed. Expression of Ki67 was measured by immunohistochemical staining. Fragmentation of deoxyribonucleic acid (DNA) was measured by comet assay. Cell death was analysed using trypan blue exclusion assay. Cell viability was measured using a Cell Counting Kit 8 (CCK8; Abcam, Cambridge, UK) assay.*

Results: *Sappanone A decreased cell viability of HOK cells and mouse salivary gland cells under ionising radiation. In addition, Sappanone A enhanced radiation-induced genomic DNA fragmentation, accompanied by impaired homologous recombination and non-homologous end joining DNA repair. Mechanistic evaluation revealed that Sappanone A counteracted radiation-induced inosine monophosphate dehydrogenase 2 (IMPDH2) activation, and that this effect could be abolished by reconstituted expression of a Sappanone A-binding defective IMPDH2 mutant.*

Conclusion: *The present study highlights a novel role played by Sappanone A in the modulation of radiosensitivity of oral epithelial cells.*

Key words: *DNA repair, ionising radiation, oral epithelial cell, Sappanone A*
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Ionising radiation can cause temporary or irreversible oral complications including oral mucositis, xerostomia, caries lesions, periodontal disease, taste dysfunction, sensory disorders and salivary gland dysfunction. Radiation-induced oral mucositis (RIOM) is the most common form of damage to the normal oral mucosa of patients who receive radiotherapy to treat head and neck cancer[1]. Patients may suffer from multiple symptoms, including severe pain and difficulties in speaking and swallowing[2]. The clinical manifestations of RIOM, such as hyperaemia and redness of the oral mucosa, usually arise 2 to 3 weeks after the initiation of radiotherapy and may progress further to oral ulcers with a pseudomembrane[3].

Double-strand breaks (DSBs) are the most severe type of damage to deoxyribonucleic acid (DNA) caused by ionising radiation, and the cell fate in such a context is largely dependent on the efficiency of DNA repair[4]. Non-homologous end joining (NHEJ) and homologous recombination (HR) are the two pivotal DSB repair mechanisms. The NHEJ pathway directly rejoins the two DSB ends by DNA ligase which does not require a homologous donor sequence. In contrast, the HR

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pathway utilises the homologous sister chromatid or homologous chromosome as a template[5]. NHEJ is a fast process in all cell cycle phases and usually dominant in G0/G1 and G2 phases, but is often considered imprecise due to frequent nucleotide deletions and insertions at repaired sites. HR is considered more accurate and is confined to the S and G2 phases for the use of a homologous chromatid[6]. Though the cellular factors involved in DNA repair have been studied extensively, the environmental factors that affect DNA repair efficiency are still relatively unknown.

Caesalpinia sappan, also known as Brazil or Sappan wood, is a species of plant belonging to the Leguminosae family that is mainly distributed in Southeast Asia. It is considered an important source that yields multiple herbal medicinal products, which are used in a variety of beverage and food formulations[7]. Several bioactive phenolic compounds have been extracted from *Caesalpinia sappan* so far, including xanthone, coumarin, chalcones, flavones, homoisoflavonoids and brazilin[7]. Among them, Sappanone A, belonging to the class of homoisoflavanones, is a bioactive ingredient isolated from the heartwood of *Caesalpinia sappan*. Sappanone A has been used as a treatment agent of allergic asthma, rheumatoid arthritis and inflammation-induced bone loss[8,9]. However, whether it affects cell radiosensitivity is relatively unclear.

In this study, we demonstrate that Sappanone A enhances ionising radiation–induced damage in oral epithelial and salivary gland cells. Furthermore, it disrupts DNA repair after ionising radiation, most likely by inhibiting guanosine nucleotide metabolism.

Materials and methods

DNA constructs and mutagenesis

The human IMPDH2 gene was amplified by polymerase chain reaction (PCR) and subcloned into the indicated vectors. A QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) was used to prepare mutant constructs. The following shRNAs were used: scramble shRNA, GCT TCT AAC ACC GGA GGT T, human IMPDH2 shRNA and TCC AGC ACA CCT CCT CGG T (recognising noncoding sequences).

Cell culture

Human oral keratinocytes (HOKs) isolated from human mucosa were purchased from ScienCell (Carlsbad, CA, USA) and maintained in Oral Keratinocyte Medium (ScienCell). For subculture, cells in a 10-cm dish were washed with phosphate-buffered saline (PBS) twice and incubated with 2 ml 0.05% T/E solution for about 5 minutes. Once the cells became completely round, 10 ml medium was added to the dish and the cells were then centrifuged at 1000 rpm for 3 minutes. The cells were resuspended with fresh culture medium and cultured in a new dish at a density of 5000 cells/cm^2. To determine the effects of Sappanone A on ionising radiation–induced damage, HOKs were incubated with 50 μM Sappanone A for 2 hours prior to 10 Gy irradiation.

Mice irradiation

An 8-Gy whole-body irradiation of 6-week-old C57BL/6 mice was performed with a ^{137}Cs gamma-ray source. A single dose of radiation was given at 1 Gy per minute. Salivary gland tissues were obtained 72 hours after irradiation. The animal maintenance and treatments were conducted according to the relevant institutional and national guidelines and regulations. The use of animals was approved by the institutional review board of West China Hospital of Stomatology.

Cell viability assay

A cell viability assay was performed using Cell Counting Kit 8 (WST-8/CCK-8) (Abcam, Cambridge, UK) and measurements were performed following the manufacturer's protocol.

Comet assay

A comet assay was performed using a single cell gel electrophoresis assay/comet assay (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol.

Analyses of DNA repair efficiency

The efficiency of NHEJ and HR repair was analysed using an I-SceI- and DR-GFP-based system, which have been studied previously[10,11]. In brief, HOKs were transfected with the DR-GFP plasmid, which was unable to express DR-GFP because of the I-SceI site. After I-SceI induced DSB in the DR-GFP locus, NHEJ repair resulted in a 0.65-kb PCR band, which is resistant to double digestion with I-SceI and BcgI, while HR repair restores the expression of GFP. Primers targeting the genomic sequence of the ACTB gene were used to measure the I-SceI cutting efficiency. To measure NHEJ repair, HOK cells were treated with lovastatin to be synchronised during the G1 phase prior to ionising radiation.

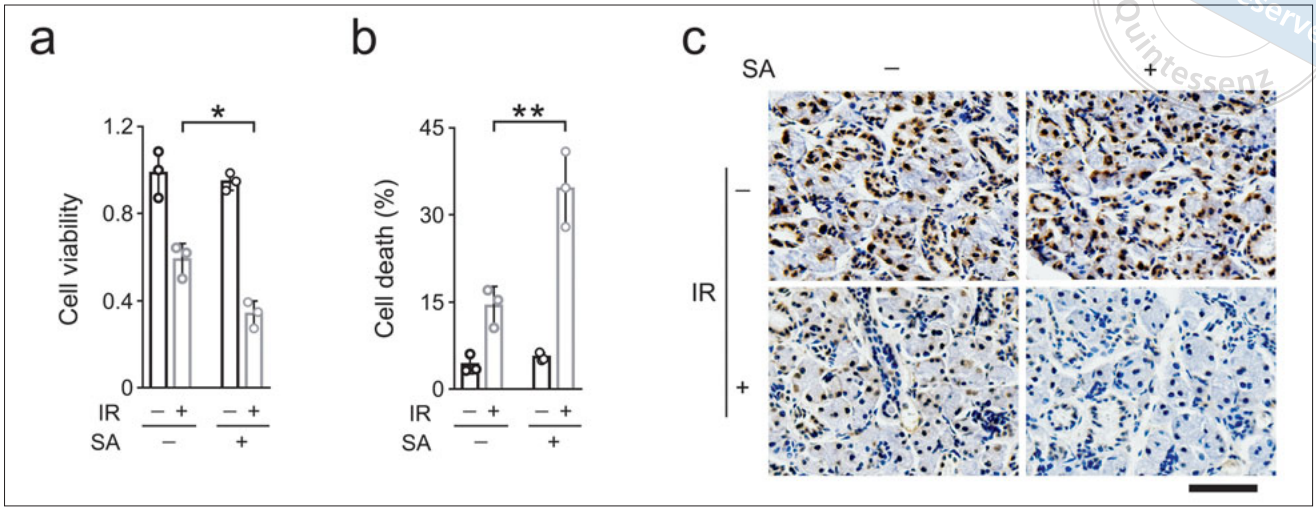

Fig 1 Sappanone A impairs the viability of oral epithelial cells under ionising radiation. **(a)** HOK cells were treated with 50 μM Sappanone A for 2 hours and challenged by 10 Gy ionising radiation. Cell viability was measured 48 hours after irradiation. The data represent the mean ± SD from three independent experiments. *$P < 0.05$. SA, Sappanone A; IR, ionising radiation. **(b)** HOK cells were incubated with 50 μM Sappanone A for 2 hours and challenged by 10 Gy ionising radiation. Cell death was measured 48 hours after irradiation. Data represents the mean ± SD from 3 independent experiments. **$P < 0.01$. **(c)** Mice (n = 7) were treated with 40 mg/kg Sappanone A. After 6 hours, the mice were exposed to 8 Gy whole-body ionising radiation. The salivary gland tissues were obtained and examined by immunohistochemical staining using an anti-Ki67 antibody. Scale bar 80 μm.

Cell death assay

Cell death was measured by trypan blue exclusion assay. Briefly, 0.05 ml of the 0.4% trypan blue solution (Thermo Fisher Scientific, Waltham, MA, USA) was mixed with 0.45 ml cell suspension. After 3 minutes, the stained cells were loaded onto a hemacytometer and the numbers of cells with or without trypan blue signal were counted.

IMPDH activity assay

IMPDH activity in the cell precipitates was measured using an IMPDH Inhibitor Screening Assay Kit (BioVision, Milpitas, CA, USA), following the manufacturer's instructions. IMPDH activity in cell lysates was measured following a previous study[12].

Statistical analysis

All data were analysed statistically using a two-tailed unpaired Student *t* test and presented as mean ± SD from three independent experiments/samples. The level of significance was set at $P < 0.05$.

Results

Sappanone A impairs the viability of oral epithelial cells under ionising radiation

As expected, exposure to ionising radiation largely reduced cell viability and increased cell death in HOK cells, and pre-treatment with Sappanone A further dampened the cell viability in the irradiated HOK cells (Figs 1a and b). In contrast, Sappanone A caused only minor effects in the unirradiated cells (Figs 1a and b). Further, in the mouse model, immunohistochemical staining showed that ionising radiation markedly reduced the expression of Ki67, a proliferating cell marker, in the salivary glands; this effect was largely amplified by Sappanone A treatment (Fig 1c). However, treatment with Sappanone A alone did not cause an obvious change to Ki67 expression in the salivary glands derived from unirradiated mice (Fig 1c). These results suggest that Sappanone A impairs the viability of oral epithelial cells under ionising radiation.

Sappanone A enhances ionising radiation–induced DNA damage in oral epithelial cells

Effective DNA repair is important to maintain cell viability upon ionising radiation–mediated DNA damage[13]. To determine the impact of Sappanone A on HR and

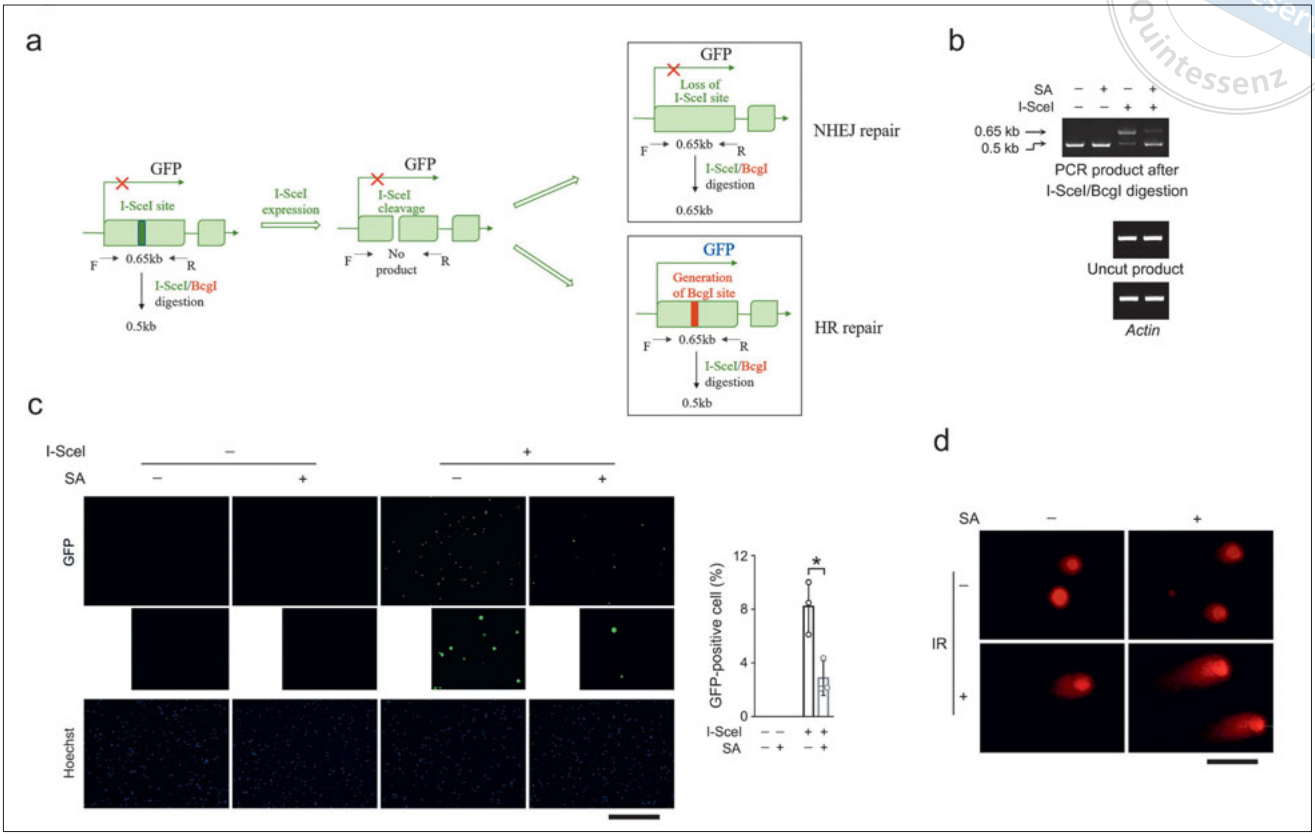

Fig 2 Sappanone A impedes DNA repair in oral epithelial cells. **(a)** A schematic for the measurement of NHEJ and HR repair. **(b and c)** HOK cells were transfected with I-SceI-expressing vector and treated with or without Sappanone A (50 μM) for 2 hours. NHEJ repair was examined 1 hour after irradiation **(b)**. HR repair was tested 72 hours after irradiation **(c)**. The data represent the mean ± SD from three independent experiments. **P* < 0.05. SA, Sappanone A. Scale bar 300 μm for original images and 100 μm for enlarged images. **(d)** HOK cells were incubated with 50 μM Sappanone A for 2 hours and challenged by 10 Gy ionising radiation. DNA fragmentation was measured 1 hour after irradiation. Scale bar 20 μm. IR, ionising radiation.

NHEJ repair, endonuclease I-SceI-mediated DSBs in an exogenously introduced DR-GFP locus was used to measure the efficiency of HR and NHEJ repair (Fig 2a)[11]. We found that treatment with Sappanone A markedly reduced the amounts of 0.65-kb PCR product, suggesting that Sappanone A impeded NHEJ repair (Fig 2b). Further, I-SceI expression induced GFP expression in about 8% of HOK cells, which could be palpably eliminated upon Sappanone A treatment (Fig 2c), hinting at an impaired HR repair. In line with this, treatment with Sappanone A aggravated the irradiation-induced DNA fragments in the HOK cells, shown by the prolonged tail-like smear in the comet assay (Fig 2d). These results suggest that Sappanone A accentuates ionising radiation–induced DNA damage in oral epithelial cells.

Sappanone A accentuates ionising radiation–induced DNA damage by targeting IMPDH2

Enhanced synthesis of nucleotide upon ionising radiation facilitates DNA repair[11]. Inosine-5′-monophosphate dehydrogenase (IMPDH)1 and IMPDH2 produce xanthosine monophosphate (XMP) from inosine monophosphate (IMP), which is the rate-limiting step for guanine nucleotide synthesis (Fig 3a)[14]. Since Sappanone A was reported as an IMPDH2-specific inhibitor[15], which was of particular interest to us, we investigated the cellular IMPDH activity in HOK cells upon radiation. As expected, cellular IMPDH activity was sharply increased 30 minutes after ionising radiation (Fig 3b), hinting at an accelerated guanine nucleotide synthesis. Intriguingly, only the enzymatic activity of IMPDH2, not IMPDH1, was elevated manyfold (Fig 3c), suggesting that an ion-

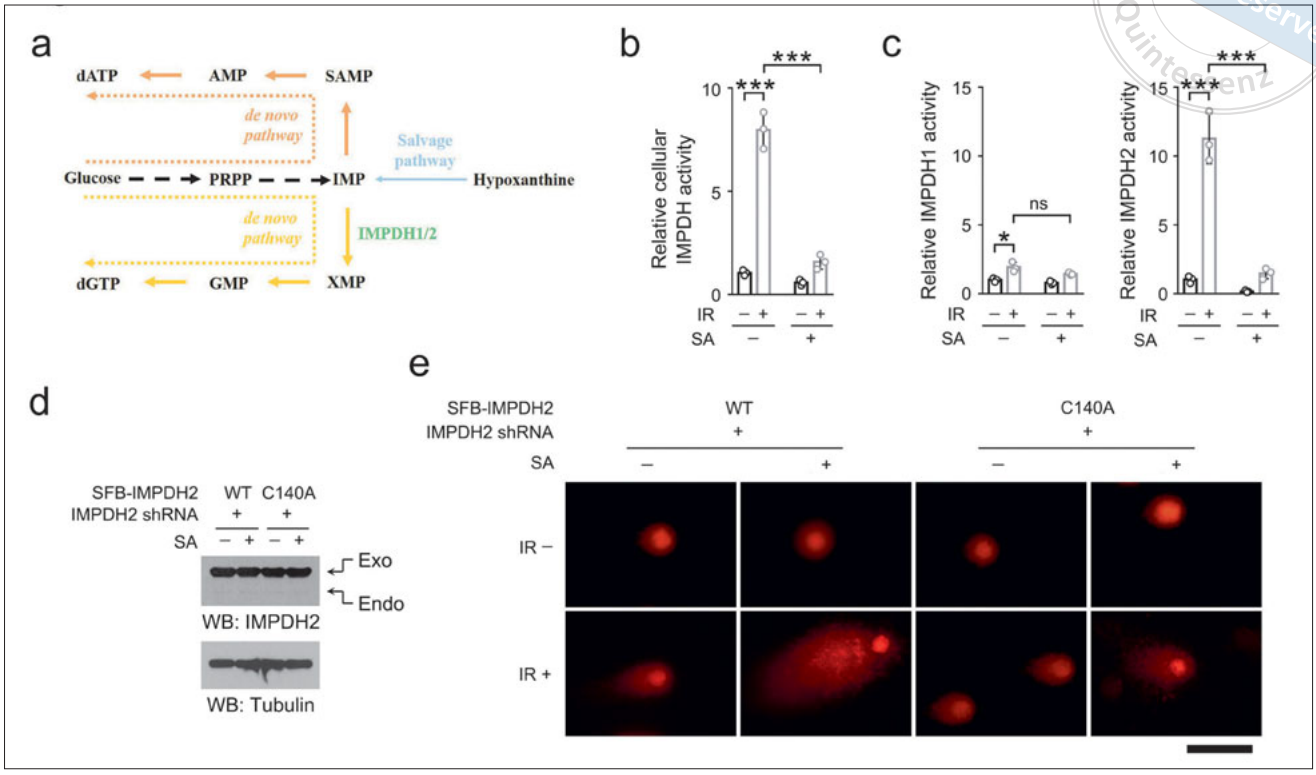

Fig 3 Sappanone A aggravates ionising radiation–induced DNA damage by binding with IMPDH2. **(a)** A schematic of purine nucleotide synthesis. **(b)** HOK cells were incubated with 50 μM Sappanone A for 2 hours and challenged by 10 Gy ionising radiation. The IMPDH activity was measured 30 minutes after irradiation. The data represent the mean ± SD from three independent experiments. ***$P < 0.001$. SA, Sappanone A; IR, ionising radiation. **(c)** HOK cells were incubated with 50 μM Sappanone A for 2 hours and then challenged by 10 Gy ionising radiation. The IMPDH1 and IMPDH2 proteins were pulled down 30 minutes after irradiation, and the activity was measured. The data represent the mean ± SD from three independent experiments. *$P < 0.05$; ***$P < 0.001$; ns, not significant. **(d)** HOK cells with expression of IMPDH2 shRNA and SFB-IMPDH2 were incubated with 50 μM Sappanone A for 2 hours. Expression of endogenous and exogenous IMPDH2 was examined by immunoblot. **(e)** HOK cells with expression of IMPDH2 shRNA and SFB-IMPDH2 were incubated with 50 μM Sappanone A for 2 hours and challenged by 10 Gy ionising radiation. DNA fragmentation was measured 1 hour after irradiation. Scale bar 30 μm.

ising radiation–induced increase in cellular IMPDH activity is mainly due to the activation of IMPDH2.

Treatment with Sappanone A prevented ionising radiation–induced IMPDH activity in HOK cell lysate markedly (Fig 3b). The increased IMPDH activity in IMPDH2 protein immunoprecipitates derived from irradiated HOK cells was consistently largely reduced in the presence of Sappanone A, whereas only minor changes were detected for IMPDH1 protein immunoprecipitates (Fig 3c). These results suggest that Sappanone A inhibits ionising radiation–induced cellular IMPDH activity in oral epithelial cells.

To determine whether IMPDH2 is the major protein target responsible for Sappanone A-regulated cell radiosensitivity, we knocked down the endogenous IMPDH2 and exogenously expressed an S protein-FLAG-Streptavidin binding peptide (SFB)-tagged wildtype IMPDH2 or IMPDH2 C140A mutant, which was defective in binding with Sappanone A (Fig 3d)[15]. Comet assay revealed that disruption of Sappanone A/IMPDH2 interaction substantially shortened the ionising radiation–induced tail-like smear after irradiation (Fig 3e). Together, these results suggest that Sappanone A aggravates ionising radiation–induced DNA damage by binding with IMPDH2.

Discussion

The oral mucosa is a vulnerable target for ionising radiation–induced damage. RIOM occurs in approximately 80% of patients receiving head and neck radiotherapy, and its incidence increases to as high a level as 100% in patients who undergo altered fractionation radiotherapy[16]. RIOM may decrease patients' quality of life sig-

nificantly and create a substantial health and economic burden. Ionising radiation can cause lethal DNA damage, resulting in apoptosis and necrosis of oral epithelial cells, which may contribute to the development of difficulties in speaking and swallowing[2].

Besides being known as a substance in traditional Chinese medicine, there is growing evidence to suggest the anti-inflammatory function of Sappanone A. Sappanone A has been proven to have inhibitory effects on nitric oxide and prostaglandin E2 in lipopolysaccharide-challenged RAW264.7 cells[17]. In a rat myocardial ischemia reperfusion injury model, Sappanone A reduced the size of myocardial infarction significantly and improved systolic and diastolic function[18]. Further, Sappanone A exhibits antioxidant activity by inducing heme oxygenase-1 protein through the nuclear factor-E2-related factor 2 (Nrf2), promoting the expression of Nrf2 downstream genes, such as NAD(P)H:quinone oxido-reductase 1 (NQO1). On the other hand, Sappanone A enhanced the phosphorylation of Nfr2 by regulating PKC and PI3K pathways[18]. The present study demonstrates that Sappanone A sensitised oral epithelial cells to ionising radiation. Treatment with Sappanone A further declined the viability in both the irradiated HOK cells and the mouse salivary gland cells. This study increases knowledge of the function of Sappanone A in regulating cellular responses in stressful conditions.

IMPDHs, which catalyse the conversion of IMP to XMP, are a rate-limiting enzyme in both the de novo and salvage biosynthesis of purine nucleotides[19]. Two IMPDHs have been discovered in humans, IMPDH1 and IMPDH2, which share 84% sequence homology but are coded by distinct genes. IMPDH1 is constitutively expressed, while IMPDH2 is often highly expressed in rapidly proliferating cell populations. Both IMPDH isoforms comprise a catalytic domain that contains cysteine residues, and a Bateman domain[15]. It has been reported that Sappanone A can selectively target Cys140 within the Bateman domain of IMPDH2, thereby reducing the enzymatic activity of IMPDH2[15]. In contrast, the corresponding Cys site does not exist in IMPDH1, which renders IMPDH1 with a low binding affinity with Sappanone A[15]. The present study has shown that ionising radiation induces a rapid elevation in cellular IMPDH activity, which is mainly caused by the activation of IMPDH2 and could be abolished upon Sappanone A treatment. Further, disruption of the association between Sappanone A/IMPDH2 by reconstituted expression of an IMPDH2 C140A mutant attenuated ionising radiation–induced DNA damage in HOK cells. Further work should be conducted to profile the alterations in purine metabolism mediated by Sappanone A in the context of irradiation.

Conclusion

In conclusion, the present study demonstrates a novel role of Sappanone A in the regulation of oral epithelial cell radiosensitivity. Sappanone A interacts with and counteracts IMPDH2 to impede DNA repair after ionising radiation, thereby further repressing the viability of the irradiated oral epithelial cells.

Conflicts of interest

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

Author contribution

Drs Xue Feng ZHAO, Zhi Yong WANG and Xue Mei QIU performed the experiments; Drs Hang ZHAO, Qian Ming CHEN and Rui LIU conceived and designed the study; Drs Qian Ming CHEN and Rui LIU wrote the paper, with comments provided by all authors.

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Comments on "Sappanone A Aggrandises Ionising Radiation–induced Damage in Oral Epithelial Cells"

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Radiation-induced oral mucosal disorders and injuries are major complications for patients undergoing head and neck radiotherapy. They are either reversible or permanent, and include oral mucositis, xerostomia, bacterial or fungal infection, taste dysfunction, sensory disorders and oral mucosal fibrosis. Among these, radiation-induced oral mucositis (RIOM) is the most common complication observed in approximately 80% of patients who undergo head and neck radiotherapy. RIOM is a form of acute inflammation that arises within the first 2 to 3 weeks after radiotherapy and is characterised by hyperaemia and redness of the oral mucosa, which can eventually progress to oral ulcers with a pseudomembrane. Patients may suffer from extreme pain when speaking and swallowing, leading to decreased food intake, significant weight loss and malnutrition, thus influencing their subsequent radiotherapy.

Traditional Chinese medicine plays an increasingly active role in the prevention and treatment of radiation-induced oral mucosal injuries. Many studies have demonstrated that traditional Chinese medicine can reduce the incidence of oral mucositis effectively and alleviate related symptoms such as xerostomia and pain, thus improving patients' prognosis. As a widely distributed medicinal material, *Caesalpinia sappan* (CS) processes anti-inflammatory and antimicrobial properties and shows potential value in the treatment of multiple inflammatory disorders.

In the article "Sappanone A aggrandises ionising radiation–induced damage in oral epithelial cells", Zhao et al report that Sappanone A, isolated from the heartwood of CS, has destructive effects on the viability of oral epithelial cells, accentuating ionising radiation–induced DNA damage. Sappanone A targets and suppresses inosine monophosphate dehydrogenase 2, a rate-limiting enzyme in nucleotide synthesis, leading to delayed DNA repair under radiation. These discoveries have deepened our understanding of the role of CS in regulating cell radiosensitivity, which will assist future pharmaceutical developments for treating RIOM.

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MiR-146a-5p Promotes Dental Stem Cells Osteo/odontogenic Differentiation through NF-Kappa B Signaling Pathway by Targeting *TRAF6*

Xin YU[1], Jian Feng LU[1], Mei Qin GAO[1], Bin XIONG[1], Wen Qian XIA[1]

Objective: *To screen miRNAs that could simultaneously regulate osteo/odontogenic differentiation of multiple stem cells, including dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAPs) and periodontal ligament stem cells (PDLSCs).*
Methods: *Differentially expressed miRNAs analysis on three miRNA microarrays data of dental stem cells undergoing osteo/odontogenic differentiation (GSE138180, GSE154466 and GSE159508) was performed, and miR-146a-5p were identified by bioinformatic prediction, dual-luciferase reporter assay and quantitative real-time polymerase chain reaction (PCR). In addition, differentially expressed genes between miR-146a-5p overexpressed group and control group (GSE79341) were applied for KEGG pathways enrichment analysis.*
Results: *MiR-146a-5p expression increased in the osteo/odontogenic differentiation of DPSCs, SCAPs and PDLSCs. Tumour necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) was identified as the target gene of miR-146a-5p. Furthermore, miR-146a-5p could influence the NF-Kappa B signalling pathway.*
Conclusion: *This study suggests that miR-146a-5p could promote differentiation in multiple dental stem cells through the NF-Kappa B signalling pathway by targeting TRAF6.*

Key words: *dental stem cells, miR-146a-5p, NF-Kappa B signalling pathway, osteo/odontogenic differentiation, TRAF6*
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Osteo/odontogenic differentiation of dental stem cells, including dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAPs) and periodontal ligament stem cells (PDLSCs), is a key process in tooth development[1]. DPSCs are mesenchymal stem cells that originate from the embryonic cranial neural crest and that are capable of self-renewal and pluripotent differentiation[2,3]. DPSCs can differentiate into osteoblasts and odontoblasts, and play an important role in dentine repair and regeneration[4]. SCAPs are derived from the apical papilla of immature teeth and can promote the formation of root dentine during root maturation[5-7]. PDLSCs are isolated from the periodontal ligament (PDL), which connects the alveolar bone to the cementum. They are also pluripotent stem cells and show great potential in the formation of new bone and other periodontal tissues[8].

To date, several studies have suggested that a variety of miRNAs are involved in the process of osteo/odontogenic differentiation of dental stem cells. For example, *miR-497-5p* influences the Smad signalling pathway by regulating *Smurf2* to promote osteo/odontogenic differentiation of SCAPs[1]. Yao et al[9] found the *miR-214-ATF4* axis to be a novel pathway for regulating the osteogenic differentiation of PDLSCs. Besides, *miR-143* inhibited DPSCs osteogenic differentiation by targeting TNF-α to block NF-Kappa B signaling path-

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way10. *MiR-146a-5p* is a frequently studied miRNA and is simply called *miR-146a* in previous nomenclature, whereas *miR-146a-3p* was named *miR-146a**[11]. *MiR-146a-5p* has been widely reported to be associated with cancers, such as liver[12], lung[13], breast[14] and oral cancers[15], as a tumour-suppressor miRNA in some cancers and an oncogenic miRNA in others.

Currently, there are few studies on shared miRNAs that regulate osteo/odontogenic differentiation of multiple dental stem cells, and the molecular mechanism remains unclear. In the present study, we explored miRNAs that could simultaneously regulate differentiation of multiple stem cells (DPSCs, SCAPs and PDLSCs), which might become a new target for promoting tooth tissue regeneration.

Materials and methods

Analysis of microarray datasets and bioinformation

The Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo) is a public database[16] that contains a large amount of gene/miRNAs expression microarray and sequencing data. We retrieved and downloaded four datasets from the database, including three miRNA microarrays datasets of dental stem cells osteo/odontogenic differentiation (GSE138180, GSE154466, GSE159508) and one gene expression datasets (GSE79341).

GSE138180 explored the roles of miRNAs in the odontogenic differentiation of DPSCs extracted from healthy premolars of 19 healthy patients aged from 15 to 25 years, whose premolars were extracted for orthodontic treatment at the Department of Stomatology, Nanfang Hospital, Southern Medical University, Guangzhou[17]. DPSCs from the differentiated group were cultured with an odontogenic differentiation medium containing 50 mg/ml ascorbic acid, 100 nmol/L dexamethasone and 10 mmol/L β-glycerolphosphate (Sigma, St Louis, MO, USA) for 14 days, while DPSCs from the undifferentiated group were cultured in basal medium with 10% foetal bovine serum (FBS).

GSE154466 applied miRNA microarrays to analyse differentially expressed miRNAs in osteo/odontogenic differentiation of SCAPs obtained from immature human third molars of patients aged 16 to 20 years for orthodontic reasons at the School and Hospital of Stomatology, Shandong University[1]. The researchers cultured SCAPs in a differentiation medium and selected two stages to obtain the stem cells: pre-osteo/odontogenic differentiation and 7 days after osteo/odontogenic differentiation, followed by RNA extraction and Affymetrix (Santa Clara, CA, USA) microarray detection[1].

GSE159508 analysed miRNA expression profile during osteogenic differentiation of PDLSCs provided by the Department of Stomatology, Nanfang Hospital, Southern Medical University, Guangzhou. PDLSCs from the differentiated group were cultured in an odontogenic differentiation medium for 14 days. Both groups had three replicates and were investigated by microarray profiling[18].

GSE79341 contained gene expression data of *miR-146a-5p* overexpressed cells using the Affymetrix GeneChip[19]. Hepatocyte-like Huh7.5.1 cells were transfected with *miR-146a-5p* mimics and control mimics, followed by total RNA extraction and purification, cDNAs hybridisation and scanning of gene chips.

The website named *Gene expression in tooth* that presented gene expression in developing dental tissues (http://bite-it.Helsinki.fi/) contains schematic illustrations of gene expression in different stages of development. These data were derived from previous studies describing the expression of genes at one or more stages of tooth development, mainly obtained by in situ hybridisation analysis to reveal the location of mRNA or by immunochemical methods to detect proteins.

Predicting the potential binding targets of miRNAs

The potential target genes of *miR-146a-5p* were predicted and identified by four databases, namely Targetscan (http://www.targetscan.org/vert_71/)[20], miRDB (http://mirdb.org/)[21], Starbase (http://starbase.sysu.edu.cn/)[22] and miRWalk (http://mirwalk.umm.uni-heidelberg.de/)[23] according to the following criteria: consistently predicted by all four databases, and had the highest target rank.

Cell culture

The HEK-293 (human embryonic kidney 293 cells), a common cell to investigate cell function and molecular regulation, was cultured in Eagle's minimum essential medium (EMEM) comprised of 10% FBS, 100 U/ml penicillin and 100 mg/ml of streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C.

Transfection and quantitative real-time PCR

HEK-293 cells, seeded into12-well culture plates, were instantly transfected with *miR-146a-5p* mimics by EndoFectin MAX (GeneCopoeia, Germantown, MD,

USA). After 48 hours, the cells were collected for RNA extraction and reverse transcribed into single stranded cDNA (Takara, Shiga, Japan). The primer sequence information of tumour necrosis factor receptor (TNFR)-associated factor 6 (*TRAF6*) is shown in Table S1 (provided on request). We performed real-time quantitative PCR by Power SYBR Green on a CFX96 PCR System (Bio-Rad, Hercules, CA, USA). Data were collected and analysed using the $2^{-\Delta\Delta Ct}$ method for qualification of the relative expression levels of *TRAF6*, with *GAPDH* as corresponding internal controls.

Construction of reporter plasmids and dual-luciferase reporter assay

The *TRAF6* 3'-UTR wild-type fragment was inserted at the NheI-SalI restriction site downstream of the luciferase gene in the pmirGLO vector. HEK-293 cells were transfected with the reporter plasmids and *miR-146a-5p* mimic. After transfection for 24 hours, the luciferase activity in the lysates was quantified by a dual-luciferase reporter assay system (Promega, Madison, WI, USA). The binding activity of *miR-146a-5p* to *TRAF6* 3'-UTR was characterised by measuring the ratio of firefly luciferase to renilla luciferase activity.

Pathway analyses and PPI network

Differentially expressed genes identified in GSE79341 were applied for Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways enrichment analysis, which was performed using clusterProfiler R software package (R Core Team, Vienna, Austria) to explore the downstream pathways affected by *miR-146a-5p* overexpression. The Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) website was used to construct a protein–protein interaction[24] network of genes in target pathways, with an interaction score of 0.4 as the cutoff criterion[25].

Statistical analysis

Differentially expressed miRNAs (DEMs) and genes (DEGs) were identified using a linear model with the GEO2R online analysis tool based on GEOquery, Limma and Umap R software packages[26]. $P < 0.05$ and $|\log2(FC)| > 1$ was taken as the criterion for screening DEMs and $P < 0.05$ for DEGs. We conducted KEGG pathway enrichment analyses of DEGs using clusterProfiler R software[27]. The volcano plots, heatmaps and Venn diagrams were created using OmicStudio tools (https://www.omicstudio.cn/tool). Luciferase activity analysis and qRT-PCR results were calculated using a two-tailed Student *t* test, with the level of significance set at $P < 0.05$.

Results

Identification of DEMs associated with osteo/odontogenic differentiation

In this study, we first separately identified differentially expressed miRNAs in three miRNA microarray datasets (GSE138180, GSE154466 and GSE159508) using GEO2R software. As a result, 185 miRNAs in DPSCs were screened out from GSE138180, 20 miRNAs in SCAPs from GSE154466 and 174 miRNAs in PDLSCs from GSE159508. The volcano plots, as well as the heatmaps, showed upregulation or downregulation of miRNAs between the two groups (Fig 1). Furthermore, we took the intersection of the results for the three datasets and finally found that *miR-146a-5p* expression increased in the differentiated groups (Fig 2), suggesting that it played an important role in dental stem cell osteo/odontogenic differentiation.

TRAF6 was identified as the target gene of miR-146a-5p

TRAF6 was consistently predicted to be the target gene of *miR-146a-5p* in the four databases, and its target rank was the first according to target scores (Table S2, provided on request). In addition, there were six possible binding sites for *miR-146a-5p* in the 3'-UTR of *TRAF6* (Fig 3a). In addition, a luciferase reporter plasmid containing *TRAF6* 3'-UTR was cloned and co-transfected with *miR-146a-5p* mimics in HEK-293 cells. Luciferase reporter gene results showed that *miR-146a-5p* reduced the activity of 3'-UTR of *TRAF6* significantly (Fig 3b). To further verify whether *TRAF6* was the target gene, we transfected *miR-146a-5p* mimics in HEK-293 cells and extracted total RNA 48 hours later to detect changes in *TRAF6* expression, which showed that increased expression of *miR-146a-5p* inhibited the expression of *TRAF6* significantly (Fig 3c). The above results suggested *TRAF6* was the target gene of *miR-146a-5p*.

Traf6 was expressed during multiple stages of mouse tooth germs

Traf6 was expressed continuously in multiple stages of mouse teeth, including the initiation, bud, cap and bell stages. In addition, *Traf6* expression signal could be

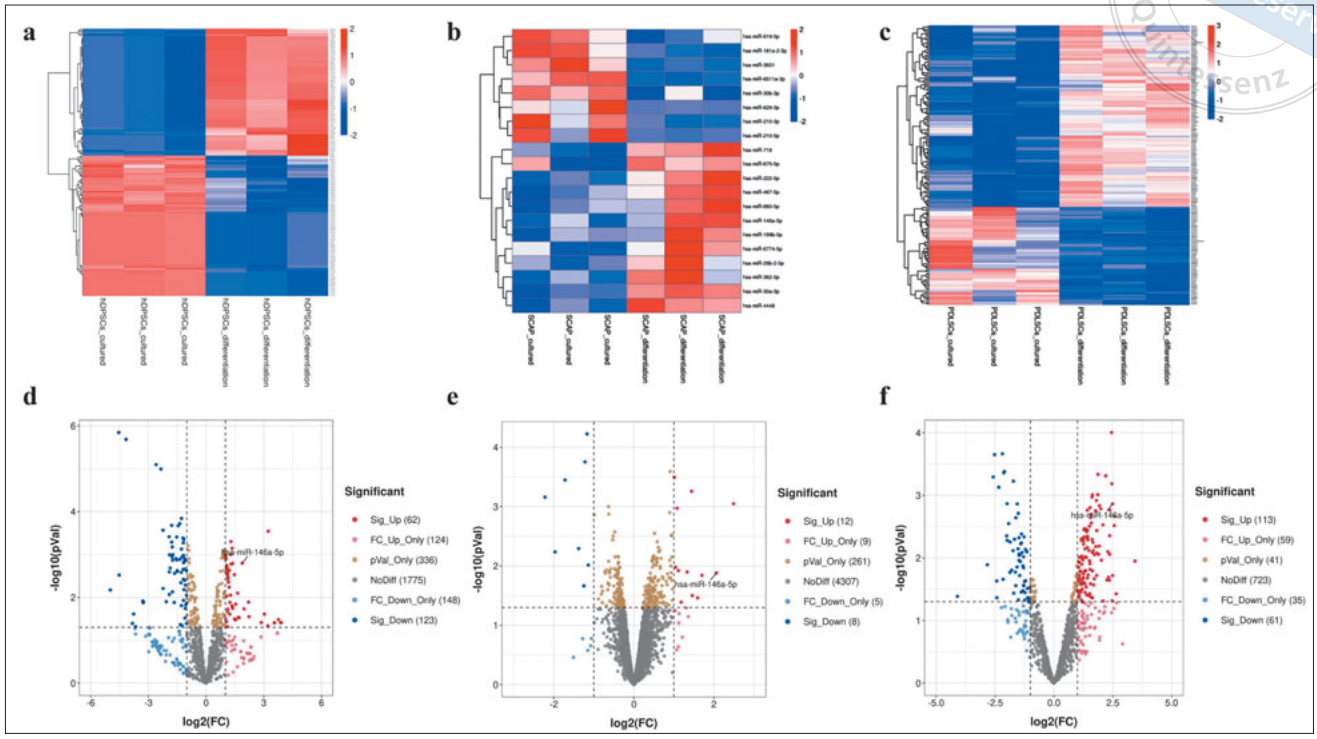

Fig 1 Heatmaps of differentially expressed miRNAs in **(a)** GSE138180, **(b)** GSE154466 and **(c)** GSE159508. The screening criteria were as follows: $|\log2(FC)| > 1$, $P < 0.05$ for miRNAs. The expression value is described by a colour scale. The intensity increased from red to blue. Each column represents one sample, and each row represents one transcript. Volcano plots of miRNAs in **(d)** GSE138180, **(e)** GSE154466 and **(f)** GSE159508. Red, blue and grey colours indicate relatively high, low and equal expression of miRNAs, respectively.

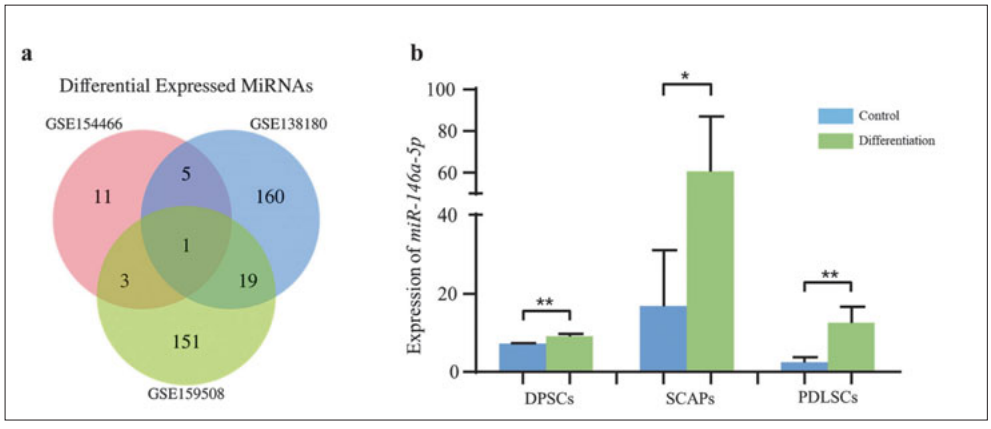

Fig 2 **(a)** A Venn diagram shows the overlapping differentially expressed miRNAs in GSE138180, GSE154466 and GSE159508. **(b)** Expression of *miR-146a-5p* was increased in the dental stem cells osteo/odontogenic differentiation groups.

detected in various tissues at each stage, especially in the dental epithelium, stellate reticulum and dental papilla (Fig 3d). The expression pattern of *Traf6* was mapped by in situ hybridisation in mouse embryonic mandibular first molar tooth germs between E11.5 and E15.5 from the study by Ohazama et al[28].

MiR-146a-5p affected the NF-Kappa B signalling pathway

As shown in the volcano plot and heatmap, we identified 2229 differentially expressed genes (duplicate and invalid gene names were removed) between *miR-146a-5p* overexpressed groups and control groups. KEGG

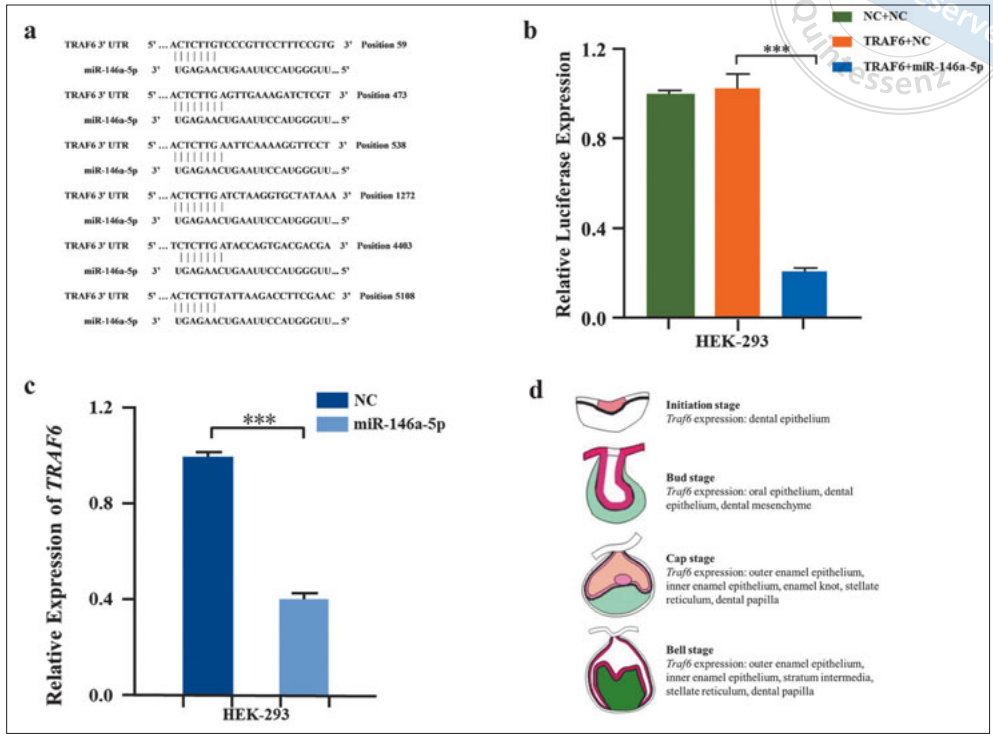

Fig 3 **(a)** The predicted binding sites for *miR-146a-5p* in the 3'-UTR of *TRAF6*. **(b)** Dual luciferase reporter assays in HEK-293 cells demonstrated that *TRAF6* was the direct target of *miR-146-5p*. **(c)** *TRAF6* mRNA expression in HEK-293 cells after transfection with *miR-146a-5p* mimics. Transcript levels were normalised to GAPDH levels (***$P < 0.001$). **(d)** *TRAF6* was expressed continuously at multiple stages in various tissues of mouse teeth.

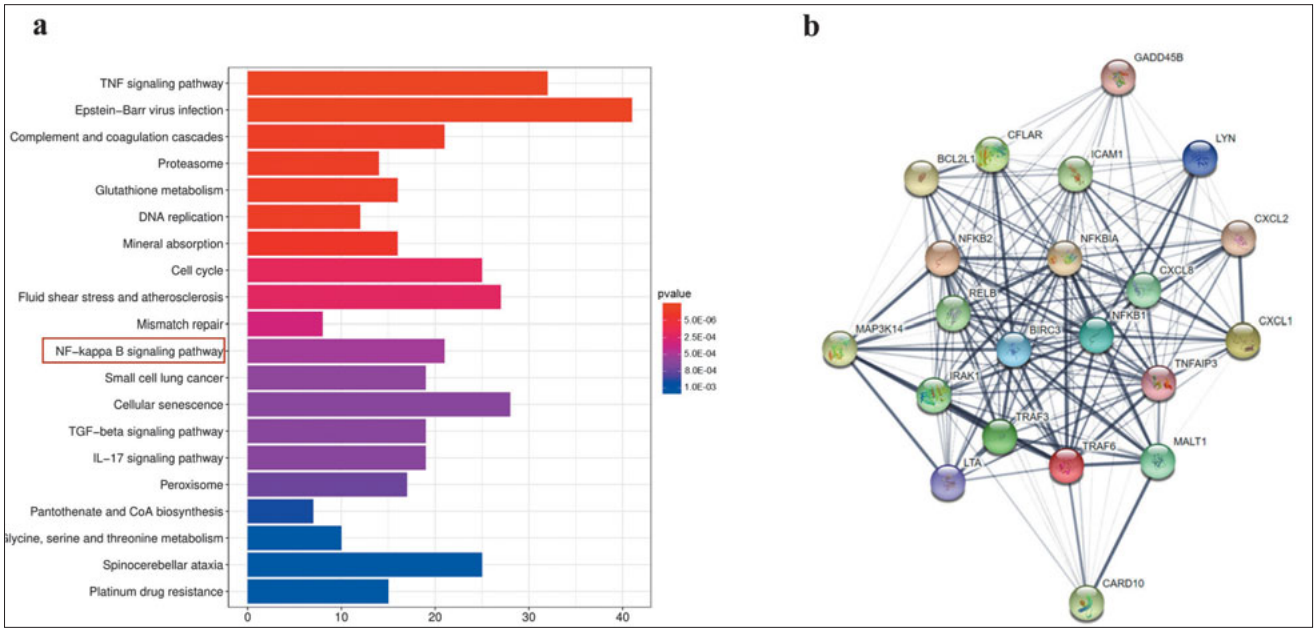

Fig 4 KEGG pathway analyses of differentially expressed mRNAs in GSE79341. **(a)** Pathways marked in the red box were reported to be associated with dental stem cell osteo/odontogenic differentiation. **(b)** The PPI network of the NF-Kappa B signalling pathway suggested *TRAF6* was the core gene of the pathway.

pathway analysis detected 48 significant pathways ($P < 0.05$), and the top 20 pathways were selected for presentation (Fig 4a). Notably, we found that the TNF, NF-Kappa B and TGF−beta signalling pathways were reported to be associated with tooth development[29-31]. In particular, it was reported that the NF-Kappa B signalling pathway significantly influenced the osteo/odontogenic differentiation ability of dental stem cells[32-35]. Meanwhile, *TRAF6* was the core gene of the NF-Kappa B signalling pathway, as shown in the protein–protein interaction (PPI) network (Fig 4b).

Discussion

In this study, we identified *miR-146a-5p* as a potential target for promoting dental stem cell osteo/odontogenic differentiation, which has been widely reported to be associated with various cancers. Interestingly, tooth development and cancer development share common molecular pathways, and almost all types of tumours are more common in families with tooth agenesis[36]. Furthermore, Pan et al[37] suggested that *miR-146a* was susceptible to non-syndromic orofacial cleft (NSOC). NSOC patients always present with dental abnormalities, especially tooth agenesis and maxillary defects[38].

Here, we further identified *TRAF6* as the target gene of *miR-146a-5p*. *TRAF6* was expressed strongly in the signal centre of the epithelial enamel knot, which was believed to regulate the morphogenesis of tooth tips in the process of mouse tooth development[38]. Besides, *TRAF6* gene silencing could enhance the proliferation of murine odontoblast-like cells (MDPC-23), which would affect the ability of odontoblast cells to form and repair dentine, suggesting that *TRAF6* was an important signalling molecule regulating the process of tooth development and dentine repair[39]. This was essentially consistent with our conclusion that the decreased expression of *TRAF6* could promote osteo/odontogenic differentiation and tooth eruption.

The classical NF-Kappa B signalling pathway was commonly believed to play an important part in the tooth organogenesis and osteo/odontogenic differentiation process. Wang et al[33] reported that inhibition of NF-Kappa B signalling pathway can reduce osteo/odontogenic differentiation of SCAPs. They also provided evidence that the 17β-oestradiol promoted osteo/odontogenic differentiation of DPSCs through activation of the NF-Kappa B signalling pathway[34]. In addition, a study found that the osteo/odontogenic capacity of PDLSCs could be improved by mineral trioxide aggregate by activating the NF-Kappa B signalling pathway[40].

Conclusion

We discovered that *miR-146a-5p* could promote osteo/odontogenic differentiation in multiple dental stem cells (DPSCs, SCAPs and PDLSCs) through the NF-Kappa B signalling pathway by targeting *TRAF6*. Our findings tended to enhance the current understanding of the molecular events regulating osteo/odontogenic differentiation and could serve as a basis for researching functional miRNAs in the differentiation of multiple dental stem cells.

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Author contribution

Dr Xin YU performed the experiments, analysed the data and drafted the article; Dr Jian Feng LU contributed to the analysis and interpretation of the data; Drs Mei Qin GAO, Bin XIONG and Wen Qian XIA designed the study, analysed the data and critically revised the manuscript.

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张孝骞

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“戒慎恐惧”，仁心仁术

一摞小本子，一个时代的高峰

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Correlation Analysis between Self-perceived Oral Health and Self-perceived General Health among Adults in China

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Objective: *To analyse the correlation between self-perceived oral health (SPOH) and self-perceived general health (SPGH) and the influencing factors.*
Methods: *In this study, we collected sociodemographic information and details about SPOH and SPGH status for Chinese adults (≥ 18 years), and a total of 2233 people were included. The data were analysed using SPSS software (IBM, Armonk, NY, USA) with a chi-square test and binary logistic regression.*
Results: *In total, 43.4% of adults' self-perceived oral health was at a "good" level and 57.9% of adults' self-perceived general health was at a "good" level. The SPOH was correlated with SPGH ($r = 0.593$, $P < 0.001$). Good SPOH was associated with younger age, no dentures, no smoking, brushing teeth twice or more a day, periodontal health, no malocclusion and decayed, missing and filled teeth (DMFT) ≤ 12. Good SPGH was associated with younger age, higher educational level, no dentures, no smoking, brushing teeth twice or more a day, periodontal health, no malocclusion and DMFT ≤ 12.*
Conclusion: *SPOH and SPGH are correlated with each other, and greater attention should be paid to oral health to promote general health.*

Key words: *adults, China, correlation, general health, oral health*
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Health is an important factor that contributes to longevity, successful ageing and satisfaction with life[1]. An increasing number of studies have found many links between oral health and general health, and people around the world are paying closer attention to the relevance of both. The World Dental Federation (FDI) defines oral health as "the health of the mouth. No matter what your age, oral health is vital to general health and well-being"[2].

Oral health is an integral component of general health[3]. Many systemic diseases and oral diseases interact with each other, and periodontitis is a risk factor for cardiovascular disease. Researchers have found that periodontitis is associated with myocardial infarction[4], periodontitis and tooth loss are associated with the occurrence of stroke[5], and severe tooth loss is significantly related to incident coronary heart disease[6]. In addition, diabetes mellitus[7,8], respiratory diseases[9], preterm birth[10] and underweight children[11] are all associated with periodontal disease.

Self-perceived general health (SPGH) can be a sensitive barometer of physiological states, and it covers a large spectrum of health conditions[12]. Many studies have shown that SPGH, with individuals asked a

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simple question to rate their overall health on a scale from excellent to poor, is a strong predictor of future morbidity and mortality[13-15]. SPGH and self-perceived oral health (SPOH) are commonly used health and oral health indicators in most national and international surveys. SPOH has also been found to be significantly associated with clinical oral health status[16], and has been suggested as a health assessment tool[17].

However, relatively few studies have explored the relationship between SPOH and SPGH status in China, and the results were often obtained from a very simple questionnaire. Based on this background, the purpose of this study was to explore Chinese adults' evaluation of their own oral health and general health, the correlation between the two, and the influencing factors.

Materials and methods

Subjects

This cross-sectional study used data from the 2020 Oral Health Observatory (OHO). The target population was residents aged 18 and above in China.

The sample size was

$$\mathbf{n} = \boldsymbol{deff}\frac{\mu^2_{\alpha/2}p(1-p)}{\delta^2}$$

deff is the design efficiency, *p* is the prevalence of dental disease in the group aged 35 to 44 years from the 4th National Oral Health Survey, μ is the level of confidence and δ is the relative error. Considering the expected response rate of 85%, the estimated sample should be composed of no fewer than 2163 people. Systematic cluster sampling was conducted by the sample of registered members of the Chinese Stomatological Association (CSA) ordered by the initials of their region, with a final sample size of 2233 people.

Data collection and methods

The questionnaire delivered to participants covered their age, sex and education and other demographic information, information related to oral health behaviour such as wearing dentures, smoking, tooth brushing and intake of sugary food, as well as SROH and SRGH, which were filled in by the participants themselves. After oral examination by dental practitioners, a record that included information about oral disease, such as periodontal health, decayed, missing and filled teeth (DMFT) and dental erosion, was completed by dental practitioners. The questionnaire and record were combined by matching the same patients' information. All the information from the online questionnaire and the record was collected using mobile phone application software.

For the questions answered by patients, the options for SPOH and SPGH were "very good", "good", "general", "poor" and "very poor", which were recoded so that "very good" and "good" were grouped under "good" and the other options were classified as "poor". The age groups were 18 to 24 years, 25 to 34 years, 35 to 44 years, 45 to 54 years and 55 years and over. DMFT was categorised into four groups based on experience of caries lesions (DMFT 0, 1 to 5, 6 to 12 and > 12). Our classification of DMFT referred to the group aged from 35 to 44 years' mean DMFT (4.54) which was rounded up to 5, from the Fourth National Oral Health Survey (2015 to 2016) in mainland China, and the median DMFT was 6 in our study. Our analysis revealed little group differences in the groups of DMFT ≤ 12, so we considered the group of DMFT > 12 as a group in which caries were severe.

Statistical analysis

All statistical analyses were performed using SPSS Statistics v. 26.0 (IBM, Armonk, NY, USA). A nonparametric test (Mann-Whitney U test) was performed to compare the indices. Chi-squared tests were then used to examine the potential correlation between SPOH and SPGH and their respective potential association between SPOH and SPGH, both as dependent variables, and the different study variables (independent variables). Multivariate binary logistic regression analysis was used to determine the related factors affecting SPOH and SPGH. All statistical tests were performed with a level of significance of $P < 0.05$.

Results

Basic information

A total of 2233 subjects were selected, 1292 of whom were women and 941 were men. In terms of education, secondary education and a bachelor's degree or above were reported most frequently (38.4% and 56.0%, respectively). Only 7.4% of participants reported having worn dentures, 26.7% smoked or had smoked previously, 79.4% brushed their teeth twice or more a day, and up to 91.3% and 83.9% consumed sugary foods and drinks, respectively. The overall periodontal health rate

Table 1 Characteristics of the study sample.

Variable		n	%
Age, y	18–24	425	19.0
	25–34	762	34.1
	35–44	428	19.2
	45–54	300	13.4
	> 55	318	14.2
Sex	Male	941	42.1
	Female	1292	57.9
Education	Primary education or less	123	5.6
	Secondary education	832	38.4
	Bachelor's degree or higher	1213	56.0
Dental prosthesis	Yes	166	7.4
	No	2067	92.6
Smoking	Yes	597	26.7
	No	1636	73.3
Brush teeth twice or more a day	Yes	1741	79.4
	No	452	20.6
Consumption of sugary food	Yes	2023	91.2
	No	194	8.8
Consumption of sugary drinks	Yes	1852	83.9
	No	355	16.1
Periodontal health	Yes	830	37.8
	No	1365	62.2
DMFT	0	160	7.6
	1-5	708	33.7
	6-12	904	43.0
	>12	330	15.7
Acid erosion	Yes	469	21.1
	No	1757	78.9
Malocclusion	Yes	343	15.4
	No	1890	84.6
Good SPGH	Yes	1290	57.9
	No	937	42.1
Good SPOH	Yes	964	43.4
	No	1262	56.6

was 37.8%, the proportion of caries-free participants was 7.6%, the prevalence of dental erosion was 21.1% and the prevalence of malocclusion was 15.4%. A total of 43.4% of participants considered their oral health to be good. In contrast, up to 57.9% thought they had good general health. All of the variables are shown in Table 1.

SPOH and SPGH

Comparison of DMFT groups showed a significant difference between DMFT > 12 and the remaining DMFT ≤ 12 groups in the univariate analysis of SPOH and SPGH, but no significant difference between the DMFT ≤ 12 groups. The variables that affected SPOH were younger age, female sex, higher education level, no dentures, no smoking, brushing teeth twice or more a day, eating sugary food, drinking sugary drinks, periodontal health, DMFT ≤ 12, no dental erosion and no malocclusion (Table 2). These variables were associated with better SPOH.

The variables that were associated with good SPGH were consistent with good SPOH, such as younger age, female sex, higher education level, no dentures, no smoking, brushing teeth twice or more a day, drinking sugary drinks, periodontal health, DMFT ≤ 12, no dental erosion, and no malocclusion (Table 3).

Correlation between SPOH and SPGH

Table 4 shows that SPOH and SPGH are positively correlated and subjects with good SPOH had good SPGH, whereas those with poor SPOH had poor SPGH. A Spearman correlation coefficient showed a moderate

Table 2 Distribution of SPOH according to the different variables. A chi square test was used.

Variable			SPOH				*P* value
			Good		Not good		
			n	%	n	%	
Social demographics	Age, y	18–24	232	55.0	190	45.0	< 0.001
		25–34	387	50.9	373	49.1	
		35–44	173	40.6	253	59.4	
		45–54	97	32.3	203	67.7	
		> 55	75	23.6	243	76.4	
	Sex	Male	353	37.6	585	62.4	0.001
		Female	611	47.4	677	52.6	
	Education	Primary education or less	26	21.3	96	78.7	< 0.001
		Secondary education	304	36.8	522	63.2	
		Bachelor's degree or higher	605	49.9	608	50.1	
Oral hygiene habits	Dental prosthesis	Yes	31	18.7	135	81.3	< 0.001
		No	933	45.3	1127	54.7	
	Smoking	Yes	172	28.9	423	71.1	< 0.001
		No	792	48.6	839	51.4	
	Brush teeth twice or more a day	Yes	827	47.6	912	52.4	< 0.001
		No	123	27.5	325	72.5	
	Sugary food consumption	Yes	887	44.0	1131	56.0	0.019
		No	68	35.1	126	64.9	
	Sugary drink consumption	Yes	848	45.9	999	54.1	< 0.001
		No	108	30.4	247	69.6	
Oral disease	Periodontal health	Yes	463	56.1	363	43.9	< 0.001
		No	487	35.8	875	64.2	
	DMFT	0	85	53.1	75	46.9	< 0.001
		1–5	369	52.3	337	47.7	
		6–12	381	42.3	520	57.7	
		> 12	78	23.8	250	76.2	
	Acid erosion	Yes	173	37.1	293	62.9	0.003
		No	788	45.0	965	55.0	
	Malocclusion	Yes	124	36.4	217	63.6	0.005
		No	840	44.6	1045	55.4	

correlation but was statistically significant ($r = 0.593$; $P < 0.05$).

Multivariate analysis

Multivariate binary logistic regression analysis showed worse SPOH in the age groups above 35 years compared to the group aged 18 to 24 years. No dentures, no smoking, brushing teeth twice or more times a day, periodontal health, no malocclusion and DMFT ≤ 12 were positively related to good SPOH (Table 5).

The influencing factors of SPGH were similar to those of SPOH. The group aged 25 to 34 years had poor SPGH compared to those aged 18 to 24 years. Higher education, no dentures, no smoking, brushing teeth twice or more times a day, sugary drink intake, periodontal health, no malocclusion and DMFT ≤ 12 were factors influencing SPGH (Table 6).

Discussion

In the present study, the same independent variables were used to assess the factors associated with SPGH and SPOH. The results showed that in Chinese adults, good SPGH and good SPOH were associated with demographic information, oral health behaviours and oral disease factors, and there were many common influencing factors between the two. An individual's self-perceived health represents a summary statement concerning the ways in which various aspects of health, subjective and

Table 3 Distribution of SPGH according to the different variables. A chi-square test was used.

Variable			SPGH				*P* value
			Good		Not good		
			n	%	n	%	
Social demographics	Age, y	18–24	281	66.4	142	33.6	< 0.001
		25–34	491	64.6	269	35.4	
		35–44	227	53.3	199	46.7	
		45–54	154	51.3	146	48.7	
		> 55	137	43.1	181	56.9	
	Sex	Male	500	53.2	439	46.8	0.001
		Female	790	61.3	498	38.7	
	Education	Primary education or less	34	27.6	89	72.4	< 0.001
		Secondary education	415	50.1	414	49.9	
		Bachelor's degree or higher	805	66.5	405	33.5	
Oral hygiene habits	Dental prosthesis	Yes	55	33.1	111	66.9	< 0.001
		No	1235	59.9	826	40.1	
	Smoking	Yes	254	42.7	341	57.3	< 0.001
		No	1036	63.5	596	36.5	
	Brush teeth twice or more a day	Yes	1098	63.2	639	36.8	< 0.001
		No	178	39.5	273	60.5	
	Sugary food consumption	Yes	1184	58.7	834	41.3	0.015
		No	96	49.5	98	50.5	
	Sugary drink consumption	Yes	1117	60.4	731	39.6	< 0.001
		No	162	45.6	193	54.4	
Oral disease	Periodontal health	Yes	546	65.9	282	34.1	< 0.001
		No	722	53.0	639	47.0	
	DMFT	0	107	66.9	53	33.1	< 0.001
		1–5	447	63.3	259	36.7	
		6–12	532	59.0	370	41.0	
		> 12	123	37.5	205	62.5	
	Acid erosion	Yes	49	53.4	217	46.6	0.027
		No	1039	59.2	715	40.8	
	Malocclusion	Yes	179	52.3	163	47.7	0.024
		No	1111	58.9	774	41.1	

objective, are combined within their perceptual framework[18]. SPOH may be an appropriate indicator for use in diverse sociocultural contexts[19]. Some findings have indicated that oral health perception may be a useful measure in public health due to its relationship with health determinants and health service use[20].

We asked subjects to evaluate their own oral and general health condition with a single intuitive question. The proportion of respondents with good SPOH was 43.4%. This was significantly lower than reported in studies conducted in Yemen (51%)[16], Canada (81.5%)[21], Nigeria (58.3%)[22] and Brazil (59.7%)[23]. The proportion of participants who considered they had good SPGH was 57.9%, a significantly higher result than that for SPOH, and higher than in Japan (36.9%)[24] but lower than in the USA (83.0%)[25]. The diversity of findings could be explained by differences in age, health knowledge and awareness, the quality of health services in different countries and personality differences in ethnic groups in different countries. The

Table 4 Correlation between responses concerning SPOH and SPGH.

		SPGH				*P* value	Spearman correlation
		Good		Not good			
		n	%	n	%		
SPOH	Good	881	91.5	82	8.5	< 0.001	0.593
	Not good	408	32.4	850	67.6		

Table 5 Ordinal regression for the effect of study variables on SPOH status.

Variable		OR	OR (95% CI)		*P* value
			Lower	Upper	
Age, y	18–24	1 (reference)	NA	NA	
	25–34	0.938	0.717	1.228	0.644
	35–44	0.665	0.489	0.905	0.009
	45–54	0.636	0.444	0.911	0.014
	> 55	0.56	0.377	0.832	0.004
Dental prosthesis	Yes	0.494	0.305	0.800	0.003
	No	1 (reference)	NA	NA	NA
Smoking	Yes	0.597	0.472	0.756	< 0.001
	No	1 (reference)	NA	NA	NA
Brush teeth twice or more a day	Yes	1.717	1.329	2.219	< 0.001
	No	1 (reference)	NA	NA	NA
Sugary drink consumption	Yes	1.592	1.198	2.114	0.001
	No	1 (reference)	NA	NA	NA
Periodontal health	Yes	1.674	1.366	2.051	< 0.001
	No	1 (reference)	NA	NA	NA
Does patient have any other oral diseases – malocclusion?	Yes	0.730	0.557	0.955	0.022
	No	1 (reference)	NA	NA	NA
DMFT	0	1.864	1.171	2.967	0.009
	1–5	1.703	1.207	2.403	0.002
	6–12	1.380	0.996	1.913	0.053
	> 12	1 (reference)	NA	NA	NA

Table 6 Ordinal regression for the effect of study variables on SPGH status.

Variable		OR	OR (95% CI)		*P* value
			Lower	Upper	
Age, y	18–24	1 (reference)	NA	NA	
	25–34	0.979	0.734	1.305	0.884
	35–44	0.670	0.488	0.920	0.013
	45–54	0.958	0.667	1.376	0.817
	> 55	0.918	0.662	1.355	0.666
Education	Primary education or less	1 (reference)	NA	NA	NA
	Secondary education	1.706	1.032	2.8200	0.037
	Bachelor's degree or higher	2.535	1.511	4.2510	< 0.001
Dental prosthesis	Yes	0.647	0.429	0.976	0.036
	No	1 (reference)	NA	NA	NA
Smoking	Yes	0.571	0.455	0.715	< 0.001
	No	1 (reference)	NA	NA	NA
Brush teeth twice or more a day	Yes	1.829	1.431	2.334	< 0.001
	No	1 (reference)	NA	NA	NA
Sugary drink consumption	Yes	1.536	1.171	2.020	0.002
	No	1 (reference)	NA	NA	NA
Periodontal health	Yes	1.253	1.015	1.547	0.036
	No	1 (reference)	NA	NA	NA
Does patient have any other oral diseases – malocclusion?	Yes	0.757	0.583	0.984	0.037
	No	1 (reference)	NA	NA	NA
DMFT	0	1.836	1.154	2.923	0.010
	1–5	1.579	1.140	2.186	0.006
	6–12	1.602	1.186	2.164	0.002
	> 12	1 (reference)	NA	NA	NA

results obtained showed that SPGH is at a slightly better level than SPOH, but still requires attention. The results suggest that more attention should be paid to oral health by improving people's oral health knowledge and behaviour through relevant policies and social publicity; in this way, we can also improve oral health.

In the multivariate analysis, subjects who smoked or used to smoke had bad SPOH and SPGH. This may be due to the fact that smoking is prone to altering oral, lung and intestinal microbiota, leading to various general diseases like periodontitis, asthma, chronic obstructive pulmonary disease, Crohn's disease, ulcerative colitis and cancer[26]. Good SPOH and SPGH status were also associated with the oral health behaviour of brushing the teeth twice or more times a day. The oral environment is a highly complex microenvironment in which a wide variety of microorganisms coexist[27]. Tooth brushing may help to maintain the oral flora balance. Some bacteria in the oral microbiota are directly associated with respiratory pathogens that cause pneumonia[28], and general inflammation can also be caused by the oral pathogen soluble antigen[29]. It was reported that some links between oral diseases and general diseases may be due to unhealthy behaviours that are harmful to both oral and general health30, for example smoking. Furthermore, studies supported that the areas in which oral disorders had the most significant impact were tooth brushing and cleaning[31,32]. Subjects without dentures were found to have better SPOH and SPGH; denture wear may be associated with previous oral disease, multiple dentition defects or full edentulism and the discomfort of wearing removable dentures. Furthermore, we found that periodontal disease, caries and malocclusion have an effect on SPOH and SPGH, which is consistent with the findings of Arantes and Frazão[19]. This result indicates that SPOH can partially reflect actual oral health status, so it can be considered as a more effective oral health evaluation index when assessing people's oral health status. Oral disease–related variables have an impact on SPGH status, and this also indicates that oral disease is partially associated with general health. This may be because periodontitis is a risk factor in the complex pathogenesis of diabetes, cardiovascular disease, kidney disease and recurrent pneumonia[33]. Caries lesions and periodontal disease can also cause some pain and tooth loss, which can affect chewing function. Oral health can impact food choices and have a negative effect on food intake, resulting in poor nutritional conditions and leading to chronic systemic disease, which in turn affects oral health[34]. Malocclusion can affect the aesthetic appearance of the face. Dental crowding in malocclusion can easily lead to plaque retention and can affect tooth cleaning, leading to caries lesions and periodontal disease.

The results showed a significant positive correlation between SPOH and SPGH. A study confirmed that oral health is an integral part of systemic health, and it is certain that oral health is part of general health[35]. We found many common risk factors related to oral health between SPOH and SPGH. This suggests that to prevent and control oral diseases and systemic diseases, we must not ignore the role of oral health–related factors and that there should not be just one method for prevention and treatment of oral or systemic diseases, but rather multidisciplinary cooperation, with oral health education included in general disease prevention and disease control projects.

Conclusion

SPOH better reflects actual oral health status and can be used as a regular indicator to predict oral health status. SPOH and SPGH have many common risk factors related to oral health, so more attention must be paid to oral health in order to promote general health.

Acknowledgements

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Conflicts of interest

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

Author contribution

Drs Yi Zhen YU, Shuo DU, Min DING and Zhi Ying CUI analysed the data; Dr Yi Zhen YU drafted the paper; Drs Yan Si and Yi Liu conceived the programme of research, checked the analysis and critically revised the manuscript. All authors approved the final manuscript for submission.

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Expressions of GLUT-1, PK-M2 and HIF-1α and Mutation Status of BRAF in Odontogenic Keratocysts

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Objective: *To investigate the expressions and clinicopathological features of glucose transporter 1 (GLUT-1), pyruvate kinase M2 (PK-M2) and hypoxia-inducible factor 1α (HIF-1α) in odontogenic keratocysts (OKCs), and to investigate the mutation status of v-raf murine sarcoma viral oncogene homolog B1 (BRAF).*

Methods: *Following a retrospective review of the clinicopathological data of 28 OKC cases, the expressions of GLUT-1, PK-M2 and HIF-1α in these tissue samples were detected through immunohistochemistry. The BRAF mutation statuses of all cases were examined using polymerase chain reaction amplification and direct sequencing.*

Results: *The expression levels of HIF-1α varied in 96.4% of OKC tissues, and there were higher positive rates of PKM2 (100%) and GLUT-1 (100%) in these tissues. None of the 28 OKC samples carried the BRAF mutation.*

Conclusion: *The positive expressions of GLUT-1, PK-M2 and HIF-1α indicate that patients with OKCs undergo anaerobic glycolysis to a certain extent, but these processes appear to be irrelevant to clinicopathological features and to the BRAF mutation.*

Key words: *BRAF gene, GLUT-1, HIF-1α, odontogenic keratocyst, PK-M2*
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Odontogenic keratocysts (OKCs) are benign odontogenic epithelial lesions. They originate from the remnants of the dental lamina, including the epithelial rests of Serres or the epithelium of the oral mucosa[1]. OKCs are of particular interest because of their aggressive behaviour and high recurrence rates[2]. Notwithstanding the numerous hypotheses regarding the pathogenesis of lesions, their exact aetiology remains unclear. Recent evidence suggests that the genetic basis for OKC development relates to clonality, abnormal cell cycle, proliferation, apoptosis, tumour suppression, matrix metalloproteinase activity and abnormal signalling pathways[3,4].

The biological behaviour and molecular pathology of OKCs remain controversial. Despite their high recurrence rates and locally aggressive behaviour[1], OKCs are derived from odontogenic epithelial elements; however, the pathogenesis of these odontogenic tumours remains unclear. The 2005 World Health Organisation

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(WHO) classification defined an OKC as an odontogenic tumour and named it a keratocystic odontogenic tumour. It was defined as "a benign intraosseous tumour of odontogenic origin, with a characteristic lining of para-keratinized stratified squamous epithelium and potential for aggressive, infiltrative behaviour"[5]. In the latest edition of the WHO classification, published in 2017, OKCs were classified as odontogenic cysts, owing to the ongoing debate regarding their neoplastic nature[6].

The pathogenesis of OKCs remains unclear. Increasing evidence shows that metabolic reprogramming may play a significant role in the occurrence and development of tumours. Even under aerobic conditions, rapidly proliferating tumour cells still use high-rate glycolysis as their main energy supply. This increases the glucose uptake and lactic acid production of tumour cells, thus enabling them to obtain the large numbers of synthetic substances needed for survival and unlimited proliferation. This phenomenon is called the Warburg effect or aerobic glycolysis[7]. Thus, the energy metabolism needs of tumour cells require a very efficient sugar transport pathway. Hypoxia inducible factor 1 (HIF-1) is a nuclear transcription factor that regulates cell oxygen homeostasis; HIF-1α is the main subunit of its activity regulation. It drives glycolysis to supply energy by mediating the transcription of glucose transporter 1 (GLUT-1) and other genes to maintain the energy metabolism of tumour cells[8,9]. At the same time, lipid-insoluble glucose can enter malignant tumour cells and participate in further metabolism, though this can only occur with the help of transporters. Thus, GLUT-1 is considered the first rate-limiting factor of glucose metabolism in malignant tumour cells. Tumours are generally highly dependent on anaerobic glycolysis; they require an increased level of glucose uptake, which explains the overexpression of GLUT-1. In all types of cancers, the overexpression of GLUT-1 has been associated with poor differentiation and poor prognosis regarding malignant tumours[10,11]. Pyruvate kinase M2 (PK-M2) is the rate-limiting enzyme in the last step of the glycolysis pathway. It cannot catalyse this last step and glucose metabolism cannot be carried out, resulting in a large number of intermediates in the pathway. Thus, these intermediates are converted into pentose phosphate, and participate in amino acid synthesis and other pathways for the synthesis of nucleic acids, proteins and other biological macromolecules needed for tumour cell growth. Pyruvate kinase catalyses the conversion of phosphoenolpyruvate to pyruvate. In recent years, researchers have found that PKM2 is closely related to the maintenance of the Warburg effect and tumorigenesis[12-14]. It has also been shown that replacing PKM2 with its isoenzyme PKM1 in tumour cells can reverse the Warburg effect, reduce lactic acid production, increase oxygen consumption and inhibit cell growth[12]. HIF-1 has also been shown to be highly expressed in many tumour cells. This may be related to the regulation of the expression of glycolysis-related genes[13]. PK-M2 can increase the transcriptional level of HIF-1, and there is a positive feedback regulation between HIF-1 and PKM2[14]. Few studies have investigated metabolism regarding OKCs, so in the present study, HIF-1α, GLUT-1 and PK-M2 were investigated regarding glucose metabolism to determine whether the Warburg effect also exists in OKCs.

Gene mutation profiles can greatly affect the pathological understanding and clinical management of OKCs in another important respect. Mitogen-activated protein kinases (MAPKs) are serine-/threonine-specific protein kinases that transduce intracellular signals in critical biological events, including cell proliferation, differentiation, survival, death and transformation[15]. In this vital pathway, the v-raf murine sarcoma viral oncogene homolog B1 (BRAF), a member of the RAF family, functions as a pro-oncogene. BRAF gene mutations lead to continuous activation of the MAPK pathway, which results in abnormal cell proliferation and tumorigenesis[16,17]. Some recent in vitro and in vivo studies[18,19] have reported that the MAPK signalling pathway contributes significantly to the pathogenesis of OKCs. The development of high-throughput deoxyribose nucleic acid (DNA) sequencing methods, such as next-generation sequencing using Illumina sequencing, has advanced studies into the MAPK signalling pathway and their association with OKC. This has revealed the BRAF V600E-mediated activation of the Ras/Raf/MAPK-extracellular signal-regulated kinase (MEK/ERK) pathway[18,19]. Many recent reports have suggested that high frequencies of gene alterations affecting the MAPK pathway exist in ameloblastomas[20]; however, previous findings regarding the BRAF V600E mutation in OKC are inconsistent. Brown et al[21] reported that none of the 19 OKC cases they analysed harboured the mutation, as assessed using BRAF V600E allele-specific polymerase chain reaction (PCR). Brunner et al[22], however, detected a wild-type BRAF variant in a patient with an OKC. França et al[18] detected the BRAF V600E mutation in one of the 28 OKC cases they analysed, using TaqMan allele-specific Qpcr. These results appear to suggest that BRAF V600E might not play a central role in OKC pathogenesis. However, Cha et al[19] detected BRAF gene mutations in 24 out of 38 cases (63.2%), indicating that this mutation might play an

important role in the pathogenesis of OKCs.

Mutated BRAF genes play an important role in driving metabolic reprogramming. The BRAF V600E mutation has been found to significantly upregulate the expression of HIF-1α in melanoma and thyroid papillary carcinoma[23,24]. In papillary thyroid cancer, BRAF mutations can also induce the overexpression of PKM2 and GLUT-1[25,26]. BRAF mutations can also induce DNA strand breaks, activate the DNA damage response pathway and upregulate the expression of GLUT-1 in non-transformed epithelial cells. This provides a tumour-promoting phenotype by enhancing glucose metabolism in cells[27]. The aforementioned studies show that the mechanism of tumour occurrence and development caused by BRAF mutations is related to the glucose pathway. To date, no studies have examined the relationship between BRAF gene mutations and metabolic reprogramming in OKC.

Thus, the purpose of the present study was to examine the expression of HIF-1α, PKM2 and GLUT-1 and determine whether there is metabolic reprogramming in OKC, and to detect the mutation of BRAF to clarify its relationship with metabolic reprogramming in OKC.

Materials and methods

Sample selection

Twenty-eight cases of OKC were included; the patients were diagnosed between 2009 and 2021 at the Department of Oral Pathology, Peking University School and Hospital of Stomatology, Beijing, China. Clinical data, including age, lesion location, disease course, symptoms, histological type, clinical findings and follow-up data, were reviewed. All fresh tissue and paraffin-embedded blocks with diagnoses of OKC were evaluated by two experienced pathologists using hematoxylin and eosin (HE) staining. This study was reviewed and approved by the Ethical Committee of Peking University Health Science Centre (no. PKUSSIRB-201840167).

DNA extraction

DNA was extracted from formalin-fixed paraffin-embedded tissue samples. In total, 24 paraffin-embedded OKC tissue samples were analysed. After the samples were deparaffinised with xylene, genomic DNA was extracted from the paraffin-embedded tissues using a QIAamp DNA Mini Kit (Qiagen, Duesseldorf, Germany), according to the manufacturer's instructions. The concentration and purity of DNA were measured using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and 70 ng genomic DNA was used for PCRs.

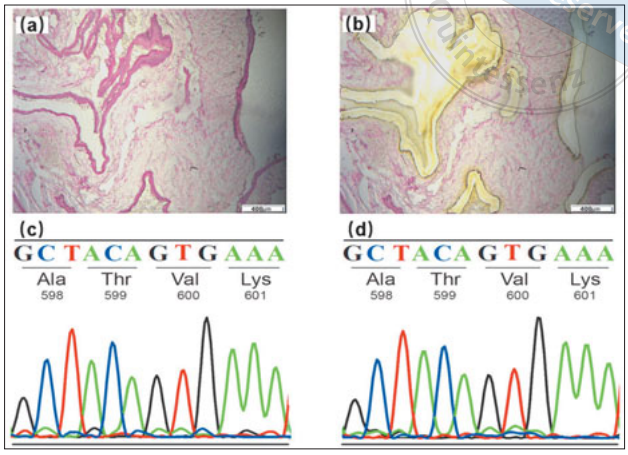

Fig 1 Laser capture microdissection and non-BRAF V600E mutations in odontogenic keratocysts.

Laser capture microdissection (LCM)

In total, 20 fresh frozen OKC samples were collected by certified medical pathologists. They were embedded in optimal cutting temperature media and HE staining was performed on the collected tissue samples to ensure the accuracy of the cases and determine the amount of epithelial tissue. Frozen sections of OKC tissues were prepared using a CM1950 cryostat (Leica, Wetzlar, Germany). The epithelial tissues of these samples were collected using laser-assisted microdissection 7000 (Leica; Fig 1). Genomic DNA of the frozen epithelial tissues was extracted using the QIAamp DNA Mini Kit according to the manufacturer's instructions. The concentration and purity of DNA were measured using the NanoDrop 8000 spectrophotometer and 70 ng genomic DNA was used for PCRs.

PCR and direct sequencing

PCR was performed using 50 μl reaction mixture, containing 25 μl Premix Ex Taq (Takara Bio, Kusatsu, Japan), 0.5 μl of each primer (approximately 10 pmol of each primer; primer sequences: forward: 5'-TGCTTGCTCTGATAGGAAAATG-3'; reverse: 5'-CCACAAAATGGATCCAGACA-3'), 22 μl nuclease-free water and approximately 70 ng template DNA. Thermocycling conditions were optimised for each primer pair and the following conditions were used: initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for

Table 1 Positivity and dilution of metabolism-related protein immunoexpression in OKCs.

Antibodies	Pretreatment	Dilution	Company	–	+	++	+++
HIF-1α	EDTA HIER	Operating fluid	ZSGB-Bio (Beijing, China)	1 (3.58%)	21 (75.00%	3 (10.71%)	3 (10.71%)
PK-M2	EDTA HIER	1:150	OriGene (Rockville, MD, USA)	0	6 (21.43%)	12 (42.86%)	10 (35.71%)
GLUT-1	EDTA HIER	Operating fluid	ZSGB-Bio	0	1 (3.58%)	10 (35.71%)	17 (60.71%)

HIER, heat-induced epitope retrieval; –, negative; +, weakly positive with less than 33% of epithelial cells positive; ++, moderately positive with 33% to 66% of epithelial cells positive; +++, strongly positive with more than 66% of epithelial cells positive.

Table 2 Demographic, clinical and pathological features of the studied samples.

Case no.	Sex	Age, y	Site	BRAF (exon 15)	HIF-1α	GLUT-1	PK-M2
1	M	49	Mandible	Wildtype	+	++	+++
2	M	15	Maxilla	Wildtype	–	+++	+++
3	F	3	Mandible	Wildtype	+	+++	+++
4	M	26	Mandible	Wildtype	+	+++	++
5	F	55	Mandible	Wildtype	+	+	++
6	M	19	Mandible	Wildtype	+	+++	+++
7	M	16	Maxilla	Wildtype	+	+	++
8	F	32	Mandible	Wildtype	+	++	+
9	F	11	Mandible	Wildtype	+	+++	+++
10	M	61	Mandible	Wildtype	+	+++	+++
11	F	27	Maxilla	Wildtype	+	+++	+++
12	M	68	Mandible	Wildtype	+	++	+++
13	M	67	Mandible	Wildtype	+	++	+++
14	M	41	Mandible	Wildtype	+	++	+++
15	F	40	Mandible	Wildtype	+	++	++
16	M	40	Maxilla	Wildtype	+	++	+++
17	F	51	Mandible	Wildtype	+	++	+++
18	F	27	Mandible	Wildtype	+++	+	++
19	M	20	Mandible	Wildtype	+++	+	++
20	M	41	Maxilla	Wildtype	+++	+	++
21	M	34	Mandible	Wildtype	++	+	+++
22	F	21	Mandible	Wildtype	+	++	++
23	F	30	Maxilla	Wildtype	+	++	+++
24	F	61	Mandible	Wildtype	++	++	++
25	M	12	Maxilla	Wildtype	++	++	++
26	M	12	Maxilla	Wildtype	+	+++	+++
27	F	25	Mandible	Wildtype	+	+++	+++
28	M	20	Mandible	Wildtype	+	+++	+++

F, female; M, male; –, negative; +, weakly positive with less than 33% of epithelial cells positive; ++, moderately positive with 33% to 66% of epithelial cells positive; +++, strongly positive with more than 66% of epithelial cells positive.

30 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 30 seconds, then final extension at 72°C for 10 minutes. The amplified products were sequenced directly using the primers employed in the original PCR. Sequencing was performed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Waltham, MA, USA). All detected mutations were confirmed via reverse sequencing, and via at least two additional independent PCR experiments.

GLUT-1, PKM2 and HIF-1α immunohistochemistry (IHC)

HE staining and IHC were performed on 3-µm thick tissue sections in all 28 cases. Negative control slides were incubated with a buffer instead of with antibodies. Sections, when available, were stained for HIF-1α, PKM2 and GLUT-1 antibodies, as listed in Table 1; the IHC reactivity for each tumour tissue sample was estimated via light microscopy. The positive rates of all markers were analysed.

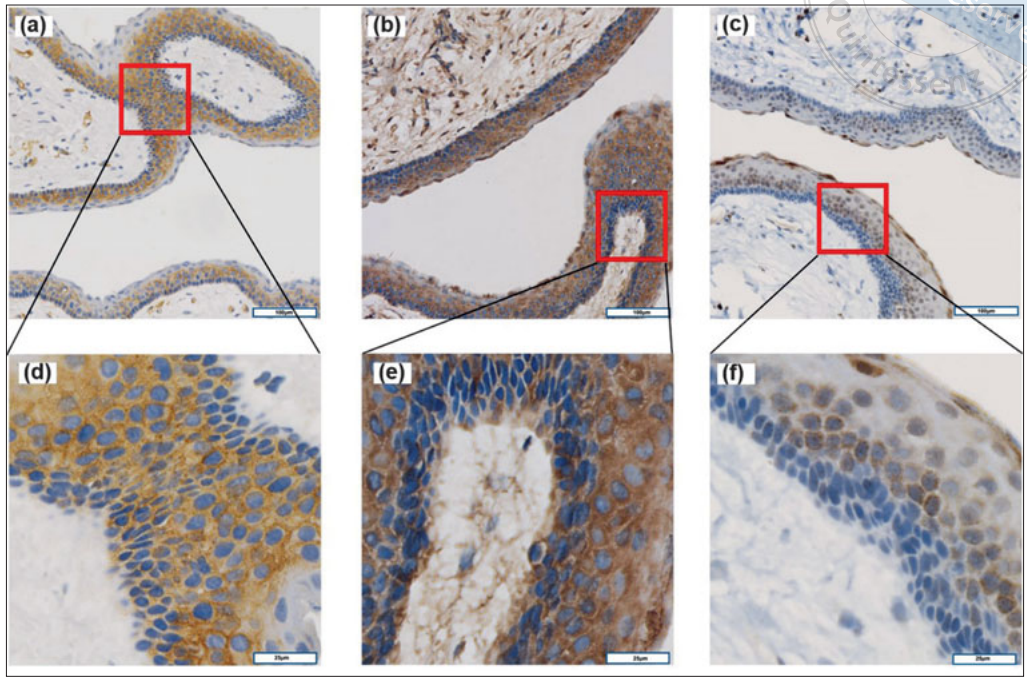

Fig 2 (a and d) The reactions with GLUT-1 show positive staining in all cases. **(b and e)** The reactions with PK-M2 show positive staining in all cases. **(c and f)** The reactions with HIF-1α show positive staining in 96.4% cases.

Statistical analysis

Categorical variables were expressed as percentages and compared using a Fisher exact test. Normally or near-normally distributed variables were presented as mean ± standard deviation (SD). Differences between repeated measures within a group were analysed using *t* tests. Statistical analysis was performed using SPSS 19.0. The level of statistical significance was set at $P < 0.05$.

Results

The demographic and clinical features of the OKC samples used in this study are shown in Table 2. The 28 patients with OKCs included 16 males and 12 females; 8 cases occurred in the maxilla and 20 in the mandible. The patients' ages ranged from 3 to 68 years (median age 28.5 years). None of the 28 paraffin tissue samples carried the BRAF gene mutation, and this mutation was also not found in the 20 fresh tissue samples from these 28 cases. Nevertheless, metabolism-related proteins showed a high positive rate, among which the positive rate of HIF-α was 96.4% and those of GLUT-1 and PK-M2 were both 100.0% (Table 1). Immunohistochemical reactivity was detected for both GLUT-1 and PK-M2 in the cytoplasm of parakeratinized epithelium 5-8 cell layers (Figs 2a, b, d and e). Immunohistochemical reactivity was detected for HIF-1α in the nuclei and cytoplasm of epithelial cells in the prickle and superficial cell layers, and to a lesser extent within epithelial cells within the basal and parabasal cell layers (Figs 2c and 2f).

Discussion

OKCs are locally aggressive lesions with a high recurrence rate and strong proliferative activity[28-30]. The majority of malignancies, including prostate cancer, glioma, hepatocellular carcinoma and other human cancers, exhibit GLUT-1, PK-M2 and HIF-1α, which may enable anabolic metabolism to support cell proliferation, neoplasm spread and recurrence[31-33]. The present study reports that GLUT-1, PK-M2 and HIF-1α are overexpressed in OKCs; these traits indicate that OKCs have substantial metabolic demands and that metabolic reprogramming may have a defining role in explaining the aggressive clinical behaviour, high growth potential and high recurrence rate of OKCs.

Although several treatment methods have been developed in addition to simple enucleation, surgical approaches are often limited owing to the high recurrence of OKCs, as well as their locally destructive consequences. In recent years, strategies for targeting glycolysis metabolic pathways during the treatment of malignant tumours have attracted much attention through the Warburg effect. Carrying out targeted glycolysis therapy in OKCs is a potentially valuable research direction.

Thus, the question of how to screen and determine the subtypes and functions of glycolytic enzymes with high expressions regarding OKC specificity is worthy of special attention. In addition, due to the heterogeneity and microenvironment variability of OKC cells, the expressions and activities of glycolytic enzymes may change. Furthermore, the therapeutic effects of a single glycolytic enzyme target may not be particularly effective. Multiple glycolytic enzyme target combination therapy is also worth exploring. The inhibition of glycolysis may not be enough to directly kill tumour cells, so adding inhibitors for other metabolic processes may prove more effective. Many clinical applications have revealed that a BRAF-targeted approach is crucial for identifying better methods to prevent, diagnose or treat ameloblastoma. Novel targeted therapies are required to treat or cure ameloblastoma effectively, and studies into BRAF-targeted therapies in ameloblastoma have yielded considerable advancements[34].

The correlation between BRAF mutations and enhanced glucose uptake, lactate generation and phosphoserine biosynthesis in colorectal cancer suggests a shift towards the glycolysis pathway[23,25,26]. Overexpression of GLUT1, PKM2 and HIF-1 was also detected in papillary thyroid cancer, with BRAF mutations[24,25]. These mutations are common in odontogenic tumours such as ameloblastomas and ameloblastic fibromas. In this study, however, no BRAF V600E mutation was detected in OKCs.

Previous research has implicated the chromosome 9q22.3-q31-mapped tumour suppressor gene PTCH1 in the development of OKCs[28,35]. PTCH1 functions as a hedgehog receptor and inhibits the hedgehog signal transduction pathway by inhibiting the oncogene SMO. A study revealed that OKCs are susceptible to PTCH1 gene mutations[28]. Inactivation of the PTCH1 gene causes its protein to lose its inhibitory effect on SMO, resulting in constitutive activation of the hedgehog pathway, which stimulates the odontogenic epithelium in the jaw to regain its proliferative potential[28]. It has been demonstrated that hedgehog pathway activation can increase PKM2 mRNA levels, thus facilitating glycolytic reprogramming observed in medulloblastoma[36]. Further research is required to determine whether PTCH1 gene mutations are associated with the metabolic abnormalities observed in OKCs.

Although the sample size of this study was small, the in-depth study of OKC-related gene profiles and metabolism-related targets is expected to explain their pathogenesis through the combined detection of these two aspects. This approach is an important target for the diagnosis and treatment of OKC.

Conclusion

In summary, the findings presented suggest that BRAF mutations do not play a significant pathogenic role in OKCs, and the role of BRAF-targeted therapy seems rather limited regarding OKCs. Metabolic reprogramming appears to play a key role in the occurrence and outcomes of OKCs. Further research is needed to establish the breadth of the mechanism and determine whether metabolic reprogramming studies for testing and therapy can support the precise medicine-based treatment of OKCs.

Conflicts of interest

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

Author contribution

Drs Zhu YOU and Jing DU contributed to the data collection and drafted the manuscript; Dr Li Li XU contributed to the data collection; Drs He Yu ZHANG and Xue Fen LI contributed to the data analysis; Drs Zhi Peng SUN and Li Sha SUN contributed to the study design and revision of the manuscript. All authors approved the final version of the manuscript.

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OPTIMAL TECHNIQUES AND SOLUTIONS

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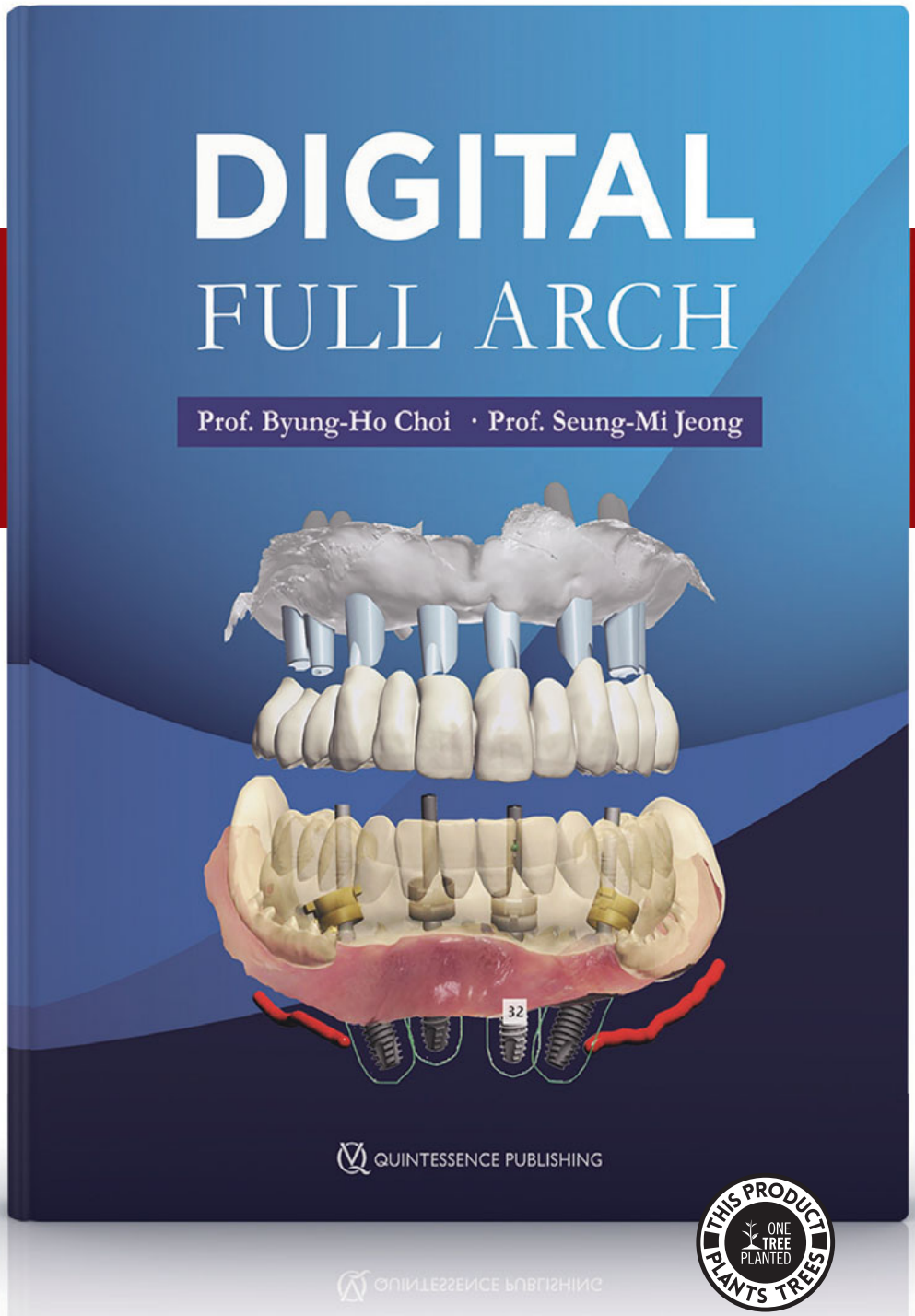

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Prefabricating Implant-supported Interim Prosthesis from CBCT Scans: a Case Report of Digital Immediate Implant Restoration

Hong Ye YANG[1,2#], Aihemaiti MUHETAER[1,2#], Ya Ke WANG[1,2], Cui HUANG[1,2]

This clinical report describes the immediate implant placement and restoration for a 47-year-old woman with a protruded and loose maxillary right central incisor. The treatment included minimally invasive extraction, flapless immediate implant placement using a fully guided surgical template, and immediate implant-supported provisionalisation. The interim anatomical prosthesis was fabricated in advance based on preoperative CBCT scans, and the digital technique made it possible to integrate data precisely from different sources. After 6 months of provisionalisation, satisfactory gingival aesthetic and functional improvements were achieved, followed by a definitive screw-retained zirconia restoration. Thus, application of a complete digital workflow could reduce chairside time and create an optimal emergence profile that matches the residual bone architecture of the extraction sites with minimal interference.

Key words: *aesthetic restoration, anterior teeth, digital dentistry, flapless surgery, immediate implant*
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After tooth extraction, alveolar bone resorption is inevitable and presents many challenges for implant restoration, especially in the aesthetic zone[1,2]. Immediate implant placement combined with immediate restoration is a preferred treatment option to preserve peri-implant bone and soft tissues which significantly shortens the treatment period, reduces the number of surgical procedures and improves aesthetics quickly[3-6]. A previous study recommended the synergistic application of flapless surgery and immediate implant restoration to further reduce the collapse of the peri-implant bone and soft tissue[7]. As such, a straightforward and noninvasive method of designing an implant-supported anatomical interim prosthesis from CBCT scans was required.

This clinical report presents a complete digital workflow of flapless and immediate implant restoration, including diagnosis and the design and fabrication of the implant-supported prosthesis.

Case report

Patient profile

The patient first presented to the Department of Prosthodontics, School and Hospital of Stomatology, Wuhan University, Wuhan, China, when she was 47 years old, with the chief complaints of tooth loosening and an unaesthetic smile. She was eager to achieve a better aesthetic and functional outcome with implant restoration. The facial examination showed that the protruded max-

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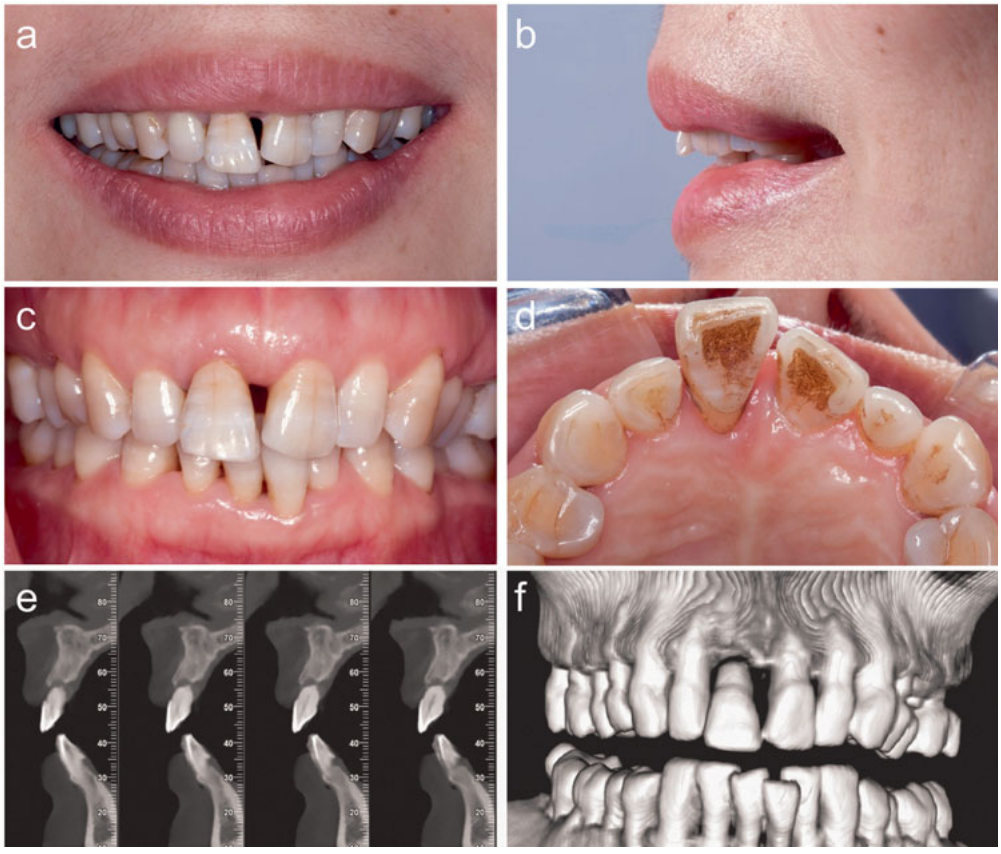

Fig 1 Pretreatment clinical examination. **(a)** Frontal smile view. **(b)** Profile smile view. **(c)** Anterior view of dentition. **(d)** Maxillary arch view. **(e and f)** CBCT slice and reconstituted images.

illary right incisor produced a disharmonious lip–tooth relationship, excessive tooth visibility at rest and black triangles during her maximum smile (Figs 1a and b). An intraoral examination identified Class 3 mobility of the right central incisor according to Miller's classification (Figs 1c and d). A CBCT evaluation further confirmed obvious resorption in the root apical zone of the maxillary right central incisor and separate from the alveolar ridge, and no inflammation was seen (Figs 1e and f). It was determined that the occlusal relationship was stable and there was sufficient restorative space.

Treatment plan

After the preoperative clinical and radiographic examination, treatment alternatives and costs were discussed. A comprehensive, detailed treatment plan was proposed, which comprised minimally invasive extraction of the maxillary right central incisor, insertion of the implant in an optimal 3D position, followed by appropriate provisionalisation, and delivery of the definitive restorations after 6 months of peri-implant tissue stability. The patient was informed of the clinical plan and informed consent was obtained prior to treatment.

Design of the surgical template and interim prosthesis

The CBCT scans generated digital imaging and communications in medicine (DICOM) files, while a 3D desktop lab scanner (iSCAN-I; Shining 3D, Hangzhou, China) was used to produce standard tessellation language (STL) files from the patient's dentition. The DICOM and STL files were merged in planning software (3Shape Implant Studio; 3Shape, Copenhagen, Denmark) to design the fully guided surgical template for implant placement (Figs 2a to c). We segmented the tooth data from the preoperative CBCT scans to design the implant-supported interim anatomical prosthesis (Fig 2d). Specifically, the emergence profile of the prosthesis was designed based on the tooth to be extracted, while the crown contour was designed with reference to the contralateral natural tooth.

It is noteworthy that, compared with the extracted tooth, the subcritical contour of the customised temporary abutment was reduced to leave enough space for the soft tissue (Figs 3a and b). The design software was also used to map the bone density around the entire circumference of the planned implant, which indicated that ideal primary stability could be achieved (Fig 3c)[8]. All the design data were sent to the dental laboratory

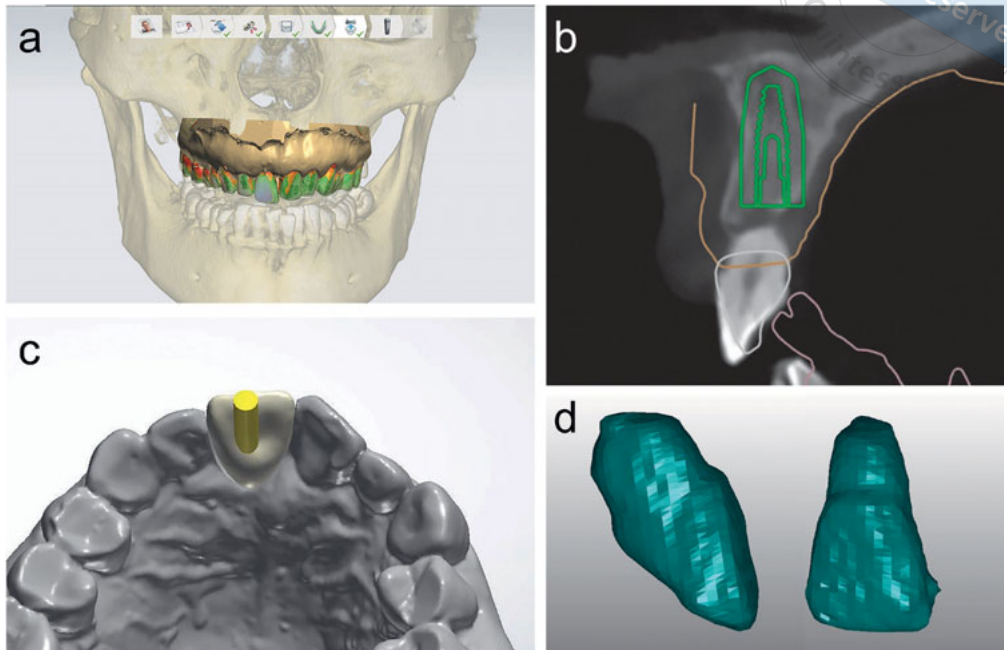

Fig 2 Design of surgical template and interim prosthesis. **(a)** Fusion of the CBCT scans and digital impression. **(b and c)** Implant placed in the optimal 3D position. **(d)** Exported tooth STL file.

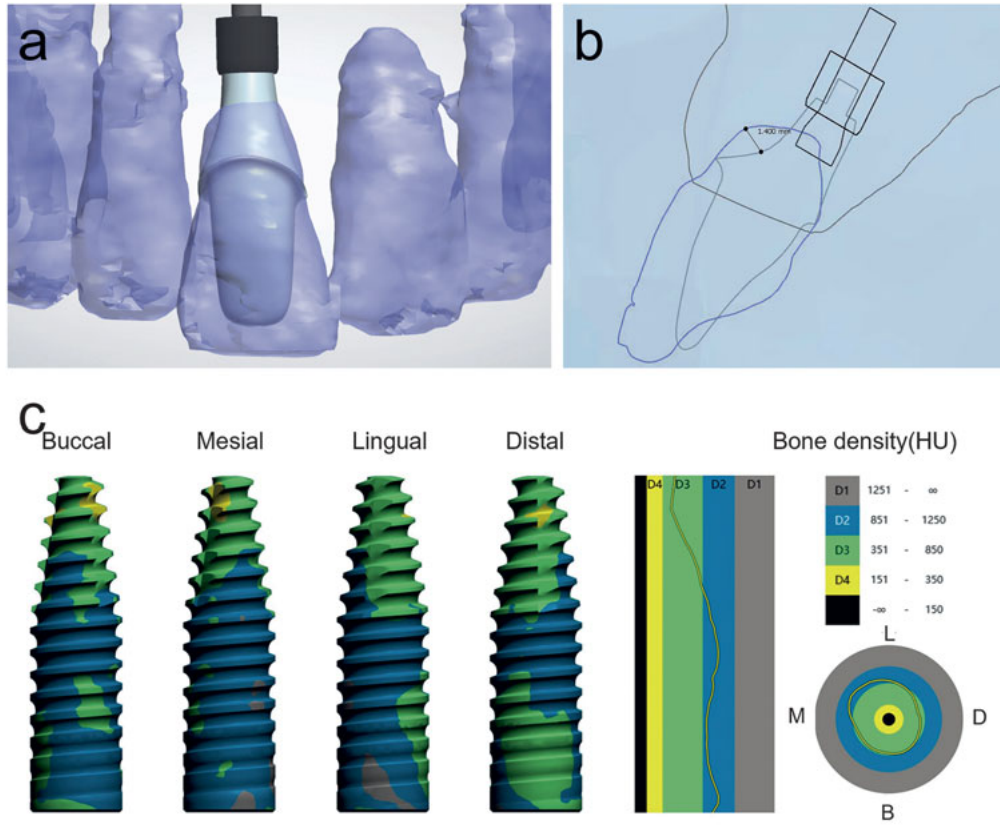

Fig 3 **(a)** Design of the temporary abutment. **(b)** The diameter of the temporary abutment was reduced on the buccal side. **(c)** Bone map around the planned implant.

for fabrication and 3D printing and the surgical template (Fig 4a) and interim prosthesis were produced. The interim prosthesis was composed of a CAD/CAM-customised titanium temporary abutment and an acrylic resin crown (Figs 4b and c) and they were cemented extraorally to form an integrated structure before use.

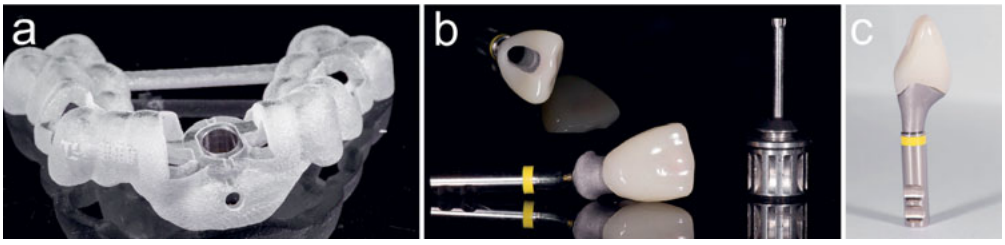

Fig 4 **(a)** Prefabricated surgical template. **(b and c)** Interim prosthesis.

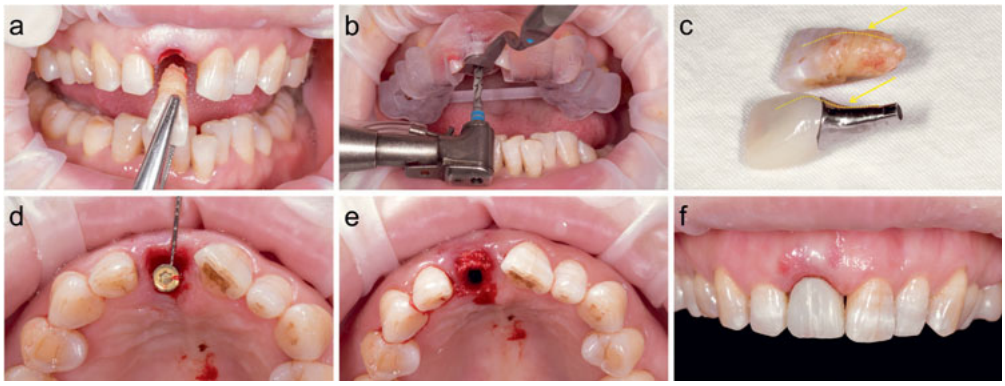

Fig 5 Surgical procedures. **(a)** Minimally invasive tooth extraction. **(b)** Fully guided implant placement. **(c)** Comparison of the extracted tooth and the temporary prosthesis. **(d)** Implant placement in a prosthetically guided position. **(e)** Bone augmentation. **(f)** Temporary restoration.

Treatment steps

Minimally invasive extraction of the maxillary right central incisor was performed under local anaesthesia, followed by socket cleaning (Fig 5a). Implant placement procedures were performed step by step according to the digital planning surgical template (Fig 5b). The self-tapping conically shaped implant (Straumann Bone Level Tapered, Basel, Switzerland) was inserted, and the primary stability reached over 35 Ncm, which allowed for immediate provisionalisation[9]. After trying-in of the interim prosthesis and confirming it matched with the actual extraction site, a healing abutment (3.6*5.0 mm) was screwed into the position to avoid the bone graft material entering the implant screw hole during the following bone augmentation procedure (Fig 5d). Guided bone regeneration (GBR) was performed using bone substitutes (Bio-Oss; Geistlich Pharma, Wolhusen, Switzerland) (Fig 5e). Finally, the implant-supported interim prosthesis was placed without occlusal contact, and this treatment can close the implant cavity to stabilise the underlying bone graft substitute and support the soft tissue contour. The screw hole was closed with Teflon and composite resin (Fig 5f).

After 6 months of provisionalisation, clinical and radiographic examination verified that the patient had preserved the contour and volume of the peri-implant bone and soft tissues, achieved gingival aesthetics and stabilised the gingival margin (Fig 6). Then, the customised impression coping was fabricated and definitive impressions were made with a polyether impression material (Impregum Penta Soft, 3M, St Paul, MN, USA) and a 3D printed customised open tray. It is notable that the labial appearance of the definitive prosthesis was designed according to the shape, contours and characteristics of the left central incisor, as well as the acrylic resin interim crowns (Fig 7). The screw-retained zirconia restoration was completed and tried-in to evaluate the aesthetic performance, occlusion and phonetics clinically. The patient was very satisfied with the final treatment outcome. The pink and white aesthetic scores were 8 and 9, respectively, according to the maxillary anterior single-tooth implants aesthetic index[10]. She was instructed to perform adequate oral hygiene. After two years of treatment, she has maintained good oral hygiene and the hard and soft tissues have remained stable.

Preoperative design and actual placement of the implant body

The data from the preoperative and postoperative CT scans were analysed using planning software. After superimposing the preoperative CBCT images on the postoperative CBCT images, 3D positional deviations, distances and angulations between the preoperative designed position and the actual placement of the implant body were measured (Fig 8).

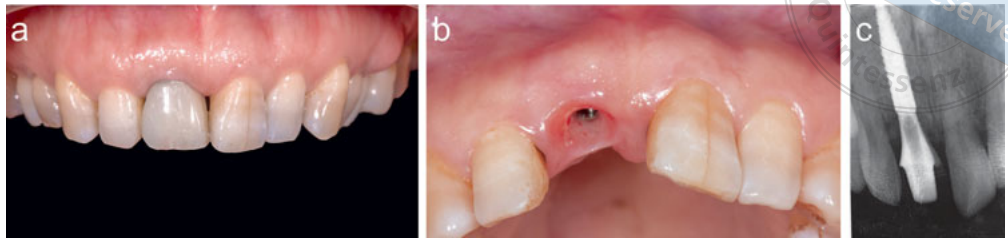

Fig 6 After 6 months of provisional restoration. **(a)** Anterior view. **(b)** Gingival emergence profile. **(c)** Periapical radiograph.

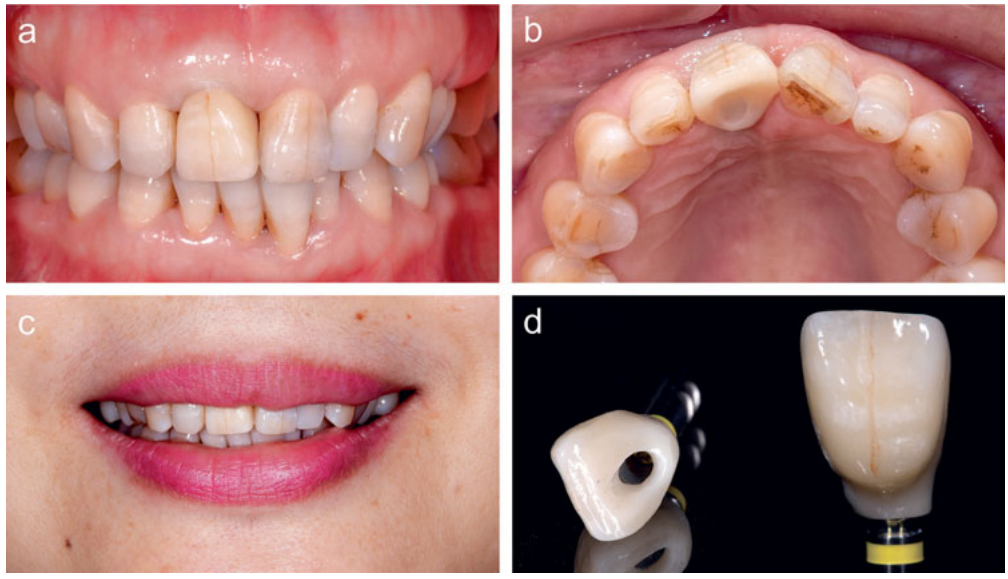

Fig 7 Posttreatment clinical examination. **(a)** Anterior view of the dentition. **(b)** Maxillary arch view. **(c)** Frontal smile view. **(d)** Definitive screw-retained zirconia prosthesis.

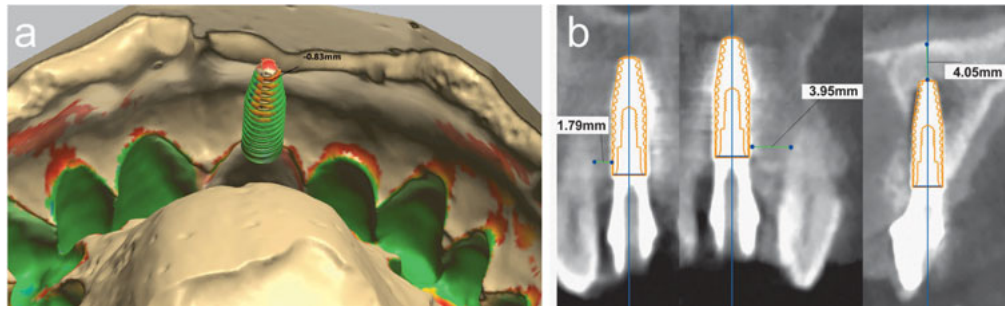

Fig 8 **(a)** Assessment of implant placement deviation. **(b)** Postoperative CBCT examination.

Discussion

According to the simplified extraction socket classification proposed by Elian et al[11], the extraction socket in the present study was Type I; that is, both the facial soft tissue and the buccal bone plate were at normal levels in relation to the cementoenamel junction. As such, the implant was placed simultaneously to tooth extraction, and immediate insertion of an interim prosthesis into the fresh extraction socket was an appropriate choice to guide wound healing and counter the physiological process of tissue resorption in this case.

Traditionally, for immediate provisionalisation, the temporary abutment is hand-tightened onto the implant, and composite resin was used directly to build up the buccal/lingual contour of the interim crown, then the mesial/distal contour and emergence profile were layered carefully extraorally. After repeated grinding, trimming and polishing, the immediate restoration can be obtained[12]. However, this method relies heavily on the dental practitioner's composite building skills, and the steps require a considerable amount of chairside time. Furthermore, there is a risk of contaminating the surgical wound during this procedure. In the present study, to overcome these problems and achieve more desirable aesthetic results, we applied a straightforward method to design an interim anatomical prosthesis based on preoperative CBCT scans. Use of the appropriate software distinguished the different components of the 3D

images developed by CBCT scans, making it possible to record the subgingival anatomy of the tooth to be extracted and the crown contour of the contralateral natural tooth at the same time[13]. The customised design of critical and subcritical contour is beneficial to achieve a better emergence profile for the definitive restoration[14].

The six critical steps in this paper for minimally invasive immediate implant placement and restoration were, in consecutive order:

1. Surgical template design and interim prosthesis preparation;
2. Minimally invasive tooth extraction;
3. Fully guided flapless immediate implant placement;
4. Guided bone regeneration;
5. Implant-supported provisionalisation;
6. Delivery of the screw-retained definitive restoration.

Immediate implant placement protocols in the aesthetic region require thorough treatment planning and step-by-step execution. Traditional implant surgery involves flap reflection to prepare the site for fixture positioning. In this case, the surgery began with a minimally invasive tooth extraction without damaging the buccal bone wall. Flapless surgery could reduce surgical discomfort, minimise aesthetic damage and preserve the contour and volume of the peri-implant tissues[15]. However, flapless surgery with digital immediate implant placement is highly technique sensitive and requires a skilled clinician. There are some ways to help beginners in implantology reduce errors. First, beginners should ensure complete seating of the surgical guide and master the correct use of the guide cassette. Second, complete osteotomy depth but under-drilling for primary stability is necessary in most immediate placement cases. Third, it is important to fill the jumping gap with slow-resorbable biomaterial to prevent bone resorption after tooth extraction. Last but not least, soft tissue augmentation is recommended for patients with a thin gingival biotype.

With the aid of digital techniques, accuracy analysis of implant placement was performed in the present case. The 3D deviation in the entry section and tip section and the maximum angle shift of the implant body was 0.85 mm, 0.83 mm and 1.2 degrees (Fig 8), respectively, which was substantially less than reported in previous studies[16]. It could be observed that there was sufficient distance between the implant body and adjacent teeth. Based on the experience in the present case, we suggest that a fully digital workflow could represent a very attractive method to increase the accuracy of implant placement and provisional restoration.

Conclusion

This clinical report presented a complete digital workflow for immediate and flapless implant placement, and a novel strategy for prefabricating an implant-supported interim prosthesis from CBCT scans was developed. This approach reduced time and created an optimal emergence profile that matched the residual bone architecture of the extraction sites with minimal interference.

Acknowledgements

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Conflicts of interest

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

Author contribution

Dr Hong Ye YANG diagnosed and treated the patient and checked the manuscript; Dr Aihemaiti MUHETAER created the treatment plan, collected the data and drafted the manuscript; Dr Ya Ke WANG treated the patient and checked and revised the manuscript; Dr Cui HUANG created the treatment plan and revised the manuscript. All authors approved the final manuscript.

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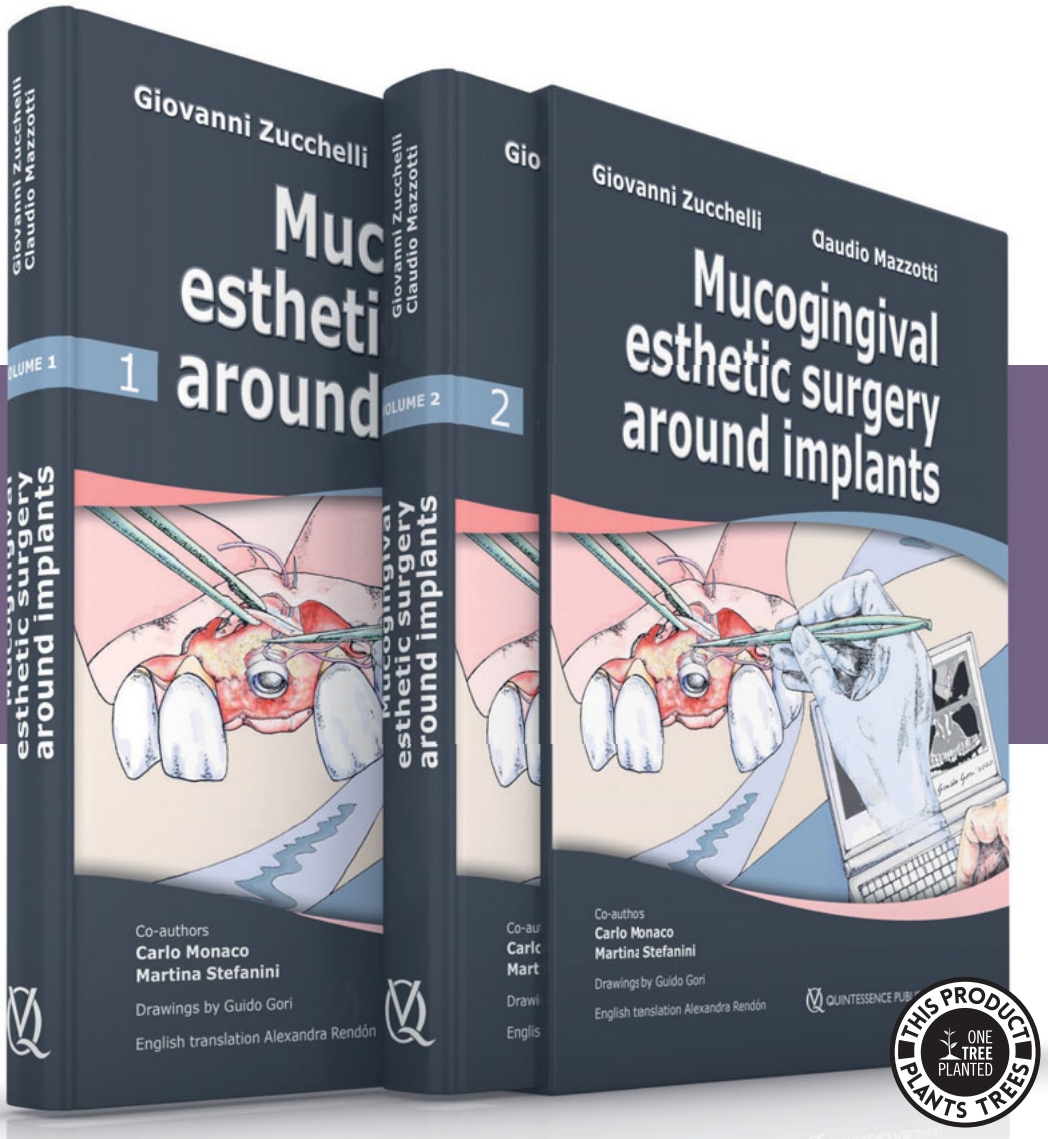

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Orthodontic and Orthognathic Treatment Combined with Surgically Assisted Rapid Maxillary Expansion in an Adult Patient with a Hyperdivergent Skeletal Pattern

Yi Neng HAN[1,2], Hao LIU[1,2], Zi Li LI[2,3], Yi Ping HUANG[1,2], Wei Ran LI[1,2]

In this case study, we report the successful treatment of a 35-year-old woman with a hyperdivergent skeletal pattern, open bite and severe transverse deficiency, exhibiting a skeletal Class III malocclusion. The treatment plan included 3D correction of these issues with surgically assisted rapid maxillary expansion (SARME) to solve the transverse deficiency, presurgical orthodontic treatment including aligning and levelling of the teeth in both arches, LeFort I osteotomy and bilateral sagittal split ramus osteotomy, and postsurgical correction of malocclusion. Orthodontic treatment was performed with labial brackets, and the patient achieved satisfactory occlusion and a significantly improved facial profile. Retention at the 1-year follow-up showed stable occlusion and arch forms with a harmonious facial profile.

Key words: *hyperdivergent skeletal pattern, open bite, orthodontic-surgical treatment, surgically assisted rapid maxillary expansion, transverse deficiency*
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Hyperdivergent skeletal patterns are often accompanied by anterior open bite, which is considered one of the most difficult orthodontic problems to correct[1]. Patients with hyperdivergent growth patterns may also have severe maxillary transverse and sagittal deficiencies. Lateral cephalometric radiographs of such patients often show a steep mandibular plane, obtuse gonial angle and increased height of the lower third of the face[2]. To correct severe skeletal deformities and establish a stable occlusion in adults, orthognathic surgery combined with orthodontic treatment is often needed to achieve an acceptable functional and aesthetic outcome.

In this case report, we describe a patient with a hyperdivergent skeletal pattern and open bite who underwent orthodontic treatment, orthognathic surgery and surgically assisted rapid maxillary expansion (SARME), which greatly improved her occlusion and facial profile.

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Case report

Diagnosis and aetiology

A 35-year-old Chinese woman presented with the chief complaint of a "long face" and an anterior open bite. She stated that she did not have any bad oral habits and had no contraindications to orthodontic treatment. The patient was diagnosed with degree II hypertrophy of the palatine tonsils. She had a convex profile, a hyperdivergent vertical skeletal pattern and lip incompetence at rest (Fig 1). The upper lip–tooth display was balanced and the mandibular midline was skewed by 1.0 mm to the patient's right. She also had marked mandibular asym-

Fig 1 Pretreatment facial and intraoral photographs.

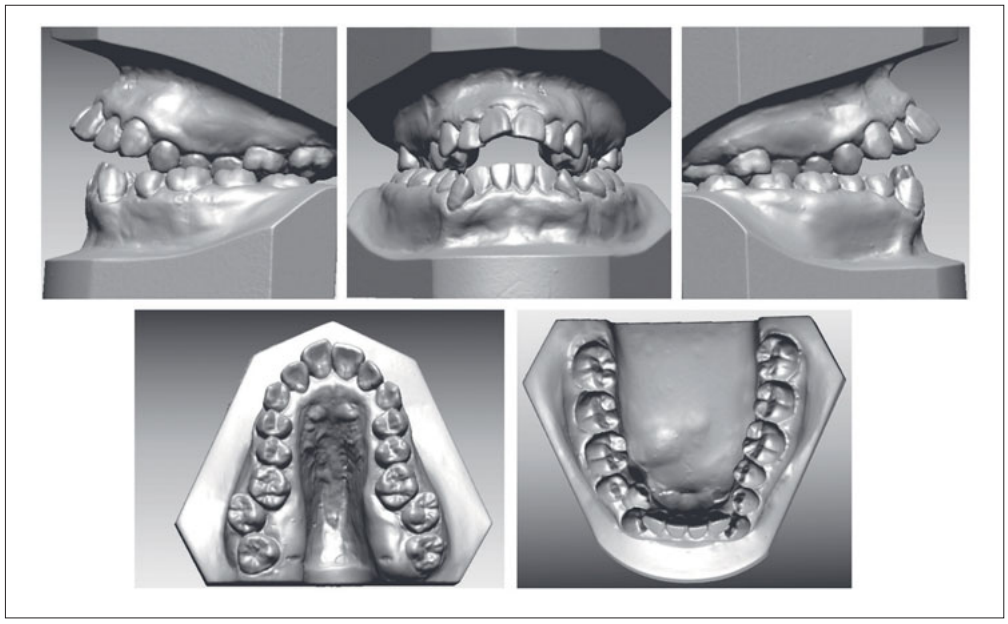

Fig 2 Pretreatment dental casts.

metry, with the chin point shifted 4.0 mm to the right. Intraoral photographs (Fig 1) revealed an Angle Class III molar relationship with a 7.0-mm open bite and a 7.5-mm horizontal overlap. The dental cast analysis showed a severe transverse discrepancy, with a maxillary intercanine width of 31.7 mm and an intermolar width of 35.8 mm. A bilateral reverse articulation of the first molars was also observed. Moderate crowding of the maxillary teeth (5.0 mm) and severe crowding of the mandibular teeth (10.0 mm) were present (Fig 2). There was no tooth size discrepancy, and there was a moderate curve of Spee. Although temporomandibular joint asymmetry was observed, the patient said she displayed no signs or symptoms of temporomandibular disorders.

Table 1 Cephalometric measurements.

Measurement		Norm* (mean ± SD)	Pre-treatment	Post- treatment	Difference
Sagittal skeletal components	SNA(degrees)	82.8 ± 4.0	82.1	81.8	1.9
	SNB(degrees)	80.1 ± 3.9	77.6	80.5	2.8
	ANB(degrees)	2.7 ± 2.0	4.5	1.3	–0.8
Vertical skeletal components	MP/SN(degrees)	32.5 ± 5.2	51.9	44.5	–7.4
	PFH(mm)	82.5 ± 5.0	76.7	75.4	–1.3
	AFH(mm)	128.5 ± 5.0	134.5	124.0	–10.5
	S-Go/N-Me (PFH/AFH)(%)	65.0 ± 4.0	57.0	60.9	3.9
Dental components	U1/NA(degrees)	22.8 ± 5.7	37.2	16.0	–21.2
	L1/NB(degrees)	30.5 ± 5.8	18.9	18.3	–0.6
	U1/L1(degrees)	125.4 ± 7.9	121.7	144.4	22.7
	U1/SN(degrees)	105.7 ± 6.3	117.1	97.8	–19.3
	LI/MP(degrees)	92.6 ± 7.0	69.3	73.3	4.0

*Reference norms for Chinese adults.

AFH, anterior facial height; ANB, A point-nasion-B point; L1/MP, mandibular incisor to mandibular plane; L1/NB, mandibular incisor to nasion-B point line; MP/SN, mandibular plane to sella-nasion plane; PFH, posterior facial height; PFH/AFH, ratio of PFH to AFH; SD, standard deviation; SNA, sella-nasion-A point; SNB, sella-nasion-B point; U1/L1, interincisal angle; U1/NA, maxillary incisor to nasion-A point line; U1/SN, maxillary incisor to sella-nasion plane.

Lateral cephalometric analysis revealed a high mandibular plane angle (MP/SN 51.9 degrees) (Table 1). There was disproportionality between the anterior lower facial height and the posterior facial height (76.7 mm and 135.5 mm, respectively), which was consistent with the patient's facial appearance. The maxillary incisors were proclined (U1/SN 117.1 degrees), whereas the mandibular incisors were lingually inclined (L1/MP 69.3 degrees). Panoramic radiographs showed complete permanent dentition with asymmetric condylar contours (Fig 3). Computed tomography (CT) showed that the labial alveolar bone was relatively thin, indicating that the inclination of the maxillary anterior teeth should be adjusted carefully to avoid periodontal damage (Fig 3). The oropharyngeal airway displayed an extremely limited volume (21882 mm^3) and minimum axial area (111.7 mm^2). CT revealed a constricted maxillary arch (J-J′) of 61.7 mm, which should be about 68.0 mm normally. Although palatine tonsillar hypertrophy may have contributed to this malocclusion, the aetiology was unclear.

In summary, the patient was diagnosed with Angle Class III malocclusion with a hyperdivergent skeletal Class II relationship, a severe open bite and transverse deficiency.

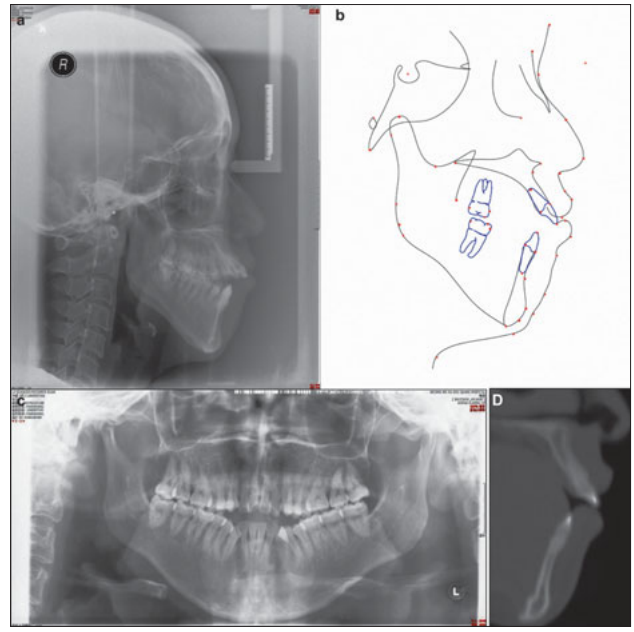

Fig 3 Pretreatment radiographs. **(a)** Pretreatment cephalometric radiograph. **(b)** Cephalometric tracing. **(c)** Panoramic radiograph. **(d)** Sagittal images from the CT.

Treatment objectives

The treatment objectives for the patient were maxillary expansion and correction of the transverse deficiency; correction of crowding in the maxilla and mandible and modification of the tapered arch forms; maxillary incisor retraction to reduce the horizontal overlap and open bite and correct the Class III molar occlusion; and correction of the mandibular asymmetry and improvement of the elongated facial profile.

Treatment plan

The severe transverse discrepancy needed to be addressed first. Conventional orthodontic expansion was ruled out because it is difficult to expand the maxilla by approximately 7 mm in an adult patient. Although max-

Fig 4 Patient after surgically assisted rapid maxillary expansion.

illary expansion during orthognathic surgery solves all skeletal problems in one surgical procedure, it increases the risks of the surgery greatly and reduces the stability of the final outcome. Thus, SARME using a hyrax appliance before presurgical orthodontic treatment was recommended. Subsequently, orthodontic treatment in combination with orthognathic surgery was performed with extraction of the maxillary premolars to reduce the proclination of the maxillary incisors and mandibular premolars and to correct the molar relationship prior to surgery. Orthognathic surgery involved LeFort I osteotomy for maxillary impaction and bilateral sagittal split ramus osteotomy (BSSRO) for mandibular rotation. The aim of treatment was to achieve an acceptable functional and aesthetic outcome. The treatment plan was fully explained to the patient, and she was aware of the risks.

Treatment progress

Two weeks before surgery, the maxillary first premolars and molars were separated using elastics. A conventional hyrax expander (9-mm expansion screw) was manufactured at Peking University, Beijing, China and bonded before surgery. Surgery was initiated using the established SARME methodology[3]. The hyrax expander was activated twice a day, creating 0.5-mm maxillary expansion daily. The amount of expansion was then evaluated clinically every other day. Two weeks days after SARME, the patient presented with 4 mm expansion of the palatine suture and a diastema between the maxillary central incisors (Fig 4). The hyrax was kept in situ for 6 months for retention based on a previous study[4].

Two mandibular second premolars were extracted and 0.022- × 0.028-inch straight wire preadjusted appliances (Shinya, Hangzhou, China) were placed in the maxilla, while segmental arches were used to distalise the first premolars to relieve the crowding of the mandibular incisors (Fig 5). Once sufficient space was obtained for the mandibular incisors, the two maxillary first premolars were extracted, the hyrax expander was removed and all the mandibular teeth were bonded with brackets for levelling and alignment. Placement of sequential nickel titanium archwires was initiated, starting with a 0.012-inch wire and ending with a 0.018- × 0.025-inch wire. To close the tooth extraction spaces, a 0.019- × 0.025-inch stainless-steel archwire was placed in the mandible using the classic sliding mechanism. Because of the thin labial and lingual upper alveolar bone, the maxillary extraction space was only used to reduce the labial inclination of the maxillary incisors without retraction of the anterior teeth, which may have led to bone fenestration or dehiscence. After achieving proper inclination of the maxillary incisors for surgery, the spaces remained, and were closed during orthognathic surgery. The patient was ready for surgery after 20 months of presurgical orthodontic treatment (Fig 6).

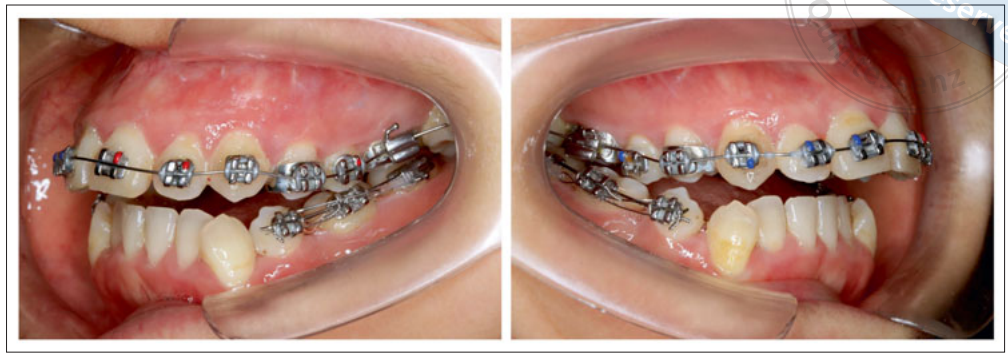

Fig 5 Brackets were bonded and segmental arches were used in the mandible to distalise the first premolars to relieve the crowding of the mandibular incisors.

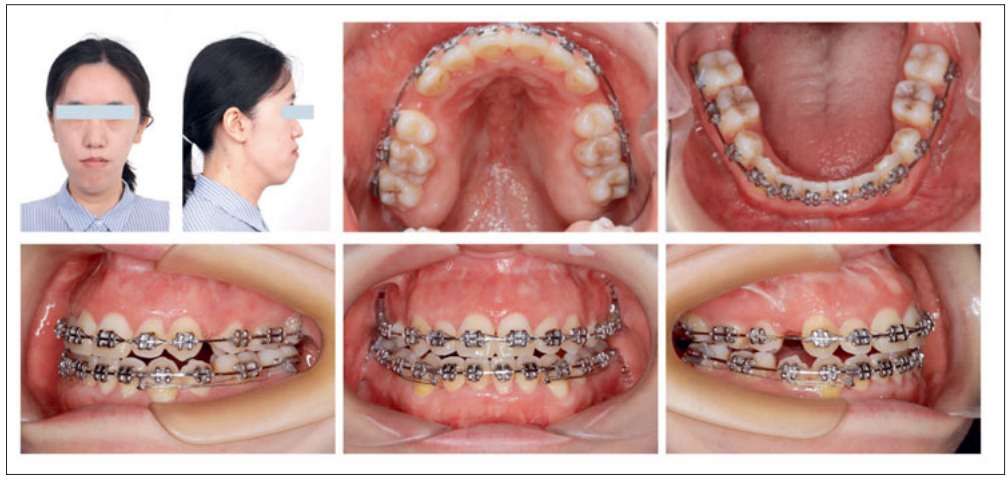

Fig 6 Preoperative facial and intraoral photographs.

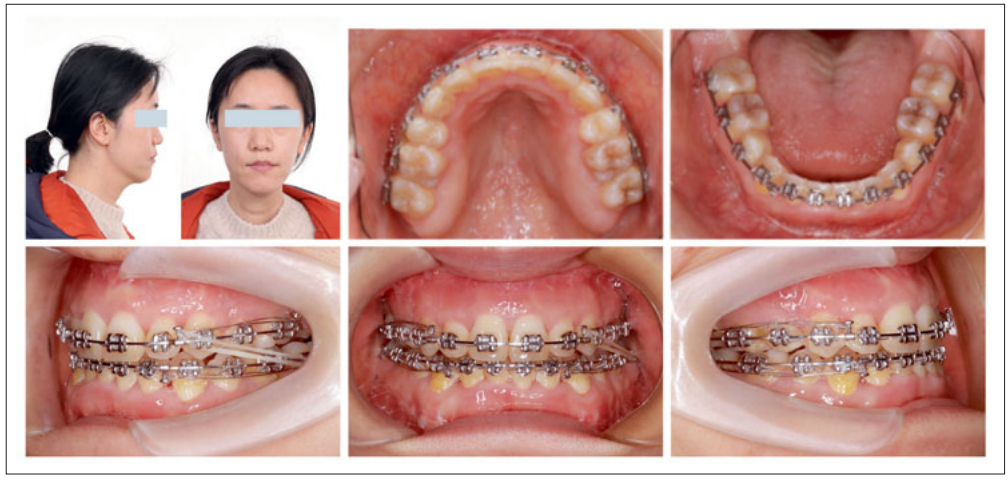

Fig 7 Postoperative facial and intraoral photographs.

A presurgical visual treatment objective (VTO) was used to guide the surgery (Fig S1, provided on request). According to the intermediate splint, the maxilla was split into four pieces: bilaterally at the tooth extraction spaces and mesial to the two central incisors. The posterior segments were raised by 5 mm and the anterior segments by 4 mm. The paranasal area was moved forwards by 2 mm, while the incisor edges remained unchanged. The intermediate splint was then replaced with the final splint. Anticlockwise mandibular rotation with BSSRO followed by rigid internal fixation was performed. One month after surgery, Class II elastics were used on the left side to treat the unilateral Class II malocclusion (Fig 7). The total treatment duration was 40 months. The severe transverse discrepancy, vertical overgrowth and sagittal protrusion were resolved. A normal horizontal and vertical overlap were achieved in both the anterior and posterior arches. The orthodontic appliances were removed after treatment completion, and invisible retainers were used for retention.

Fig 8 Posttreatment facial and intraoral photographs.

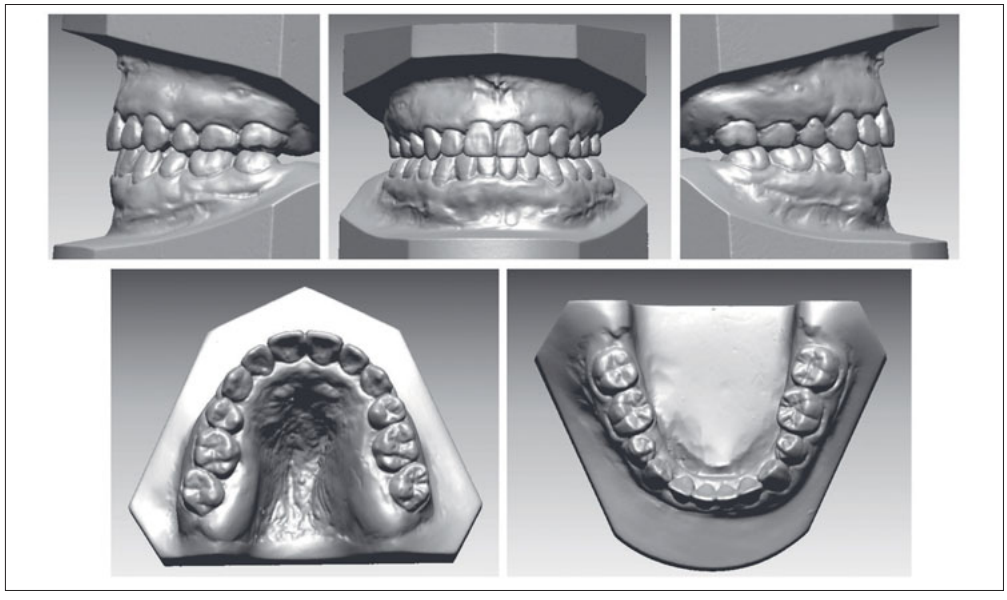

Fig 9 Posttreatment dental casts.

Treatment results

After treatment, extraoral photographs demonstrated that the hyperdivergent skeletal pattern had improved significantly, with a reduction in lip incompetence noted at rest (Fig 8). The decrease in facial height led to an improved facial profile. The anticlockwise rotation of the mandible decreased the angle of the mandibular plane. The maxillary incisor protrusion was corrected. Intraoral photographs showed significant correction of the arch shapes and well-aligned dentition (Figs 8 and 9). The open bite and horizontal overlap were corrected,

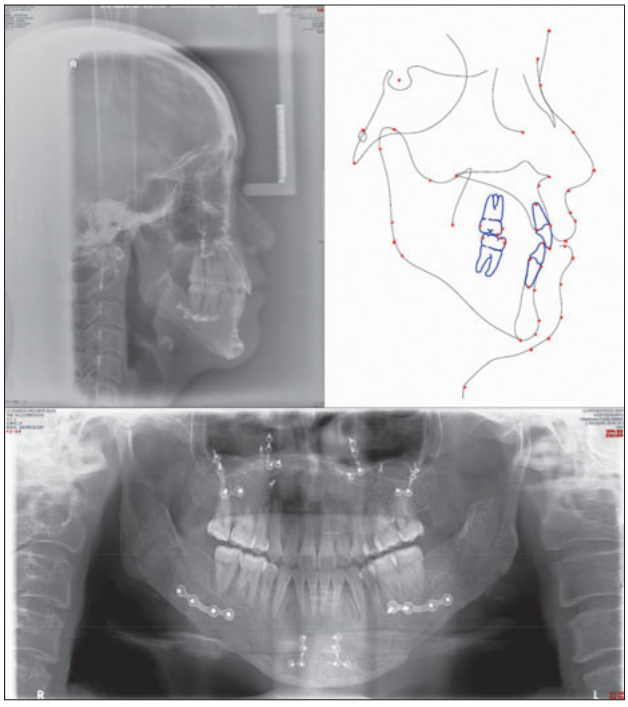

Fig 10 Posttreatment radiographs. **(a)** Posttreatment cephalometric radiograph. **(b)** Cephalometric tracing. **(c)** Panoramic radiograph.

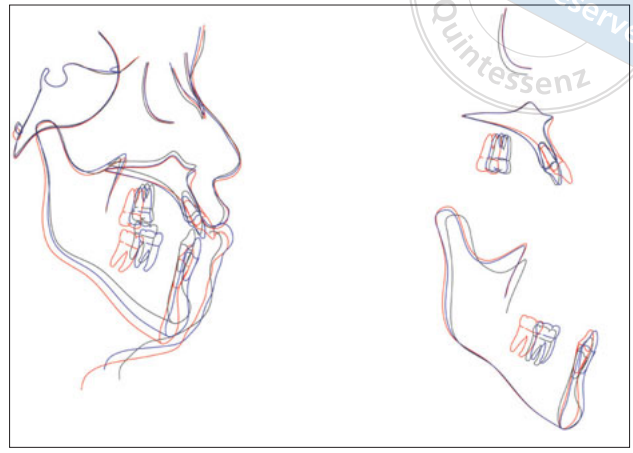

Fig 11 Pretreatment (red), preoperative (blue) and posttreatment (black) cephalometric superimpositions.

Table 2 Comparison of the means of maxillary dimensions before and after treatment.

Measurement	Pre-treatment (T0)	Post- treatment (T1)	1-year follow-up (T2)	T1–T0	T2–T0
Intercanine width (mm)	31.7	34.3	34.6	2.6	2.9
Inter–first molar width (mm)	35.8	46.2	46.1	10.4	10.4

Table 3 Comparison of maxillary width before and after treatment.

Measurement	Pre-expansion (T0)	Post-expansion (T1)	T1–T0
Maxillary width J-J' (mm)	61.7	67.1	6.3

and Class I molar and canine relationships were established. After treatment, the patient presented with a harmonious facial profile with decreased facial convexity.

The posttreatment panoramic radiographs demonstrated root parallelism in all the teeth, and there was no obvious root or alveolar bone resorption (Fig 10). Cephalometric analysis showed a significant decrease in MP/SN by 7.4 degrees and in U1/SN by 19.3 degrees (Table 1). The superimposition of the lateral cephalograms on the anterior cranial base from the pretreatment, preoperative (after presurgical orthodontics) and posttreatment phases demonstrated anticlockwise mandibular rotation with a decreased anterior facial height, although the patient still had a hyperdivergent vertical skeletal pattern. The superimposition on the palatal plane displayed molar mesialisation and incisor retroclination compared to pretreatment tracing (Fig 11). The patient showed less lip incompetence, and the soft tissue profile was markedly improved due to the decrease in vertical facial height and maxillary incisor retraction.

The intermolar width increased by 10.4 mm and the intercanine width increased by 2.6 mm (Table 2). In addition, CT showed that the maxillary skeletal base width had increased by 6.3 mm (Table 3).

Quantitative measurement of the oropharyngeal airway volumes was performed according to the following defined borders: anterior border, the vertical plane passing through the posterior nasal spine (PNS); superior border, the horizontal plane passing from the PNS; posterior border, the posterior pharyngeal wall; and inferior border, the horizontal plane passing from the most anterior and inferior point of the third vertebra. The minimum

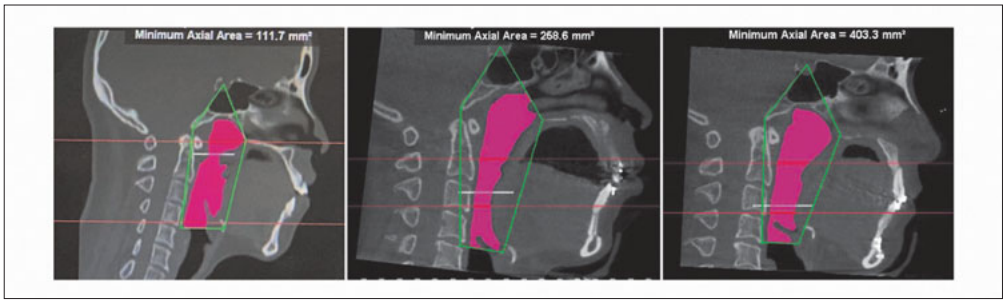

Fig 12 Pretreatment, post-SARME and postoperation oropharyngeal airway changes.

Table 4 Airway changes.

Measurement	Pre-treatment	Post-SARME	Post-treatment
Minimum axial area (mm^2)	111.7	258.6	403.3
Oropharyngeal airway volume (mm^3)	21882.0	23614.0	27283.9

axial areas increased by 146.9 mm^2 after SARME and by 296.1 mm^2 after mandibular advancement surgery. The volume of the oropharyngeal airway increased by 1732.0 mm^3 after SARME and 5401.9 mm^3 after mandibular surgery (Fig 12 and Table 4).

One year after completion of orthodontic treatment, the occlusion and arch forms were stable with a harmonious facial profile (Fig 13 and Table 2).

Discussion

The patient presented with a hyperdivergent skeletal pattern combined with an anterior open bite and transverse discrepancy. Orthodontic treatment was combined with a properly designed and executed surgical intervention to achieve this goal. Treatment of such patients requires 3D consideration due to their complexity.

In such cases, transverse discrepancies must be solved first. Rapid maxillary expansion (RME) is an effective clinical procedure for correcting transverse maxillary deficiency and increasing the arch length in adolescents. However, adult patients present with ossification of the midpalatal suture due to completed skeletal growth, and conventional RME has limited effects and can even cause periodontal defects and gingival recession[5]. SARME was first used in orthodontic treatment in 1938 to solve transverse discrepancies. Its advantages of SARME are improved nasal airflow, reduced buccal corridor when smiling and created space. In addition, SARME shows long-term stability of alveolar, intercanine, and intermolar widths according to a meta-analysis[3]. In the present patient, SARME successfully expanded the maxilla without obvious maxillary molar elongation, which may have led to clockwise rotation of the mandible and a worse facial profile. Considering the relapse of SARME over time, the hyrax expanders were removed after 6 months of use.

The effects of SARME on pharyngeal enlargement, which further improved nasal respiratory function, have been reported widely[6,7]. Some studies have reported that after SARME, respiratory parameters improved slightly during sleep, and the mandibular advancement procedure enlarged the airway significantly[8]. In the present patient, CT results showed that SARME enlarged the minimum axial area of the airway significantly, from 111.7 to 258.6 mm^2 (Table 4). The SARME procedure had a positive impact on the airway volume, provided the tongue with available space, improved the patient's respiratory function significantly and reduced lip incompetence.

A skeletal open bite is one of the most challenging malocclusions to treat because of the high frequency of relapse[9]. Conventional orthodontic treatment with molar intrusion using temporary anchorage devices (TADs) to achieve a compromised result without skeletal correction has been reported to result in relapse in approximately 10% to 30% of cases[10]. Orthognathic surgery is used widely to treat severe skeletal open bite; however, a meta-analysis showed that vertical relapses, including a decrease in vertical overlap and an increase in the intermaxillary angles and mandibular plane, were characteristics of numerous patients who received combined orthodontic surgical therapy regardless of surgery type[11]. Proffit et al[12] found that patients who underwent maxillary surgery only were less prone to relapse (7% decrease in vertical overlap) than those who received maxillomandibular surgery (12% decrease in vertical overlap). However, a recent meta-analysis reported that maxillomandibular surgery produced the most beneficial postoperative increase in vertical overlap and was more stable than maxillary or mandibular surgery[13]. Moreover, overcorrection of vertical overlap

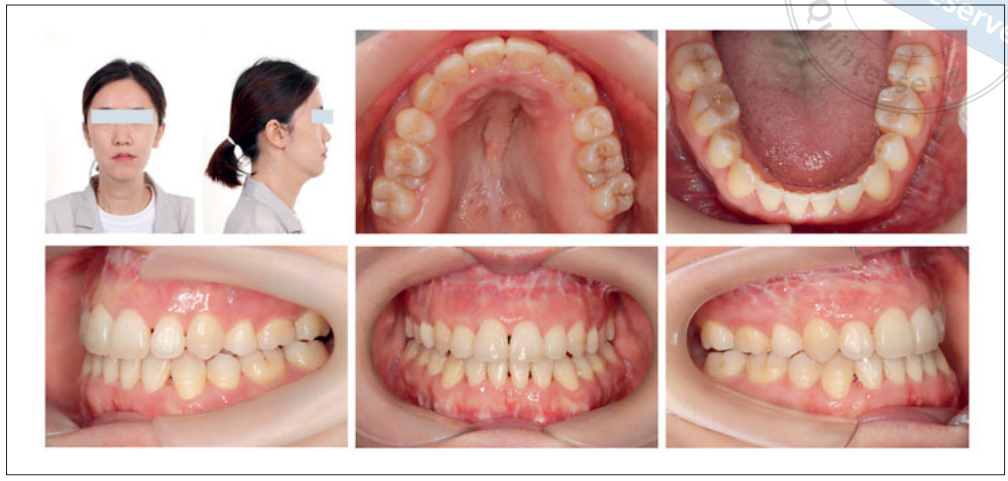

Fig 13 Postretention facial and intraoral photographs (1-year follow-up).

is often needed to reduce the risk of relapse. In this patient, maxillomandibular surgery was performed and a slightly deeper vertical overlap was achieved, which remained favourable at the 1-year follow-up.

To address the sagittal problems, the periodontal risks of alveolar bone fenestration and dehiscence should be considered. Thus, in this case, segmental arches were first used to reduce the proclination of the mandibular incisors and avoid periodontal issues. Moreover, the mandibular incisors were not retracted to close the extraction space before surgery; instead, we kept the space and closed it during orthognathic surgery, which posed little risk to periodontal health. Finally, BSSRO significantly alleviated the patient's obstructive sleep apnoea (OSA). OSA usually affects quality of life and can even increase the risk of death[14]. Previous studies have shown that maxillomandibular advancement surgery is helpful in patients with OSA. Mandibular advancement of more than 10 mm effectively enlarges the pharyngeal spaces, especially the oropharynx and hypopharynx, and has a positive effect on OSA patients[15]. In the present patient, enlargement of the oropharyngeal airway volume was significant, especially in the minimal axis area, which may significantly improve the patient's sleep quality.

Recently, 3D virtual planning has been used increasingly in orthognathic surgery, allowing to the clinician to simulate surgery and visualise the postoperative outcomes in the soft and hard tissues. Such technology was not used in the treatment planning for this patient, which can be considered a limitation of this study.

Conclusion

Orthodontic treatment combined with SARME and orthognathic surgery should be considered in patients with a hyperdivergent skeletal pattern and skeletal anterior open bite. 3D problems should be considered to achieve this goal. SARME can produce fewer negative effects on the teeth and is suitable for adults who have already completed their skeletal growth. BSSRO combined with Lefort I needs to be performed to reduce the high mandibular plane angle open bite in adult patients. The surgery can lead to a favourable anticlockwise mandibular rotation and optimal horizontal and vertical overlap. One year after surgery, no relapse was observed; however, to assess the long-term stability of this treatment modality, a longer follow-up period is needed in the future. Moreover, this case report demonstrated the harmonious cooperation between an orthodontist and oral and maxillofacial surgeons to achieve a successful functional and aesthetic outcome.

Conflicts of interest

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

Author contribution

Dr Yi Neng HAN took part in the orthodontic treatment and drafted the manuscript; Dr Hao LIU took part in the orthodontic treatment; Dr Zi Li LI and his team performed the surgical treatment; Dr Yi Ping HUANG took part in the orthodontic treatment and revised the manuscript; Dr Wei Ran Li performed the orthodontic treatment and approved the final manuscript.

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