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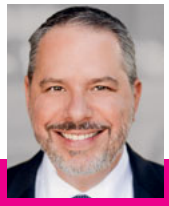

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Growth Factors in Periodontal Complex Regeneration

Fazele ATARBASHI-MOGHADAM[1], Maryam REZAI RAD[2], Sorane SIJANIVANDI[2], Pouya KHODAYARI[2], Masoumeh MAHMOUM[2]

The ultimate goal of periodontal treatments is the regeneration of all lost periodontal tissues including bone, cementum and the periodontal ligament (PDL). Until now, the clinical methods for periodontal regeneration have been associated with significant failure or incomplete success. Various studies have reported the promising effects of growth factors/cytokines on periodontal regeneration. Growth factors/cytokines include proteins or steroid hormones that bind to cellular receptors, known as signalling molecules, and that trigger cellular responses that eventually stimulate cell proliferation and differentiation. The present review aims to provide an overview of the main growth factors that play an important role in and have been used in the regeneration of periodontal components.

Key words: growth factors, cementogenesis, osteogenesis, periodontal regeneration, stem cells

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The supporting structure of the teeth, i.e, the periodontium, is composed of alveolar bone, root cementum, the periodontal ligament (PDL) and the gingiva which cover other components[1]. After completion of tooth crown development, root formation begins and dental follicle stem cells (DFSCs) differentiate to fibroblasts, cementoblasts and osteoblasts to form the PDL, cementum and alveolar bone, respectively[2]. These structures function as a unit[1] in which the principal PDL fibre connects the cementum to the alveolar bone and provides an apparatus to control all forces and support the dentition[1,3]. Destructive chronic inflammation of these supporting tooth structures, known as periodontitis, eventually results in tooth loss[4]. The ultimate goal of periodontal treatments is the regeneration of all lost periodontal tissue[4]. Until now, the clinical methods for periodontal regeneration have been associated with significant failure or incomplete success, and most have been technique sensitive[5].

Growth factors/cytokines include proteins or steroid hormones that bind to cellular receptors, known as signalling molecules, and result in cellular responses that eventually stimulate proliferation and differentiation[6,7]. Various studies have reported the promising effects of these signalling molecules on periodontal regeneration[3,6]. The present review aimed to provide an overview of the main growth factors that play an important role in and have been used in the regeneration of periodontal components.

Table 1 summarises the included studies and Fig 1 illustrates the main growth factors that play an important role in the regeneration of each periodontal component.

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Table 1 Growth factors used in periodontal complex regeneration.

Location	Growth factor	Effect
Bone	BMPs	BMP-7 induces PDLSCs and DFSCs towards the osteoblast/cementoblast in a dose- and time-dependent manner[14].
		BMP-7 increases the expression of osteoblastic genes in human gingiva-derived MSCs[16].
		BMP-9 promotes osteogenesis in DFSCs[17].
		BMP-2, 6 and 7 increase biomineralisation of hPDLSCs[18].
		The most pronounced induction of biomineralisation of hPDLSCs occurs in BMP-6[18].
	PTH	Regulates the differentiation of DFSCs into osteoblasts[19,21].
	IGF	Increases the volume of newly formed bone following tooth extraction[26]. Promotes osteogenic differentiation and osteogenesis but decreases the odontogenic differentiation and dentinogenesis capacity[62].
	Vit D	Stimulates osteoblastic differentiation with the subsequent increase of bone mineral matrix deposition[27,30].
Cementum	EMD	Affects both proliferation and differentiation of PDLSCs[32-34]. Induces DFSCs towards the cementoblast phenotype[38]. Induces the formation of cementum-like structures on teeth affected by periodontal disease[40].
	PTH	Is essential for cementoblast differentiation[54,55]. Improves the stability of tooth movement by promoting periodontal regeneration[56].
	CP-23	Differentiates both DFSCs and PDLSCs to the cementoblast lineage[52,53].
	BMP	BMP-7 mediates cementogenesis of PDLSCs and DFSCs in a dose- and time-dependent manner[14,49]. BMP-3 inhibits BMP-2 mediated osteoblastic differentiation and enables maintenance of the PDL and root cementum[44,47].
PDL	FGF2	Decreases the expression of osteo/cementogenic markers in hPDLCs[59,70]. Increases the expression of teno/ligamentogenic markers in PDLSCs[59]. Induces PDLSCs towards fibroblastic differentiation and inhibits mineralisation[60]. Stimulates and maintains the fibroblastic feature in hPDLSCs[59].
	TGF-β	TGF-β1 suppresses the proliferation of PDL cells and contributes to fibroblastic differentiation[71], and increases tenomodulin but decreases scleraxis[59]. TGF-β3 enhances periodontal tissue regeneration significantly[67].
	PDGF	PDGF-BB enhances mitogenesis and matrix biosynthesis in PDLSCs[74,77], and enhances alveolar bone formation and cementogenesis in large periodontal bone defects[75].

BMP, bone morphogenic protein; CP-23, human cementum protein 1 (also CEMP-1); DFSCs, dental follicle stem cells; EMD, enamel matrix derivative; FGF2, fibroblast growth factor 2; hPDLSCs, human PDL stem cells; IGF, insulin-like growth factor; MSCs, mesenchymal stem cells; PDGF, platelet-derived growth factor; PDLScs, PDL stem cells; PTH, parathyroid hormone; TGF-β, transforming growth factor β; Vit D, vitamin D.

Growth factors used in alveolar bone regeneration

Bone morphogenetic proteins (BMPs)

Bone morphogenetic proteins (BMPs) are multifunctional growth factors that are so-called due to their osteoinductive properties and are part of the transforming growth factor-beta (TGF-β) superfamily which enhances mineralisation in several tissues[8]. The effect of BMPs in bone formation occurs through their involvement in the differentiation of mesenchymal stem cells (MSCs) and osteoprogenitors to osteoblasts[9]. BMPs can also recruit endogenous MSCs and osteoblasts to defect sites[10]. Over 20 types of BMPs have been shown to differentiate MSCs into osteoblasts; however, the most potent factors among them include BMP-2, 4, 6, 7, 9 and 13[9,11]. In a study by Cheng et al[12] on different types of BMPs on the osteogenic activity of MSCs and osteoblastic cells, it was shown that BMP-2, 6 and 9 may play an important role in inducing osteoblast differentiation of MSCs. BMPs have been found to regulate the differentiation of MSCs mainly through Smad proteins[13]. Moreover, BMPs can function in the form of heterodimers, including BMP-4/7, 2/7 and 2/6/99.

Açil et al[14] found that BMP-7 induces osteoblast/cementoblast differentiation of PDL stem cells (PDLSCs) and DFSCs in a dose- and time-dependent manner. In a similar study, they indicated that compared to DFSCs, PDLSCs exhibited a slightly higher response to BMP-7[15]. Lee et al[16] evaluated the effects of short-term application BMP-7 on human gingiva–derived MSCs and recorded increased expression of target genes for osteoblastic differentiation. Li et al[17] showed that DFSCs transfected with BMP-9 could significantly promote osteogenesis. Hakki et al[18] demonstrated the regulatory effect of BMPs on the proliferation, mineralisation and expression of bone/mineralised tissue-

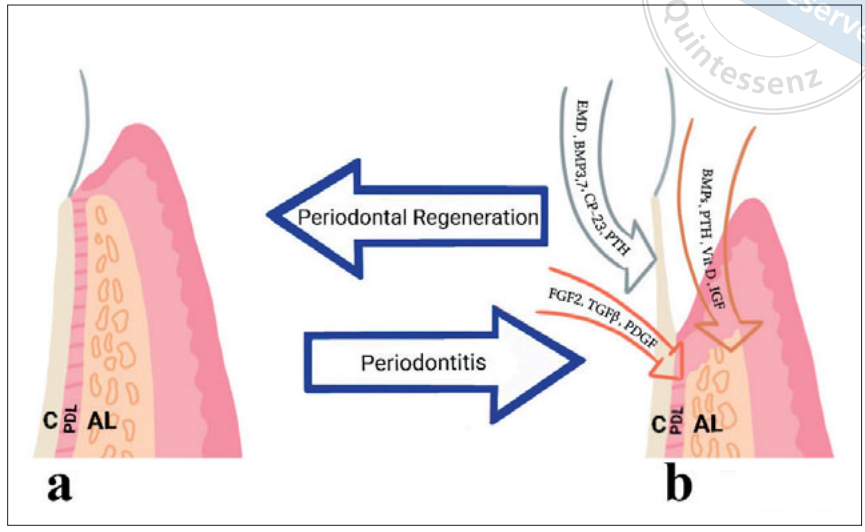

Fig 1 The main growth factors that play an important role in the regeneration of each periodontal component. **(a)** Healthy periodontium; **(b)** destroyed periodontium due to periodontitis. The red arrow shows growth factors related to PDL regeneration, the grey arrow indicates growth factors related to regeneration of cementum, and the brown arrow shows growth factors related to alveolar bone regeneration. AL, alveolar bone; BMPs, bone morphogenic proteins; C, cementum; CP-23, human cementum protein 1 (also CEMP-1); EMD, enamel matrix derivative; FGF2, fibroblast growth factor 2; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; PTH, parathyroid hormone; TGF-β, transforming growth factor β; Vit D, vitamin D.

associated genes in human PDLSCs in a time and dose-dependent manner. They suggested that BMP-2, 6 and 7 are potent regulators for gene expression and biomineralisation of human PDLSCs; however, the effect of BMP-6 on mediating the mineralisation of human PDLSCs was superior to BMP-2 and 7[18].

Parathyroid hormone (PTH)

Both parathyroid hormone (PTH) and PTH receptor (PTHrp) are important signals that regulate osteoblastic differentiation[8]. PTHrp is required for several different regulations of enchondral bone development, differentiation of bone precursor cells and development of craniofacial tissues.

The dental follicle, which is involved in tooth eruption and alveolar bone regeneration, has been found to express a large amount of PTH[19]. Klingelhöffer et al[19] showed that PTH participates in the early phase of osteogenic differentiation in DFSCs. The PTH-PTHrp autocrine signal maintains the physiological cell fates of DFSCs to establish the functional periodontal attachment apparatus and orchestrates tooth eruption[20]. Moreover, Pieles et al[21] reported that PTH supports the expression of BMP-2, which is strongly involved in the osteogenic differentiation of DFSCs.

Insulin-like growth factor (IGF)

Insulin-like growth factor (IGF) is signalling through type 1 receptor that stimulates cell proliferation, function and survival of osteoblasts[22]. IGF-1 can regulate osteogenic differentiation in an endocrine, paracrine or autocrine manner which is regulated by a family of six IGF binding proteins (IGFBPs). IGFBP-3 and 5 have been shown to stimulate the actions of IGF-1, whereas IGFBP-1, 2, 4 and 6 are known inhibitors of IGF 1 in bone[23].

In a study by Wang et al[24], stem cells from the apical papilla (SCAPs) treated with IGF-1 showed an increase in osteogenic differentiation; however, the odontogenic differentiation and dentinogenesis capacity of SCAPs was reduced significantly. A similar study by Feng et al[25] showed that IGF-1 triggers early-stage osteogenic differentiation and maintains the later-stage osteogenic differentiation of dental pulp stem cells (DPSCs). IGF-1 was also administrated following tooth extraction in a diabetic rat model, and the results showed that IGF-1 not only increased new bone formation but also normalised the expression of glucose transporter 1 in diabetic rats[26].

Vitamin D (Vit D)

Vitamin D (Vit D) is crucial for bone mineralisation as well as maintenance of calcium homeostasis. It has also been shown to play an important role in the proliferation and differentiation of MSCs to osteoblasts[27,28]. Vit D is an important regulator of Runt-related transcription factor 2 (RUNX2), with which it cooperates in inducing the expression of osteocalcin, which is also a key protein that regulates osteoblastic differentiation[29]. Posa et al[30] showed that Vit D treatment increases osteogenic differentiation of tooth bud stem cells (DBSCs).

Growth factors used in cementum regeneration

Enamel matrix derivative (EMD)

Enamel matrix derivative (EMD) is an enamel matrix extract that mainly contains amelogenins, which have been shown to be involved not only in formation of enamel but also in that of the periodontal attachment[31]. Several studies have illustrated the effect of EMD on the proliferation and differentiation of PDLSCs[32-34]. Davenport et al[32] showed that in the presence of EMD, human PDL fibroblasts differentiate to cells more similar to cementoblasts than fibroblasts. The amelogenins can form an insoluble extracellular matrix that has a high affinity for collagens and hydroxyapatite[35,36], and histological evaluation has shown that it can form acellular cementum which is essential for PDL fibre attachment[37]. Kenmour et al[38] found that EMD induced human DFSCs towards the cementoblastic phenotype through BMP-dependent pathways. Moreover, two in vivo studies confirmed that new cementum was formed following administration of EMD[39,40]. Bosshardt et al[40] showed that EMD can induce the formation of cementum-like structures on teeth affected by periodontal disease.

BMPs

BMPs are best known for their potential in osteoblastic differentiation[41,42]; however, several studies have indicated the effect of BMP-7 and BMP-3 on cementoblastic differentiation[41,43-48]. Bozic et al[43] showed the BMP-7 mechanism induces differentiation and mineralisation of cementoblasts, and does so via inducing procollagen COOH-terminal proteinase enhancer 1 (PCPE1) and BMP-1. Torii et al[49] showed that BMP-7 mediates cementogenesis of PDLSCs via activation of protein tyrosine phosphatase-like, member A/cementum attachment protein (PTPLA/CAP) and cementum-derived protein (CEMP1). Similarly, it has been shown that BMP-7 can induce cementoblast differentiation of PDLSCs and DFSCs in a dose- and time-dependent manner[14]. In the analysis of tooth development by Aberg et al[50], it was reported that BMP-3 is involved in cementum development. Another study showed that BMP-3 inhibits BMP-2–mediated osteoblastic differentiation[44]. The negative regulation of BMP-3 in mineralisation enables the maintenance of the PDL between bone and root cementum[47].

Cementum-derived protein (CEMP1)

CEMP1, also known as CP23, is well-known as a key marker for cementoblast differentiation; however, it has been shown to be expressed not only in cementoblasts, but also in PDLSCs[51]. An immunohistological evaluation by Alvarez-Pérez et al[52] showed the distribution of CEMP1 throughout the entire root surface, including acellular and cellular cementum, cementocytes and cells located near the blood vessels in the PDL. Further studies showed that the application of CEMP1 on both DFSCs and PDLSCs differentiate them to the cementoblast lineage, indicating that CEMP1 is a key protein in cementoblast differentiation[52,53].

PTH

PTH, which is involved in mediating several important biological actions, such as endochondral bone development, promotes cementogenesis in a protein kinase A (PKA) and extracellular signal-regulated mitogen-activated protein kinase 1/2 (ERK1/2)-dependent manner[50,54]. It has been speculated that PTH could promote cementoblastic differentiation and cementogenesis[50]. Most of the cells expressing PTH are in the dental follicle and on the root surface. The deletion of this peptide receptor (PPR) in these progenitors leads to failure of eruption and significantly truncated roots lacking PDLs. PPR is likely to orchestrate cementoblast differentiation of the progenitors, as the PPR-deficient cells fail to form the acellular cementum, and rather form irregular cellular cementum on the root surface. This phenotype can be interpreted as accelerated and disordered differentiation[55]. Li et al[56] showed that intermittent PTH administration could promote cementogenesis and regeneration of the tooth root caused by resorption.

Growth factors used in PDL regeneration

Fibroblast growth factor 2 (FGF2)

Fibroblast growth factor 2 (FGF2), also known as the basic FGF, is a heparin-biding cytokine that plays a role in the inflammatory phase as an anti-inflammatory cytokine, and the proliferative phase of wound healing[57]. Its angiogenic and fibrous tissue forming activity, along with its ability to stimulate proliferation and differentiation of MSCs, make it suitable to be used in wound healing and periodontal regeneration[57,58].

Hyun et al[59] showed have shown that FGF2 acts as a signalling molecule and increases the expression of scleraxis and tenomodulin, early and late teno/ligamentogenic markers, respectively, in human PDLCs[5] and that FGF2 decreases the expression of osteo/cementogenic markers on hPDLCs and has an antagonist effect on BMPs. A similar observation was made by

Murakami et al[60], who found that FGF2 decreased collagen 1 (COL1) expression and calcification. It therefore appears that FGF2 guides human PDLSCs towards fibroblastic differentiation and inhibits mineralisation; however, administration of FGF2 in combination with BMP-2 was found to enhance bone regeneration both in vitro and in vivo[61,62].

Nagayasu-Tanaka et al[63], in a dog model, showed that periodontal regeneration in the presence of FGF2 was revealed to promote disappearance of blood clots and granulation tissue formation, which are replaced rapidly with new bone; thus, bone formation is more accelerated. Besides, the vascularised connective tissue with tight collagen fibres on the root surface extends coronally from the existing PDL and forms new cementum and PDL with Sharpey's fibres. Rapid dense connective tissue formation in the presence of FGF2 maintains gingival tissue at higher levels and consequently creates a regenerative space and inhibits further periodontal collapse. The clinical application of FGF2 shows its effectiveness in bone regeneration of periodontal defects; however, there is still a lack of clinical attachment[64,65].

TGF-β

TGF-β is a superfamily of growth factors with multifunctional effects. Its role in wound healing occurs through its effect on cell proliferation, differentiation and migration. Three isoforms, i.e., TGF-β1, 2 and 3, with significant homology have been detected for TGF-β. It has been reported that the isoforms 1 and 3 which signal through the same receptor complex, TGF-β receptor type II (TβRII)[66], play roles in periodontal regeneration[67,68].

TGF-β1 has long been recognised as a prerequisite for the differentiation of myofibroblasts that play a key role in the remodelling and reconstruction of connective tissue by the secretion and organisation of the extracellular matrix and by endowing tissue with contractile forces[69]. Moreover, Fujii et al[70] showed the exclusive distribution of TGF-β1 throughout the PDL tissues and proved that the amount of TGF-β1 in PDL tissues is greater than that in pulp tissues or alveolar bone tissues, indicating the important physiological role played by TGF-β1 in PDL cells.

TGF-β1 has been shown to suppress the proliferation of PDL cells, while its upregulation of actin alpha 2 (ACTA2), COL1 and fibrillin-1 encoding gene (FBN1) contributes to their fibroblastic differentiation[71]. Besides, TGF-β1 has been shown to increase tenomodulin but decrease scleraxis[59]. Hence, subsequent administration of TGF-β1 after FGF2 on the differentiation of PDLSCs into fibroblastic cells has been suggested to accelerate the regeneration of functional periodontium[71]. TGF-β3 is of particular interest due to its association with dermal wound and tendon healing promotion without fibrotic scar formation[72]. TGF-β3 has been shown to enhance the proliferation and early differentiation of MSCs into osteoblasts, chondrocytes, adipocytes and tendon cells[66]. In a study by Moshaverinia et al[72], in a mouse model, PDLSCs and gingival stem cells encapsulated in TGF-β3–loaded RGD-modified alginate microspheres showed the successful differentiation of given cells into tendon-like tissue.

Platelet-derived growth factor (PDGF)

Platelets can produce and release growth factors and cytokines involved in angiogenesis, inflammation and immune response which eventually enhance tissue repair. PDGF is one of the growth factors stored in platelets[73] that are actively involved in tissue regeneration and wound healing[74]. Various studies have confirmed the effect of PDGF on the proliferation of PDL fibroblasts[75-77]. Three different forms of PDGF, i.e., PDGF-AA, PDGF-AB and PDGF-BB, have been identified[74,78]. Of these, the efficacy of PDGF-BB in both soft and hard tissue regeneration of the periodontium has been demonstrated most clearly. Studies have shown that PDGF-BB is the most effective form, enhancing PDL cell mitogenesis and matrix biosynthesis[74,77]. Jin et al[75] also demonstrated enhanced alveolar bone formation and cementogenesis in large periodontal bone defects using gene therapy with a mode of PDGF-BB delivery in vivo. Furthermore, the use of PDGF-BB in combination with IGF[79] or FGF[74] has been shown to improve periodontal regeneration.

Conclusion

Optimal periodontal regeneration includes restoration of all periodontium components, i.e., alveolar bone, cementum and PDL. Knowledge regarding specific growth factors and cytokines involved in regeneration of each of them may serve as a basis for development of the therapeutic methods targeting all periodontium components. Future studies in this field should be dedicated to designing a combination of these factors in a single smart delivery system and evaluating the effectiveness of their periodontal complex regeneration.

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Conflicts of interest

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

Author contribution

Drs Fazele ATARBASHI-MOGHADAM and Maryam REZAI RAD contributed to the data analysis; Drs Soran SIJANIVANDI, Pouya KHODAYARI and Masoumeh MAHMOUM were involved in the data gathering. All authors contributed to the writing and editing of the manuscript.

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Maxillary Sinus Floor Augmentation with Two Different Inorganic Bovine Bone Grafts: an Experimental Study in Rabbits

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Objective: *To compare the sequential healing of maxillary sinuses grafted with two different xenogeneic bone substitutes processed at either a low (300°C) or high (1200ºC) temperature.*
Methods: *A sinus augmentation procedure was performed bilaterally in 20 rabbits and two different xenogeneic bone grafts were randomly used to fill the elevated spaces. Healing was studied after 2 and 10 weeks, in 10 rabbits during each period.*
Results: *After 2 weeks of healing, very small amounts of new bone were observed in both groups, and were mainly confined to close to the sinus bone walls and osteotomy edges. After 10 weeks of healing, new bone was found in all regions, with higher percentages in those close to the bone walls and to the osteotomy. In this period of healing, the proportion of new bone in the 300°C group was 20.0% ± 4.3%, and in the 1200ºC group it was 17.2% ± 4.3% (P = 0.162). In the 1200ºC group, translucent, dark fog-like shadows in regions of the grafts were hiding portions of new bone (interpenetrating bone network).*
Conclusion: *Both biomaterials provided conditions that allowed bone growth within the elevated space, confirming that both biomaterials are suitable to be used as a graft for sinus floor augmentation.*
Key words: *animal study, bone healing, histology, sinus floor augmentation, sinus membrane*
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Maxillary sinus augmentation is a widely used procedure to restore the bone volume lost in the posterior maxilla in patients who require implant-supported rehabilitation[1].

Several studies have shown that the sinus mucosa tends to return to its original position if implants or biomaterials are not inserted into the space created by its elevation[2,3]. To counteract the volume shrinkage, the use of biomaterials[4], implants[5-9] or devices[10-13] has been suggested. Autogenous bone is still considered the filler of choice for sinus floor augmentation[14]; however, high rates of volumetric resorption of autogenous bone have been reported[15,16], in addition to the surgical morbidity associated with the donor bed[17]. Several grafting materials have been used to fill the augmented maxillary sinus, among which xenogeneic bone granules, derived from different animal species, have been studied in the literature[4]. Among the xenogeneic graft materials, a deproteinised bovine bone mineral (DBBM) processed at a low temperature (300°C) has been used in several clinical[18-23] and animal studies[2,3,15,24-26]. This xenogeneic bone presents slow resorption and excellent osteoconductive properties[24,27-29]. Another deproteinised bovine bone produced only from the pure mineral bovine bone phase and sintered at a high temperature

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(> 1200°C) has been used for sinus floor augmentation[30-35]. This xenogeneic graft has also been employed in animal experiments, using different models and sites such as chambers prepared in skinfolds on the back of hamsters[36], critical defects in the rabbit ulna[37], circumferential defects around dental implants in minipigs[38] or critical-size calvarium defects in albino rats[39]. An animal model using rabbits for maxillary sinus augmentation surgery has been shown to be the most appropriate for experiments, mainly due to its similarity to the human anatomy; both have wide and accessible cavity, well-defined ostium and a mucociliary system with the same characteristics[40,41].

A comparative clinical study of Bio-Oss (Geistlich, Wolhusen, Switzerland) and Cerabone (Botiss Biomaterials, Zossen, Germany) used in maxillary sinus augmentation surgery found that Cerabone had larger particles (1:2.7) and a less intense gradual release of calcium ions[30]. Moreover, in a radiographic evaluation conducted after 4 years, a more pronounced volumetric loss was observed for Bio-Oss compared to Cerabone[30]. An in vitro study reported a higher level of hydrophilicity for Cerabone compared to Bio-Oss[42].

In histological analyses, maxillary sinuses of rabbits filled with DBBM presented a good amount of newly formed bone, providing maintenance of the space created within the elevated area[2,3,24]. There are no reports in the literature involving maxillary sinus augmentation in animals that compared the results of healing using Bio-Oss and Cerabone as fillers. Thus, the aim of the present study was to compare the sequential healing of maxillary sinuses grafted with two different xenogeneic bone substitutes processed at either a low or high temperature.

Materials and methods

Ethical statements

The experimental protocol was approved by the Ethical Committee of the Faculty of Dentistry of Ribeirão Preto, University of São Paulo, São Paulo, Brazil on 8 April 2019 (protocol #2019.1.113.58.1). The article was written according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The Brazilian rules for animal care were followed strictly.

Animal sample

A total of 20 adult male New Zealand white rabbits weighing approximately 3.5 to 4.0 kg and aged 5 to 6 months were used in the present study following a previously published methodology[43].

Study design and sample calculation

A randomised split-mouth design was used to eliminate interferences between subjects in the same group. Sinus augmentation was performed bilaterally and two different bovine xenogeneic bone materials, sintered at 300ºC (Bio-Oss group) or 1200ºC (Cerabone group), were used to fill the two augmented spaces. Two healing periods were analysed, namely 2 and 10 weeks, with 10 animals each period.

The sample size was determined considering data from a previous study available at the time of sample size calculation[28], applying alpha = 0.05, power = 0.8 and a correlation between measures of 0.5. Thus, for this configuration, the sample size was 10 animals per group (two groups, n = 10) to enable the authors to find statistical significance between the experimental groups.

Biomaterials

Bio-Oss is a DBBM with sinterisation at 300º, porosity of 75% to 80%, pores of 20 to 200 µm and a mean particle size of 0.5 to 1.0 mm[44]. Cerabone, meanwhile, is formed completely by hydroxyapatite from bovine cancellous bone, with sinterisation at 1200°C, porosity of 65% to 80%, pores of 600 to 900 µm and a mean particle size of 0.5 to 1.0 mm[44].

Randomisation and allocation concealment

Randomisation between groups and periods of healing was performed electronically (randomization.com) by an author who was not involved in the selection and handling of animals and/or surgical procedures (SPX) on 5 May 2019. The treatment allocations were secured in opaque sealed envelopes and revealed to the surgeon (VFB) immediately after completion of the osteotomy and sinus mucosa elevation.

Surgical procedures

The surgical procedures were performed by one expert surgeon (VFB), preceded by injection of acepromazine maleate (1.0 mg/kg; Acepran, Vetnil, Louveira, SP, Brazil), xylazine (3.0 mg/kg; Dopaser, Hertape Calier, Juatuba, MG, Brazil) and ketamine hydrochloride (50 mg/kg: Ketamin Agener, União Química Farmacêutica Nacional, Embu-Guaçú, São Paulo, Brazil). Through an incision in the nasal dorsum, the nasal bone was exposed, and osteotomies were created bilaterally to the naso-incisal suture (Fig 1a). The sinus mucosa was elevated and the subantral spaces were randomly filled

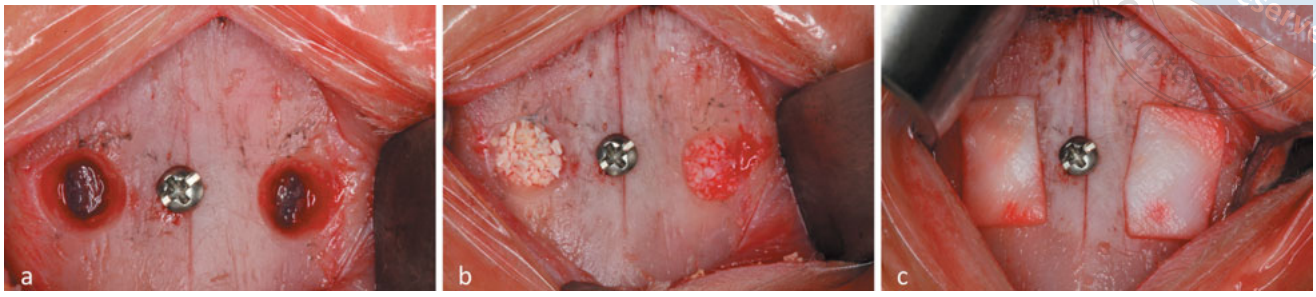

Fig 1 Clinical view of the nasal dorsum and the two osteotomies lateral to the naso-incisal suture. **(a)** Both osteotomies prepared; a small screw was placed in the naso-incisal suture between the centres of the osteotomies to assist the subsequent histological processing; **(b)** xenogeneic bone grafts placed within the elevated space: Cerabone on the left and Bio-Oss on the right; **(c)** collagen membranes placed on the osteotomies.

with similar amounts of the two biomaterials (Fig 1b). The osteotomies were subsequently covered using a collagen membrane (Bio-Gide, Geistlich; Fig 1c), and the wounds were closed with sutures.

Maintenance care

The animals were housed in individual cages placed in climatised rooms with access to food and water ad libitum. The biological functions and the wounds were monitored by veterinarians over the whole period of the experiment.

Euthanasia

The rabbits were first anaesthetised following similar procedure exposed above and then euthanised in a closed transparent acrylic box containing gas carbon dioxide (CO_2). The region of interest was harvested and fixed in 10% formaldehyde.

Microcomputed tomography (microCT) evaluations

A 1172 microcomputed tomography (microCT) system (Bruker, Kontich, Belgium) was used to take microCT scans of the specimens. The parameters used were 9.92 μm isotropic pixel, 60 KV/165, 134 μA, filter Al 0.5 mm, exposure time 596 ms, rotation step 0.4 degrees, frame average 4, and random movement 10. The analyses were performed using DataViewer (Bruker).

Histological preparation

Precision cutting/grinding equipment (Exakt, Apparatebau, Norderstedt, Germany) was used to prepare two histological slides from each biopsy specimen after dehydration, inclusion in resin (LR White Hard Grid, London Resin, Berkshire, UK) and polymerisation. The small screw placed in the naso-incisal suture was used as a reference. The two slides were stained with either toluidine blue or Stevenel's blue and alizarin red.

Histometric evaluations

Photomicrographs were taken under the microscope (Leica DMLB, Wetzlar, Germany) using a digital camera (Digital Sight DS-2Mv, Nikon, Tokyo, Japan).

The following regions were analysed within the elevated space (Fig 2): medial bone wall (M), lateral bone wall (L), sub-sinus region (S), central area (C) and osteotomy region (A).

A grid of 80 squares was superimposed on the images of the histological slides using the software Image J 1.50i (National Institutes of Health, Bethesda, MD, USA) and a point-counting procedure at 100× magnification was adopted for morphometric measurements[45]. New bone (not covered by graft shadows), interpenetrating bone network (IBN; see-through bone graft in the Cerabone group) and residual xenogeneic bone were assessed. Total bone was calculated as the sum of new bone and IBN. Percentages were calculated with respect to the total area of the region evaluated and a mean value was obtained for the five regions. Before taking the measurements, calibration with another expert examiner (SPX) was performed until an inter-class correlation coefficient $k > 0.9$ was achieved for tissue recognition.

Data analysis

The primary variable was the mean total bone percentage in the full elevated space. The data from the various

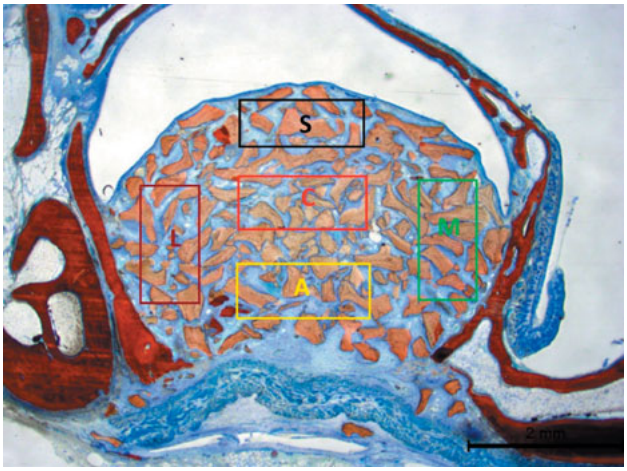

Fig 2 References scheme. Five regions of the sinus were identified: the osteotomy region (A), the central area of the graft (C), the sub-sinus region, subjacent to the sinus mucosa (S), and the regions close to the medial (M) and lateral bone walls (L).

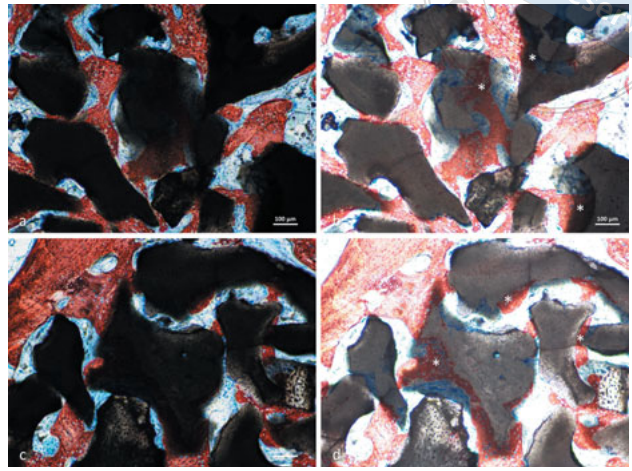

Fig 3 Photomicrographs of ground sections of Cerabone sites after 10 weeks of healing. Note the translucent dark fog-like shadow regions that, after increasing the light intensity, revealed new bone (IBN). **(a and c)** Normal light intensity; **(b and d)** high light intensity. The white asterisks indicate examples of IBN regions.

regions were used to establish a more detailed description of bone formation.

Prism 9.1.1 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses. The normal distribution of the variables was assessed with a Shapiro-Wilk test for both paired and unpaired variables. Either a paired *t* test or a Wilcoxon test was used to evaluate the differences between the Bio-Oss and Cerabone groups. Differences between the two healing periods were evaluated using either an unpaired *t* test or a Mann-Whitney test.

A Spearman two-tailed correlation coefficient was applied to measure the strength of the correlation between the outcomes of the histological and microCT analyses. GraphPad Prism 9.1.1 was used. The correlation coefficient and *P* values were reported. $P < 0.05$ was considered statistically significant.

The tables listed mean values, standard deviations, *P* values, medians and 25% and 75% percentiles, whereas in the text only mean values were reported.

Results

Descriptive histological evaluation

In the histological analysis, the surface of the biomaterial was well defined for the Bio-Oss granules whereas for Cerabone, translucent dark fog-like shadows gave the granules an undefined periphery. In some instances, especially at the 10-week period, these shadows hid the presence of new bone that was unveiled when light intensity was increased when the photomicrographs were taken under the microscope (IBN; Figs 3a to d). After 2 weeks of healing, very small amounts of new bone were observed in both groups, mainly confined to close to the sinus bone walls and osteotomy edges, whereas the other regions were practically devoid of new bone (Figs 4a and b). The graft material occupied almost half of the elevated space.

Ridges of new bone sprouting from the sinus bone walls towards the centre of the elevated space incorporated the nearest granules in both the Cerabone (Fig 5a) and Bio-Oss (Fig 5b) groups. The granules furthest from the bone walls were instead surrounded by soft tissue, presenting a dense layer of fibroblast-like cells disposed along the graft surface. Some osteoclast-like cells were also seen on the surface of both biomaterials (Figs 5a and b). The osteotomy was still covered by the collagen membrane (Figs 4a and b) involved in degradation processes. New bone was forming from the edges of the osteotomies, aiming to close the defect (Figs 5c and d). A few granules were found in some specimens beyond the osteotomy in both groups.

After 10 weeks of healing, new bone was found in all regions, with higher percentages recorded in regions close to the bone walls and the osteotomy (Figs 6a and b). The granules presented a higher grade

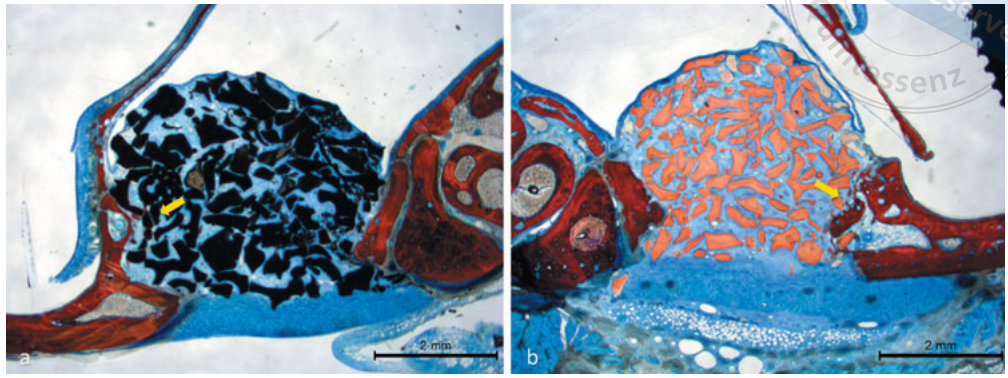

Fig 4 Photomicrographs of ground sections after 2 weeks of healing. **(a)** Cerabone site; **(b)** Bio-Oss site. Stevenel's blue and alizarin red staining were used. The yellow arrows indicate new bone forming from the mesial bone walls of the sinus on both sides.

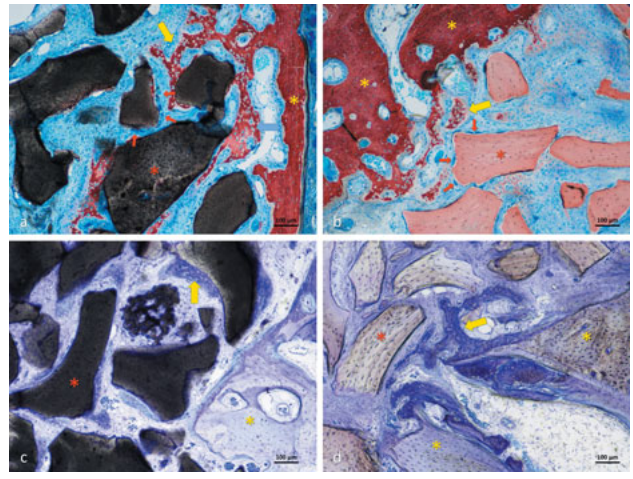

Fig 5 Photomicrographs of ground sections after 2 weeks of healing. New bone formed from the bone walls surrounded the closest **(a)** Cerabone and **(b)** Bio-Oss granules. New bone forming from the osteotomy edge at the **(c)** Cerabone and **(d)** Bio-Oss sites. **(a and b)** Stevenel's blue and alizarin red staining; **(c and d)** toluidine blue staining. The yellow arrows indicate new bone, the yellow asterisks mark old parent bone, the red asterisks show biomaterial and the red arrows indicate osteoclasts.

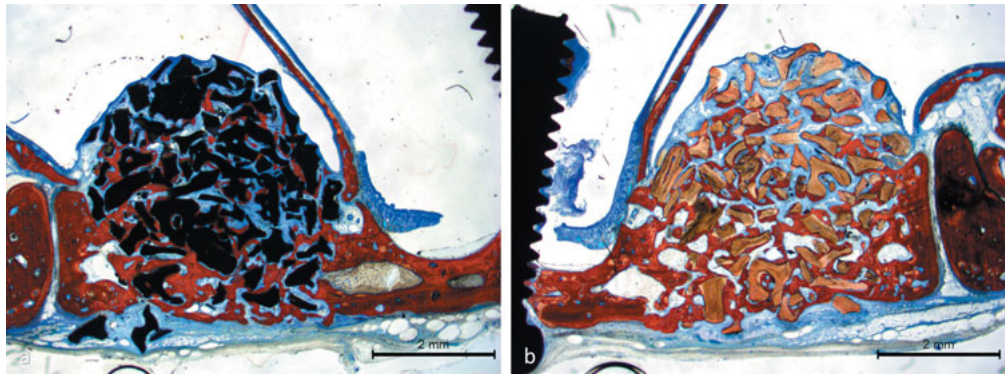

Fig 6 Photomicrographs of ground sections after 10 weeks of healing. New bone was found in all regions examined at both **(a)** Cerabone and **(b)** Bio-Oss sites. Stevenel's blue and alizarin red staining were used.

of incorporation into newly formed bone compared to the previous healing period (Figs 7a and d). The regions subjacent to the sinus mucosa presented the lowest amounts of new bone. Most of the osteotomies were closed by newly formed bone with signs of remodelling. The degradation of the collagen membrane was not completed yet (Figs 6a and b). Some granules were found beyond the osteotomy and several perforations of the sinus mucosa were observed for both biomaterials.

Histometric evaluation

After 2 weeks of healing (Table 1), IBN was almost absent such that both new bone and total bone fractions were 0.7% and 0.3% for the Bio-Oss and Cerabone groups, respectively ($P = 0.098$). The residual graft fraction was 43.4% for the Bio-Oss group and 48.8% for the Cerabone group ($P = 0.037$).

New bone was mostly located close to the sinus bone walls (Table 2). A slight tendency towards higher bone

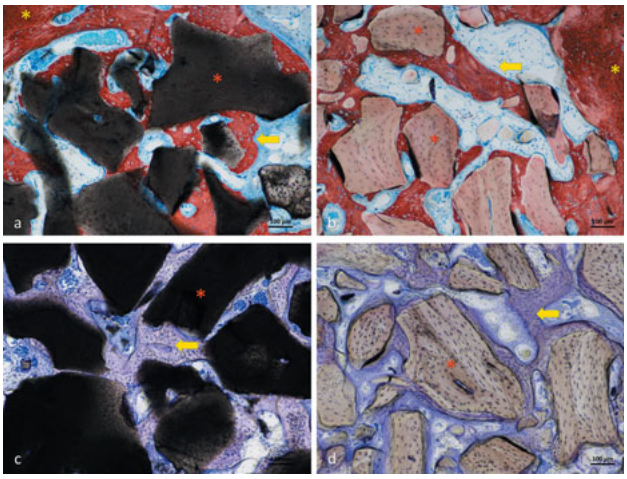

Fig 7 Photomicrographs of ground sections after 10 weeks of healing. New bone incorporated the granules of both biomaterials and formed bridges that interconnected the granules. **(a and c)** Cerabone and **(b and d)** Bio-Oss granules. **(a and b)** Stevenel's blue and alizarin red staining; **(c and d)** toluidine blue staining.

formation was seen in the Bio-Oss group compared to the Cerabone group in all regions. The submucosa region was virtually devoid of new bone in both groups. None of the differences between groups were statistically significant considering the various regions.

After 10 weeks of healing (Table 1), small fractions of IBN were found in the Cerabone group (1.3%). Total bone was found in proportions of 20.0% in the Bio-Oss group, and 17.2% in the Cerabone group ($P = 0.162$). The residual graft decreased with respect to the previous healing period to 36.4% and 35.3% ($P = 0.846$) in the Bio-Oss and Cerabone groups, respectively (Fig 8). In both groups, the differences between periods in total bone and residual graft percentages were statistically significant ($P < 0.05$).

In both groups, the regions presenting the greatest amount of new bone were those close to the window, followed by those close to the sinus bone walls

Table 1 Percentage of new bone and graft remnants within the full elevated area.

Time point	Grafting material	New bone, %			IBN, %		
		Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value
2 weeks	Bio-Oss	0.7 ± 0.8	0.4 (0.2; 0.6)	0.098	0.0 ± 0.0	0.0 (0.0; 0.0)	NA
	Cerabone	0.3 ± 0.2	0.3 (0.1; 0.4)		0.0 ± 0.1	0.0 (0.0; 0.0)	
10 weeks	Bio-Oss	20.0 ± 4.3	21.4 (18.1; 22.1)	0.041	0.0 ± 0.0	0.0 (0.0; 0.0)	NA
	Cerabone	15.8 ± 4.0	14.5 (13.8; 19.6)		1.3 ± 0.6	1.5 (1.2; 1.5)	

$P < 0.05$.

Table 2 Total bone percentages after 2 weeks of healing. The various regions of the augmented area were evaluated.

Grafting material	Full augmented area			Next-to-window			Central		
	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value
Bio-Oss	0.7 ± 0.8	0.4 (0.2; 0.6)	0.159	0.3 ± 0.5	0.0 (0.0; 0.4)	0.250	0.3 ± 0.8	0.0 (0.0; 0.0)	0.750
Cerabone	0.3 ± 0.3	0.3 (0.1; 0.5)		0.0 ± 0.0	0.0 (0.0; 0.0)		0.1 ± 0.1	0.0 (0.0; 0.0)	

Table 3 Percentage of new bone after 10 weeks of healing. The various regions of the augmented area were assessed.

Grafting material	Full elevated area			Next-to-window			Central		
	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value
Bio-Oss	20.0 ± 4.3	21.4 (18.1; 22.1)	0.162	31.5 ± 8.1	32.4 (28.0; 33.7)	0.018	13.8 ± 8.3	13.4 (7.8; 21.6)	0.585
Cerabone	17.2 ± 4.3	15.8 (14.1; 21.1)		21.9 ± 7.8	22.9 (21.0; 24.6)		12.2 ± 5.1	12.8 (8.6; 15.3)	

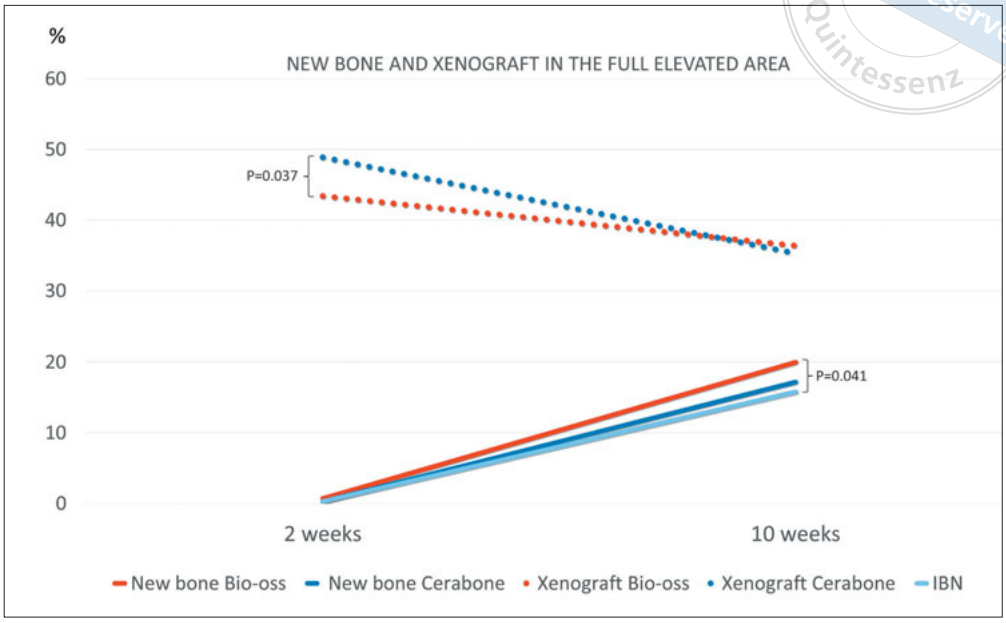

Fig 8 New bone formation and xenogeneic bone graft resorption between 2 and 10 weeks of healing.

(Table 3). In these regions, the total bone percentage was higher in the Bio-Oss group; however, only the difference at the next-to-window wall region was statistically significant ($P = 0.018$). The lowest percentage of new bone was found in the submucosa regions, followed by the central region.

microCT evaluation

The evaluation was performed at two different thresholds of grey levels to identify bone tissue from xenogeneic bone graft, namely 60 to 80 and 70 to 100. After 2 weeks of healing (Figs 9a to c), the tissue vol-

	Total bone, %			Residual graft, %		
	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value
	0.7 ± 0.8	0.4 (0.2; 0.6)	0.159	43.4 ± 6.2	45.6 (40.0; 47.6)	0.037
	0.3 ± 0.3	0.3 (0.1; 0.5)		48.8 ± 4.3	50.0 (49.2; 51.5)	
	20.0 ± 4.3	21.4 (18.1; 22.1)	0.162	36.4 ± 4.3	36.4 (35.0; 38.2)	0.846
	17.2 ± 4.3	15.8 (14.1; 21.1)		35.3 ± 10.5	39.7 (33.5; 41.5)	

	Submucosa			Lateral wall			Medial wall		
	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value
	0.0 ± 0.0	0.0 (0.0; 0.0)	1.000	1.0 ± 1.2	0.8 (0.0; 1.3)	0.231	1.7 ± 2.2	0.5 (0.1; 2.6)	0.214
	0.0 ± 0.0	0.0 (0.0; 0.0)		0.4 ± 0.5	0.2 (0.0; 0.7)		1.0 ± 1.3	0.3 (0.0; 1.7)	

	Submucosa			Lateral wall			Medial wall		
	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value	Mean ± SD	Median (25%; 75%)	*P* value
	6.9 ± 6.1	5.4 (3.0; 9.6)	0.112	22.1 ± 10.5	21.2 (15.7; 28.6)	0.529	25.7 ± 6.8	27.0 (21.6; 29.1)	0.242
	9.7 ± 5.6	11.5 (4.7; 13.6)		19.4 ± 8.5	18.6 (14.8; 25.4)		22.6 ± 8.3	24.3 (18.8; 29.7)	

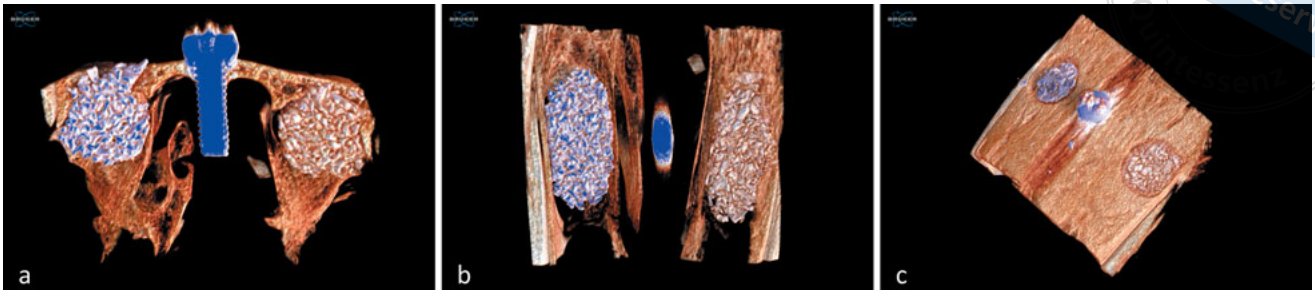

Fig 9 MicroCT 3D images representing healing in the grafted sinus after 2 weeks. **(a)** Coronal; **(b)** from the sinus; **(c)** from the nasal bone. Cerabone grafts are on the left and Bio-Oss grafts are on the right side of the image.

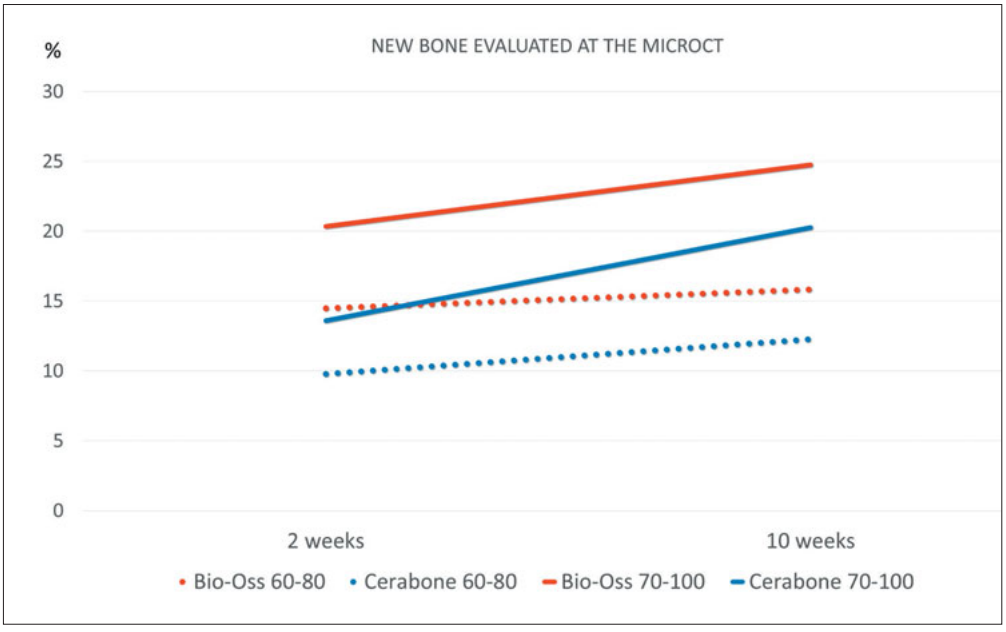

Fig 10 Percentage of new bone evaluated in the microCT analysis using different grey levels: 60 to 80 and 70 to 100.

umes of the elevated spaces were 78.52 ± 8.89 mm^3 and 80.44 ± 13.12 mm^3 for the Bio-Oss and Cerabone groups, respectively ($P = 0.208$). The grey level of 60 to 80 yielded 14.48% ± 2.29% and 9.79% ± 1.27% ($P < 0.0001$) of new bone in the Bio-Oss and Cerabone groups, respectively (Fig 10). Applying a grey level of 70 to 100, the respective proportions of new bone were 20.35% ± 3.37% and 13.60% ± 2.09% ($P < 0.0001$).

After 10 weeks of healing (Figs 11a to c), the tissue volumes of the elevated spaces were 84.49 ± 9.78 mm^3 and 92.96 ± 12.0 mm^3 for the Bio-Oss and Cerabone groups, respectively ($P = 0.057$). The grey level of 60 to 80 disclosed 15.83% ± 1.82% and 12.28% ± 0.96% ($P < 0.0001$) of new bone in the Bio-Oss and Cerabone groups, respectively. Applying a grey level of 70 to 100, 24.76% ± 2.50% was recorded in the Bio-Oss group, and 20.26% ± 1.57% in the Cerabone group ($P < 0.0001$).

Correlation between 10-week histological and microCT analyses

Applying a grey level of 60 to 80 for bone tissue evaluation, a weak positive correlation for the Bio-Oss group (0.091; $P = 0.811$) and a weak negative correlation for the Cerabone group (−0.333; $P = 0.349$) were found. Using a grey level of 70 to 100, a strong positive correlation for the Bio-Oss group (0.709; $P = 0.027$) and a weak positive correlation for the Cerabone group (0.212; $P = 0.560$) were found.

Discussion

The present study aimed to compare the sequential healing of maxillary sinuses grafted with two different xenogeneic bone substitutes sintered at either a low or high temperature. After 10 weeks of healing, the histological

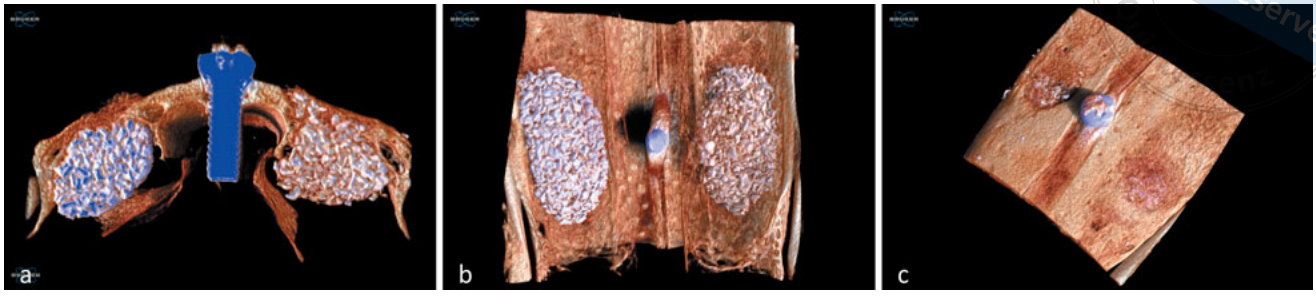

Fig 11 MicroCT 3D images representing healing in the grafted sinus after 10 weeks. **(a)** Coronal; **(b)** from the sinus; **(c)** from the nasal bone. Cerabone grafts are on the left and Bio-Oss grafts are on the right side of the image.

evaluation showed that a tendency towards higher bone formation was found in the Bio-Oss group compared to the Cerabone group; however, the difference was not statistically significant. In both groups, the highest percentages of new bone were observed in the regions close to the bone walls and the osteotomy.

In the Cerabone group, several graft particles presented dark regions with fog-like shadows that hid the hard and soft tissues. These tissues were shown more clearly under the microscope when overexposing the image to the light whereas, when adjusting the focus, no improvement was made in the identification. This method made it possible to identify new bone hidden by the shadows and was illustrated in other articles in which biphasic beta tricalcium phosphate/hydroxyapatite (β-TCP/HA) were used for sinus floor augmentation in sheep[46] and rabbits[47] and recently in human biopsy specimens to unveil bone covered by shadows around Cerabone[48]. The total amount of bone reported represented the sum of new bone outside the dark shadows and that covered by those shadows (IBN). The structure of the IBN recalled that of an "interpenetrating polymer network"[49] and consequently, a similar name was adopted.

After 2 weeks of healing, the percentage of new bone in the full elevated area was < 1% in both groups. The inclusion of an early period of healing in the analysis of the present study enabled the differences in the regions close to the source of new bone to be evaluated and, at the same time, provided the opportunity to assess dimensional variations over time. After 2 weeks of healing, the postsurgical oedema that occurs after sinus floor augmentation[50-53] appeared to be resolved. The resorptive processes were still in an early phase, resulting in little effect on dimensional changes. During this healing period in both groups, bone was formed from the lateral sinus walls while, in the submucosa area, no new bone was found. This is in agreement with several other reports that showed the absence of participation of the sinus mucosa in bone formation during this early period of healing[8,9,54-55].

After 10 weeks, new bone proportions increased in both groups in all regions included in the analyses. In this healing period, the proportion of new bone was 20.0% in the Bio-Oss group and 17.2% in the Cerabone group. A comparison of the results of healing between Bio-Oss and Cerabone after sinus floor augmentation was also performed in a clinical study[31]. Biopsy specimens were harvested after 6 months from the augmented sinus, and the proportion of new bone was 41.4% in the Bio-Oss group and 39.2% in the Cerabone group; however, the difference was not statistically significant[31]. These proportions of new bone were approximately double for both groups compared to the present study. The difference in percentage might be related to the different model used, but also to the fact that the biopsy specimens in the animal study comprised all regions of the sinus, including those with very little bone such as that subjacent to the submucosa. In the previous study, only the region close to the base of the sinus was included in the analysis[31], i.e., closer to an important source of new bone[50,51].

In another clinical study, the analysis performed on biopsy specimens harvested after 8 months from sinus augmentation revealed higher proportions of new bone in the Cerabone group (29.1%) compared to the Bio-Oss group (24.6%)[56]. Again, the difference was not statistically significant.

In the present study, in both groups, the highest amounts of new bone were located close to the bone walls and the osteotomy. Both biomaterials allowed new bone formation and, after 2 weeks, the first signs of incorporation of the neighbouring granules were already noted close to the bone walls. After 10 weeks, incorporation of the granules was observed in all regions. This event has been described in other experimental

studies[24,27,28]. In an experiment on sinus augmentation in rabbits, the sequential events of bone-to-graft contact at DBBM granules were analysed after 7, 14, 20 and 40 days of healing[24]. After 7 days, small amounts of bone were found close to the bone walls, whereas the DBBM granules were surrounded by soft tissue containing fibres and fibroblast-like cells arranged in layers in direct contact with the biomaterial surface, and after 14 days, several DBBM granules were covered by newly formed bone; however, the majority were surrounded by dense tissues, similar to those observed after 7 days of healing, and the regions between granules were occupied by loose tissue, poor in cells but rich in vessels[24]. In the following healing periods, further granules were enclosed by newly formed bone, and the surfaces not covered by bone presented dense tissues[24]. The regions including those between granules, first occupied by loose tissues, underwent a transformation into primitive bone marrow[24].

In the present study, after 10 weeks of healing, new bone in the whole area of the Bio-Oss group reached a proportion of 20.0%. This result is similar to that reported in another study in which Bio-Oss granules were treated with Argon plasma or left untreated before being used to fill the subantral space in rabbits[26]. The percentages of new bone after 10 weeks were 23.5% in the plasma group and 21.3% in the untreated group. The histological analyses were also performed in the bone wall and central regions of the sinuses[26]. The proportion of new bone in the group not treated with argon plasma was 26.3% in the bone wall region, and 13.2% in the central regions[26]. These data are consistent with those of the present study, with the proportion of new bone being approximately 22.0% to 26.0% in the bone wall region and 13.8% in the central region.

In both groups in the present study, the submucosa region exhibited lower amounts of new bone compared to the other regions examined, namely 6.9% and 9.7% in the Bio-Oss and Cerabone groups, respectively. Other similar experiments on sinus augmentation in rabbits reported data on bone formation in this region[57-61]. The presence of new bone subjacent to the sinus mucosa cannot exclude its contribution to bone formation; however, if this contribution does exist, it is limited. It is also necessary to consider that several perforations of the sinus mucosa were observed with relation to the biomaterial particles in both groups. It has been shown in a rabbit model that the presence of sinus mucosa perforations compromised new bone formation in the adjacent regions within the sinus[62].

The difference in percentage between 2 and 10 weeks of healing was 7.0% for Bio-Oss and 13.5% for Cerabone. This means that part of the biomaterial was resorbed or lost through the osteotomy or the sinus mucosa. Indeed, several osteoclastic-like zones were observed around both biomaterials at the 2-week evaluation period whereas after 10 weeks, osteoclastic-like cells were rarely observed. This observation agrees with other reports that showed a progressively decreasing percentage of osteoclasts within the augmented space over time[54,55].

The two bovine cancellous bone grafts used in the present experiment as fillers for sinus augmentation were processed at different temperatures, namely 300°C for Bio-Oss and 1200°C for Cerabone. The process carried out at a high temperature produces macroporous particles with increased crystallinity which might result in slower resorption of the graft, and decreases the microporosity of the surface which might also reduce the osteoconductivity[63-65]. In the present study, however, despite the use of similar volumes of biomaterial in all sinuses, slightly higher volumes were found after 10 weeks compared to 2 weeks of healing. This in turn means that the volumes were maintained over time or even increased, perhaps for bone apposition.

The microCT showed higher percentages of new bone in the Bio-Oss group compared to the Cerabone group ($P > 0.0001$). The data yielded from the microCT analysis were not completely in agreement with those from the histological analysis, especially for the 2-week period. These differences might be ascribed to the fact that histology is a 2-dimensional analysis that represents only a central, limited portion of the sinus that includes the osteotomy. The microCT analysis instead assesses the whole volume that also includes regions located distally and mesially to the osteotomy, in contact with the nasal bone, that represent a further source for bone formation; however, another aspect that should be considered is that the microCT analysis might make it difficult to discriminate between bone and xenogeneic bone graft, yielding contradictory outcomes compared to the histological assessments, especially in the earliest periods of healing[25,66]. Indeed, three out of four correlations evaluated between the bone percentage after 10 weeks in the Bio-Oss and Cerabone groups were weak, whereas only that for the Bio-Oss group applying a grey level of 70 to 100 was strong positive.

With regard to the limitations of the present study, the dark fog-like shadows present in some regions of Cerabone granules should be mentioned. This event, perhaps due to the high porosity of the biomaterial, the stain characteristics or the slow degradation of the biomaterial[67], might obstruct identification of new bone and decrease the percentage of new bone detected with

Cerabone. The model used represents another limitation of the study considering the dimensions of the sinus and the lower thickness of the sinus mucosa compared to humans[54]. Moreover, healing in rabbits has been shown to be faster compared to humans[68]; thus, any inferences about humans must be taken with caution.

Conclusion

The present study illustrated that both biomaterials provided conditions that allowed bone growth within the elevated space and confirmed that both biomaterials are suitable to be used as graft materials for sinus floor augmentation. The overexposure to the microscope light in the histological preparation might help to identify the tissues veiled by the dark shadows surrounding Cerabone particles.

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Conflicts of interest

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

Author contribution

Drs Vitor FERREIRA BALAN, Daniele BOTTICELLI, David PEÑARROCHA-OLTRA and Samuel Porfirio XAVIER contributed to the conceptualization of the study; Vitor FERREIRA BALAN and Eduardo PIRES GODOY contributed to surgical procedures; Dr Vitor FERREIRA BALAN contributed to the histological and microCT analyses; Drs Vitor FERREIRA BALAN, Daniele BOTTICELLI and Katsuhiko MASUDA contributed to the data analysis; Drs Daniele BOTTICELLI and Samuel Porfirio XAVIER contributed to the supervision of the project; Drs Vitor FERREIRA BALAN and Daniele BOTTICELLI contributed to the manuscript draft; Drs David PEÑARROCHA-OLTRA, Katsuhiko MASUDA, Daniele BOTTICELLI and Samuel Porfirio XAVIER contributed to finalising the article. All authors have read and approved the published version of the manuscript.

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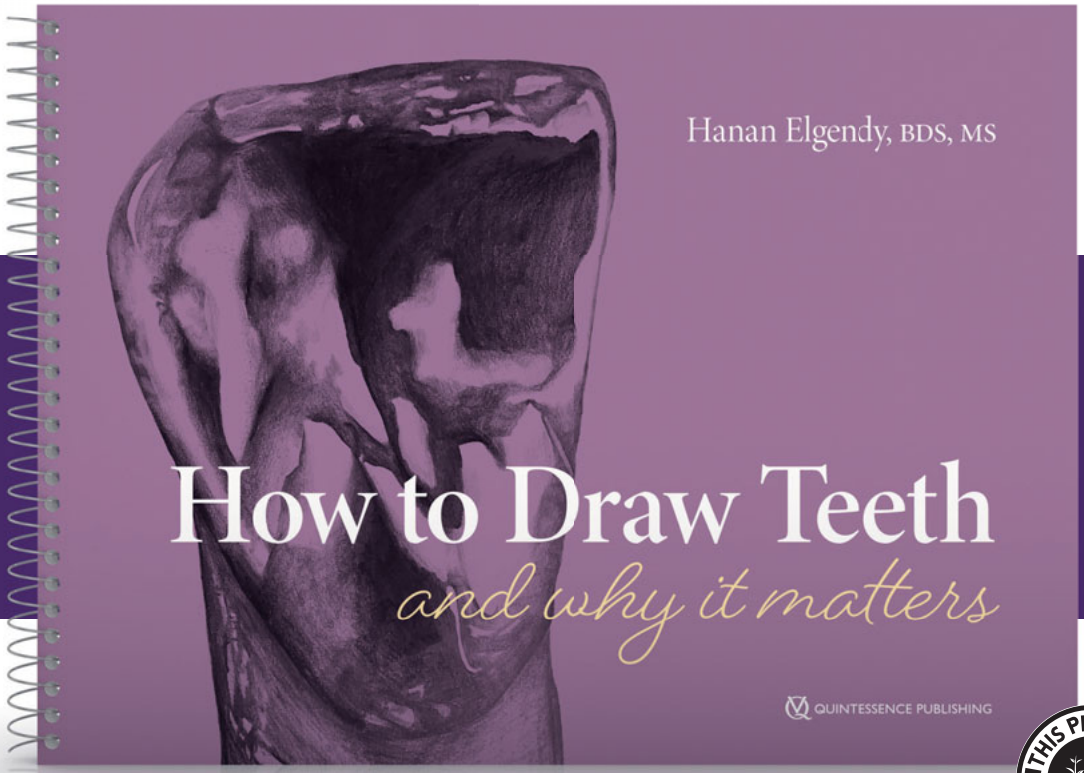

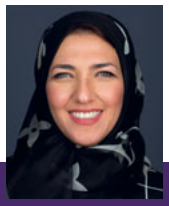

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Structure and Composition of Candidate Phyla Radiation in Supragingival Plaque of Caries Patients

Song JIANG[1], Jie NIE[1], Yu Xing CHEN[1], Xiao Yan WANG[1], Feng CHEN[2]

Objective: *To investigate the composition and abundance of candidate phyla radiation (CPR) in the oral cavity in caries patients and a healthy population.*
Methods: *The raw macrogenomic sequencing data for a total of 88 subjects were downloaded from the National Centre for Biotechnology Sequence Read Archive (NCBI SRA) public database according to the public data usage specifications. Trimmomatic (Department for Metabolic Networks, Potsdam, Germany) and Bowtie 2 (University of Maryland, College Park, MD, USA) were used to quality control and dehost the host sequences. Species annotation was made using Kraken2 (Johns Hopkins University, Baltimore, MD, USA) and Bracken (Johns Hopkins University) based on the reference database. According to the results of the species annotation, the species-significant differences and species correlation of caries and healthy oral microbiota in species composition and microbiota diversity were analysed to study the distribution and abundance differences of CPR in the oral environment.*
Results: *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Fusobacteria were the main components. The relative abundance of TM7 (Candidatus Saccharibacteria) and GN02 (Candidatus Gracilibacteria) of CPR is second only to the aforementioned five bacteria, indicating that CPR is an important part of the oral microbiota. TM7 and GN02 were common to both the caries patients and healthy patients and were detected in all samples, suggesting that CPR is the 'core microbiome'. There was a correlation between CPR and a variety of oral microbiota, among which the positive correlation with Capnocytophaga was the strongest, suggesting that Capnocytophaga might be the potential host bacteria of CPR.*
Conclusion: *CPR is an indispensable part of the oral microbiota. It is the 'core microflora' of the oral cavity and may play an important role in the stability and function of the oral microecological environment. Capnocytophaga may be the potential host bacteria of CPR.*
Key words: *candidate phyla radiation, caries, core microbiome, metagenomics, oral microbiota*
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Dental caries is a common and frequently occurring oral disease that affects the health of residents in China. According to statistics from the Global Burden of Disease Study (GBD) from 1990 to 2016, it was estimated that there were 2.4 billion untreated dental caries patients worldwide, with the highest prevalence of oral disease in the world[1]. Dental caries is an infectious oral disease, and its occurrence and development are closely related to oral microorganisms. In recent years, as research into the microbiota and metabolomics has progressed, scholars have begun to recognise that the microorganisms in the dental plaque biofilm on the human tooth surface as a whole participate in the occurrence and development of dental caries and have proposed the "ecological plaque hypothesis"[2,3]. This hypothesis suggests that the changes in dental plaque microecology are the causes of the onset and pathogenesis of dental caries. The microbial community in a healthy oral environment is maintained in a relatively balanced state, and microorganisms and the environment interact and restrict each other to avoid the occurrence of diseases; however, when the microbial community changes beyond its resistance threshold, the relative balance is broken, causing ecological imbalance of the flora and finally leading to the occurrence of dental caries, periodontal disease and other oral diseases. Dental caries is a disease caused by multiple factors, and its pathogenic bacteria are not a single bacterium, but multiple microorganisms[4]. Understanding and elucidating the pathogenic mechanism of oral microorganisms in dental caries is of great importance for the development of disease prevention and treatment techniques.

Since the oral cavity is an open environment and in continuous contact with the external environment, the composition of the oral microbiota is diverse. A large number of complex microorganisms colonize different sites in the mouth. Candidate phyla radiation (CPR) is a newly discovered large flora including more than 75 phylum bacteria, and accounts for an important part of one quarter of the whole bacterial domain[5]. An increasing number of studies have shown that CPR is also common in oral microbial communities, including TM7 (*Candidatus Saccharibacteria*), GN02 (*Candidatus Gracilibacteria*) and SR1 (*Candidatus Absconditabacteria*)[6,7]. CPR cells are small in size and lack a large number of essential genes related to bacterial survival, which makes them difficult to obtain by conventional laboratory culture. Very little is known about their biological characteristics and role in the occurrence and development of dental caries. CPR may have a potentially pathogenic association with a variety of oral diseases.

At present, research on oral CPR is focused mainly on the oral microbial community of periodontitis. The abundance of oral CPR in periodontitis patients is significantly higher than that in the healthy population and is related to the severity of periodontitis[8-15]. Previous studies related to caries and CPR are rare, and all of them used amplicon sequencing of 16S rRNA for their analysis and some suggested that CPR might be related to the occurrence of dental caries[16-18]. He et al[19] obtained the first culturable strain of TM7 by co-culture and proved the characteristics of its parasitic bacteria. Studies have shown that there is a complex interaction between TM7 and its host bacteria, which can enhance the biofilm formation ability of the host bacteria and help it to avoid the immune system of the human body. These characteristics of CPR may also be related to the occurrence and development of dental caries, but research is still insufficient. Previous studies on the aetiology and mechanism of dental caries have not focused on this newly discovered CPR, and a large number of second-generation sequenced genomic data have not been explored fully[20-22]. As interest in the exploration of unknown microorganisms has grown, ab initio analysis and reuse of metagenomic sequencing raw data have become important research topics, greatly improving understanding of the microbial world. The application of metagenomic data to explore the correlation between CPR in the oral cavity and the occurrence and development of dental caries further deserves to be studied in greater depth.

Materials and methods

Data acquisition

Medline/PubMed database (up to 2020.1) was searched with "metagenomic AND caries", "Candidate Phyla Radiation AND caries" and "Saccharibacteria AND caries" as the search terms. A total of 75 studies related to metagenomic and caries research were reviewed. Of these, 20 were excluded during preliminary screening. After browsing the literature abstracts and excluding studies using 16S rRNA sequencing, three studies that used shotgun metagenomics to study caries microorganisms were finally obtained[20-22]. The authors browsed the data availability statement of the literature, in which the original sequencing data of one literature was not public and could not be obtained[21]. The original sequencing data from the other two studies were uploaded to the NCBI database for open use. The literature that could not be obtained was excluded, and the full text of the

remaining two studies was read. The results of one of the studies did not report the existence of CPR[22]. Another study reported the existence of two types of CPR, TM7 and GN02[20], but was not examined further. This study was selected as the source of the original metagenomic sequencing data[20].

The original metagenomic sequencing data of a total of 88 subjects were downloaded from the NCBI SRA public database, and the accession numbers were SRR6865436 to SRR6865523 in accordance with the public data usage specifications. Dental plaque samples were collected from participants of the University of Adelaide Craniofacial Biology Research Group (CBRG) and the Murdoch Children's Research Institute (MCRI)'s Peri/Postnatal Epigenetic Twins Study (PETS)[20]. The PETS (n = 193) and CBRG (n = 292) cohorts were composed of twins aged 5 to 11 years old. The number of pairs selected for metagenomic sequencing was constrained by budget; thus, a subset of the broader clinical cohort was subsampled[20]. In the original study, oral examination of the subjects was conducted by trained and experienced clinicians. The International Caries Detection and Assessment System (ICDAS II) was used to evaluate caries and was employed to diagnose caries lesions from the initial stage of enamel caries lesions to dentine caries lesions and the final stage of the latter. Subjects with enamel or dentine caries lesions were diagnosed as caries patients, whereas those without enamel or dentine caries were considered as healthy patients. The subjects did not brush their teeth the night before or on the day of plaque collection. A sterile swab was used to wipe the plaque samples thoroughly along the gingival edge and tooth. The sterile swab was then placed in a microcentrifuge tube containing 500 μl bacterial RNA protectant, and the samples were immediately stored in a refrigerator at –80°C for DNA extraction. The DNA in the samples was then extracted with lysozyme. Phenol/chloroform isoamyl alcohol was used for extraction and ethanol was employed for precipitation. The metagenomic sequencing library was prepared using a NEBNext DNA kit for Illumina (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions, then 300 cycles of the metagenomic library were sequenced using a NextSeq 500 kit (Illumina, La Jolla, CA, USA) according to the manufacturer's specified standards to obtain the original metagenomic sequencing data.

Data quality control

The SRA Toolkit (version 2.10.8) was used to convert the downloaded raw data (*.sra files) into the data format required for subsequent metagenomic analysis routines (*.fastq files) to obtain raw reads. The quality of raw reads was assessed using FastQC (version 0.11.7, Babraham Bioinformatics, Cambridge, UK). Trimmomatic[23] (version 0.36), Department for Metabolic Networks, Potsdam, Germany) was used to cut adapters and primers and filter low-quality sequences from raw reads of each sample (parameter set to ILLUMINACLIP: adapters/TruSeq2-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36). Bowtie 2 (version 2.3.5.1) University of Maryland, College Park, MD, USA) was used to compare the sequences after quality control with the human reference genome hg38, and all sequences from human hosts were filtered to obtain clean reads containing only all microbial genome information[24]. FastQC (version 0.11.7) was used again to assess the quality of clean reads, and MultiQC[25] (version 1.9, Stockholm University, Stockholm, Sweden) was employed to summarise raw and clean reads quality, respectively.

Analysis of species composition

According to the Kraken2 reference database, clean reads from all samples were annotated using Kraken2[26] (version 2.0.8) (parameter set to --confidence 0.2). After classifying the species annotation results obtained from Kraken2, Bracken[27] (version 2.5.3) was used to estimate the abundance of each taxonomic level of metagenomic samples by Bayesian reestimation[28]. Using R language (R Core Team, R Foundation for Statistical Computing, Vienna, Austria), the bar charts of two groups of species composition at the phylum level and species level were drawn based on the Bracken corrected results. Using R, Venn diagrams were drawn based on species annotation results (reads threshold > 1000). Endemic or common species at species level were analysed for the caries group and healthy group. The top 20 species with relative abundance were selected for sample clustering analysis, and sample clustering was realised according to the species composition of the samples. R language was used to draw the clustering heat map of the samples, so as to study the similarities between them.

Analysis of bacterial diversity

According to the species abundance-sample matrix, principal component analysis (PCA) scatter plots and 3D maps were drawn using R language. Principal coordinates analysis (PCOA) was carried out on species abundance based on Bray-Curtis. R was used to draw scatter plots and 3D maps, and analysis of similarities

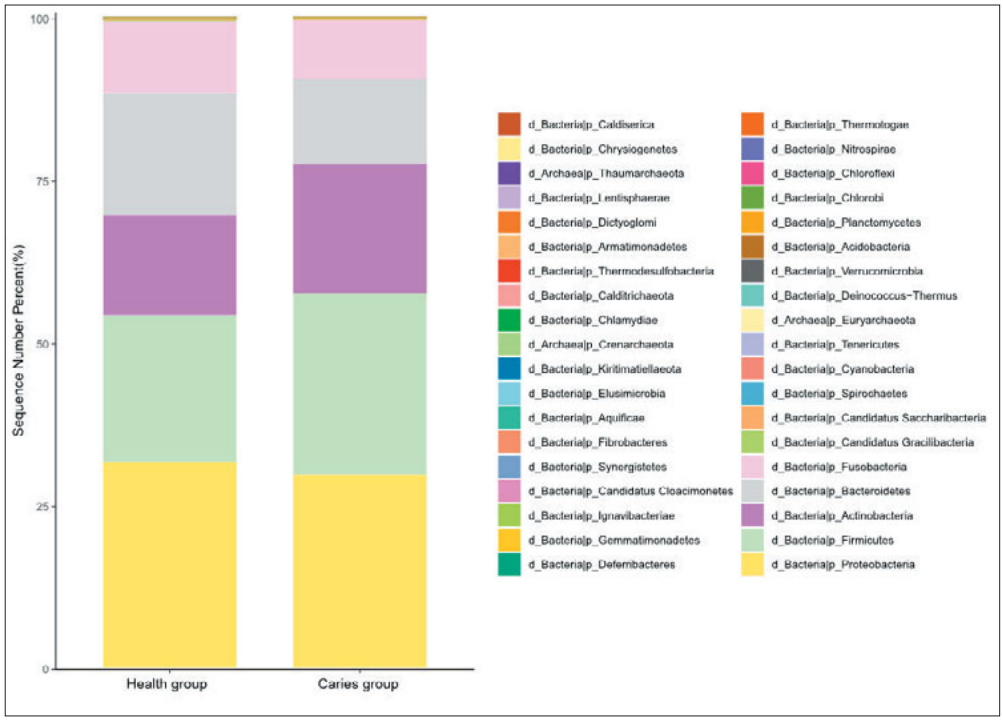

Fig 1 Relative distribution of the caries group and healthy group at one level. *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria* accounted for more than 99% of the total sequence.

(ANOSIM) was employed to calculate β diversity between groups to compare the differences in microbial community composition.

Significant analysis of species differences

Linear discriminant analysis (LDA) was used to calculate the differences in the abundance of species at different taxonomic levels between the two groups. LDA effect size (LEfSE) was used to determine the labelled species of the two groups. The LDA score threshold for difference identification was set as 2.0, and the evolutionary branch diagram and LDA bar chart were drawn using R[29].

Analysis of correlation network

The species abundance for the caries group and healthy group was counted, and the top 40 species with the highest abundance in the two groups were screened for a Spearman correlation analysis. When correlation coefficient $|R| > 0.4$ and $P < 0.01$, it is believed that there is correlation between the two bacteria, and the correlation network diagram is drawn using R.

Results

Data overview

Espinoza et al[20] performed metagenomic shotgun sequencing on a total of 88 dental plaque samples from 44 pairs of twins and uploaded the raw data to the NCBI SRA database for open use. Of the 88 subjects, 58 with caries were classified into the caries group and 30 without caries were classified into the healthy group.

The original sequencing data were downloaded from the NCBI database according to the accession numbers SRR6865436 to SRR6865523 (in line with the standards for the use of data in public databases). After format conversion, a total of 96 Gb of paired double-ended sequence data were obtained, and each sample included an average of 5.52 million sequence fragments (1.1 Gb). After removal of low-quality sequences, primers and linkers and screening of human gene sequences, 47.8% of sequence data remained.

Species composition

Bacteria, archaea and viruses were detected in 88 samples, of which bacteria accounted for 99.90%, including 38 phyla. The main bacteria in the caries group were the same as those in the healthy group, with *Proteo-*

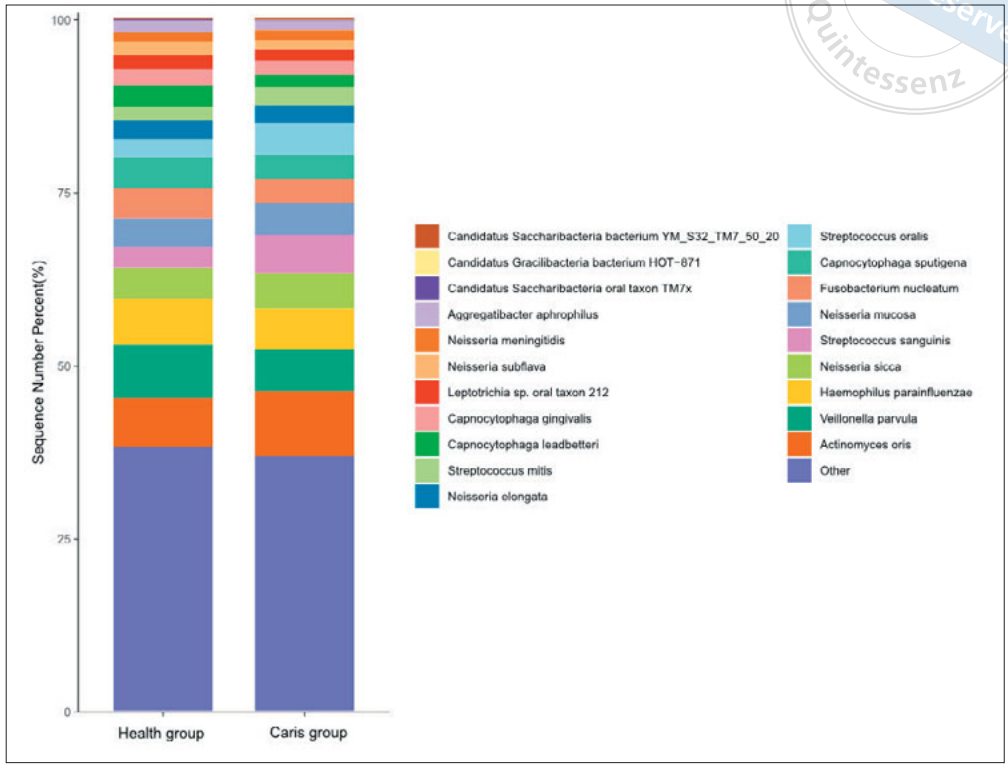

Fig 2 Relative distribution of the caries group and healthy group at the species level. *Actinomyces oris*, *Veillonella parvula*, *Haemophilus parainfluenzae* and *Neisseria sicca* are the species with relatively high abundance in both groups.

bacteria, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria* as the main bacteria, accounting for more than 99% of the total sequences. The percentages of TM7 and GN02 belonging to CPR were second to those of the aforementioned five phyla; however, we did not find another bacterium SR1 belonging to CPR.

In the caries group, the dominant phylum was *Proteobacteria* (relative abundance 29.67%), followed by *Firmicutes* (27.96%), *Actinobacteria* (19.78%), *Bacteroidetes* (13.14%) and *Fusobacteria* (8.93%), GN02 (0.15%) and TM7 (0.12%). In the healthy group, the dominant phylum was Proteobacteria (relative abundance 31.70%), followed by *Firmicutes* (22.56%), *Bacteroidetes* (18.72%), *Actinobacteria* (15.37%), *Fusobacteria* (11.10%), TM7 (0.18%) and GN02 (0.09%). Figure 1 shows the horizontal relative distribution for the two groups.

At species level, the five most dominant bacteria relative to each other in the caries group were *Actinomyces oris* (relative abundance 9.42%), *Veillonella parvula* (6.11%), *Haemophilus parainfluenzae* (5.82%), *Streptococcus sanguinis* (5.52%) and *Neisseria sicca* (5.12%). In addition, we found three species of CPR bacteria belonging to the TM7 and GN02 phylum, namely TM7x, *Candidatus Saccharibacteria* bacterium YM_S32_TM7_50_20 and *Candidatus Gracilibacteria* bacterium HOT-871. The relative abundance of *TM7x*

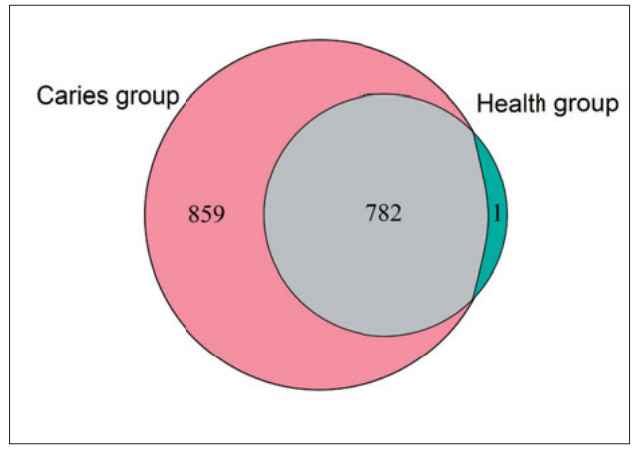

Fig 3 Common species at the species level in the caries group and healthy group. The overlapping part indicates the species composition shared by the groups, and the non-overlapping part indicates the species composition unique to each group.

belonging to the TM7 phylum was 0.12%, and that of *Candidatus Gracilibacteria* bacterium HOT-871 belonging to the GN02 phylum was 0.15%. The five most dominant bacteria in the healthy group were *Actinomyces oris* (relative abundance 7.14%), *Veillonella parvula* (7.67%), *Haemophilus parainfluenzae* (6.64%), *Neisseria sicca* (4.57%) and *Fusobacterium nucleatum* (4.49%). The relative abundance of *TM7x*

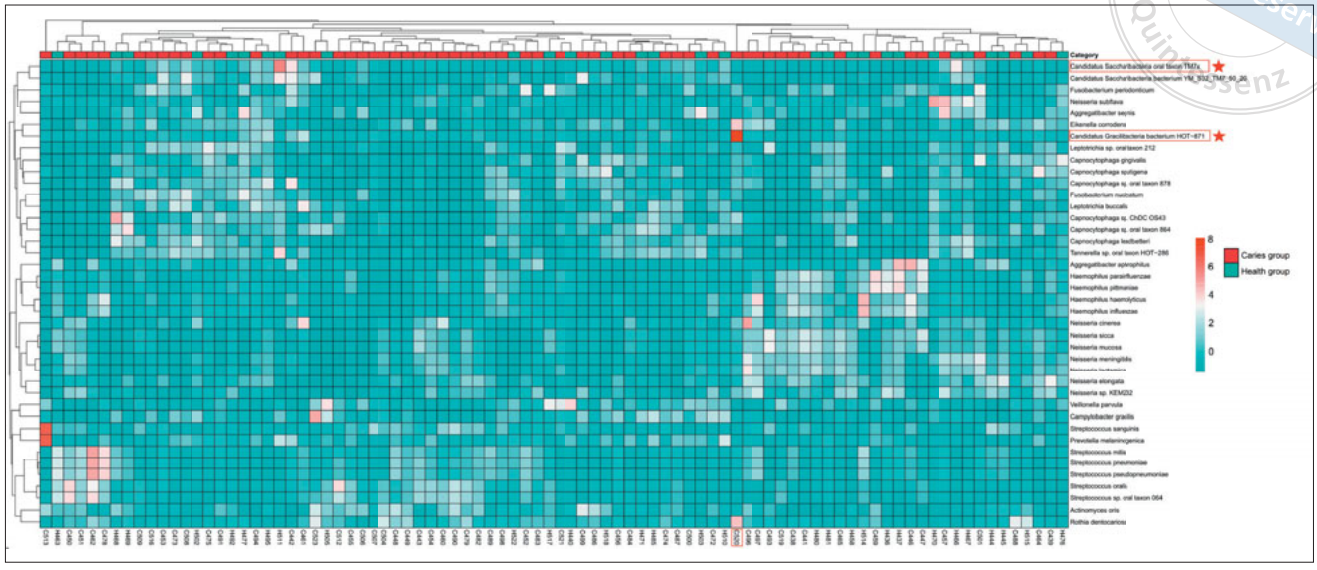

Fig 4 Results of heat map clustering at the species classification level. The horizontal axis is sample name information and also includes grouping information, and the vertical axis is species annotation name (species level in this figure). The cluster tree on the left is the similarity cluster of species distribution in each sample, the sample cluster tree on the top, and the heat map in the middle is the heat map of the relative abundance of species.

was 0.17% and that of *Gracilibacteria bacterium HOT-871* was 0.09%. Figure 2 shows the horizontal relative distribution of two groups of species.

The species in the caries group and the healthy group were analysed and a Venn diagram (Fig 3) was drawn to find the endemic and common species between the two groups. The results showed that there was a total of 782 species in the caries group and the healthy group, of which *TM7x* and *Graciliberia bacterium HOT-871* were species in common in the two groups. There were 859 endemic species in the caries group, among which *Streptococcus influenzalis*, *Arthrobacter sp. ATCC 21022* and *Streptococcus phage Dp-1* were more abundant, whereas only one endemic species, *Enterobacteriaceae bacterium ENNIH2*, was found in the healthy group.

Clustering analysis was performed on the first 20 bacteria with higher abundance in the samples (Fig 4). The results showed that there was a good clustering relationship between the samples in the caries group and the healthy group. *Neisseria*, *Streptococcus* and *Capnocytophaga* showed a good clustering relationship, respectively. Some of the caries samples showed a high abundance of CPR. It is noteworthy that the abundance of *Graciliberia bacterium HOT-871* in the C520 sample of the caries group was extremely high. The patients in this sample developed dentine caries and were not treated, suggesting that *Graciliberia bacterium HOT-871* might be related to caries activity.

Structural similarity of the microbiome

PCA was used for ordination analysis based on the original species composition matrix (Fig 5) and PCoA was used for analysis based on the distance matrix calculated from the species composition (Fig 6) to assess the similarity of the bacterial community structures between the two groups. The point distributions between the caries group and the healthy group showed a trend of cross-coverage, and there was no significant difference between the groups. The species composition of individual samples in the caries group was significantly different to that of other samples, but this did not affect the similarity of the overall flora structure of the two groups.

Significant differences in bacteria

LEfSe helped to identify the bacteria that were most likely to show significant differences at each classification level between the caries group and the healthy group (Figs 7 and 8). *Firmicutes* were the marker bacteria at the gate level in the caries group, whereas in the healthy group it was *Bacteroidetes*. The taxonomic abundance at the level of one class, two orders, two families and two genera in the caries group was relatively high. At the species level, *Streptococcus sanguinis*, *Streptococcus oralis* and other *Streptococcus* bacteria were enriched significantly in the caries group. In the healthy group, the abundance was higher at the level of

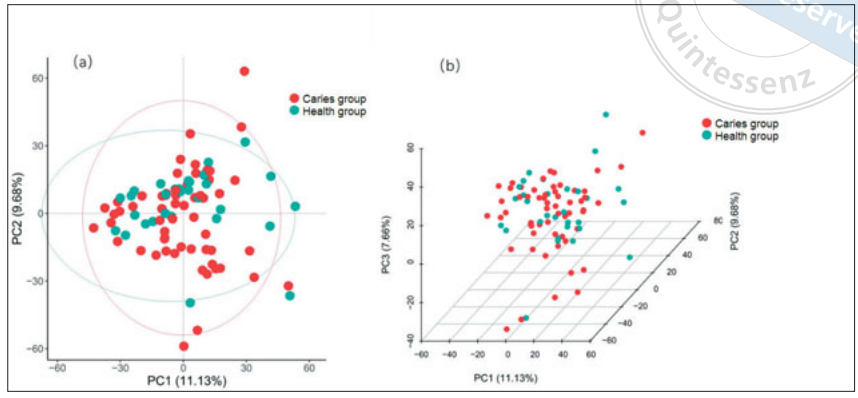

Fig 5 PCA scatter plot for the caries group and healthy group. **(a)** PCA two-dimensional scatter plot. Each point represents a sample; the red points represent caries group samples and the green points represent healthy group samples. PC1 is the first principal component, indicating that it contributes 11.13% to the sample difference, and PC2 is the second principal component, indicating that it contributes 9.66% to the sample difference. **(b)** PCA 3D scatter plot with PC3 as the third principal component, indicating that the contribution to sample difference is 7.66%.

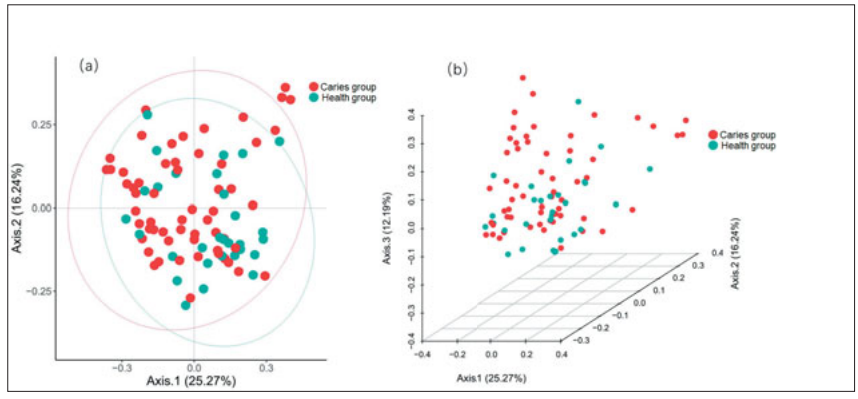

Fig 6 PCoA scatter plot for the caries group and healthy group. **(a)** PCoA two-dimensional scatter plot shows that each point represented a sample, with the red points representing the samples of the caries group and the green points representing the samples of the healthy group. Axis.1 was the first principal component, representing 25.27% and Axis.2 was the second principal component, representing 16.24% of the sample difference. **(b)** PCoA 3D scatter plot with Axis.3 as the third principal component, representing a contribution to the sample difference of 12.19%.

one class, two orders, five families and nine genera in the horizontal classification. In addition, the abundance of *Capnocytophaga* such as *Capnocytophaga leadbetteri* and *Capnocytophaga sputigena* at the species level was high.

Correlation network of bacteria

The results of the correlation analysis of the interaction between different microorganisms in the two groups are shown in Fig 9. Bacteria in the caries group and the healthy group showed different species correlations. The correlation between bacteria in the healthy group was more complex and aggregated. The larger the node, the

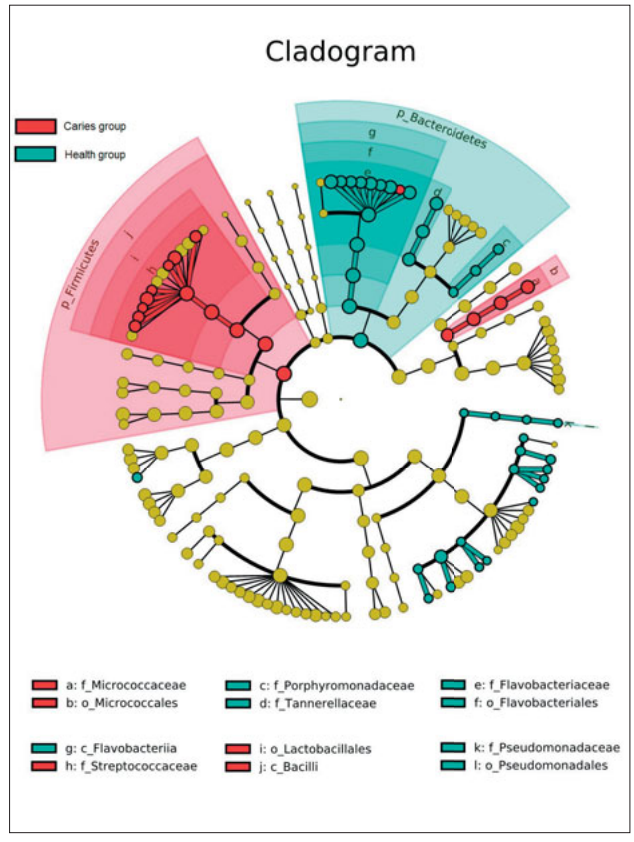

Fig 7 Cladogram for the caries group and healthy group. The coloured nodes from the inner ring to the outer ring represent the level of classification from gate to species. Different colours indicate that there were significantly different taxa between the two groups. Red indicates significantly increased bacteria in the caries group, and green indicates significantly increased bacteria in the healthy group.

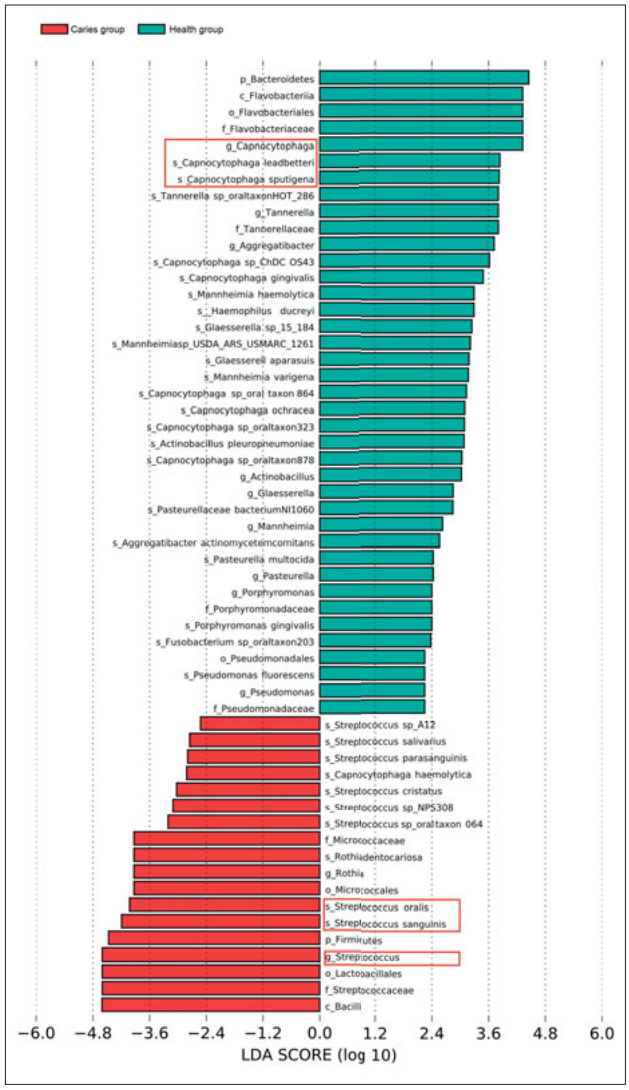

Fig 8 The column diagram of LDA was analysed by LEfSE in the caries group and healthy group. The bacteria in both groups were ranked according to the LDA score of different species at each classification level.

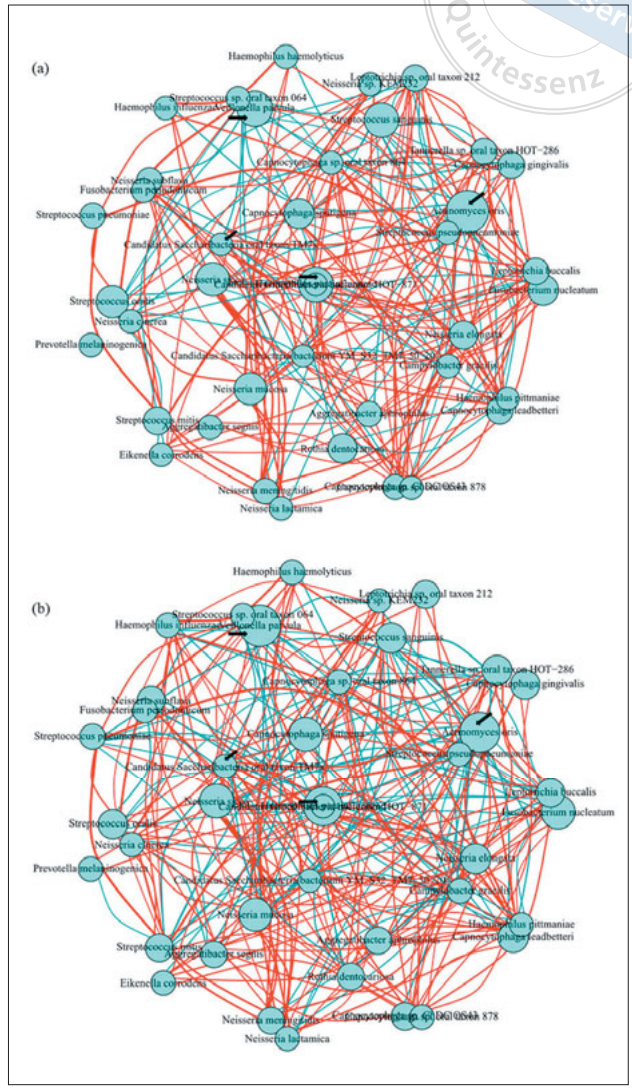

Fig 9 Flora correlation network in the **(a)** caries group and **(b)** healthy group. Nodes represent a type of bacteria. The larger the node, the more bacteria it is related to. Each link indicates a significant correlation between the two bacteria (correlation coefficient $|R| > 0.4$, $P < 0.01$, Spearman correlation analysis). Red indicates a positive correlation and blue indicates a negative correlation.

more connections it has with other nodes, indicating that the corresponding bacteria have more correlation with other bacteria. The results of this study showed that two bacteria, *Actinomyces oris* and *Veillonella parvula*, showed the most complex association with other bacteria in the caries group and the healthy group, and these were also the two bacteria with the highest relative abundance. There was a strong positive correlation between the bacteria of TM7 and GN02, and a complex correlation with a variety of other bacteria of the *Streptococcus*, *Capnocytophaga*, *Fusobacterium* and *Leptotrichia*.

Discussion

CPR is an important component of the oral flora

In this study, we used metagenomics to analyse the microbial composition and structure of caries patients and healthy patients, which was more accurate than previous studies using amplicon sequencing. All the samples detected the presence of three types of microorganisms, namely bacteria, archaea and viruses, including 38 phyla of bacteria, mainly *Proteobacteria*, *Firmicutes*,

Bacteroidetes, *Actinobacteria* and *Fusobacteria*, which were similar to previous studies[16,30,31] and showed that the oral microflora of the human body was relatively stable.

The results of this study showed that the abundance of TM7 and GN02 was second only to that of the major microflora, indicating that CPR were an important component of the oral microflora. The results also indicated that the CPR at species level were mainly *TM7x* of TM7 and *Candidatus Gracilibacteria bacterium HOT-871* of GN02, both of which had high taxonomic abundance at the species level of the oral microflora, further proving the importance of CPR in the oral microbial community. *TM7x* and *Candidatus Gracilibacteria bacterium HOT-871* were detected in all samples, with a 100% detection rate indicating that CPR is common in the oral cavity and constitutes an important component of the oral microbiota. Studies have shown that CPR is a highly diverse bacterium that exists in all walks of life in nature and can exist in a variety of natural environments around the world, including fresh water, seawater, hot springs, swamps[32] and soil[33]. CPR is also detected in many parts of the human body, including the skin[34], the distal oesophagus[35] and the intestine[36], and is particularly common in the oral cavity[37]. CPR may have the ability to maintain growth in states of both health and critical illness[9].

The clustering analysis of samples in this study showed that the samples from the caries group and the healthy group showed a good clustering relationship, respectively. A similar bacterial cluster was observed between some samples from the caries group and the healthy group, indicating that the disease state might not significantly affect the bacterial species composition. *Neisseria*, *Streptococcus* and *Capnocytophaga* showed a good clustering relationship, respectively, indicating the similarity of survival conditions between the bacteria of the same genus. There was a difference in the relative abundance of species between the samples from the caries group and the healthy group, and the abundance of CPR in some samples from the caries group was significantly higher than that in other samples from the healthy group, indicating that CPR might have a potential pathogenic relationship with caries. Affected by the disease state, living habits, diet and drinking water conditions, the relative abundance of oral CPR in individuals will differ. This result of species classification with similar composition and different abundance supports the 'ecological plaque hypothesis', and caries is caused by the destruction of microbial homeostasis in the body, rather than the activities of specific microorganisms[38].

CPR is the 'core microbiome' of the oral cavity

The present results showed that around 50% bacteria in the caries group were common to the healthy group at the species level. Compared with the healthy group, there were more specific bacteria in the caries group, while only one specific bacterium was found in the healthy group. In general, a 'core microbiome' is defined as a group of members shared by a microbiota present in all or most of the different tissue sites in a human[39]. Analysis of species common to all samples using a Venn diagram is a basic method for finding the 'core microbiome'[40,41]. The oral 'core microbiome' was initially discovered by analysing multiple oral sites in three adults using 454 pyrosequencing technology[42]. Through further studies, scholars have confirmed the existence of the core microbiome and studied and expanded their overall composition[43,44]. *Firmicutes* is considered the 'core microbiome' in the oral cavity. The difference analysis of LEfSe in this study showed that bacteria of *Firmicutes* and *Streptococcus* in the caries group were significantly enriched, which was in line with the results of a previous study[45]. *Streptococcus* uses its sugar metabolism acid-producing and acid-resistant ability to change its microbial ecological environment and affect the colony structure, which may further lead to the occurrence and development of dental caries[45]. In addition, the results suggest that bacteria of the *Rothia* and *Micrococcaceae* may also be associated with dental caries.

The results of this study showed that TM7 and GN02 were common species in the caries group and the healthy group, and were detected in all samples, indicating that TM7 and GN02 belong to the 'core microbiome' of the oral cavity and might play an important role in the stability and function of the oral microecological environment, which was consistent with the results of previous studies[20,46,47]. Whether they are the core microbiota of caries remains to be further studied. Based on the level of the existing database annotations, the LEfSE difference analysis shows that there is no significant difference in the bacteria of TM7 and GN02 in the caries group and the healthy group at the overall level; however, it was found that the abundance of CPR was significantly increased in some samples. In this study, after in-depth analysis of the CPR obtained by genomic assembly and box division, it was revealed that there was a difference in the level of unknown strains of CPR between the caries group and the healthy group.

There is a complex correlation between CPR and a variety of oral microorganisms

Correlation network analysis reveals the potential correlation between the oral microflora and indicates the possible synergistic and antagonistic interactions between different microorganisms. Unique bacterial interactions are exhibited in oral colonies from different disease states. Analysis of the correlation between the microbiota in the caries group and the healthy group clearly revealed the complex relationship between the oral microbiota. The bacteria in the healthy group showed a richer and more complex correlation, suggesting that some microbial relationships in the caries group might be destroyed and lead to an ecological imbalance in the process of dental caries. This is consistent with the view expressed in a previous study that acidic conditions are produced due to an increase in fermentable carbohydrates, resulting in a change in the nutritional status of the microbial colony and disruption of microbial interactions that maintain the balance of microbial communities in a healthy state[48].

The results of this study showed a strong positive correlation between TM7 and GN02 in the caries group and the healthy group, indicating that there was a close survival relationship between the CPR. TM7 and GN02 in the two groups had a positive correlation with a variety of *Capnocytophaga*. *Capnocytophaga* is the 'core microbiome' of the oral cavity and exists widely in oral colonies. Previous studies have shown that *Capnocytophaga* are involved in the formation of dental plaque biofilms[49], which are significantly enriched in patients with gingivitis[50], dental caries[51] and oral cancer[52]. The strong positive correlation between CPR and *Capnocytophaga* suggested that *Capnocytophaga* might be a potential host bacterium of CPR, and the ability of CPR to assist host bacteria in avoiding the human immune system and promoting the formation of host biofilm might increase the role of their host bacteria in the occurrence and development of diseases[53]; however, whether the CPR truly have a parasitic relationship with the *Capnocytophaga* needs to be verified by isolation and culture in the laboratory. In addition, there was a complex correlation between *Actinomyces spp.*, *Veillonella parvula* and other bacteria. Meanwhile, *Actinomyces spp.* and *Veillonella parvula* were the most abundant bacteria in the two groups, suggesting that they might be important for oral bacterial interaction and homeostasis.

In this study, the diversity of colony structure showed that the abundance and diversity of bacterial communities in the caries group were similar to those in the healthy group, which was consistent with the results of some previous studies[31,54]. Other studies have shown that there are significant differences in the diversity of oral microorganisms between healthy controls and caries patients, indicating that there may be differences between oral flora ecosystems in different disease states[16,55,56]. The results of the colony structure may be influenced by sample size, sequencing method, individual differences and a number of other factors. Metagenomics methods can achieve greater sequencing depth than 16S amplicon sequencing methods, while increasing the number of species annotated and relatively reducing the difference in colony diversity between subgroups. In view of the lack of research on the relationship between metagenomics and caries microorganisms, more studies are needed to further clarify the structural characteristics of the colonies.

Conclusion

In this study, we analysed the difference in the composition, distribution and abundance of CPR in the oral environment of caries patients and healthy people by metagenomic bioinformatics, based on the problem of the correlation between CPR and the potential pathogenesis of dental caries. CPR is a common species in caries and healthy oral cavities with a high detection rate and relative abundance, indicating that it is an important component of the oral microflora and the 'core microflora' of the oral cavity, which may play an important role in the stability and function of the oral microecological environment. There was a correlation between CPR and a variety of oral microorganisms, and the positive correlation with *Capnocytophaga* was strongest, suggesting that *Capnocytophaga* might be the potential host bacteria of CPR. TM7 and GN02 were significantly increased in some caries samples, but the correlation between CPR bacteria and caries and its possible pathogenic mechanism still need to be studied in greater depth. Further development of culture mediums suitable for CPR and acquisition of more clinical isolates to explore the mechanism of CPR participating in the pathogenesis of dental caries will be one of the research directions of oral CPR in the future.

Conflicts of interest

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

Author contribution

Dr Song JIANG developed the experimental strategy, analysed the data and drafted the manuscript; Dr Yu Xing CHEN assisted in analysing the data and writing the manuscript; Drs Jie NIE, Xiao Yan WANG and Feng CHEN refined the strategy and mentored the work. All authors approved the final manuscript.

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Age Estimation using Panoramic Radiographs by Transfer Learning

Chuang Chuang MU[1], Gang LI[1]

Objective: *To assess the accuracy of transfer learning models for age estimation from panoramic photographs of permanent dentition of patients with an equal sex and age distribution and provide a new method of age estimation.*
Methods: *The panoramic photographs of 3000 patients with an equal sex and age distribution were divided into three groups: a training set (n = 2400), validation set (n = 300) and test set (n = 300). The ResNet, EffiecientNet, VggNet and DenseNet transfer learning models were trained with the training set. The models were subsequently tested using the data in the test set. The mean absolute errors were calculated and the different features extracted by the deep learning models in different age groups were visualixed.*
Results: *The mean absolute error (MAE) and root mean square error (RMSE) of the optimal transfer learning model EfficientNet-B5 in the test set were 2.83 and 4.59, respectively. The dentition, maxillary sinus, mandibular body and mandibular angle all played a role in age estimation.*
Conclusion: *Transfer learning models can extract different features in different age groups and can be used for age estimation in panoramic radiographs.*
Key words: *age estimation, deep learning, forensic odontology, panoramic radiograph, transfer learning*
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Age estimation based on panoramic radiographs is essential in forensic science and anthropological research. Several methods have been proposed for age estimation in children and adolescents. Among these, those proposed by Demirjian et al[1] and Nolla et al[2] are adopted frequently. Their methods define the different stages of tooth development by the first appearance of calcified points to the closure of the tooth apex in panoramic radiographs and score them accordingly, and the scores are then converted to a dental age from 3 to 17 years. Willems et al[3] modified the scoring system proposed by Demirjian et al[1] and applied it to Belgian children, achieving relatively high accuracy in age estimation. Cameriere et al[4] established a regression model according to age with measurements of open apices in different teeth. The estimated age obtained from this model has a residual error of 0.035[4]. For adults, age estimation is based on the negative correlation between age and pulp/pulp chamber size. Mittal et al[5] measured the lengths and widths of six anterior teeth at different levels in 152 panoramic radiographs and performed regression analysis for age estimation. The standard error from this regression model was 7.97 between chronological and estimated age[5]. Cameriere et al[6] analysed the pulp/tooth

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area ratios of the premolars in panoramic radiographs of 606 patients. The mean absolute errors (MAEs) obtained from the regression model established in the study ranged from 4.43 to 6.02 (at a 95% confidence interval)[6]. Manual extraction of tooth development features for age estimation is used mainly in traditional algorithms, but this is time-consuming and complicated.

Deep learning, an artificial intelligence technique, has been widely applied to the automatic extraction of image features. This has led to an improvement in traditional dental imaging tasks[7-10]. Vila-Blanco et al[9] and Guo et al[11] used end-to-end neural networks based on panoramic radiographs for age estimation, with promising results. The data sets in their studies consisted primarily of samples from patients aged under 25 years. Whether deep learning performs equally well for other dental images, however, is unknown. Transfer learning[12] is a common deep learning technique that is used for similar tasks in computer vision. In this process, one pretrained model can be used as the starting point for another model aimed at another similar task. Thus, the purpose of the present study was to use different transfer learning models for age estimation in panoramic radiographs and explore the extracted features in different age groups.

Materials and methods

A data set containing 3000 panoramic radiographs was collected retrospectively from the database of Peking University School and Hospital of Stomatology. The sample consisted of 1500 females and 1500 males, aged between 12 and 71 years. All the patients' ages were confirmed in the hospital's patient information system. Their age and sex distributions are shown in Table 1.

Image acquisition

The panoramic radiographs were all acquired using panoramic radiograph equipment (Hyperion X9, MyRay, Cefla, Imola, Italy) with exposure parameters of 60~85 kV, 1~10 mA and 13.0 seconds according to patient size.

The panoramic radiographs were reviewed by a professional oral and maxillofacial radiologist. The inclusion criteria were as follows:

- no fractures or large defects in either the maxilla or mandible;
- no space-occupying lesions;
- no primary teeth;
- no systemic diseases or developmental delays.

Transfer learning models

In this study, ResNet[13], EfficientNet[14], VggNet[15] and DenseNet[16] models pretrained on ImageNet[17] (Tampa, FL, USA) images were used to extract features in panoramic radiographs for automatic age estimation. ImageNet is a large-scale hierarchical image database.

Image processing and augmentation

All 3000 panoramic radiographs were divided into three sets: a training set (n = 2400, 80%), a validation set (n = 300, 10%) and a test set (n = 300, 10%). The division was conducted by keeping age and sex equal within the three sets. All the panoramic radiographs had a 24-bit colour depth and a height and width of 2500 × 1248 pixels. The images were padded while preserving the aspect ratio and resized to 224 × 224 pixels to satisfy the pretrained model input size. Random horizontal flip was the only image augmentation technique used due to the fixed image acquisition parameters for the panoramic radiographs used in the present study.

Model training and evaluation

The fully connected layers of pretrained models were modified to target directly presenting the estimated age. The softmax function was used to change the activation function in the last fully connected layer into a probability distribution, defined as follows:

$$p_i = \frac{\exp(x_i)}{\sum_{n=1}^{N} \exp(x_n)},$$

where ρ_i represents the probability.
The estimation age was then calculated as:

$$\hat{y} = \sum_{i=1}^{K} i\, p_i,$$

where ŷ represents the estimation age.
The loss function of the model was set to the sum of the softmax loss and the mean-variance loss[18], then the training set was fed into the model and the epoch was set as 200, with an early stop strategy if the loss result did not improve after 20 successive epochs. The network performance could be assessed with the validation set for hyperparameter adjustment and to determine the optimal deep learning model.

MAEs and RMSEs have been commonly used in age estimation studies[4,6,9,11]. Thus, this study applied MAE

and RMSE to evaluate the accuracy of age estimation with the transfer learning models in the test set. The estimated ages in different age and sex groups were recorded for further analysis.

The MAE is the mean difference between the estimated and chronological age:

$$MAE = \frac{1}{N}\sum_{n=1}^{N} |\hat{y}_n - y_n| ,$$

where ŷ and y represent the estimated and chronological age, respectively, and n is the counter for the images.

The RMSE is the standard deviation (SD) of the residuals (estimated errors between the estimated and chronological age):

$$\mathrm{RMSE} = \frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2,$$

Statistical analysis and feature visualization

A Bland-Altman plot was used to compare the chronological and estimated age returned by the optimal transfer learning model. Class activation mapping[19] (CAM) was employed to visualise the attention regions for age estimation. Anatomically significant areas in the maps were marked and analysed.

Results

The MAEs and RMSEs of the transfer learning models for age estimation with the test set are displayed in Table 2. Different pretrained models had different accuracies in age estimation. The optimal model in this study was EfficientNet-B5, with an MAE of 2.83 and RMSE of 4.59, while the maximum MAE for Vgg19 and ResNet101 was 5.26 and the maximum RMSE for ResNet101 was 7.19. Further results from EfficientNet-B5 for different age groups and sexes with the test set are shown in Table 3.

The plots for chronological age versus estimated age by EfficientNet-B5 with the test set (n = 300) are shown in Fig 1. The points are close to the diagonal line, especially those representing ages under 41 years. Points falling directly on the diagonal line indicate a perfect match between chronological and estimated age.

The Bland-Altman plot was created using SPSS Statistics 24.0 (SPSS, Chicago, IL, USA). The differences between chronological age and the age estimated using EfficientNet-B5 are shown in Fig 2. The mean difference, chronological age minus estimated age, was 0.0 years and the SD was 4.59. Thus, the lower 95% limit was 0.00 − 1.96 × 4.59 = −9.00 years and the upper 95% limit was 0.00 + 1.96 × 4.59 = 9.00 years. A total of 285 points out of 300 were within the 95% limitsof agreement. Thus, this model provides reliable estimates of agreement in this study.

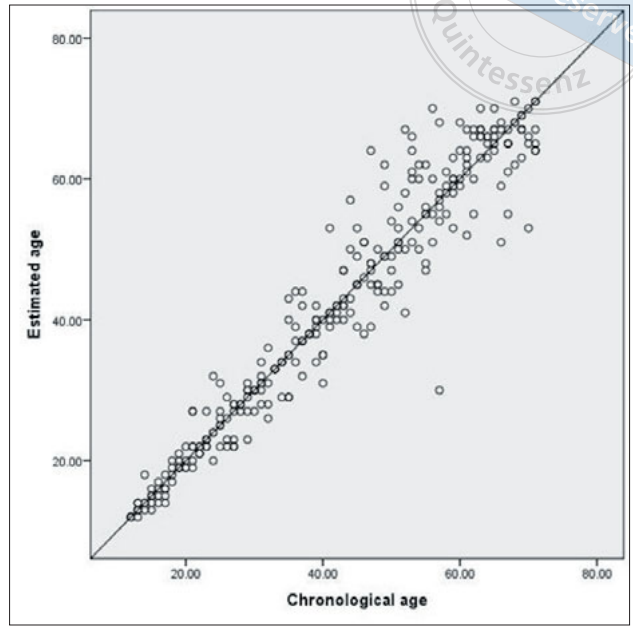

Fig 1 Chronological age versus estimated age.

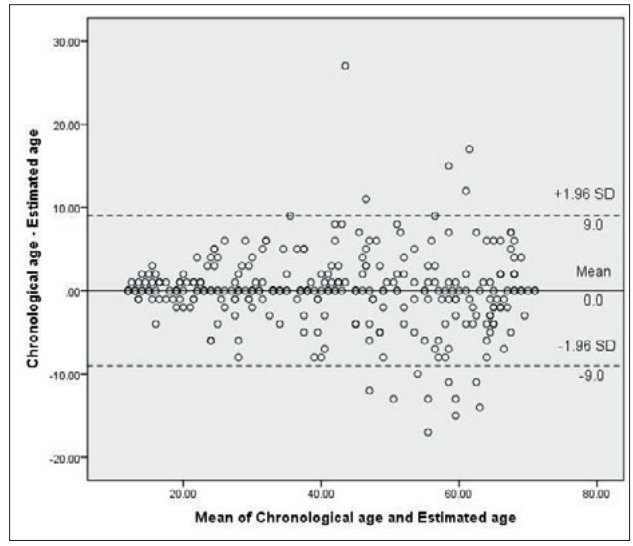

Fig 2 Differences between chronological and estimated ages versus mean of chronological and estimated ages, with 95% limits of agreement (broken lines).

The class activation mapping results for different age groups are shown in Fig 3. Differently coloured areas represent the weights according to the colour bar. The colour maps for the groups aged 12 to 21 years (Figs 3a and b) and 22 to 31 years (Figs 3c and d) lie more in the dentition than in the other parts of the panoramic

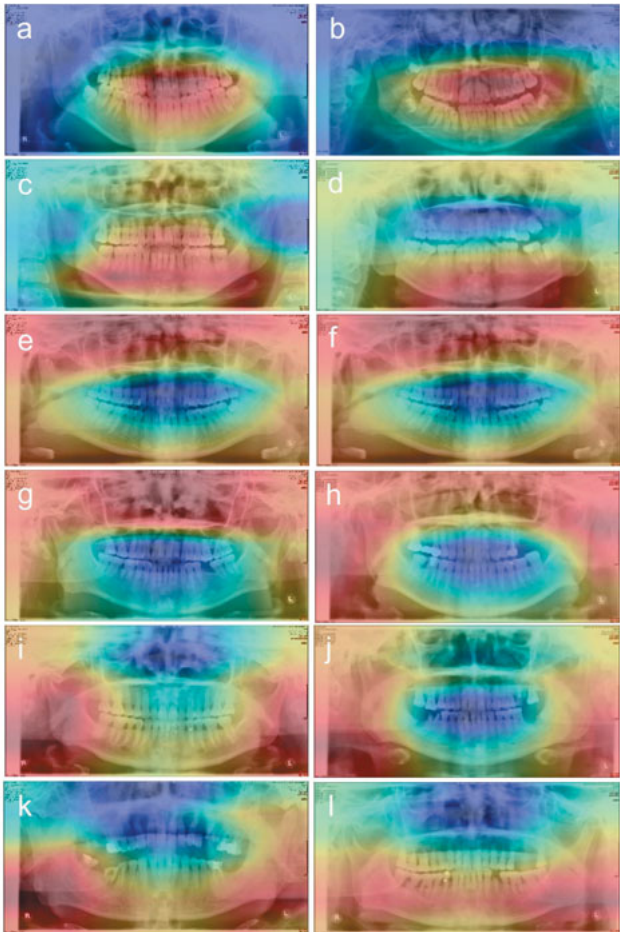

Fig 3 Class activation mapping results for the different age groups.

radiographs. The maxillary sinus was of greater concern for the groups aged 32 to 41 (Figs 3e and f) and 42 to 51 years (Figs 3g and h). The mandibular body and mandibular angle were more important in the groups aged 52 to 61 (Figs 3i and j) and 62 to 71 years (Figs 3k and l).

Discussion

In this study, transfer learning models based on ResNet, EfficientNet, VggNet and DenseNet were established using panoramic radiographs for two reasons. First, these models have been verified on ImageNet and have demonstrated good classification performance, and second, they have been applied in similar related studies[11,20,21].

The minimum and maximum MAEs and RMSEs of the transfer learning models used for age estimation with the test set were 2.83 (EfficientNet-B5) and 5.26 (Vgg19 and ResNet101), 4.59 (EfficientNet-B5) and 7.19 (ResNet101), respectively. Other transfer learning models also showed different levels of accuracy in age estimation. These models are trained, validated and tested on the same data; thus, the differences in the models' architecture cause the differences in the final accuracies. Deep learning is a black box, and the principal differences between different models' architectures need to be further studied by computer vision, and customised architecture for tooth age may perform better.

Table 1 Age and sex distribution of the data set.

Age (y)	Male	Female	Total
12–21	250	250	500
22–31	250	250	500
32–41	250	250	500
42–51	250	250	500
52–61	250	250	500
62–71	250	250	500
Total	1500	1500	3000

Using the optimal EfficientNet-B5 model, women in the group aged 22 to 31 years had the smallest estimation error (MAE 0.96, RMSE 1.52), whereas men in the group aged 52 to 61 years had the largest (MAE 5.12, RMSE 7.03). In general, the estimation error between chronological age and estimated age increased as age increased, as shown in Table 3. The Bland-Altman plot (Fig 2) shows that the differences increased with the mean, which is consistent with Table 3 and Fig 1.

The results of the class activation mapping suggest that in different age groups, different anatomical structures are considered. In the younger age groups (12 to 21 and 22 to 31 years), the extracted features were primarily in the dentition, which is consistent with traditional methods. In the middle age groups (32 to 41 and 42 to 51 years), the feature areas moved to the maxillary sinus. Jun et al[22] analysed the volume of the maxillary sinus using high-resolution computed tomography (CT) and found that maximum growth was reached in the fourth decade of life in men and in the third decade of life in women. Aktuna et al[23] found that maxillary sinus volume decreases as age increases, and the present results confirm these findings. The mandibular body and mandibular angle were emphasised in the older age groups (52 to 61 and 62 to 71 years). This coincides with the findings from the study by Upadhyay et al[24], in which the investigators used physicoforensic anthropometry and lateral cephalometric methods to measure 185 subjects and obtained results showing a decrease in the mandibular angle as age increased. The finding that the deep learning models extracted these features shows that these anatomical structures also have the potential to aid age estimation.

Table 2 MAE and RMSE for different pretrained models.

Model	Both sexes		Female		Male	
	MAE	RMSE	MAE	RMSE	MAE	RMSE
ResNet18	2.95	4.69	2.99	4.96	2.92	4.42
ResNet50	3.78	5.98	3.93	6.21	3.68	5.75
ResNet101	5.26	7.19	5.10	7.11	5.43	7.28
Vgg16	4.51	5.96	4.40	5.96	4.63	5.97
Vgg19	5.26	6.85	5.48	7.29	5.04	6.36
EfficientNet-B1	4.59	6.23	4.60	6.38	4.59	6.07
EfficientNet-B3	3.87	5.44	3.85	5.70	3.89	5.16
EfficientNet-B5	2.83	4.59	2.83	4.79	2.83	4.38
DenseNet121	3.15	4.81	3.00	4.79	3.30	4.84

Table 3 MAE and RMSE with different age groups and sexes for EfficientNet-B5.

Age group	Both sexes		Female		Male	
	MAE	RMSE	MAE	RMSE	MAE	RMSE
12–21 y	1.06	1.70	1.08	1.78	1.04	1.62
22–31 y	1.64	2.56	0.96	1.52	2.32	3.28
32–41 y	2.42	3.87	2.76	3.96	2.08	3.77
42–51 y	3.86	5.22	3.68	5.56	4.04	4.85
52–61 y	4.78	7.01	4.44	6.99	5.12	7.03
62–71 y	3.52	5.06	4.08	6.05	2.96	3.84
Total	2.83	4.59	2.83	4.79	2.83	4.38

Vila-Blanco et al[9] used 2289 panoramic radiographs to establish two convolutional neural network models for age estimation. The MAEs obtained from the two models were 3.19 ± 4.32 and 2.84 ± 3.75, respectively. The data set was unbalanced and, most importantly, the subjects involved were aged mostly under 20 years (1381/2289)[9]. This may have caused bias in the age estimation for adults. Guo et al[11] collected 10,257 panoramic radiographs from patients aged 5 to 24 years and developed end-to-end neural networks to compare their predictions with the manual method (by Demirjian et al[1]). Their results proved that conventional neural network models can surpass the manual method in age classification; however, in their study, the subjects were all aged under 25 years, and they did not provide the MAE or explore the ability of the neural networks to extract features at other ages, as was done in this study.

Although we collected panoramic radiographs from patients with equal distributions of age and sex to avoid confounding factors that might impact the results of such a study as far as possible, limitations still exist. First, only a few pretrained deep learning models were used, and other neural network architectures and pretrained models were not compared, thus the differences across different model architectures need to be explored further. Second, deep learning usually requires a large-scale data set. Although the data set used in the present study was notably large and similar in size to other studies, it was still smaller than the typical datasets used for facial tasks[20].

Conclusion

Transfer learning models can be used for age estimation with panoramic radiographs. Differences between the different sexes and age groups were also observed and presented. Class activation mapping showed that different anatomical features were used for age estimation in different age groups. The role of these features in age estimation needs to be studied further.

Conflicts of interest

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

Author contribution

Dr Chuang Chuang MU contributed to data preparation, established models and drafted the manuscript; Dr Gang LI contributed to design, supervised the study and revised the manuscript.

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A MicroCT Study on Mineral Change over Time Associated with Demineralisation in Primary Teeth

Ke Chung WU[1], Jung Wei CHEN[1], So Ran KWON[1]

Objective: *To evaluate the change in demineralisation depth (DD)and mineral density (MD) over time in primary teeth exposed to a demineralisation protocol with microcomputed tomography (microCT).*

Methods: *Caries lesions were artificially induced on the labial surfaces of 9 primary incisors by way of a demineralisation protocol using 0.1 M lactic acid with 10% methylcellulose gel for 7 and 14 days. The specimens were scanned with microCT and CTAn software (Bruker, Billerica, MA, USA) was used to analyse the changes in DD and MD. Statistical analyses were performed using SPSS software (IBM, Armonk, NY, USA). Repeated analysis of variance (ANOVA) test and Pearson bivariate correlation were used and the level of significance was set at $P < 0.05$.*

Results: *The DD ranged from 0.00 to 0.99 µm (mean ± standard deviation [SD] 0.70 ± 0.43 µm) at baseline, 11.18 to 29.5 µm (18.15 ± 5.23 µm) at 7 days and 18.00 to 55.30 µm (34.20 ± 8.70 µm) at 14 days. The MD for all specimens (n = 9) ranged from 1.48 to 1.76 g/cm^3 (1.65 ± 0.08 g/cm^3) at baseline, from 1.47 to 1.74 g/cm^3 (1.62 ± 0.08 g/cm^3) at 7 days demineralisation and 1.33 to 1.72 g/cm^3 (1.54 ± 0.13 g/cm^3) at 14 days. There were statistically significant differences in DD ($P < 0.001$) and MD ($P = 0.016$) between different durations of demineralisation.*

Conclusion: *DD and MD change with time after being exposed to demineralising solution. MicroCT is a nondestructive method that allows repeated MD evaluations of the same sample.*

Key words: *demineralisation, lesion depth, microcomputed tomography, mineral density, primary teeth*

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It is well established that acid attack of tooth enamel can cause a reduction in mineralisation and mechanical strength. In the oral environment, demineralisation of the enamel surface is reversible through ions in saliva and the effect of fluoride[1]; however, there is a gap in knowledge regarding the remineralisation potential of different remineralisation agents relative to density change and demineralisation depth (DD). Thus, a laboratory model using microcomputed tomography (microCT) to assess changes in mineral density (MD) and DD may improve understanding of initial caries progression in primary teeth and how to treat them in a minimally invasive manner.

There is strong evidence that caries in the primary dentition is predictive of later caries experience[2]; however, primary teeth may be more susceptible to initial caries progression compared to permanent teeth for several reasons. First, primary teeth have much thinner enamel. The mean thickness of the buccal enamel on the maxillary central incisors is 0.787 mm for primary

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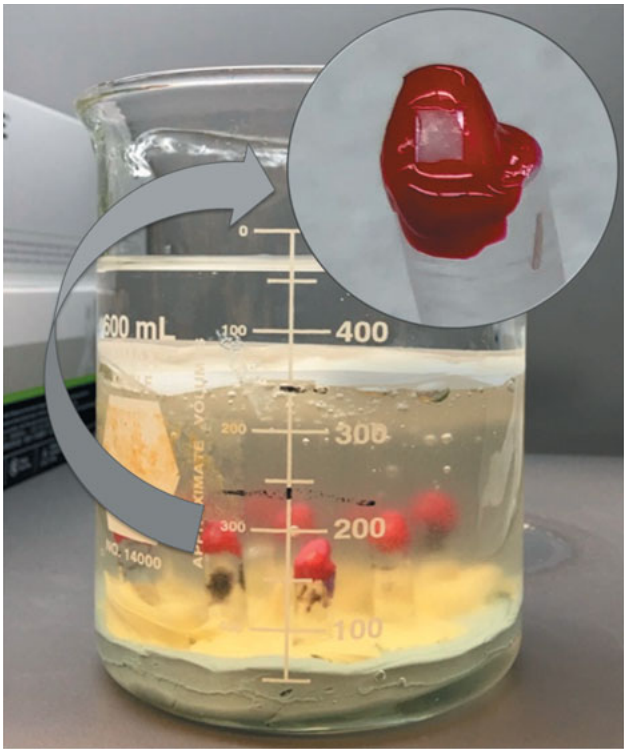

Fig 1 Experimental design. Mounted specimen in 10% methylcellulose gel and 0.1 M lactic acid on filter paper; insert: specimen mounted on acrylic rod with a 2 × 2 mm^2 exposure window.

teeth[3] compared to 0.945 mm in permanent teeth[4]. Second, primary teeth have a thicker aprismatic layer that exhibits low porosity[5]. Third, primary teeth exhibit far greater porosity overall, making them more prone to caries-like attacks[6]. Last, the interprismatic fraction and prism-junction density are also greater in primary teeth[6]. However, only one study has have evaluated enamel density changes after acid attacks related to early caries progression in primary teeth[7].

Laboratory demineralisation methods create standardised and reproducible caries lesions. Artificial caries have been created on primary teeth mainly using two demineralisation protocols. The most common methodof creating artificial caries lesions is using a demineralisation solution containing 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid with the pH adjusted to 4.4 to 5.0[8]. The direct immersion of primary teeth samples in the solution for 4 days yields an artificial lesion of 60 to 200 µm in depth[9,10]. Another method involves using 8% methylcellulose gel and 0.1 M lactic acid. After 3 to 14 days, lesion depth of 110 µm was noted[11]. Both methods were proven to be reliable in other studies[12,13].

Despite the widespread use of light microscopy to measure demineralisation and remineralisation, its main limitations include the need to section the samples and the fact that the two-dimensional view does not enable measurement of changes in density[6]. However, several methods have also been used to measure caries depth, including microCT[10] and scanning electron microscopy (SEM)[14]. MicroCT is a modified version of medical computed tomography (CT) and is used to assess the mineral concentration at micron levels, thus differentiating between sound and carious dentine. The new, cutting-edge technique enables nondestructive visualisation of dental structures in 3D and provides an easy way to monitor the progression of caries lesions without destroying the samples[15].

The present study aimed to evaluate the change in DD and mineral density (MD) over time in primary teeth exposed to a demineralisation protocol as measured using microCT. The null hypotheses tested were:

1. There would be no difference in DD depending on the amount of time exposed to the demineralisation agent.
2. There would be no difference in MD depending on the amount of time exposed to the demineralisation agent.
3. There would be no correlation between MD and DD.

Materials and methods

This study was approved by the Institutional Review Board of Loma Linda University, CA, USA (IRB#5180417).

Specimen preparations

Extracted/exfoliated primary incisors (N = 9) without restorations or caries were collected and stored in 0.1% sodium azide solution. Enamel-dentine blocks with an approximate size of 4 × 4 × 3 mm^3 were cut using a water-cooled diamond-edged blade and mounted on acrylic rods with the labial surface facing upwards. The tooth surfaces were then painted with acid-resistant nail varnish (Maybelline, New York, NY, USA) to leave a 2 × 2 mm^2 window to be exposed to the demineralisation agent (Fig 1).

Demineralisation protocol

A well-established demineralisation protocol using 0.1 M lactic acid with 10% methylcellulose gel was used[11]. The tooth specimens mounted on rods were stabilised in a 600 ml beaker with polyvinylsiloxane. 10%

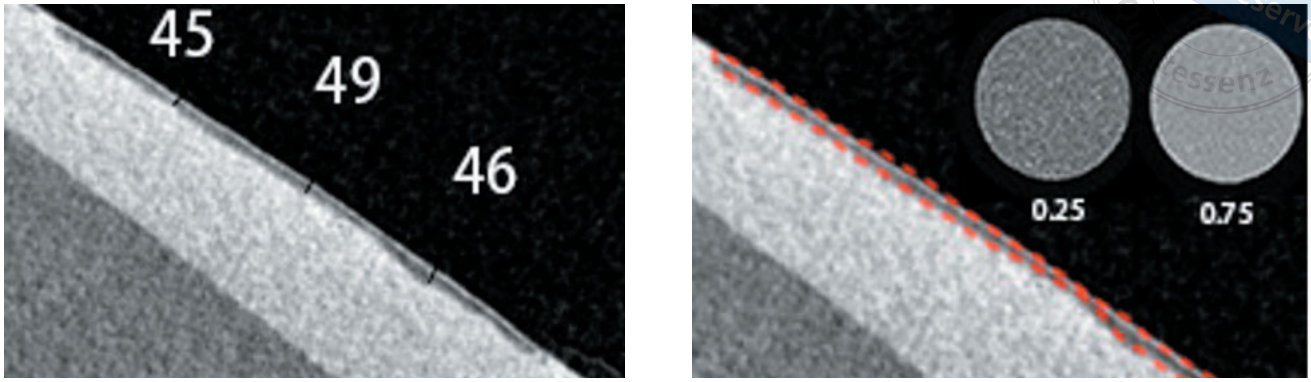

Fig 2 MicroCT images. **(a)** Lesion depth after 14 days of demineralisation; **(b)** region of interest set up (dotted line) for mineral density measurement and reconstructed images of phantoms at 0.25; 0.75 g/cm^3.

methylcellulose gel was poured onto the rods so that the specimens were covered with gel. The beaker was kept in the refrigerator for 4 hours for the gel to solidify. A filter paper was placed on top of the gel and 200 ml of 0.1 M lactic acid adjusted to pH 4.6 was poured over it (Fig 1). The specimens were kept for 7 days at 37°C then removed, rinsed with deionised water, kept in artificial saliva and scanned with microCT. This procedure was repeated for the 14-day measurements.

MicroCT parameters

A desktop radiograph microCT system SkyScan1272 scanner (Bruker, Billerica, MA, USA) was used to evaluate DD (μm) and enamel MD (g/cm^3) compared to the baseline measurements. Phase-contrast enhancement allowed object details as small as 4.0 μm to be detected. The enamel-dentine blocks mounted on acrylic rods were scanned with X-rays generated by a sealed microfocus X-ray tube (tungsten anode) at 85 keV and 100 mA with an integration time of 60 minutes. The samples were rotated over 180 degrees at rotation steps of 0.4 degrees. A 1.0-mm-thick aluminum filter was placed in front of the detector to remove low-energy X-rays. Scanning was performed under 100% humidity to avoid dehydration of the specimens, which were placed in a small container with wet gauze on top of them. A positioning jig was used for repeated measurements and precise superimposing of images.

Image analysis

Each specimen was reviewed and the parameters showing clearly defined margins and best contrast between enamel and dentine were recorded. When reduced noise and artificial defects in the images were observed, the critical value was chosen by one operator, then the mean of the values was calculated and the same parameters were used to reconstruct each image. Reconstruction of 800 to 900 images per specimen per scan was performed using NRecon software (Bruker). All images were reconstructed with a beam-hardening correction of 40%, smoothing of 2 and a ring artefact correction of 4. DataViewer software (Bruker), which provides tools for intensity-based image registration for both two and three dimensions, was used for visualisation of the image. The evaluated specimens served as their own controls for the assessments. The sagittal views of baseline, 7-day and 14-day images were superimposed using the 3D registration function in DataViewer. CTAn software (Bruker) was used to analyse the mineral concentration. Phantoms with different densities (0.25; 0.75 g/cm^3) were scanned with the same parameters and were used to calibrate the enamel density (Fig 2b). A linear calibration curve relationship between greyscale (linear attenuation coefficient, μ) and MD could then be generated from these phantoms. The calibration standards needed to fulfil basic requirements were that the X-ray attenuation must reflect the absorbance of the interested object and cover a representative range of MDs, and the calibration phantoms must be homogeneous at the spatial resolution of the scanner. After calibration, the images were analysed between baseline and after treatment to measure DD, and changes in MD were determined (Fig 2). The demineralisation area was separated from sound enamel and other tissue using the region of interest function (Fig 2b). DD was measured in the section from the middle of the nail varnish window. From that section, DD was determined by calculating the mean of the measurement from the one-third, one-half and

Table 1 Descriptive summary of DD and MD at different time points (mean ± SD).

Variable	Baseline	7 days	14 days	*P* value
DD, µm	0.70 ± 0.43[a]	18.15 ± 5.23[b]	34.2 ± 8.7[c]	< 0.001*
MD, g/cm^3	1.65 ± 0.08[a]	1.62 ± 0.08[ab]	1.54 ± 0.13[b]	0.016*

*Different superscript letters within rows indicate a significant difference determined by repeated measures ANOVA, $P < 0.05$.

two-thirds points of the window. MD was determined by the mean value of fifty sections from the middle of the nail varnish window.

Statistical analysis

Descriptive and inferential statistics were performed using SPSS Statistics 25 software (IBM, Armonk, NY, USA). Repeated analysis of variance (ANOVA) tests were used to evaluate the differences in DD and MD over time. A Pearson bivariate correlation was run to determine the relationship between MD at different times and DD and MD. The level of significance was set at $P < 0.05$.

Results

DD by exposure time to demineralisation agent

DD measured at different time points is summarised in Table 1. It ranged from 0.00 to 0.99 µm (mean 0.70 µm), 11.18 to 29.50 µm (mean 18.15 µm) and 18.00 to 55.30 µm (mean 34.20 µm) at baseline, 7 days and 14 days, respectively. There was a statistically significant difference between DD at the different time points as determined by repeated measures ANOVA (Pillai trace $P < 0.001$). The range and distribution of DD by time point are illustrated in Fig 3.

MD by exposure time to demineralisation agent

MD measured at different time points is summarised in Table 1. It ranged from 1.48 to 1.76 g/cm^3 (mean 1.65 g/cm^3), 1.47 to 1.74 g/cm^3 (mean 1.62 g/cm^3) and 1.33 to 1.72 g/cm^3 (mean 1.54 g/cm^3) at baseline, 7 days and 14 days, respectively. There was statistically significant difference in MD between the different time points, as determined by repeated measures ANOVA (Pillai trace $P = 0.016$). After multiple pairwise comparisons, there was a statistically significant difference between MD at baseline and 14 days ($P = 0.035$), whereas there was no difference between baseline and 7 days ($P = 0.654$) and 7 days and 14 days ($P = 0.089$).

Correlation between MD and DD

A Pearson bivariate correlation was run to determine the relationship between MD and DD. There was a strong negative correlation between them, which was statistically significant ($r = -0.406$, $P = 0.035$).

Discussion

The present study explored the applicability of microCT to evaluate changes in DD and MD in primary teeth after exposure to a specific demineralisation protocol. To the best of the authors' knowledge, this is the first study to use microCT and evaluate changes over time on the same primary teeth. Based on the results, we rejected our null hypotheses as there were differences in DD and MD depending on the length of time exposed to demineralisation agents. This supports that microCT methods can be used to detect changes in DD and MD over time in the same specimens, and can therefore be a valid and reliable method for future research into primary teeth.

For primary teeth, artificial caries depth ranged from 26 µm created by lactic acid with pH 4.5 by 7 days to 350 µm created by acetic acid with pH 3.9 and a soaking time of 10 days[16,17]. The main advantage of using a gel method is the indirect contact of the demineralisation solution facilitated by the gel that may simulate the oral environment that presents a pellicle or biofilm on top of the tooth structure. Compared to a previous study that recorded a mean DD of 36.1 ± 8.2 µm measured by transverse microradiography (TMR) and microscopy after 7 days' exposure to lactic acid and 6% hydroxyethyl cellulose with pH 4.5[16], the present results showed less lesion depth, namely 19.11 ± 5.49 µm after 7 days of demineralisation. This may be attributed to factors such as the use of a denser concentration of methylcellulose gel (10%) and immersion in a demineralising solution with a slightly higher pH (pH 4.6) in the present study. DD varied from previous studies due to different demineralisation protocols, length of soaking time and type of teeth used[16,17].

The MD of sound enamel of primary incisors in the present study (mean 1.65 ± 0.08 g/cm^3) was similar to a previous study measuring mandibular primary anter-

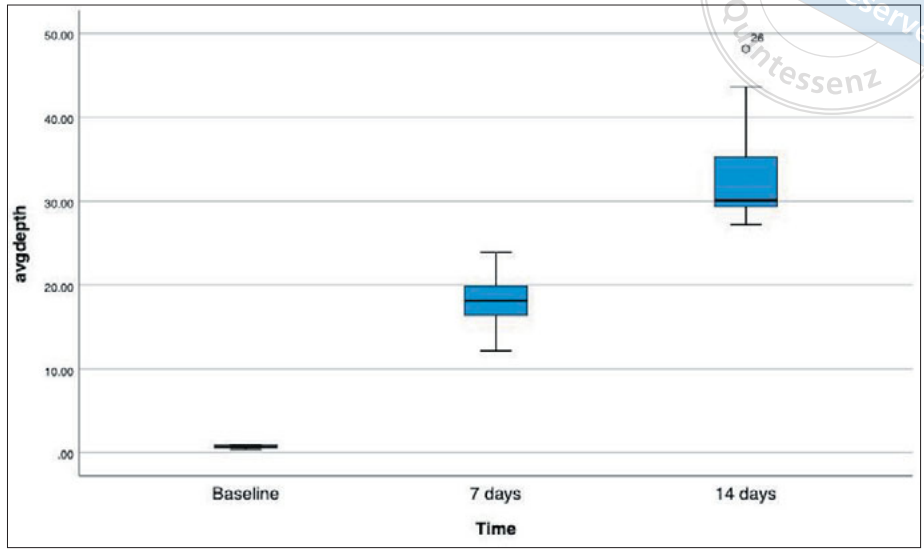

Fig 3 Changes in DD over time.

ior teeth using microCT (mean 1.68 g/cm^3)[18]. Kecuk et al[19] measured the MD of permanent premolars using microCT and reported a mean of 2.32 g/cm^3. This result was similar to other microCT studies measuring permanent premolars (2.43 g/cm^3)[20] and third molars (2.74 ± 0.19 g/cm^3)[21]. It is reasonable to expect higher MD for permanent teeth compared to primary teeth due to the increased porosity of primary tooth enamel[6]. Loss of MD of 1.85 g/cm^3 was measured using microCT from permanent premolars after 21 days' demineralisation with pH 4.5 lactic acid solution, containing 6% methylcellulose and 500 mg/l hydroxyapatite[20]. With a different demineralisation protocol with 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4 and 0.05 M acetic acid with pH 4.4, mineral loss of 1.38 ± 0.21 g/cm^3 was noted with permanent third molars after 96 hours of exposure[21]. The present results showed less MD loss (0.11 g/cm^3 after 14 days of demineralisation) compared to previous studies; this may be due to the demineralisation protocol involving use of methylcellulose gel for milder artificial caries progression.

It is noteworthy that previous studies only measured either DD or MD and none compared the two parameters. The present authors found a negative correlation between DD and MD. DD became deeper as caries progressed and the MD of the whole demineralised area decreased. As a result, progression of DD may predict loss of MD; however, the upper limit of DD in our study was 55.3 μm, so the correlation of DD and MD is not generalisable to depths beyond our upper limit.

Light microscopy is used widely to measure caries depth in many diagnostic studies of caries lesions[6,8]; however, it analyses 3D alterations using a two-dimensional method. As such, some characteristics can be lost during sample preparation, which can be particularly critical for analysis of caries depth[15]. TMR is considered the gold standard for determining the mineral content and MD change of dental enamel[22]. It is a reliable technique, but the cross-sectional sample preparation is difficult and makes it impossible to take repetitive scans of the same specimen[23].

MicroCT provides 3D images with no sample preparation required, and repeated scanning is possible, with the enamel surface of the lesion not being damaged during scanning[24,25]. From the 3D images provided by microCT, changes in depth, surface area, volume and density of the tooth structure can be calculated. The disadvantages of microCT are the long amounts of time needed to form a 3D model of each sample and the high cost of the process. Different settings of microCT scanners from different studies may affect the results. This is therefore a technique-sensitive method with a steep learning curve for image processing.

The present study has inherent limitations that warrant interpretation. Because no similar studies on primary teeth had been conducted previously, to the best of the authors' knowledge, the sample size was determined based on a pilot study. During the pilot study, specimen fracture was noted after prolonged microCT scanning; this may have been due to the dryness during the scanning time. Modification was done by placing a wet gauze over the specimens while scanning to maintain 100% humidity. Future studies should be conducted with a longer demineralisation time and with deeper

artificial caries lesions being analysed in both sagittal and horizontal directions; this would enable a 3D map showing caries progression over time to be created.

Conclusion

Within the limitations of this study, we concluded that the microCT method makes it possible to evaluate the MD of the same sample repeatedly and in a way that is comparable to the traditional method. DD increased with exposure time and MD changed over time after exposure to a demineralisation agent.

Conflicts of interest

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

Author contribution

Dr Ke Chung WU contributed to the conception and design of the study and drafted the manuscript; Dr Jung Wei CHEN contributed to the acquisition, analysis and interpretation of data; Dr So Ran KWON contributed to the conception and design of the study; Drs Jung Wei CHEN and So Ran KWON critically revised the manuscript for important intellectual content. All the authors gave their final approval of the manuscript for publication.

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Relationship between Chewing Sugar-free Gum and Dental Caries Status in China

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Objective: *To investigate the relationship between chewing sugar-free gum (SFG) and dental caries status in China.*

Methods: *A total of 860 teenagers (aged 12 to 15 years) and 490 adults (aged ≥ 18 years) were recruited using a multistage stratified cluster method from economically developed areas (Beijing, Guangdong) and less economically developed areas (Hubei, Xinjiang). Each participant completed a questionnaire including oral health-related knowledge of SFG and chewing habits of SFG and agreed to undertake a clinical assessment. Potential factors associated with chewing conditions were analysed through a chi-square statistical test. A negative binominal regression analysis was performed to quantify the relationship between dental caries and consumption of SFG.*

Results: *The overall percentage of the survey population who consumed SFG was 43.4%, and SFG-related knowledge and awareness was only 19.4%. For decayed, missing and filled permanent teeth (DMFT), the mean value was 1.63 ± 2.41 and 2.29 ± 3.65 in the chewing group and non-chewing group, respectively. According to the negative binominal regression analysis, the caries status in the SFG chewing group was better than in the non-chewing group (adjusted prevalence rate ratio [PRR] 0.73; 95% confidence interval [CI] 0.62–0.87).*

Conclusion: *The chewing condition and oral health-related knowledge and awareness of SFG is low. Chewing SFG is related to a better dental caries status, so regular consumption of SFG should be recommended when promoting oral health.*

Key words: *caries prevention, China, sugar-free gum*

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Dental caries is a common chronic bacterial infectious disease that has a severe effect on both oral and general health in humans[1,2]. According to reports from the World Health Organisation (WHO), oral disease has become one of the important contributing factors compromising quality of life[3]. The data on the global burden of disease from the Lancet showed that the prevalence of permanent dental caries ranked first among 328 main diseases worldwide[2]. The 4th National Oral Health Survey (NOHS) in China showed that the prevalence of dental caries was 41.9% and the mean value for decayed, missing and filled permanent teeth (DMFT) was 1.04 in the permanent dentition among individuals aged 12 to 15 years, and in individuals aged 35 to 44 years, the prevalence was 89.0% and the mean DMFT value was 4.54 in the permanent dentition[4]. There is still a relatively high prevalence of dental caries in teenagers and adults in China; thus, suitable strategies for caries prevention are urgently needed.

A number of studies have observed that chewing sugar-free gum (SFG) has an inhibitory effect on dental caries by stimulating the secretion of saliva, mechanically removing plaque and acting as an agent for antibac-

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terial ingredients[5-7]. There is evidence of a causal relationship between chewing SFG and caries reduction[8,9]. Reinhard et al[10] conducted a survey on chewing SFG use and the annual SFG consumption in China ranked the fourth lowest in 25 industrialised countries, far behind that in Switzerland, Sweden, the United States and other Western countries; however, there is limited information about SFG chewing habits in China. As such, the aim of the present study was to investigate the chewing condition of SFG in China and explore the relationship between chewing SFG and dental caries through a cross-sectional survey.

Materials and methods

The study was revised and approved by the Peking University School of Stomatology Institutional Review Board (no. PKUSSIRB-201942018).

Study design and sample selection

Eastern provinces (Beijing, Guangdong) of economically developed areas and the central (Hubei) and western province (Xinjiang) of less economically developed areas were included. A probability proportional to size (PPS) design was used to select one urban area and one rural area from each province at random after division of the urban and rural areas in each province. The PPS method was used to select one middle school and one neighbourhood community from an urban district or a village community in a rural district. Individuals aged 12 to 15 years were invited to participate in the survey and adults (aged ≥ 18 years) were recruited consecutively from neighbourhood or village communities using cluster sampling. The inclusion criteria were as follows:

- aged 12 to 15 years or 18 years and over;
- with at least one fully erupted permanent molar;
- in good general health.

Individuals with serious systemic diseases, enamel hypoplasia, fluorosis and tetracycline teeth, occlusal dysfunction, such as bruxism at night, tooth clenching, a history of related allergies, incomplete fractured teeth or dens evaginatus were excluded. The sample size was calculated using the formula ($n = (\mu^2(\alpha/2)\pi(1 - \pi)/\sigma^2)$ based on the prevalence of dental caries reported by the results of the 4th NOHS in China[4]. Allowable error was controlled at the level of 0.1. Considering the anticipated response rate of 90%, a total of 860 young people (aged 12 to 15 years) took part in the survey with their and their legal guardians' consent, and 490 adults (aged ≥ 18 years) signed the consent form prior to participating.

Data collection

According to the criteria of the 4th NOHS[11], all the subjects received an oral health examination through visual examination combined with probing under artificial light using plane mouth mirrors and a Community Periodontal Index (CPI) probe. Caries status was recorded in accordance with the WHO criteria[12]. In each province, three trained licensed dental practitioners who had been calibrated by the training of the 4th NOHS under WHO guidelines performed the examination. The kappa values were 0.80~0.96.

A structured questionnaire covering areas including socioeconomic and demographic information, knowledge of SFG, SFG chewing habits, knowledge about and attitude towards oral health, and oral health promoting behaviours were recorded. For the 12- to 15-year-olds, schoolteachers and interviewers co-organised and illustrated the content of the questionnaire, then the participants answered all the questions by themselves in the classroom, and for the adults, the structured questionnaires were recorded by trained interviewers, using a face-to-face interview method.

Data analysis

The caries status related to demographic characteristics and SFG chewing habits were displayed by descriptive analysis. Factors that may be related to the chewing condition were analysed using a chi-square statistical test. Furthermore, the mean DMFT value was compared across all categories of involved factors using non-parameter tests (Mann-Whiney test for two-categorised variables and Kruskal-Wallis test for factors with three or more categories) as the DMFT value was not normally distributed. Detailed information regarding the grading standard of each variable is presented in a supplemental table (provided on request).

For multivariable analysis of the DMFT value, due to the overdispersion (variance exceeds the mean) and the result of the Vuong test[13], a negative binominal regression model was preferred to estimate the prevalence rate ratio (PRR). Two models were constructed to measure the crude and adjusted effects of SFG consumption on DMFT values. In model 1, SFG consumption was introduced as the only independent variable, then factors related to the mean DMFT with statistical significance at the 0.05 level were added in model 2.

All statistical analyses were performed using SPSS Statistics v.25.0 (IBM, Armonk, NY, USA) and STATA SE 15.0 (Stata, College Station, TX, USA). The *P* values reported were two-tailed, and statistical significance was set at 0.05.

Table 1 Dental caries status related to subjects' demographic characteristics by age group.

Variable		Teenager		Adult		Overall	
		Number of subjects (%)	Mean DMFT value	Number of subjects (%)	Mean DMFT value	Number of subjects (%)	Mean DMFT value
Total		860 (100)	1.14	490 (100)	3.50	1350 (100)	2.00
Sex	Male	417 (48.6)	0.93	196 (40)	3.04	613 (45.5)	1.60
	Female	441 (51.4)	1.35	294 (60)	3.81	735 (54.5)	2.33
Region	Less developed area	426 (49.5)	1.04	246 (50.2)	3.99	672 (49.8)	2.12
	Developed area	434 (50.5)	1.24	244 (49.8)	3.01	678 (50.2)	1.88
Nationality	Han	711 (83.1)	1.09	412 (84.3)	3.10	1123 (83.5)	1.83
	Minorities	145 (16.9)	1.43	77 (15.7)	5.66	222 (16.5)	2.90
Residential area	Urban	536 (64.3)	1.11	326 (67.5)	3.85	862 (65.5)	2.15
	Rural	298 (35.7)	1.20	157 (32.5)	2.69	455 (34.5)	1.72

Note that some values in the cells do not add up to the total number of participants due to missing values including sex, nationality and residential area, but the percentages for each variable were calculated using the actual number without missing values.

Table 2 SFG chewing habits and knowledge of SFG in different age groups in China.

Variable		Teenagers, n (%)	Adults, n (%)	Overall, n (%)
Chewing frequency	Hardly/never	433 (50.8)	325 (66.9)	758 (56.6)
	1–3 times a month	197 (23.1)	83 (17.1)	280 (20.9)
	Once a week	73 (8.6)	31 (6.4)	104 (7.8)
	2–6 times a week	74 (8.7)	23 (4.7)	97 (7.2)
	Once a day	41 (4.8)	18 (3.7)	59 (4.4)
	At least twice a day	35 (4.1)	6 (1.2)	41 (3.1)
Knowledge of SFG	Not acquired	693 (81.1)	384 (79.7)	1077 (80.6)
	Acquired	162 (18.9)	98 (20.3)	260 (19.4)

Results

A total of 860 12- to 15-year-olds and 490 adults were included in the study. An overview of dental caries status related to demographic characteristics is provided in Table 1.

Table 2 illustrates that a total of 43.4% of subjects reported that they chewed SFG at varying frequencies and the SFG-related knowledge and awareness was only 19.4%. Only 7.5% chewed SFG on a daily basis. The mean DMFT values were 1.63 ± 2.41 and 2.29 ± 3.65 in the chewing group and non-chewing groups, respectively (Table 3).

Table 4 shows possible influencing factors for the SFG chewing habits in the different age groups. The chewing condition was apparently associated with sex, region, nationality, oral health-related knowledge of SFG use of dental floss, sugar consumption habits and intention of the most recent dental visit. The proportion of individuals who chewed SFG was higher in teenagers whose parents had a high level of education. In adults, those with a high level of education were more likely to chew SFG.

Table 5 explores the potential correlative factors for the mean DMFT value in different age groups. The results exhibited that five variables were significantly associated with the DMFT value: sex, nationality, consumption of SFG, history of dental visits in the past 12 months and the intention of the most recent visit. Adults from economically developed areas reported a lower DMFT value.

Table 6 presents a multivariate negative binomial regression analysis of the mean DMFT value. In model 1, the mean DMFT value in the chewing group was obviously lower than that of the non-chewing group, with a crude PRR of 0.71 (95% confidence interval [CI] 0.61–0.84). After factors associated with the mean DMFT value were introduced into model 2, the PRR was adjusted to 0.73 (95% CI 0.62–0.87). People who chewed SFG (PRR 0.73, 95% CI 0.62–0.87), were male (PRR 0.71, 95% CI 0.60–0.85), of Han nationality (PRR 0.65, 95% CI 0.51–0.83) and not having had a dental visit in the past 12 month (PRR 0.82, 95% CI 0.70–0.98) were less likely to have caries.

Table 3 Oral health status of subjects by SFG chewing habits.

Variable	Chewing group			Non-chewing group		
	Teenagers	Adults	Overall	Teenagers	Adults	Overall
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DT	0.98 ± 1.46	1.29 ± 2.07	1.07 ± 1.65	0.82 ± 1.55	1.74 ± 2.28	1.22 ± 1.95
MT	0.00 ± 0.05	0.30 ± 1.06	0.09 ± 0.57	0.01 ± 0.16	0.76 ± 2.67	0.33 ± 1.79
FT	0.21 ± 0.79	1.17 ± 2.24	0.48 ± 1.42	0.27 ± 0.90	1.37 ± 2.81	0.74 ± 2.04
DMFT value	1.20 ± 1.72	2.77 ± 3.38	1.63 ± 2.41	1.10 ± 1.79	3.87 ± 4.74	2.29 ± 3.65

DT, decayed teeth; FT, filled teeth; MT, missing teeth.

Table 4 Possible influencing factors for the SFG chewing condition in different age groups.

Variable		*P* value		
		Teenagers	Adults	Overall
Social demographics	Sex (male/female)	0.295	0.004	0.003
	Region (less developed area/developed area)	0.037	0.058	0.006
	Nationality (Han/others)	0.015	0.520	0.015
	Parental education level (low/medium/high)	0.003	NA	NA
	Educational level (low/medium/high)	NA	0.017	NA
Knowledge of, attitude towards and practice of oral health	Attitude (negative/positive)	0.895	0.432	0.613
	Knowledge (low/high)	0.058	0.111	0.076
	Knowledge of SFG (yes/no)	0.009	0.038	0.002
	Frequency of tooth brushing (low/high)	0.156	0.145	0.177
	Use of dental floss (yes/no)	< 0.001	< 0.001	< 0.001
	Use of fluoride toothpaste (yes/no/don't know)	0.046	0.168	0.284
Sugar consumption habits[a]		< 0.001	< 0.001	< 0.001
Use of dental services	Dental visit in the past 12 months (yes/no)	0.139	0.742	0.736
	Intention[b]	0.007	0.004	< 0.001

NA, not applicable. [a]Sugar consumption habits were classified based on frequency as low frequency, moderate frequency, relatively high frequency and high frequency. [b]Intention of use of dental services was grouped into four main categories: don't know, treatment, consultation and prevention.

Table 5 Analysis of mean DMFT values according to exposure variables for different age groups.

Variables		*P* value		
		Teenagers	Adults	Overall
Social demographics	Sex (male/female)	0.004[a]	0.004[a]	< 0.001[a]
	Region (less developed area/developed area)	0.203[a]	0.042[a]	0.718[a]
	Nationality (Han/others)	0.041[a]	0.004[a]	0.009[a]
	Parental education level (low/medium/high)	0.379[b]	NA	NA
	Educational level (low/medium/high)	NA	0.057[b]	NA
Knowledge of, attitude towards and practice of oral health	Attitude (negative/positive)	0.127[a]	0.784[a]	0.313[a]
	Knowledge (low/high)	0.246[a]	0.306[a]	0.091[a]
	Knowledge of SFG (yes/no)	0.342[a]	0.142[a]	0.262[a]
	Frequency of tooth brushing (low/high)	0.291[a]	< 0.001[a]	0.974[a]
	Use of SFG (yes/hardly/never)	0.234[a]	0.006[a]	0.007[a]
	Use of dental floss (yes/no)	0.543[a]	0.645[a]	0.298[a]
Use of dental services	Dental visit in the past 12 months (yes/no)	0.008[a]	0.091[a]	0.001[a]
	Intention[c]	0.400[b]	< 0.001[b]	< 0.001[b]

NA, not applicable. [a]Mann-Whitney test for two-categorised variables. [b]A Kruskal-Wallis H test was used for analysis of these factors with three or more categories. [c] Intention of use of dental services was grouped into four main categories: don't know, treatment, consultation and prevention.

Table 6 Multivariate negative binominal regression analysis of the mean DMFT value and SFG chewing habits.

Variable		Model 1[a]	Model 2[b]
		PRR (95% CI)	PRR (95% CI)
Use of SFG	No	1.00 (reference)	1.00 (reference)
	Yes	0.71 (0.61–0.84)***	0.73 (0.62–0.87)***
Sex	Female	NA	1.00 (reference)
	Male	NA	0.71 (0.60–0.85)***
Nationality	Other	NA	1.00 (reference)
	Han	NA	0.65 (0.51–0.83)***
Dental visit in the past 12 months	Yes	NA	1.00 (reference)
	No	NA	0.82 (0.70–0.98)*

[a]Use of SFG was included as the only independent variable. [b]Factors associated with the mean DMFT value were added to model 1.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

NA, not applicable.

Discussion

The present study identified the level of use of SFG in the survey population. A total of 43.4% of subjects reported that they chewed SFG gum with varying frequencies and SFG-related oral health knowledge and awareness was only 19.4%. The proportion of people in the surveyed population who chew SFG is apparently lower than in some western countries[14-16]. The survey population also had less awareness about the role played by SFG in preventing dental caries compared to that of fluoride (55.7%) and pit and fissure sealant (45.9%). Chewing SFG was also found to be significantly associated with dental caries. According to the 4th NOHS in China, although oral health behaviours have improved in the past 10 years, most citizens still do not brush their teeth twice daily and barely use dental floss, and there were still a large number of untreated caries[4]. Due to the insufficient resources for oral health, inequalities in their distribution and the high economic burden of oral disease[17,18], it is essential to seek oral health behaviours that are easily accessible to individuals, besides the promotion of frequent tooth brushing and flossing.

SFG is an easily accessible and acceptable commodity with a wide variety of flavours for consumers to choose from. Moreover, people can chew SFG almost anytime and anywhere. Not only can chewing SFG relieve stress[19-23] and improve halitosis[24], but the oral care benefits of chewing SFG have also been recognised and supported by a number of regulatory bodies such as the European Association[25], the European Food Safety Authority[26], the FDI[27] and other national dental associations around the world. In recent years, studies on the health economics of SFG have indicated that chewing SFG is a cost-effective method of caries prevention[10,15,28]. The present study focuses mainly on SFG chewing habits and its association with dental caries in China.

In the present study, only 43.4% of the participants chewed SFG, and its preventive effect on caries was not well understood by the public. Sex, region, nationality, parents' level of education, knowledge of SFG, use of dental floss, sugar consumption habits and the intention of dental visits were shown to be related to SFG chewing habits. Among them, the SFG chewing rate was higher in men, people from economically underdeveloped areas and ethnic minorities, which may be due to the greater work- and lifestyle-related pressure experienced by these populations, as studies have found that chewing gum can relieve stress and improve concentration[19-23]. Information on this as a healthy habit may also encourage people to make a change[29], and this may be why people with greater oral health-related knowledge were more likely to chew SFG. Those who used dental floss had a higher chewing rate, perhaps because participants with better oral health promoting behaviours tend to chew SFG. Furthermore, those who consumed sugary foods frequently were more likely to chew SFG, suggesting that the sweetness of SFG may be one of the essential factors that attracts people. The chewing proportion varied widely in adolescents whose parents had different levels of education and in adults with different levels of education. Both adolescents whose parents had a higher level of education and adults with a higher level of education had higher chewing rates. It may be that people with a higher level of education have more access to correct information about oral health, which improves their attitude towards oral health care. A study found that children's oral health behaviours can be influenced by those of their parents[30]; thus, for schoolchildren and teenagers, oral health education should be added into the school curriculum, and also more effort

should be made to strengthen caregivers' oral health knowledge, such as through oral health classes for parents. Those who had a dental visit for prevention or consultation may pay more attention to oral health care and were found to be more likely to chew SFG.

Some studies have shown that those who chew SFG regularly had a lower severity of dental caries[5,6], but researchers had only studied the effect on children (aged 6 to 9 years) in China[31,32]. There is a lack of studies on teenagers and adults, who are the main consumers of SFG. Mean DMFT values reflect lifetime experience of dental caries and the severity of caries in the population examined. After adjusting for factors related to the mean DMFT value, the present study has provided convincing evidence that chewing SFG is a related factor for dental caries.

Some studies have shown that dental visits are one of the important determinants of dental caries experience[33,34]. In the present study, use of dental services was not found to have an influence on SFG consumption but was a crucial factor associated with dental caries experience. In agreement with previous studies[33,34], those who had a history of dental visits had a higher DMFT value, indicating that treatment-related care is still more common in China than prevention-orientated care. Most individuals only seek help from dentists when suffering from a toothache or other symptoms. On the one hand, it may be that the public do not realise the importance of caries prevention, especially through professional dental care, which leads indirectly to a substantial increase in the cost of caries treatment, creating a huge economic burden[18,35]. According to the WHO, dental caries has become the fourth most expensive chronic disease to treat in the world[1]. On the other hand, the limited oral health resources and services in China prevent residents from accessing professional dental care. However, dental visits cannot be considered a risk factor because the act of attending a dental visit is the result of caries experience[36].

The present study has some limitations. First, the prevalence and mean DMFT value for permanent dental caries in teenagers were higher than that reported in the 4th NOHS in China, whereas the same indicator in adults was lower[4]. This may be because compared with the 4th NOHS, fewer areas were included in the present survey and the sample size was smaller, and urban and rural areas were distributed unevenly. Second, the present study was cross-sectional, so causality between caries and related factors including chewing SFG could not be obtained directly. Randomised controlled trials about the effect of the mastication time and volume of SFG chewed on dental caries could be designed in the future.

Conclusion

Based on the current status of dental caries in China, relevant departments could consider chewing SFG as a possible supplement to the existing caries prevention strategies. However, it is important to emphasise that chewing SFG is no substitute for traditional oral health practices, such as tooth brushing and flossing. Under the ambitious goal of "Healthy China 2030", the Oral Health Plan (2019-2025) should be implemented to protect the public's oral health.

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Conflicts of interest

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

Author contribution

Drs Chun Zi ZHANG, Shuo DU, Wen Hui WANG, Jian LIU, Chao YUAN and Yi Zhen YU analysed the data; Drs Chun Zi ZHANG and Shuo DU drafted the paper; Drs Yan SI and Shan Shan ZHANG conceived the programme of research and critically revised the manuscript. All the authors read and approved the final version for submission.

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A Scoping Review about Migrants' Oral Health in South-South Contexts

Andrés M MURILLO-PEDROZO[1], Elena RONDA-PEREZ[2], Eliana MARTINEZ-HERRERA[3-5], Andrés A AGUDELO-SUAREZ[1,2]

Objective: *To gather the available scientific evidence about the oral health of migrants in south-south contexts.*
Methods: *A scoping review methodology was applied through a comprehensive search in databases of scientific and grey literature: PubMed/Medline, Scopus, LILACS, EMBASE, Google Scholar and the International Centre for Migration, Health and Development. A descriptive analysis of the characteristics of the selected studies was conducted.*
Results: *The search yielded 23 papers. Seventeen studies (17/23, 73.9%) were conducted on the Asian continent and 91.3% (21/23) were cross-sectional. Studies were focused on oral health problems such as dental caries and periodontal disease with diverse findings when comparing immigrants with natives. Some studies found poor oral health indexes in migrants. Migrants face barriers to dental health services. Other oral health variables addressed in the studies were oral health–related quality of life, beliefs, knowledge and practices in oral health. Determining factors related to oral health were evidenced, such as migration status, sociodemographic, cultural, psychological, living, economic and material conditions, social support, oral health practices and previous oral and general health status. Studies reported conceptual and methodological gaps and limitations that must be considered when interpreting the results.*
Conclusion: *According to the scientific evidence, immigrant populations in south-south migratory contexts show poor oral health indicators, and this translates into social vulnerability in this group. Further research is needed to increase the scientific body about the social and contextual determinants in oral health and understanding of the social construction of this phenomenon.*
Key words: *dental health services, emigrants, immigrants, oral health*
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Oral health is acknowledged as an important public health topic that, according to the World Dental Federation (FDI), "is multi-faceted and includes the ability to speak, smile, smell, taste, touch, chew, swallow, and convey a range of emotions through facial expressions with confidence and without pain, discomfort, and disease of the craniofacial complex"[1]. This concept, although closely associated with the absence of disease, should be interpreted within the framework of the broader concept of good general health[2]. Considering physical, mental and social well-being as a unit provides a holistic view of health, offering the opportunity to study health from

multiple perspectives, recognising that it is conditioned not only by issues, such as access to health services, but also by a variety of circumstances in which people are born, grow, live, work and age, known as the social determinants of health[3].

Migration is a phenomenon that impacts social, economic, demographic and health aspects in the origin and host countries. Although migration as a social phenomenon has numerous causes, most people migrate as an economic and labour project (these are "migrant workers") to support themselves and their families, although this can be intertwined with other political, academic or health reasons[4]. For the migrant population, social determinants influence the well-being of individuals in different ways and at different points in their migration process: the phase prior to migration, transfer, arrival, integration and, for some, their return[4].

When studying migration and health, it is important to understand that migrants are not a homogeneous group. As such, their needs or social determinants vary, generating different axes of inequality among migrants that have repercussions on their health status and quality of life[5-7]. When migration occurs in unfavourable circumstances from the pre-migration moment, through the migration process and according to the conditions in the destination country, migrants are under a potential axis of health inequity[5].

For some, migration can increase exposure to health risks, as in the case of migrant workers employed in precarious conditions and with little access to health care, but for others it may improve their health, for example when people flee a situation of persecution and fear of violence for a safe environment[4]; some who migrate may even find a better state of health than those who remain in the place of origin, a phenomenon known as the healthy migrant effect[8].

In the case of oral health, some studies have reported unfavourable oral health indicators due to carrying a heavier burden of disease from pre-migration processes, social factors and barriers to access to dental services[9,10], pointing to different axes of significant inequity in oral health[11]. Similarly, other authors have described how the state of oral health in immigrants deteriorates over time, reflecting that the healthy migrant effect is temporary[6].

It is currently estimated that there are around 272 million international migrants worldwide, almost two-thirds of whom are labour migrants[4]. A person who resides in a country other than their country of origin is recognised as a migrant; however, some who have never migrated are also considered migrants, such as children born abroad to migrant parents, usually known as second-generation migrants[4,12].

Although the United States of America continues to be the most frequent destination country, the number of migrant workers has been decreasing slightly in high-income countries and increasing in others. In 2017, 68% of migrant workers settled in high-income countries, 29% in middle-income countries and 3.4% in low-income countries. The change between 2013 and 2017 is notable: high-income countries experienced a decrease of 7 percentage points from 75% to 68%, while upper-middle–income countries registered an increase of 7 percentage points from 12% to 19% in the same period[4].

This clear shift in destination countries for some migrant workers may be due in part to economic growth in upper-middle–income countries, to changes in labour immigration regulations in high-income countries, or both factors[4]. As the number of migrants increases globally, so too does the number of research publications analysing migratory processes. However, the majority of research is conducted in developed countries from the perspective of their being a destination country; that is, the classic migration process in the south-north direction is most often analysed, i.e., migrants from developing countries moving to developed countries[4,5].

Understanding that migration is not uniform throughout the world but responds to economic, geographic, demographic and other factors that determine migration patterns, migration 'corridors', intraregional migration or south-south migration[4], and that research on oral health in these contexts is scarce[5], the aim of the present scoping review was to gather the available scientific evidence about the oral health of migrants in south-south contexts.

Materials and methods

A scoping review was conducted following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) model for scoping reviews (http://www.prisma-statement.org/Extensions/ScopingReviews). We also consulted and adopted the methodology proposed by the Joanna Briggs Institute[13] and the Arksey and O'Malley methodological framework for scoping studies[14]. According to the inclusion criteria proposed for systematic reviews, the protocol for scoping reviews should not be registered in the International Prospective Register of Systematic Reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/#aboutregpage).

Table 1 Search strategy.

Database	Search strategy
Scopus	((TITLE-ABS-KEY (immigration) OR TITLE-ABS-KEY (immigrants))) AND ((TITLE-ABS-KEY ("oral health") OR TITLE-ABS-KEY ("dental caries") OR TITLE-ABS-KEY ("periodontal diseases") OR TITLE-ABS-KEY ("dental care") OR TITLE-ABS-KEY ("dental health services")))
PubMed	Search (((((("Emigration and Immigration"[Mesh])) OR "Human Migration"[Mesh]) OR "Undocumented Immigrants"[Mesh]) OR ("Emigrants and Immigrants"[Mesh])) OR ("Transients and Migrants"[Mesh]))) AND ((((((("Dental Health Services"[Mesh]) OR "Dental Care"[Mesh]) OR "Periodontal Diseases"[Mesh]) OR "Dental Caries"[Mesh]) OR "Dental Health Surveys"[Mesh]) OR "Oral Health"[Mesh])
Embase	((immigration:ab,ti OR immigrants:ab,ti) AND ('oral health':ti,ab,kw OR 'dental caries':ti,ab,kw OR 'periodontal disease':ti,ab,kw OR 'dental care':ti,ab,kw OR 'dental health service':ti,ab,kw))
LILACS	tw:((inmigración OR inmigrantes) AND (salud oral OR caries dental OR enfermedad periodontal OR servicios de salud oral)) AND (db:("LILACS" OR "BBO" OR "BINACIS")) AND (year_cluster:[2010 TO 2020])

Research question

In accordance with the research purposes, the population, concept and context (PCC) question was as follows: What scientific evidence is available about the oral health situation in migrant populations in south-south contexts and their social determinants?

- population: migrant groups in south-south contexts;
- concept: oral health and its social determinants;
- context: south-south movement.

In this study, south-south migration studies were defined as those including migrants proceeding from Asia (excluding Japan), Africa and Latin America (with these same destination regions), and excluding destination countries in North America, Europe, Australia and Oceania.

Search process for identifying relevant studies

A comprehensive search was conducted of peer-reviewed and grey literature to locate publications relevant to the research topic. Four electronic databases were included: PubMed/Medline, Scopus, LILACS (Latin-American and Caribbean Health Sciences Literature) and Embase (the Excerpta Medica Database). The search was complemented by adding Google Scholar and the International Centre for Migration Health and Development (https://icmhd.ch/). The detailed search strategy used in the databases employing medical subject headings (MeSH) terms and keywords is shown in Table 1.

The search was focused on original research studies published in Spanish, English and Portuguese, and no time range was applied. Theses and other academic degree works relating to the aim of this review were also included. Letters to the editor, editorials, systematic and theoretical reviews, summaries of conferences, historical papers and book summaries were excluded.

Study screening and selection

Two reviewers (AMMP and AAAS) searched independently to identify titles and abstracts of potentially eligible studies. Articles with information in the abstract that fit the eligibility criteria were included, and the papers were selected for a full reading. The reviewers also checked the reference lists of the selected papers for studies not identified in the initial searches, and all papers selected for inclusion in the review were processed for data extraction. No software was used for screening and selection of the studies. To guarantee the quality process, the authors conducted a pilot test with five articles and calculated a simple concordance index, with a score of 85%.

Collating, summarising and reporting findings

The following variables were described for each study: author, country, year of publication, conceptual framework used in the justification of the study, type of study, data collection methodology, type and size of sample, country of origin, migration status characteristics, central topics (health services accessibility, dental caries, periodontal disease, other oral pathologies, risk factors), main findings, limitations/gaps, conclusions, recommendations and general comments.

Results

The initial search resulted in 462 records (458 obtained by database searching and 4 by additional searches). After removal of duplicates, 364 records were selected for title and abstract review, 329 of which were excluded, and 35 articles remained for full text reading. Ultimately, 23 publications were included[15-37]. The reasons for exclusion are shown in Fig 1.

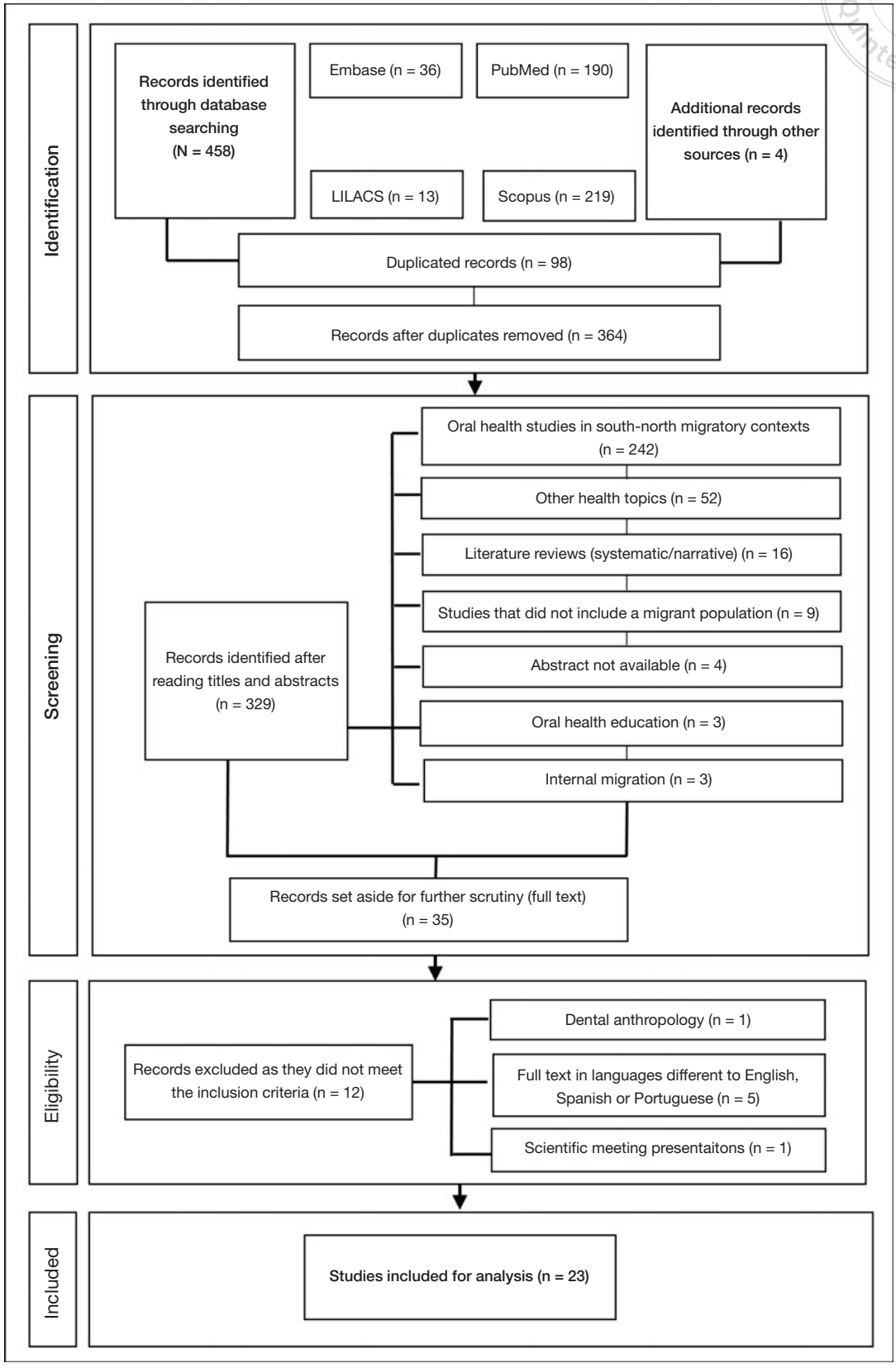

Fig 1 Study selection process.

General characteristics of the studies

Regarding the countries where the studies were conducted, the majority were carried out in Asia (n = 17; 73.9%)[15-31], and the remaining studies (n = 6; 26.1%) in Latin America[32-37]. It is important to highlight that there were no studies conducted in African countries. The studies were conducted with samples of Asian and Latin-American origin. When the theoretical framework to justify the study is considered in the background, the approach employed in 52.2% (n = 12)[15-18,20,23-25,28,31,32,36] used the Inequalities in Oral Health (IOH) model, 30.4% (n = 7)[15,18,24,29,34,35,37] employed the Social Determinants of Health model[3,38], and 4.3% (n = 1) mentioned a model related to accessibility to health services[21], specifically the Aday and Andersen model[39]. One study[30] (4.3%) mentioned a conceptual framework related to the relationship between acculturation and oral health[40]. Five studies (21.7%) did not specify a clear conceptual framework in the study background[19,22,26,27,33]. Regarding the methodological designs of the studies, 91.3% (n = 21) were cross-sectional[16-21,23-37], and the remaining two (8.7%) used a cohort design[15] and an intervention study[22]. The data collection methods were diverse; six studies (26.1%) used surveys or questionnaires[16,19,21-23,25], seven (30.4%) carried out mainly clinical examinations[17,27,29-31,35,37] and ten (43.5%) combined both methodologies[15,18,20,24,26,28,32-34,36]. Finally, the studies considered different age groups: 43.5% (n = 10) were carried out on minors[16,17,20,24,28,31,33-36], 47.8% (n = 11) were conducted on adults[15,18,19,21-23,25-27,29,30], and 8.7% (n = 2) included both age groups[32,37].

Oral health status: dental caries and periodontal disease

Regarding oral health clinical indicators, 69.6% (n = 16) of the studies performed a clinical examination for caries and/or periodontal disease[15,18,20,24,26-37] and one (4.3%) evaluated the presence of enamel defects in the canines[17]. Five studies (21.7%) reported higher rates of caries in migrants in comparison with natives[20,24,28,31,33]. One study (4.3%) reported an analogous decayed, missing and filled permanent teeth (DMFT) index but with a higher number of decayed teeth in migrants than those that had been filled in natives[32]. Two studies (8.7%), both carried out on schoolchildren in Chile, calculated the DMFT indices of primary teeth and permanent teeth and found higher indices and a higher prevalence of dental caries in the native population[34,35], with some slight differences according to the indicator used and other sociodemographic factors. In the case of migrant adults, the frequency of untreated dental caries was 54.8% in one study conducted by Lee et al[27] in 2019, and 90.3% in another study conducted on foreign domestic workers by Gao et al[18] in 2013. A study on caries and psychological distress in Ethiopian immigrants in Israel found that in subjects with psychological distress, dental caries exceeded the values for subjects not in distress by nine times[15]. Finally, a study conducted in India found a significant association between the prevalence of dental caries and dietary habits in migrants[30].

Nine studies (39.1%) evaluated periodontal health[15,18,24,27,29,32,34,35,37], four of which (17.4%) compared periodontal indices of migrants with their counterparts using various measurements[24,32,34,35]. Three studies (13%) used the Oral Hygiene Index (OHI); one reported worse indexes in migrants[32] and the other two reported worse indicators in natives[34,35]. One study (4.3%) reported a higher frequency of "gingival bleeding" in natives and "healthier gums" in immigrants[24]. One study (4.3%) examined gingival inflammation (GI) in both primary and permanent teeth of patients, and in both cases, inflammation was higher in migrants[35]. These studies were conducted on schoolchildren. The other five studies (21.7%) reported gingival health only in migrants[15,18,27,29,37]. The presence of dental calculus was reported in three studies (13%), with frequencies of 29.7%[27], 53.0%[18] and 67.3%[37]. Likewise, one of them (4.3%) reported the presence of superficial and deep periodontal pockets in 39% and 7%, respectively, of the migrants studied[18]. Lastly, a study relating gum disease to psychological distress found that in subjects with psychological distress, periodontal disease increased by 40%[15].

Other oral health aspects evaluated in the studies

Eight studies (34.8%) included in this scoping review explored other conditions related to oral health[16,17,19,21-23,25,32]. One study conducted in Israel by Davidovich et al[16] compared oral hygiene habits in preschool children from an Ethiopian background with natives in low socioeconomic neighbourhoods. A difference was reported, despite the fact that the parents of Ethiopian descent had lived in Israel for at least 20 years[16]. Another study conducted in Israel by the same authors on the same sample of immigrants focused on the concept of infant oral mutilation (IOM), mainly the removal of incipient canine teeth in babies[17]. This practice was more frequent in immigrants than in native Israelis[17]. A study conducted in Taiwan showed that the level of caries-related knowledge, attitudes and oral health behaviours was lower in immigrant mothers than in natives[19].

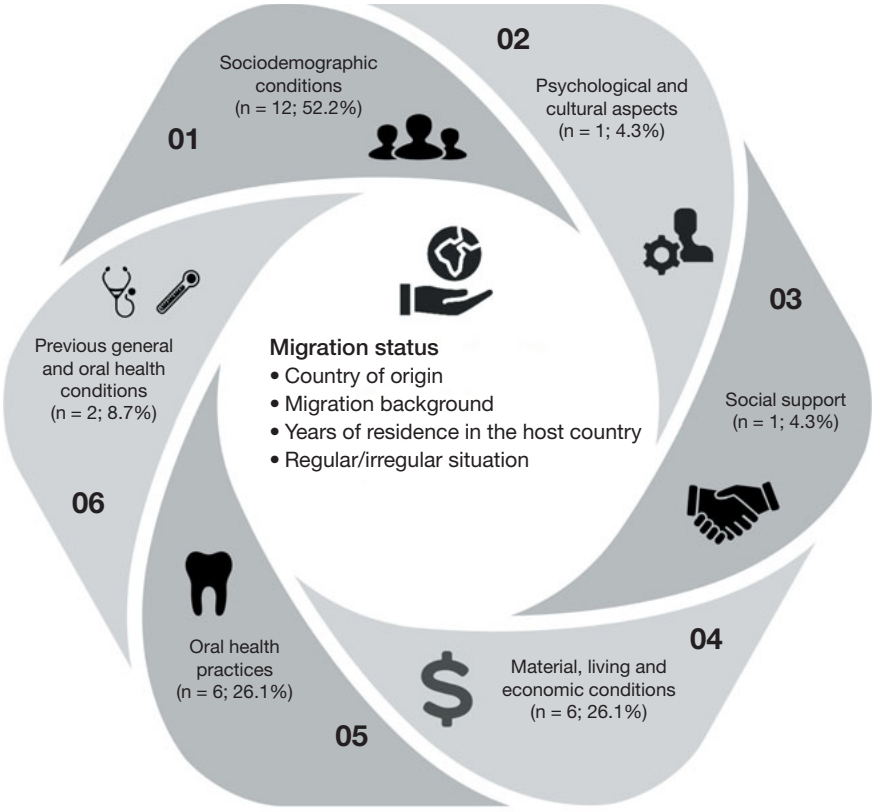

Fig 2 Factors and variables related to oral health considered in the included studies (n = 23). Percentages are not mutually exclusive.

Use of and access to oral health services was explored in a study carried out in adults in Israel and disparities in primary dental care were found based on immigrant and ethnic minority status, a situation that depends on sociodemographic factors[21]. Research conducted in migrant women from multicultural families in Taiwan showed that they were aware of the importance of oral health, but their opportunity to receive education through oral-health–related programs was limited[23]. As such, these migrant women experience some barriers to access to dental care. Another study conducted by Hsu et al[22] demonstrated the effect of a lay health advisor (LHA) and an oral health training curriculum on improvement of oral hygiene behaviours and access to dental care for immigrant children.

Oral health beliefs and behaviours were explored by Sivakumar et al[25] in Tibetan immigrants, and they found that oral health hygiene practices were high in study participants (i.e., tooth brushing). Although the perceived seriousness of oral health was high among the migrant participants, the perceived benefit of dental treatment was relatively low[25]. Cultural aspects related to migratory processes are involved and they need to be examined more deeply in future studies. Finally, oral health–related quality of life (OHRQoL) was analysed in a study conducted in Chile but no statistically significant differences were found between groups (Chilean natives and Peruvian immigrant women)[32].

Variables and determinant factors of oral health status and access to health services

Figure 1 shows the variables considered when analysing the studies included in this scoping review. Migratory status is related in all the studies, complemented by elements such as years of residence in the country of origin, whether migrants' residence and work status had been regularised, and migratory background (parents' country of origin in studies conducted on children). Other factors related to oral health and access to health services considered more frequently in the analysis of findings were sociodemographic aspects[15,18,19,21,26,29-34,37], psychological/cultural aspects[15], social support[15], material, living and economic conditions[18,21,23,26,31,33], oral health practices[16,18,20-22,30] and previous general/oral health conditions[21,35].

Limitations and gaps reported in the studies

The studies reported some methodological limitations. These conceptual and methodological gaps should be identified and mitigated in further research to increase the reliability of findings about the relationship between migration and oral health in south-south contexts.

- Type and size of the sample: This determines the difficulty of generalising the results to the entire immigrant population in the countries where the studies were carried out. Some studies were done with small samples, and in many cases, participants were recruited by convenience method or snowballing[18-22,24,25,28,32,36].
- Use of self-reported questionnaires: In many cases, immigrants and natives may have under- or overestimated their true situation when reporting[19,21,25,28,34,35].
- Methodological design of the study: The cross-sectional nature of studies does not allow for the establishment of causal relationships. Moreover, some studies did not use control groups to make comparisons[20,27,28,36].
- Lack of information on other variables of interest that would complement the objectives of the study: Considering the availability of the data, the studies acknowledged the lack of social and contextual variables that could explain the relationship of migration to indicators of oral health and access to health services[17,26,31,36].
- Selection bias for the proxy variable of migration status: One study defined the migrant status of a child based on the family's household registration information[31], and some families living for several years in the host country may have been assigned to the native group.
- Seven studies did not declare limitations in their methodology[15,16,23,29,30,33,37], which affects the quality of the report.

Discussion

Main findings

This scoping review, focused on migration and oral health in south-south contexts, included 23 articles characterising epidemiologically pathologies such as caries and periodontal disease and evaluating use of and access to dental health services, quality of life, habits, knowledge and practices in oral health and hygiene. In general terms, the results showed the situation of vulnerability in which the immigrant population finds itself, which translates into less favourable oral health indicators when compared with the native population, and such migrant populations face barriers to accessing health services. These involve factors specific to the individual and social factors related to the country of origin and host. To the best of the present authors' knowledge, this is the first compilation of scientific evidence that explores the oral health situation and its determinants in such migratory contexts.

Scientific evidence of factors determining oral health in migrants

Diverse results were found in the present review of oral health indicators. Some differences were found in the results of clinical examinations, mainly regarding dental caries[41]. When comparing the frequency of dental caries in natives and immigrants, the findings are consistent with those reported in the literature indicating that the latter have higher rates of caries than their native counterparts, and natives have a greater number of filled teeth[28]. Interestingly, two studies conducted in Chile found that in the school population, Chileans had a higher incidence of caries than immigrants[34,35]. In the case of periodontal disease, the results were also dissimilar, but some studies found worse periodontal health in migrants than in the native population[32,35]. Although the literature comments extensively on the healthy migrant effect, in time, migrants acquire the same morbidity profile as the local population, and in some cases, there is evidence of greater prevalence of poor oral health and less use of health services.

Migratory status is a social determinant that influences disparities and inequities in the state of oral health[5,41]. Immigrants have a series of unmet basic needs, which leads to them being considered as a particularly vulnerable group[11]. The unsatisfactory state of oral health in this population suggests that social factors influence this condition and that, in addition to the acculturation phenomena[42], they may be affected more frequently in comparison to other social groups. These determinants act from the pre-migratory phase; the conditions in the country of origin affect migrants throughout the entire migratory process until their installation in their new country of residence[5]. Occasionally, migrants' state of health may not have been negatively affected when their migratory process was favourable and they may even present better health indices than the native population, but after a certain period of residence in the new country, there is a trend towards deterioration of their health status, demonstrating the temporary nature of the healthy migrant effect[6].

The literature also establishes that the migratory process can have an impact on mental health related to challenges migrants face in the adaptation process in the social, labour and educational spheres[43]. Symptoms of psychological distress may impact general and oral health, as reported in a study included in the present review[15]. Another study showed the impact of oral health conditions on individual psychological dimensions[44]. Closely linked to subjective well-being is the variable called social support, since migration involves many changes in daily life for individuals and this can affect oral health indicators, the profile of use of health services, and behaviours and practices[45]. This is borne out by the results found in a systematic review focusing on the issue of immigrants and ethnic minorities; however, this scoping review identified only one study on the impact of social support on oral health evaluated in Ethiopian immigrants residing in Israel[15].

Taking other indicators into account, OHRQoL has predominated in scientific approaches in recent decades, especially in examining the impact of variables that influence the subjective perception of well-being compared to physical, social, psychological and functional dimensions[46]. A systematic review of the subject in question showed that the impact on OHRQoL for immigrants with less time living in the host country or children of a foreign mother was more negative[47]. However, only one study carried out in Chile evaluated this condition and found no statistically significant correlations, although it is worth noting that the research was conducted in a specific population of pregnant women[32]. Similarly, some studies evaluated knowledge and practices in oral health[16,19,25]; the results were strongly associated with the geographic context where they were conducted and according to the particular experiences of each population group and their intervening cultural factors, whereas others related to the availability of health promotion and education programmes[48].

Health services constitute an intermediate determinant that impacts disparities and inequities in health[49]. Only two studies in this scoping review focused on this aspect[21,22]. This is a vital point, as the most vulnerable people who require better health care are those who receive the least. This has been called the inverse care law, and it has been studied in other geographical contexts in relation to access to dental care[50]. However, studies included in this scoping review did not investigate elements relating to availability of policies and programmes to provide oral health care to the migrant population in these contexts.

Scope and limitations of this scoping review

It is striking that almost three-quarters of the scientific investigation on migration and oral health in south-south contexts has been conducted on the Asian continent, with research in Latin America making up the remaining quarter, while no studies conducted in Africa were found. This may be due to two factors: the fact that migration within or between these continents is mainly south-north, and that scientific investigation in this region is still scarce or has not been published in high impact or indexed journals and therefore could not be included in this examination of intraregional migration.

Further, the fact that over 90% of the studies were cross-sectional and with a purely descriptive scope indicates that this issue is still in an exploratory and descriptive phase without progressing to seeking alternative analyses. Despite being in an early stage, only study designs with quantitative approaches are supported by surveys, clinical examinations or both, whereas qualitative studies allowing a deeper interpretation of south-south migration and the phenomena that underlie it are not included. Such research may enhance understanding of the social construction of health-disease phenomena in a holistic and comprehensive way.

In the theoretical models, the determinants and social inequalities in health were the conceptual framework most frequently applied. The descriptive and/or exploratory scope of the chosen methodological designs reviewed afforded a better understanding and discussion of the findings in light of the scientific literature. However, in conducting the analysis and presenting results in accordance with the objective and the research question, some limitations and knowledge gaps are evident. Although the methodological procedures to conduct this scoping review were followed carefully through a comprehensive search in different databases, possible grey literature or unpublished studies in the analysed context should not be overlooked. No quality assessment of the included studies was conducted since the objective of this review was exploratory in nature.

Conclusion

The scientific evidence suggests that the migrant population in south-south migratory contexts is especially vulnerable socially, and this translates into poor oral health indicators; however, definitive research is still in the process of development, and studies in other geographic contexts and concerning the use of other methodologies that allow recognition, analysis and understanding of the social and contextual factors in both origin and host

countries of the immigrant populations are particularly lacking. A larger body of evidence may be useful in implementing public policies and strategies based on social reality to improve access to health services and inclusive strategies in education, health promotion and disease prevention.

Conflicts of interest

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

Author contribution

Drs Andrés M MURILLO-PEDROZO and Andrés A AGUDELO-SUAREZ participated in the conception and project design. All the authors participated in the data analysis, critical review and approval of the final version. All the authors take responsibility for and guarantee all the aspects included in the paper.

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Polyetheretherketone Framework for Implant-supported Full-arch Fixed Dental Prostheses in a Periodontitis Patient with a 6-year Follow-up: a Case Report

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Dental implants are widely used in the rehabilitation of patients with edentulous jaws caused by periodontitis. The success of implants is closely related to their framework material and patients' periodontal health. Polyetheretherketone (PEEK) is a kind of high polymer material that has broad prospects as the framework for full-arch dental prostheses, but long-term follow-up data are lacking. The present clinical report demonstrates the use of a PEEK framework for the construction of an implant-supported full-arch fixed dental prosthesis for a patient diagnosed with periodontitis. With the guidance of biological width, a provisional retained restoration was achieved to create the emergence profile, resulting in a 3D printed PEEK framework with good aesthetics and biological functions.

Key words: *gingival modification, high polymer framework, implant-supported fixed prosthesis, staged extraction*

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Periodontitis is a chronic infectious disease of the oral cavity and is the principal cause of tooth loss in the elderly[1]. Dental implants are an alternative to bridges or removable partial dentures in the rehabilitation of patients with periodontal disease; however, periodontitis is considered high risk for peri-implantitis[2], which may result in treatment failure. It has been well reported that the periodontal status adjacent to implants is an important factor that affects long-term implant outcomes[3]. Accordingly, implant treatment can only be successful in patients with periodontitis after they undergo periodontal therapy and achieve a stable periodontal status. In patients with periodontitis who have undergone periodontal treatment and are in their maintenance phase, the rate of clinical success of immediate implant placement and immediate provisional restoration is as high as 98.3%[4].

Peri-implant gingival morphology is closely related to the aesthetics and biological function of implant restoration. Similar to the biological width (~2 mm) around natural teeth, the width (~3 mm) from the top of the peri-implant mucosa to the first point of bone–implant contact is also referred to as biological width around implants. This width is formed to resist external stimulation and provide a stable soft and hard tissue relationship around implants[5]. Gingival modification to form biological width in implant is therefore essential for implant prostheses, and is still a major challenge. Nowadays, digital technology can effectively analyse the relationship between the soft and hard tissues of the implant, which will be the future trend towards

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gingival shaping in the complete implant-supported fixed denture[6].

Traditionally, implant-supported full-arch fixed dentures are manufactured with metal alloy frameworks; acrylic resin or porcelain teeth are then laid over the casted framework[7]. Such frameworks are mostly fabricated from cobalt-chromium alloy. Although the alloy has sufficient strength to withstand occlusal forces, it is not resistant to corrosion[8]. Cobalt-chromium frameworks have subsequently been replaced by titanium and zirconia frameworks that provide favourable biocompatibility, exceptional wear resistance and excellent corrosion resistance[8-10]. Unfortunately, titanium and zirconia frameworks have shown high stiffness[11,12], which may lead to conduction of occlusal loading. The force around implants is greatly increased when the implant is connected with a rigid framework. The stress concentration is considered a risk factor for prosthesis failure and peri-implant bone loss. Due to the drawbacks of the aforementioned materials, a new generation of material is required for the preparation of implant-supported prosthetic frameworks.

Polyetheretherketone (PEEK) is a high temperature thermoplastic, semi-crystalline polymer with a high melting temperature[12,13], which is widely suitable for CAD/CAM fabrication of various dental protheses[14]. It has good biocompatibility and favourable resistance to wear and fatigue. In addition, PEEK can be easily chemically modified. This enables simple adjustment of its mechanical properties in accordance with requirements[12]. BioHPP (Bredent, Senden, Germany) is a PEEK-based, ceramic-reinforced high-performance polymer. It exhibits excellent polishing properties and wear resistance, with low plaque affinity[15], and is therefore a good choice for the preparation of denture frameworks in patients with periodontitis.

The use of PEEK polymer material in dentistry has yet to gain momentum. In particular, the literature is sparse on long-term clinical studies of the use of PEEK in clinical dental practice. This case report presents a 6-year follow up of a patient with periodontitis who received restoration with an implant-supported full-arch fixed dental prosthesis fabricated with a PEEK framework.

Case report

A 61-year-old, non-smoking man was referred to our department, with the chief compliant of mobility in his mandibular teeth. The patient had been suffering from loose teeth and masticatory dysfunction for 5 years, with teeth lost in both the anterior mandible and maxilla.

The intraoral examination identified generalised, severe plaque accumulation and heavy calculus deposits. Periodontal pocket depth ranged from 4 to 7 mm on probing. In the maxilla, the left central incisor had been lost and the right central and left lateral incisors exhibited class I mobility (i.e., < 1 mm horizontal movement) according to Miller's classification. In the mandible, the right central incisor had been lost; all the remaining teeth were mobile with class III mobility (i.e., > 2 mm horizontal or vertical mobility), with the exception of the left and right premolars, which exhibited class II mobility (> 1 mm horizontal movement) (Table 1, Fig 1a).

The radiographic examination showed severely resorbed alveolar bone due to periodontitis, with horizontal bone resorption to the middle of the roots in the maxilla. Bone resorption was more extensive in the mandible, with bone loss up to two-thirds of the root lengths. The mandibular right second molar, left canine and left first and second molars suffered from asymptomatic apical periodontitis with periapical radiolucencies (Fig 1b). The patient had a thick gingival biotype. There were 10 to 12 mm of available inter-ridge restorative space in the edentulous region for prosthesis construction (i.e., Class III vertical restorative space)[16].

Several prosthodontic treatment plans were presented to the patient, including a removable denture, an implant-retained overdenture and an implant-supported fixed prosthesis. After understanding the advantages and disadvantages associated with each plan, the patient opted for an implant-supported fixed prosthesis in the mandible and a fixed partial denture in the maxilla.

The initial therapy involved controlling the periodontitis with periodontal treatment. The latter included oral hygiene instruction, scaling and root planning, according to the European Federation of Periodontology Clinical Practice Guidelines[17]. The maxillary teeth were restored as planned after periodontal treatment; however, all the mandibular teeth showed a poor prognosis after treatment. Herein, the mandible was planned to be restored with implant-supported full-arch fixed dental prostheses, after adequate communication with the patient.

CBCT (Orthophos XG 3D; Dentsply Sirona, Charlotte, NC, USA) was performed with a radiological diagnostic prosthesis, according to the dual-scan procedure outlined in the scanning protocol[18]. The obtained DICOM file was matched with data acquired from an intraoral scanner (D2000 3D Scanner, 3Shape, Copenhagen, Denmark). The implant template was designed using implant planning software (GuideMia Technologies, Los Alamitos, CA, USA) and fabri-

cated using a 3D printer (ProJect MJP 3600 Dental, 3D Systems, Rock Hill, SC, USA) with surgical guide resin (VisiJet MP200, VisiJet M3 Stoneplast, SD Systems (New York, NY, USA).

All the mandibular teeth were extracted 1 month prior to implant surgery, except for the canines and second molars. After delivery of local anaesthesia, an implant guide supported by the teeth and mucosa was used for preparation of the implantation fossae. The mandibular canines were then extracted. An incision was made on the mandibular alveolar crest by raising a full-thickness flap. Six 3.3 mm diameter bone-level implants (Straumann, Basel, Switzerland) were placed. Insertion torque of 35 Ncm was placed on the prosthetic screws (Fig 2).

An implant-level impression was made immediately after implant placement. The multiunit impression copings were splinted together using stainless-steel bars and auto-polymerising acrylic resin (GC Pattern Resin, GC, Tokyo, Japan). Immediate impressions were taken using an addition silicone impression material (Virtual Heavy Body, Ivoclar Vivadent, Schaan, Lichenstein). The interocclusal centric relationship was registered using a conventional occlusal wax rim. The mandibular second molars were extracted after the record was taken. The accuracy of implants was checked using CBCT (Fig 3). An interim restoration, consisting of individual artificial teeth and heat-polymerised acrylic resin (Trevalon, Dentsply Sirona), was designed and fabricated using CAD/CAM (GuideMia Technologies) (Fig 4). The ridge of the pontic was designed to be laid 2.3 mm above the alveolar crest, and the rest of the pontic was designed following the morphology of the alveolar bone. The interim restoration was delivered immediately after the surgical operation to restore masticatory function and aesthetics during the recovery period.

Prior to construction of the definitive restoration, the morphology of the pontic site and mucosa around implants of the interim restoration were modified according to the oral soft tissue 3 and 6 months after implant surgery. After that, information on gingival morphology was collected by scanning the final version of the modified interim restoration (D2000 3D Scanner, 3Shape). This information was duplicated to the permanent prosthesis. A definitive impression was taken with addition silicone impression material (Virtual Heavy Body and Virtual Light Body, Ivoclar Vivadent) using splinted open-tray impression copings and the final interocclusal relationship was recorded. The definitive prosthesis consisted of a fixed implant-supported prosthesis with individual artificial resin

Table 1 Pretreatment periodontal chart.

Prognosis		Q			F			Q			F			Q			F			Q			F			Q			F			Q
Plaque		II			I			I			I			I			I			I			I			I			I			I
Mobility		I			-			I			-			I			-			I			-			-			-			I
BOP		+			-			+			-			-			+			+			-			+			+			+
PPD, mm	BS	5	3	6	3	2	3	2	2	3	3	2	2	3	2	2				2	4	4	2	3	2	3	4	3	2	3	5	6	3	4
	PS	5	3	4	3	2	4	2	2	3	2	2	2	2	1	2				2	2	3	2	2	3	3	3	3	4	3	6	5	3	4
Tooth number		17			15			12			13			11			21			22			24			25			26			27	
		47			45			42			43			41			31			32			34			35			36			37	
PPD, mm	LS	6	3	7													3	4	3	3	2	4	4	3	4	5	3	4	7	3	3	7	4	5
	BS	5	5	6													3	3	3	3	3	4	4	2	3	4	3	5	6	3	7	6	5	7
BOP		+			+			+			+			+			+			+			++			+			+			+	
Mobility		III			II			III			II			III			III			III			II			II			III			III	
Plaque		II			I			II			II			II			II			II			I			I			I			II	
Prognosis		H			P			H			P			H			H			H			P			H			H			H	

BOP, bleeding on probing; BS, buccal side; F, fair; H, hopeless; LS, lingual side; P, poor; PPD, periodontal probing depth; PS, palatal side; Q: questionable.

I or II for plaque indicates the Plaque Index, and for mobility it indicates the degree of tooth mobility.

+ means that BOP is positive and - means it is negative.

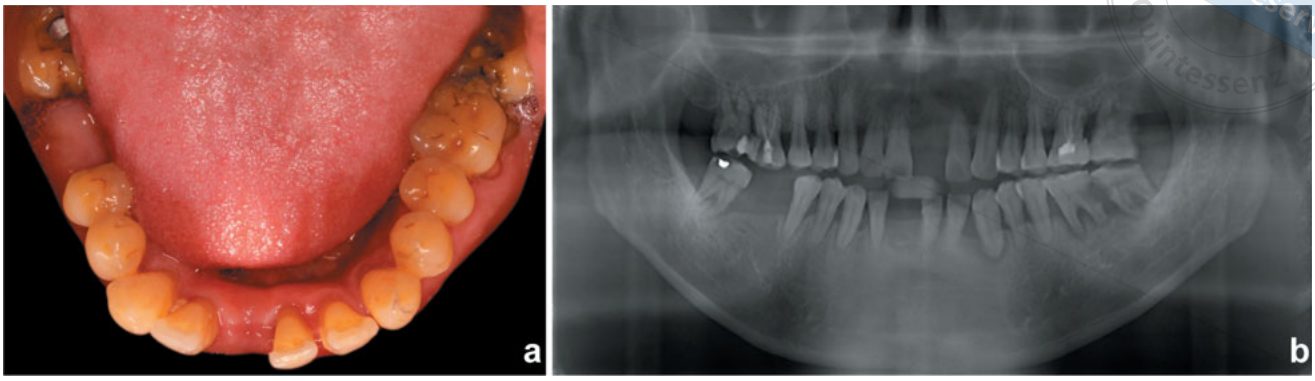


Fig 1 Pretreatment photographs: **(a)** intraoral view of the mandible and **(b)** panoramic radiograph of the initial dental status.

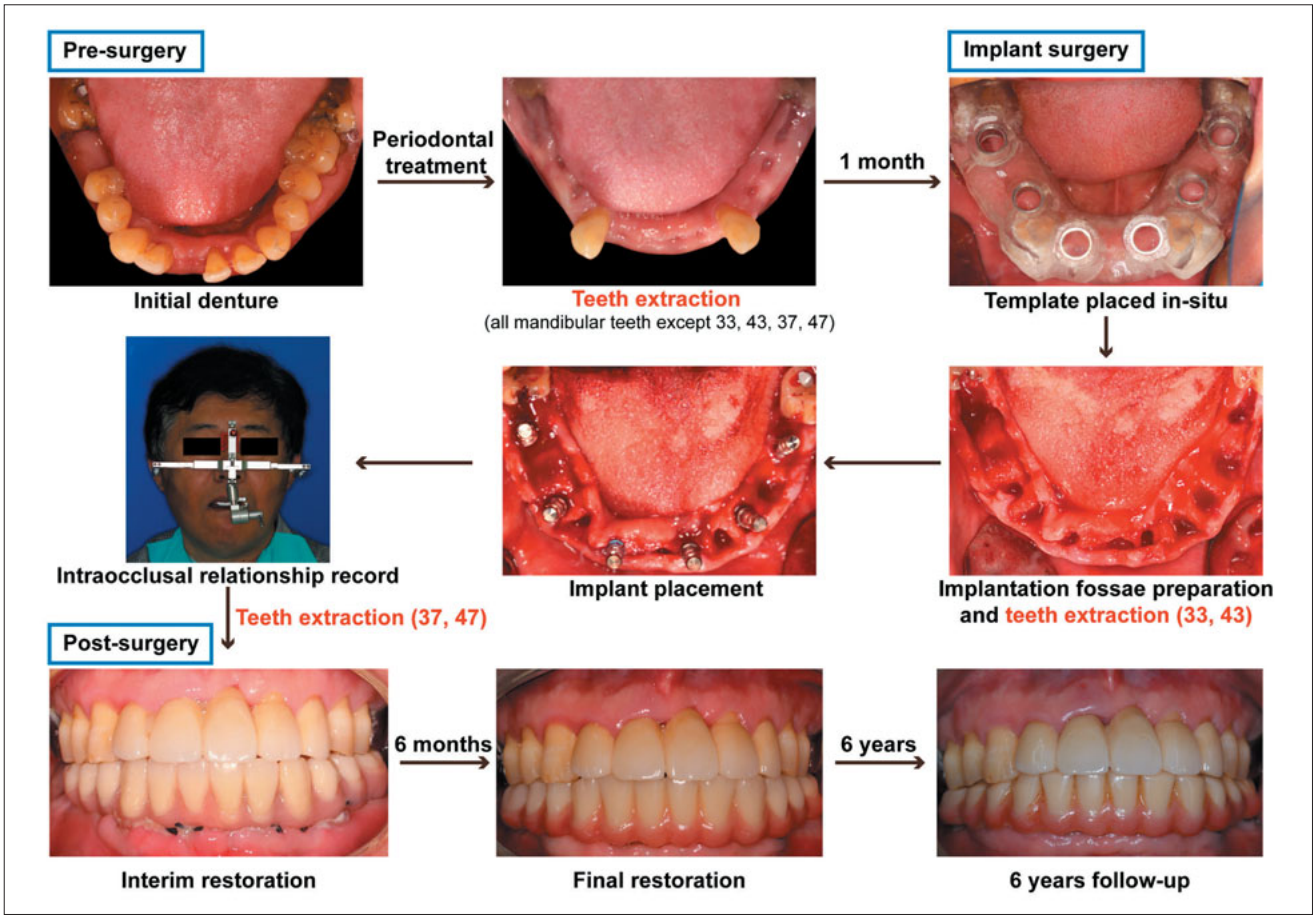
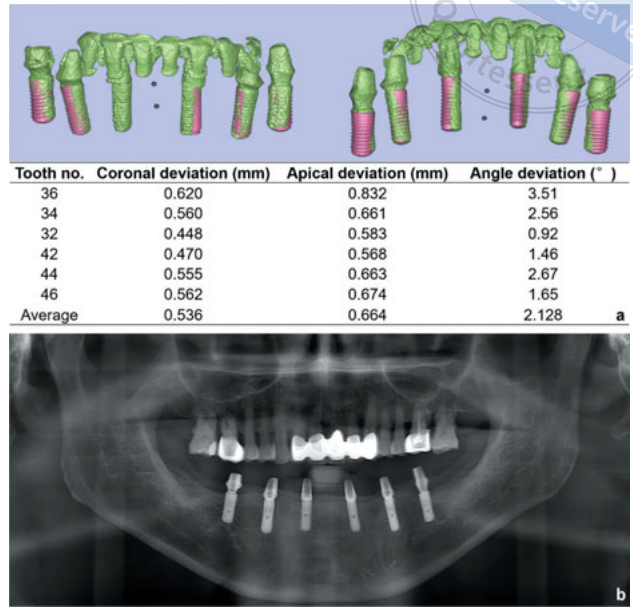


Fig 2 Treatment procedure.

teeth (neo.lign, Bredent), and polymethyl methacrylate (PMMA) veneers (neo.lign) laid over a PEEK framework (breCAM.BioHPP, Bredent) (Fig 5). The adhesive procedures were as follows. First, the PEEK framework was conditioned as per the instructions. It was airborne-particle abraded at a pressure of 2 to 3 MPa using 110 µm aluminium oxide wetted with primer (visio.link, Bredent) followed by light-curing, then covered by a lamella of theopaquer (Opaquer combo.lign, Bredent), polymerised in the light-curing unit. Meanwhile, the PMMA veneer was also airborne-particle abraded and primer conditioned using the same protocol with the PEEK framework. After that, the veneer was cemented to the framework using the tooth shade cement (combo.



Tooth no.	Coronal deviation (mm)	Apical deviation (mm)	Angle deviation (°)
36	0.620	0.832	3.51
34	0.560	0.661	2.56
32	0.448	0.583	0.92
42	0.470	0.568	1.46
44	0.555	0.663	2.67
46	0.562	0.674	1.65
Average	0.536	0.664	2.128

a

Fig 3 **(a)** Deviation of implant placement from the design and **(b)** panoramic radiograph after final implantation.

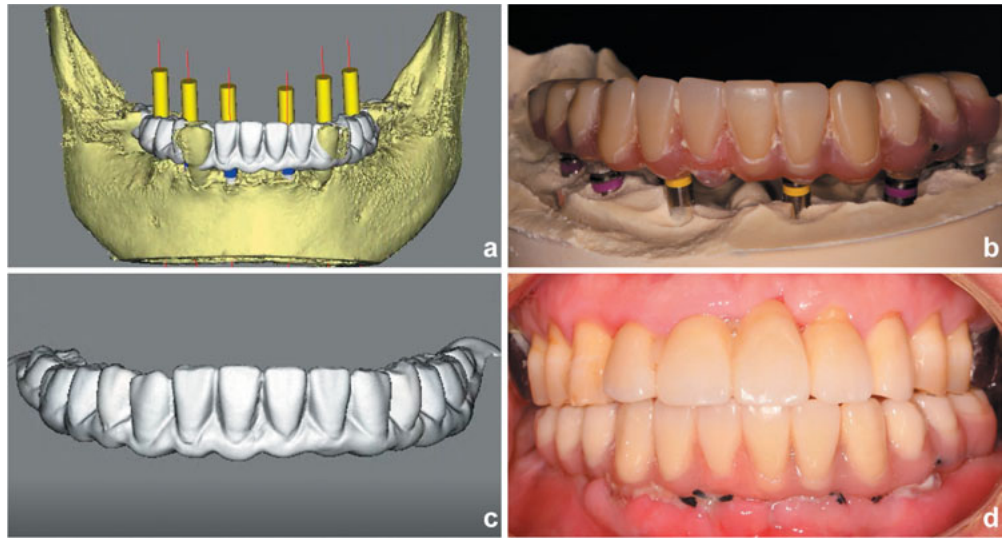


Fig 4 **(a to c)** CAD/CAM of the interim prostheses, **(d)** panoramic intraoral view after restoration of the interim prostheses.

lign, Bredent) and subsequently polymerised using the light-curing unit.

After the entire implant procedure, an occlusal splint was made to protect the protheses. Over the 6-year follow-up, the patient's periodontal condition remained stable with no signs of inflammation or bleeding. The mandibular implants were firm and stable, with negligible bone resorption (Table 2 and Fig 6). There was no swelling or recession of the adjacent gingivae. The patient indicated that his chewing efficacy was significantly improved after insertion of the prosthesis and was satisfied with the aesthetic results; however, veneer collapse was found on the posterior area of the mandibular protheses. According to our clinical experience, the veneer damage in the left posterior region of the protheses was considered to have worn off, while in the right region it was thought to have chipped off. Even though veneer collapse occurred, the implant could still be considered a 6-year success, based on the commonly accepted criteria of implant success[19].

Discussion

Periodontitis causes attachment loss and alveolar bone destruction, ultimately resulting in tooth loss. Patients with periodontitis usually have poor oral hygiene and extensive plaque accumulation and are prone to peri-

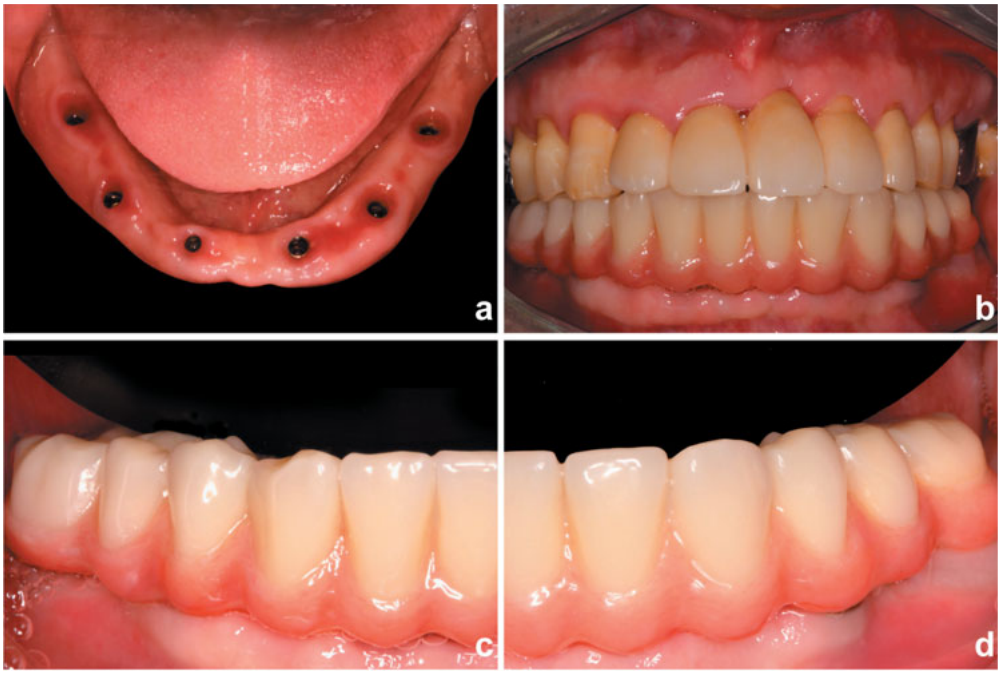


Fig 5 **(a)** Occlusal, frontal and lateral views on the day of delivery showing healthy peri-implant mucosal conditions and **(b to d)** the final reconstruction in situ.

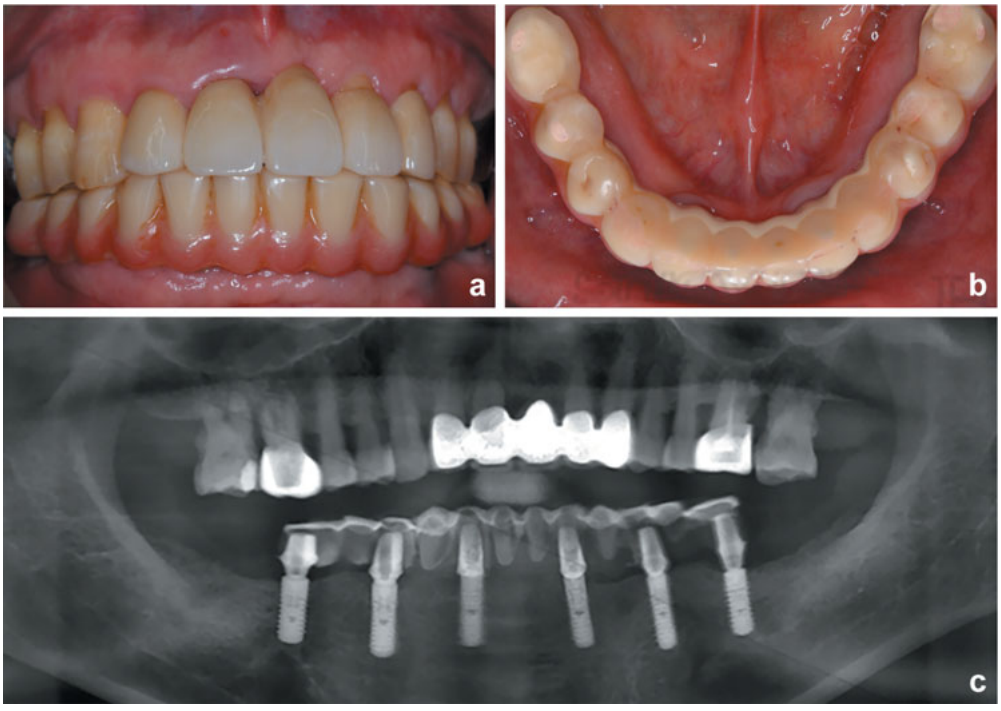


Fig 6 Posttreatment photographs from the 6-year follow-up: **(a)** intraoral panoramic view, **(b)** occlusal view of the prosthesis and **(c)** panoramic radiograph.

implantitis[2]. Multiple investigations have illustrated that poor periodontal status adjacent to implants results in implant treatment failure[3,20]. Preoperative periodontal treatment is therefore necessary to achieve a healthy periodontal condition prior to implant surgery. According to the European Federation of Periodontology Clinical Practice Guidelines[17], the treatment continued with bleeding on probing < 10%, shallow probing depths of ≤ 4 mm and no 4-mm pockets with bleeding on probing. Such a periodontal condition is considered a prerequisite for implant placement. Besides, the patient should be subjected to a stringent periodontal maintenance scheme over the entire implant treatment procedure to achieve a favourable long-term outcome[17].

After initial periodontal treatment, teeth with excessive mobility and severe bone resorption must be extracted prior to construction of the implant-supported fixed denture[17,21]. According to the Third International Team for Implantology (ITI) Consensus Conference, there are three protocols for determining the timing of implant placement, i.e., immediate, early and conventional[22]. Immediate implant placement in patients with periodontitis is often associated with greater bone loss and a high failure rate[23]. Conversely, conventional implant placement requires a lengthy treatment period. Accordingly, the early implant placement protocol was utilised in the present case. In this protocol, implants are placed approximately 4 to 8 weeks after tooth extraction in the absence of pathology around the implant, to optimise primary healing of the soft tissue and bone. After implant placement, immediate loading was utilised because the strategy restores the compromised aesthetics and function immediately after implant surgery, with reduced treatment time[24,25]. Long-term data on immediate loading of implant-supported prostheses has shown that such a loading strategy has an acceptable success rate and clinical outcome[26,27].

Gingiva modification is commonly used in implants in the anterior maxilla to provide a satisfactory aesthetic. The gingiva modification in the present case was not only due to the patient's high aesthetic demands, but also to the biological width around implants. Maintenance of adequate keratinised mucosa around implants and underneath the pontic area is essential for long-term survival of implants. The peri-implant mucosal tissue and bone establish a stable biological dimension after implant insertion, generating a biological width that is similar to that of the natural dentition. The newly formed biological width is essential to prevent accumulation of plaque biofilms and their bacterial by-products[28]. It has been reported that a minimum peri-implant mucosa width of 2.26 mm is required to establish a proper biological width and house the prosthetic interface[28]. Mucosal thickness affects marginal bone stability and bone resorption may occur with reduced thickness of peri-implant mucosa. The soft tissue at the pontic site should therefore be manipulated carefully to enable satisfactory implant treatment. The anatomy of the peri-implant mucosa could be obtained through conventional impressions and provisional restorations working as implant impression copings. In recent years, with the development of digital impressions, intraoral scanning technology has been introduced in image collection and analysis. The digital information provides high-quality images of the relationship between soft tissue and bone and has

Table 2 Periodontal chart of 6-year follow-up.

Plaque		+	-	-	+	-	-	-		-	-	-	-	-	+
Mobility		I	-	-	-	-	-	-		-	-	-	-	-	I
BOP		-	-	-	-	-	-	-		-	-	-	-	-	+
PPD, mm	BS	4 3 5	4 3 3	3 2 2	2 3 3	2 2 2	3 2 3	2 1 2		2 2 1	2 3 2	3 3 2	3 3 4	4 3 3	5 3 4
	PS	5 3 4	5 3 3	4 3 2	3 2 4	2 2 2	2 2 3	2 1 2		2 2 3	2 2 2	3 2 3	3 3 3	4 3 6	5 3 4
Tooth number		17	16	15	14	13	12	11	21	22	23	24	25	26	27
		47	46 (implant)	45	44 (implant)	43	42 (implant)	41	31	32 (implant)	33	34 (implant)	35	36 (implant)	37
PPD, mm	LS		3 3 2		3 2 4		2 2 2			2 1 2		3 2 3		3 3 3	
	BS		3 2 3		3 3 3		2 2 3			2 2 2		3 2 3		3 2 2	
BOP			-		-		-			-		-		-	
Mobility			-		-		-			-		-		-	
BR, mm			0.43		0.53		0.52			0.41		0.81		0.56	

BOP, bleeding on probing; BR, bone resorption; BS, buccal side; LS, lingual side; PPD, periodontal probing depth; PS, palatal side.
I or II for plaque indicates the Plaque Index, and for mobility it indicates the degree of tooth mobility.
+ means that BOP is positive and - means it is negative.

become an essential tool in prothesis design[6]. In the present case, a provisional restoration with a margin located 2.3 mm above the alveolar bone was designed and fabricated by CAD/CAM according to the patient's computed tomography data. We combined the digital impression and provisional restoration, forming a suitable biological width around the implants.

As a new generation of implant material, PEEK has the following advantages. First, it is white in colour and eliminates the greyish hue of metal frameworks to produce a more aesthetic outcome[29]. The material is comparable to bone in terms of mechanical and physical properties[12]. Hence, the PEEK framework here was combined with the high-strength resin teeth, aiming to reduce the stress concentration caused by natural maxillary teeth to reduce relative complications. It is also resistant to wear and has a high survival rate[13]. Because PEEK is insoluble in water, a PEEK framework does not have a metallic taste. For these reasons, a PEEK-based prosthetic framework has a high level of patient acceptance[29]. In addition, PEEK is highly biocompatible and causes fewer inflammation effects and less plaque accumulation[15].

There are still some limitations concerning the applications of PEEK. First, PEEK blanks are a greyish-brown or pearl-white opaque colour and are unsuitable for monolithic aesthetic dental restorations, especially in the anterior region[13]. Thus, veneering is required, but bonding to veneering composite resin materials remains a challenge because of the complex chemical structure of PEEK. Besides, in contrast to titanium, PEEK has very limited inherent osteoconductive properties[30]. Although unmodified PEEK is considered as a bio-inert material, there is no conclusive evidence of the osteoconductive effects of PEEK in vivo and in vitro. Moreover, protheses with a PEEK framework are still at risk of mechanical complications.

In the present case, the veneer collapse happened in the posterior region after a 6-year follow-up. Veneer fracture is the most common mechanical complication for protheses with a PEEK framework, which emphasises the imperfect bonding between the framework and PMMA veneer[11]. As PEEK is characterised by an inert surface, surface pretreatment is crucial for successful bonding with the veneer[31,32]. It was reported that the shear bond strength between pure PEEK substrate (without surface pretreatment) and PMMA veneer was only 0.7 to 18.2 MPa, and that after surface pretreatment, the shear bond strength between the veneer and PEEK can be increased to about 30 MPa or more[33]. The surface pretreatments include airborne-particle abrasion, silica coating and laser, among which airborne-particle abrasion was proven to provide superior pretreatment of PEEK[34]. Besides, the bond strength is also related to the adhesive system. The prosthesis using the visio.link adhesive system, which contains methyl methacrylate and 2-propenoic acid reaction products with pentaerythritol and diphenyl (2,4,6,-trimeth-ylbenzoyl)-phosphine oxide[35], was proven to show a favourable survival rate[36]. Thus, in the present case, the PEEK surface was airborne-particle abraded and treated with a viso.link prime and an opaquer catalyst (Bredent) to improve bonding between the methacrylate veneer and the PEEK framework. In addition, an implant protective occlusion splint was applied to protect the protheses by reducing the tension from the occlusal force on it[37]. The occlusal splint could help move stress forward towards the bone structure to protect the protheses and maintain implants for the long term[38]. The patient did not wear the occlusal splint as advised, and veneer collapse still happened. Thus, further studies are required to eliminate the occurrence of veneer collapse.

Microcracks are another commonly occurred mechanical complication encountered with the PEEK framework[11], which might be related to the fatigue behaviour of PEEK[33]. To address this drawback, some reinforced materials were introduced to improve the fatigue performance of PEEK. Appropriate design and standardised manufacturing might also be conductive to preventing cracks, including enough inter-ridge space, proper thickness of frameworks, and so on[11]. Thus, further studies should emphasise the relationship between the design of the PEEK framework and its integrity.

The limitations of the present study include the fact that this was a case report with only one patient, and that it was conducted 6 years ago. At that time, the relevant technology was not sufficiently developed. The 3D printing of PEEK frameworks was not developed enough, which may have led to deficiencies in framework production, and the multiunit abutment (Screw Retained Abutment; Straumann) was not available in China 6 years ago. As such, we could only choose implant-level impression, which may have caused unsatisfactory precision. Further research should focus on long-term studies with a bigger sample size to fully confirm the validity of the PEEK framework applied in patients with periodontitis.

Conclusion

In the present case, a patient with chronic periodontitis received a PEEK framework containing an implant-supported full-arch fixed prosthesis after appropriate periodontal treatment. At the 6-year follow-up, the patient

was satisfied with the prosthesis and there was no recurrence of periodontitis or peri-implantitis. Within the limitations of this study, full-arch protheses with PEEK framework can represent a good alternative treatment choice for patients with periodontitis. To further evaluate the clinical effectiveness of this treatment option, studies with a larger sample size and over a longer period are required.

Conflicts of interest

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

Author contribution

Dr Jing WANG contributed to the surgery, data collection and concept; Dr Jun Ting GU contributed to the original draft preparation and data interpretation; Drs Meng MENG and Chen Yu WANG contributed to the surgery and data analysis; Dr Ji Hua CHEN and Dr Li Na NIU contributed to the manuscript revision, funding acquisition and project administration.

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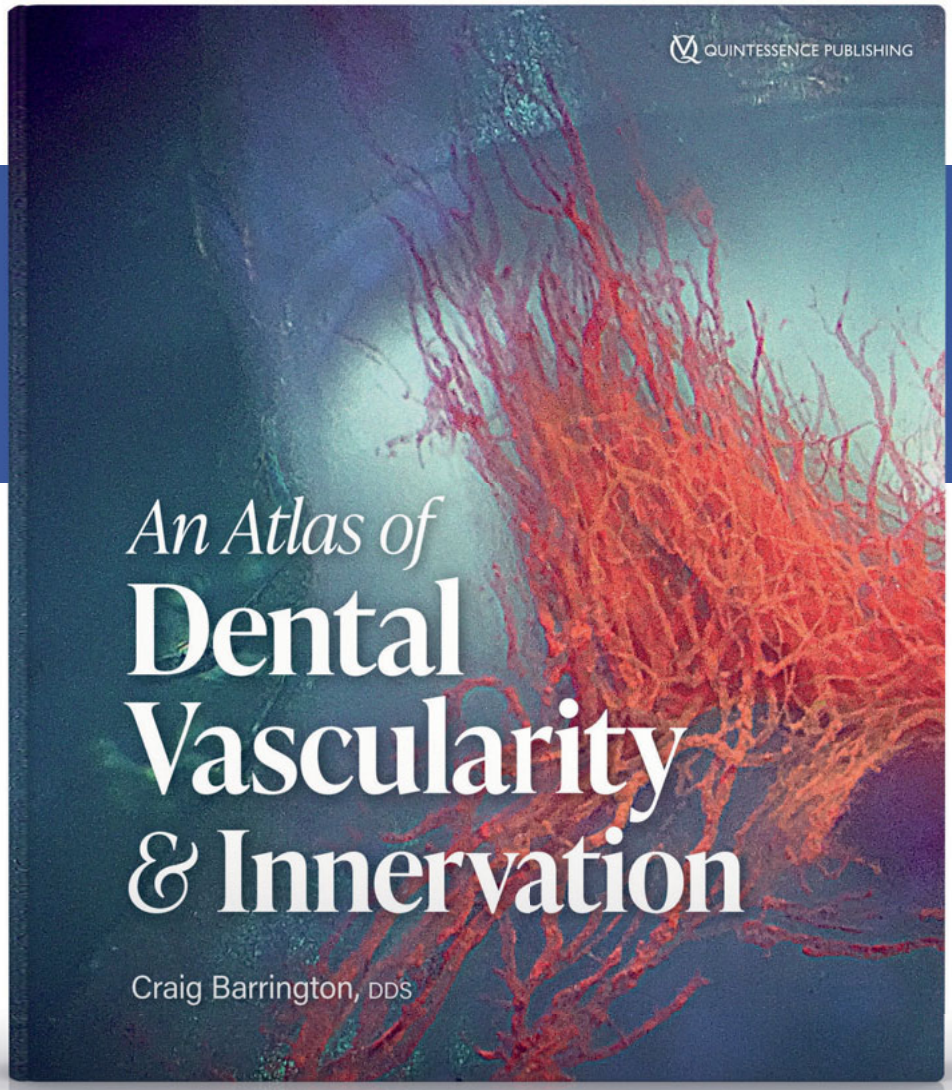

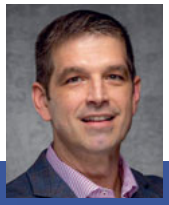

Craig Barrington

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