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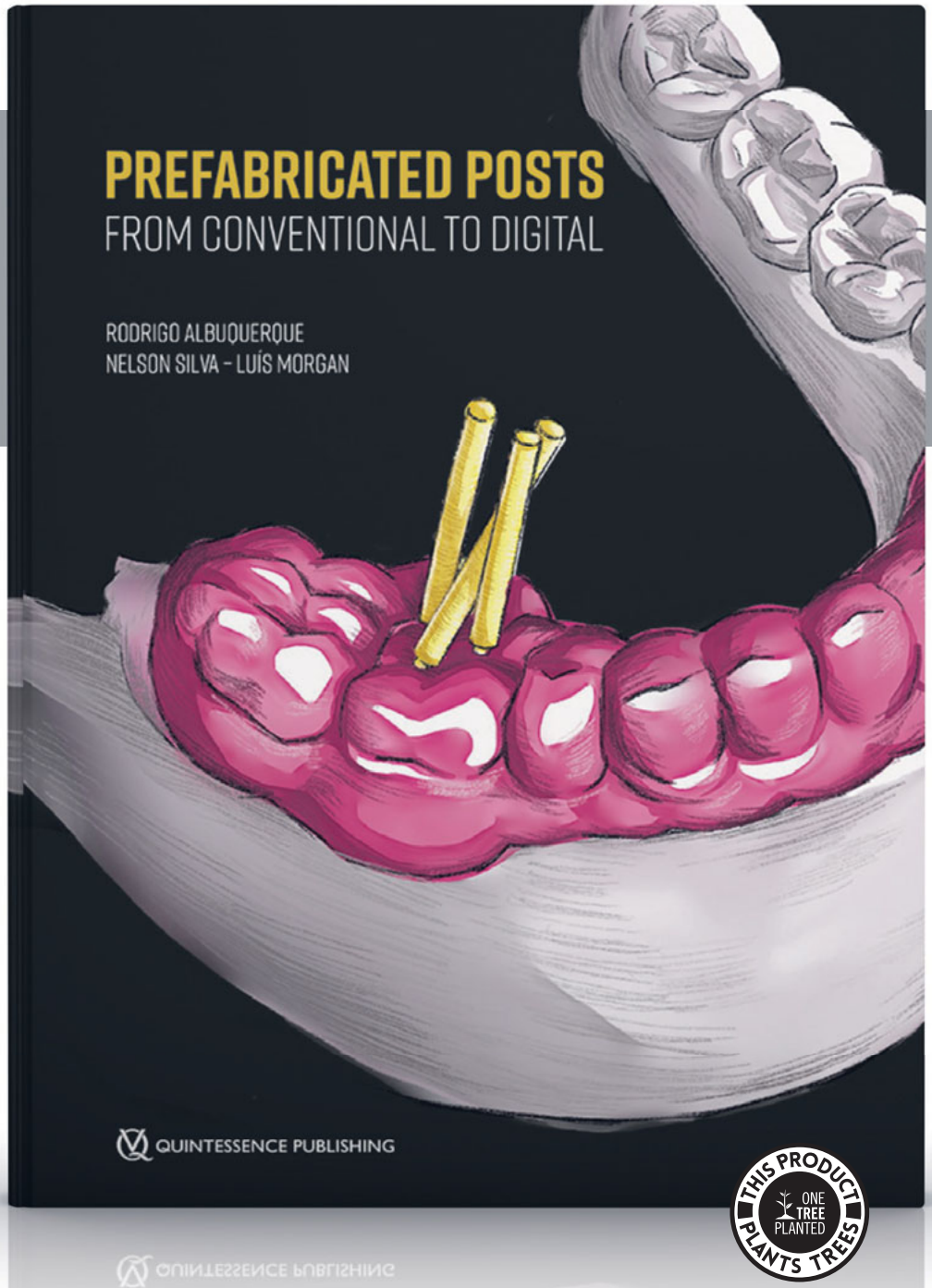

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Extracellular Matrix Remodelling of the Periodontium under Orthodontic Force

Qian Yao YU[1], Yi Ping HUANG[1,2], Wei Ran LI[1,2]

As the biological mechanisms of orthodontic tooth movement have been explored further, scholars have gradually focused on the remodelling mechanism of the extracellular matrix (ECM) in the periodontal ligament (PDL). The ECM of the PDL consists of various types of collagens and other glycoproteins. The specific process and mechanism of ECM remodelling during orthodontic tooth movement remains unclear. Collagen I and III, which constitute major components of the PDL, are upregulated under orthodontic force. The changes in the contents of ECM proteins also depend on the expression of ECM-related enzymes, which organise new collagen fibre networks to adapt to changes in tooth position. The matrix metalloproteinase family is the main enzyme that participates in collagen hydrolysis and renewal and changes its expression under orthodontic force. Moreover, ECM adhesion molecules, such as integrins, are also regulated by orthodontic force and participate in the dynamic reaction of cell adhesion and separation with the ECM. This article reviews the changes in ECM components, related enzymes and adhesion molecules in the PDL under orthodontic force to lay the foundation for the exploration of the regulatory mechanism of ECM remodelling during orthodontic tooth movement.

Keywords: *biomechanics, extracellular matrix, orthodontic tooth movement, periodontal ligament, remodelling*

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The biological mechanism of orthodontic treatment involves moving teeth to a new position by way of orthodontic force, accompanied by periodontal tissue remodelling in the process. Periodontal tissue consists of periodontal ligament (PDL), alveolar bone, cementum and gingiva. PDL is a specialised fibrous connective tissue that fixes teeth to alveolar bone through Sharpey fibres. It maintains the structural integrity of periodontal tissue, provides protection and support for the masticatory system and is also the biological basis for orthodontic tooth movement.[1] PDL dysfunction leads to an imbalance in the periodontal microenvironment, causing subsequent root resorption and alveolar bone loss. Previous studies have shown that the removal of PDL results in ankylosis of teeth and restriction of tooth movement.[2] As the main site of mechanical signal transduction and subsequent molecular biological reactions, the PDL is an indispensable part of the mechanism of orthodontic tooth movement.

The extracellular matrix (ECM) is a key component of the noncirculating cell microenvironment. As well as providing structural support, the ECM contributes to maintaining cell viability, regulating intercellular adhesion and transmitting mechanical forces.[3] Studies have found that the ECM derived from PDL cells (PDLCs) promotes cell proliferation and migration and maintains cell stemness.[4,5] Cells respond to mechanical and biochemical signals from the ECM and thereby change their biological behaviours, such as survival, migration and differentiation. Cells also secrete cytokines and growth factors to regulate the ECM environment.[6] The PDL is mainly composed of the ECM and cells within it. The interaction between cells and the ECM constitutes the biological mechanism of orthodontic tooth movement; however, the regulatory mechanism behind the

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ECM changes in the PDL during orthodontic processes remains unclear. This review aims to summarise and review existing studies, explore the change patterns of ECM components and related molecules in the PDL during orthodontic tooth movement, and lay the foundation for exploring the periodontium remodelling mechanism during orthodontic tooth movement.

ECM components of PDL

PDL is mainly composed of cells and ECM. The cell populations comprise fibroblasts, endothelial cells, Malasser epithelial cells, osteoblasts and osteoclasts, cementoblasts, immune cells and cells associated with the sensory system. Among these, fibroblasts, often known as PDLCs, are the main cell type, accounting for approximately 20% to 30% of the PDL volume.[1] PDLCs attach to collagen through focal adhesions and participate in the alignment of collagen fibre nets.

ECM components of PDL include collagens and other noncollagenous glycoproteins. The collagen fibre bundles in the PDL are mainly composed of collagen I and III. Collagen V is embedded in fibre bundles or interstices.[7] Previous studies have shown that collagen I, III and V make up 75%, 20% and 5% of PDL, respectively.[7,8] In recent years, Thant et al[9] performed laser microdissection and lipid chromatography-tandem mass spectrometry to comprehensively analyse the PDL tissue–specific proteome and found that the major collagen types of PDL were type I (86.5%), type III (4.5%), type XII (4.5%) and type V (1.7%) based on the spectral count. The slight difference in the proportion of each major collagen component in various studies is potentially due to factors such as protein extraction and measurement methods and species differences. In addition to the above collagens, there are also small amounts of collagens IV, VI, VIII and XII in the PDL, which jointly participate in the formation of a mature fibre network. Recently, many proteomic studies also found collagen II in rat and mouse PDLCs[10,11], which has been proven to be upregulated under cyclic tension stretch and hypoxic conditions.[12] Collagen II allows the ECM to accommodate more water molecules to dissipate stress[13], but its specific function in the PDL still needs to be further explored.

Changes in ECM components under orthodontic force

The rapid ECM remodelling of the PDL determines its unique adaptability. The coordinated breakdown and synthesis of ECM components take the forms of turnover and remodelling. ECM turnover refers to the dynamic stability of structural organisation, whereas remodelling reflects the adaptive changes in the shape and function of the ECM.[14] The two forms often coexist in the process of orthodontic tooth movement. While maintaining the width and normal function of the PDL, the ECM forms a periodontal microenvironment that is conducive to orthodontic tooth movement by rapid remodelling. The exploration of changes in ECM components in the PDL under different types of forces would facilitate understanding of the process of ECM microenvironment formation.

Collagens

Collagen is the main fibre component of the PDL. Similar to other ECM proteins, collagen is synthesised and modified in the endoplasmic reticulum, transported to the endoplasmic reticulum and the Golgi intermediate compartment in a COPII-dependent manner, and then passes through the cell. Procollagen is secreted into the extracellular space, where it is cleaved and assembled into fibrous structures.[15] At the end of the 20th century, scholars proposed that the PDL remodelling mechanism induced by orthodontic treatment is similar to the wound healing process, accompanied by increased collagen metabolism.[16] Turnover and remodelling of collagens in the PDL under orthodontic force is an important physiological process in orthodontic tooth movement. Changes in the expression levels of collagens in previous studies are summarised in Table 1.

At the macroscopic level, Thant et al[9] used second harmonic generation (SHG) imaging and the CT-FIRE automatic tracking algorithm to detect individual collagen fibres and found that the number, width and length of fibres on the compressed side of the PDL decreased significantly after 14 days of orthodontic force application, whereas no significant change was observed on the tension side. At the microscopic level, the synthesis and degradation of various types of collagens vary with each other under different types of mechanical forces at different times and in different sites in past studies.

Collagen I and III are the main components of the ECM in the PDL. Previous studies have demonstrated that collagen I is mainly responsible for maintaining tissue strength, whereas collagen III contributes to maintaining tissue elasticity and relieving the tension force placed on the PDL during orthodontic tooth movement.[17] Remodelling of the two collagens is dominant in PDL remodelling during orthodontic treatment. Most research has reported that collagen I and III first increase and then decrease in the PDL after orthodon-

tic force application. As early as 1984, Duncan et al[18] verified through animal models that after 3 to 5 days of orthodontic force application, the content of collagen III in the PDL increased significantly. Subsequently, Bumann et al[16], Militi et al[19] and Anastasi et al[20] extracted human premolars after applying orthodontic force and found that collagen I gradually increased on the compression side within 14 to 30 days of force application. Redlich et al[21]and Hacopian et al[22] reported that compression upregulated the expression of collagen I via in vitro experiments as well. Upregulation of collagen III under compression was also reported.[16] Regarding tensional force, Pei et al[2] demonstrated that tension promoted collagen remodelling in the ECM and increased the secretion of collagen I in PDLCs. Howard et al[23] and Kook et al[24] also found that the expression of collagen I and III in PDLCs was upregulated under tension. For in vivo experiments, Militi et al[19] and Anastasi et al[20] observed that collagen I increased on the tension side of extracted human premolars at an early stage, before decreasing gradually. However, unlike most other studies, Wescott et al[25] and Nemoto et al[26] reported downregulation of the short-chain collagen genes *COL3A1* and *COL1A1* in hPDLCs under tension.

In the early stage of collagen remodelling, although the synthesis of collagen I and III generally show an upward trend, studies also found that the rate of increase of the two is not consistent. The deposition of collagen III is more significant than that of collagen I, which leads to an increase in the ratio of collagen III to I. Over time, the content of collagen I gradually increases to maintain a balanced ratio of the two.[17] Li et al[27] compared orthodontic tooth movement between diabetic rats and normal rats. They found that in normal rats, the ratio of collagen III to I gradually increased in the early stage, then decreased, and gradually returned to the baseline level on the 14th day. In the diabetes group, the ratio of the two continued to increase and did not show a downward trend, suggesting that diabetes causes abnormal collagen remodelling during orthodontic tooth movement.[27] In another study, Li et al[28] used Sirius red staining and immunohistochemistry to examine the regional distribution of collagen III and I in PDL after applying orthodontic force and found that the ratio of collagen III to I increased, especially in the apical region, which corresponds to the stress concentration area. The above studies suggest that in the early phase of orthodontics, the elasticity of the PDL increases to adapt to tooth movement, especially in the compression area. Then, as the ratio of collagen III to I increases to normal levels, the hardness and toughness of the PDL gradually recover.

The hydrolysate of collagen IV can promote angiogenesis, thereby improving the proliferation, stemness and osteogenic differentiation of PDL stem cells.[29] In aged oral fibroblasts, the expression of collagen IV decreased, and the arrangement of ECM collagen changed from network to line, suggesting the role of collagen IV in the arrangement of the collagen fibre network.[30] In previous studies, the expression of IV collagen in the PDL mainly showed a downward trend under mechanical force. Anastasi et al[20] reported that the synthesis of collagen IV was significantly decreased on both the compression side and the tension side of human teeth after orthodontic treatment. Militi et al[19] found that the expression of collagen IV first decreased and then rose slightly on the tension side within 30 days of force application, and on the compression side, collagen IV decreased and then returned to the baseline level. The downregulation of collagen IV partly explains the disordered arrangement of collagen fibres under orthodontic force.

Collagen VI is closely related to the posttranslational modification of collagen fibres, participates in collagen fibre assembly and 3D structural organisation, and increases the stiffness of collagen I fibres.[31] It was reported that the PDL in the cervical area was harder and more viscoelastic than that in other areas, the abundance of collagen VI was higher in the cervical area, and the collagen fibre network was denser.[10] Saminathan et al[32] found a significant downregulation of the expression of the short-chain collagen gene *COL6A1* after applying cyclic tension. Thant et al[9] demonstrated that the proportion of collagen VI in the PDL decreased on the compression side in orthodontically treated mice. Bumann et al[16] reported an increase in collagen VI on the compression side as well, and the changes were not significant on the tension side in this study. The changes in the expression of collagen VI under orthodontic forces remain to be clarified.

At present, there are few studies related to other types of collagens. Bumann et al[16] found that the secretion of collagen V increased under compression. Ma et al[33] also reported that stretching force upregulated the expression of *COL5A1* in PDLCs. In the study conducted by Saminathan et al[32], the expression of *COL8A1* was downregulated in PDLCs under tension. Regarding collagen XI, studies demonstrated consistent results that stretching force downregulates *COL11A1* in PDLCs.[25,32,33] Thant et al[9] found that collagen XII increased on the tension side of the PDL in mice. Further research is needed on the changes and roles of collagen in ECM remodelling.

Table 1 Collagens outcome from all included studies.

Study	Force type	Methods	Object	Outcome	
				COL-I	
Howard et al[23]	Tension	ELISA	hPDLCs	Upregulated significantly (5%, 24 h), non-significant (10%, 24 h)	
Redlich et al[21]	Compression	RT-PCR	hPDLCs	Upregulated significantly (within 60 min), non-significant (90 min)	
Wescott et al[25]	Tension	RT-PCR	hPDLCs	NR	
Nemoto et al[26]	Tension	RT-PCR	hPDLCs	Downregulated significantly (within 7 d)	
Kook et al[24]	Tension	RT-PCR	hPDLCs	Upregulated significantly (within 12 h)	
Hacopian et al[22]	Compression	RT-PCR	hPDLCs	Upregulated significantly (30 min)	
Saminathan et al[32]	Tension	RT-PCR	hPDLCs	NR	
Ma et al[33]	Tension	RT-PCR	hPDLCs	NR	
Pei et al[2]	Magnetic stretch	IHC	hPDLCs	Upregulated significantly (within 15 d)	
Duncan et al[18]	NR	Radiolabelling	Mice	NR	
Li et al[27]	Compression and tension	IHC	Sprague-Dawley rats	Upregulated significantly (7 d), non-significant (14 d), non-significant (within 14 d)	
Li et al[28]	Compression tension	Picrosirius red staining, IHC	Sprague-Dawley rats	Downregulated significantly (7 d), downregulated significantly (7 d)	
Thant et al[9]	Compression tension	Picrosirius red staining, IHC	C57BL/6J mice	Downregulated significantly (14 d), non-significant (14 d)	
Bumann et al[16]	Compression tension	ELISA	Extracted human premolars	Upregulated significantly (14 d), non-significant (14 d)	
Anastasi et al[20]	Compression tension	IHC	Extracted human premolars	Upregulated significantly (within 14 d), upregulated significantly (3, 72 h), downregulated significantly (7, 14 d)	
Militi et al[19]	Compression tension	IHC	Extracted human premolars	Upregulated significantly (within 30 d), upregulated significantly (1 d), downregulated significantly (7, 14 d), non-significant (30 d)	

Significance compared to unstimulated control.

ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; NR, not reported.

	COL-III	COL-IV	COL-VI	Other collagens
	Upregulated significantly (5%, 24 h)	NR	NR	NR
	NR	NR	NR	NR
	Downregulated significantly (12 h)	NR	NR	*COL2A1* upregulated significantly (12 h)
	NR	Upregulated significantly (within 7 d)	NR	NR
	NR	NR	NR	NR
	NR	NR	NR	NR
	NR	NR	NR	*COL6A1* downregulated significantly (12 h), *COL8A1* downregulated significantly (12 h), *COL11A1* downregulated significantly (24 h)
	NR	NR	NR	*COL5A1* upregulated significantly (24 h), *COL11A1* downregulated significantly (6 h)
	NR	NR	NR	NR
	Upregulated significantly (3 d)	NR	NR	NR
	NR Upregulated significantly (3, 7 d), non-significant (14 d)	NR	NR	NR
	Upregulated significantly (7 d), upregulated significantly (7 d)	NR	NR	NR
	Non-significant (14 d), non-significant (14 d)	NR	Upregulated significantly (14 d), non-significant (14 d)	COL-II non-significant (14 d), COL-XII upregulated (14 d), COL-II non-significant (14 d), COL-XII non-significant (14 d)
	Upregulated significantly (14 d), non-significant (14 d)	NR	Upregulated significantly (14 d), non-significant (14 d)	COL-V upregulated significantly (14 d), non-significant (14 d)
	NR	Downregulated significantly (3, 72 h), upregulated significantly (7, 14 d), downregulated significantly (3, 72 h), upregulated significantly (7, 14 d)	NR	NR
	NR	Downregulated significantly (1, 7, 14 d), upregulated significantly (21, 30 d), downregulated significantly (within 30 d)	NR	NR

Noncollagenous glycoproteins

In addition to collagens, there are other glycoproteins in the ECM. Most of them are multifunctional macromolecules that combine with cells and other ECM components to affect biological behaviours such as cell survival, proliferation and migration; however, the remodelling pattern of these noncollagenous glycoproteins under orthodontic force remains unclear.

Fibronectin is a common ECM glycoprotein. It coats type I and type III collagen fibres in the PDL, covers the gap between adjacent collagen fibrils and cell membranes, and facilitates cell migration and osteogenic differentiation.[34] The regulation of fibronectin reflects the process of damage and remodelling of the periodontal fibre network. The changes in fibronectin content also adapt to the process of orthodontic tooth movement by regulating cell migration and osteogenic activity. Howard et al[23] first reported the upregulation of fibronectin in PDLCs under stretching force. Subsequently, Militi et al[19] found that after applying orthodontic force to human teeth, the expression of fibronectin decreased at first and then increased to the baseline level on the tension side, whereas its expression on the pressure side increased constantly within 30 days after the force was applied. Similarly, Anastasi et al[20] also found that fibronectin increased on the compression side and decreased on the tension side of the moved teeth; however, He et al[35] reported that compression caused a downregulation of fibronectin expression. After applying centrifugal force to PDLCs, Theilig et al[36] also observed a decrease in fibronectin expression accompanied by a decrease in tenascin and laminin. It is known that tenascin can inhibit cell adhesion to the ECM by binding fibronectin.[37] Elastin is a component of the elastic fibre networks in the ECM, which is conducive to the rapid recovery of the original shape after external force pulling. Currently, only one study mentions that its monomeric form, tropoelastin, was downregulated under tension force.[23] The pattern of changes in other glycoproteins under orthodontic force remains to be defined.

Changes in ECM-related enzymes under orthodontic force

The degradation of ECM collagen fibre bundles and other protein components occurs mainly through intracellular phagocytosis and extracellular enzymatic degradation. Previous studies have shown that PDL fibroblasts can phagocytose collagen. There are cysteine proteases in their lysosomes, which participate in the degradation of phagocytosed collagen.[1] Svoboda et al[38] demonstrated that the turnover rate of collagen in periodontal tissue was related to the amount of collagen ingested by fibroblasts. Nakamura et al[39] showed that inhibition of autophagy induced collagen accumulation in the cytoplasm and led to a decrease in collagen production by PDLCs. Under healthy and stable PDL conditions, the aforementioned intracellular phagocytosis is conducive to more precise and selective degradation of collagen; however, under force application and inflammation conditions, this pathway appears cumbersome and delayed and is easily influenced by cell states. The release of extracellular collagenolytic enzymes is beneficial for rapid and extensive regulation.[40] Matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) and ADAM metallopeptidase with thrombospondin (ADAMTS) are common ECM-related enzymes that all participate in periodontal tissue remodelling processes.

MMPs and TIMPs

MMPs are a family of calcium- and zinc-dependent endopeptidases known to contain 23 types in the human genome. According to the arrangement of their structural domains and substrate preferences, they are divided into six categories: collagenase (MMP-1, 8, 13 and 18), gelatinase (MMP-2 and 9), stromelysins (MMP-3 and 10), matrilysins (MMP-7 and 26), membrane-type MMP (MMP-14, 15, 16, 17, 24 and 25), and other types (MMP-12, 19, 20, 22 and 27).[41] To sustain homeostatic ECM conditions, MMPs are mainly regulated through their expression, zymogen processing activation and the endogenous inhibitor TIMPs. Most studies have focused on the expression changes of MMPs and TIMPs under orthodontic force. Changes in the expression levels of MMPs in previous studies are summarised in Table 2.

MMP-8 is the most abundantly expressed in periodontal tissue and is also one of the main collagenases responsible for collagen I and III degradation. Grimm et al[42] and Nettelhoff et al[43] found that MMP-8 was upregulated in PDLCs under compressive force; however, the results are diverse under tensional force. Ma et al[33] and Saminathan et al[32] found that the expression of the MMP-8 gene was significantly downregulated under tension, whereas Jacobs et al[44] reported the opposite upward trend.

MMP-1 and MMP-2 are common collagenases in periodontal tissue as well. Researchers applied tension to PDLCs for periods ranging from 1 hour to 7 days and found that the expression of MMP-1 was upregulated.[24,26,45,46] Under compression, others found simi-

lar results that the expression of MMP-1 increases in PDLCs.[21,22,47,48] In addition to traditional compression and tension, Zheng et al[49] found that the expression of MMP-1 was significantly upregulated in PDLCs under shear force. Regarding experiments in vivo, Zhang et al[50] and Bildt et al[51] observed that the content of MMP-1 in the gingival crevicular fluid from the compression side and the tension side were both upregulated after orthodontic treatment.

Concerning MMP-2, Bolcato-Bellemin et al[45], Tantilertanant et al[46] and Chen et al[52] found that the expression of MMP-2 in PDLCs was increased under tension, and the results recorded by Lisboa et al[53] showed a downregulation of MMP-2 expression under compression. Zheng et al[49] demonstrated that shear force downregulated MMP-2 in PDLCs in a short period of time and then upregulated MMP-2 after 8 hours. Studies collected gingival crevicular fluid from patients undergoing orthodontic treatment, and the experimental results showed an increase in MMP-2 content, which was consistent with the tension and compression sides.[50,51,54] These studies suggest that under compression, MMP-1 and MMP-8 show an upregulation trend, while MMP-1 and MMP-2 are upregulated under tension. These MMPs increase and jointly participate in the hydrolysis and remodelling of collagen fibres in the PDL.

MMP-9 mainly degrades collagen IV, V, XI and other glycoproteins. Most studies have reported the upregulation of MMP-9 content in gingival crevicular fluid during orthodontic treatment.[50,51,55-57] MMP-3 mainly degrades noncollagenous glycoproteins. Only three studies have reported the relationship between MMP-3 and orthodontic force. Nemoto et al[26] found that MMP-3 expression is downregulated in PDLCs under tension, whereas Tantilertanant et al[46] reported the opposite trend. Under compression, Lisboa et al[48] found that MMP-3 was downregulated. Apart from the mentioned MMPs, a study reported the responses of MMP-10, MMP-11, MMP-14, etc.[41]; however, there are limited data and significant differences.

TIMPs are matrix metalloproteinase inhibitors that can nonspecifically bind to MMPs to inhibit their activity. There are four types of TIMPs (TIMP-1, 2, 3 and 4). Under compression, Redlich et al[21] found that TIMP-1 and TIMP-2 were upregulated; however, Grimm et al[42] and Nettelhoff et al[43] observed that the expression of TIMP-1 decreased instead. Regarding tensional force, most studies have shown that TIMP-1 and TIMP-2 are significantly upregulated at both the mRNA and protein levels in PDLCs.[33,44,45] Bildt et al[51] and Grant et al[55] found that the expression of TIMP-1 and TIMP-2 was significantly upregulated in gingival crevicular fluid regardless of the tension side or the compression side. Jacobs et al[58] also noted a decrease in the expression of TIMP-2 under shear force.

Although there is heterogeneity in the above studies, it is obvious that most types of MMPs and TIMPs showed upregulation trends under orthodontic force and participated in the ECM remodelling of PDL. The process has spatiotemporal differences and is influenced by mechanical force types, involving various collagen and glycoproteins, forming a complex regulatory network. The specific mechanisms still require more detailed and comprehensive experiments and exploration.

Other enzymes

The ADAMTS family is involved in the maturation of collagens and the hydrolysis of ECM. Saminathan et al[32] and Ma et al[33] found that *ADAMTS1* and *ADAMTS8* in PDLCs were upregulated under tension; however, due to the relatively low expression abundance of the ADAMTS family in periodontal tissue, studies in this field are relatively lacking at present. Thant et al[9] observed that mechanical force leads to an increase in the production of other collagen modifying enzymes, such as proline hydroxylase, lysine hydroxylase and transglutaminase, which collectively affect the formation of intermolecular crosslinking and alter tissue structure and mechanical properties.

Changes in ECM adhesion molecules under orthodontic force

The expression changes of ECM-related adhesion molecules can reflect the separation and reattachment that occur during cell rearrangement under mechanical forces. The two processes were kept in a dynamic balanced condition. Integrins are the main adhesion molecules in the PDL and can also serve as mechanical signal receptors, play a mechanical transduction role, mediate various biochemical reactions within cells and promote adaptive changes in cells. Other adhesion molecules include intercellular cell adhesion molecule-1 (ICAM1), osteonectin and tenascin C. There is relatively little research on the changes and functions of various adhesive molecules in the PDL under mechanical force.

Integrins

Integrin is a transmembrane mechanoreceptor that connects ECM components and the intracellular skeleton

Table 2 MMP outcomes from all included studies.

Study	Force type	Methods	Object	Outcome	
				MMP-1	
Bolcato-Bellemin et al[45]	Tension	RT-PCR	hPDLCs	Upregulated significantly (12 h)	
Redlich et al [21]	Compression	RT-PCR	hPDLCs	Upregulated significantly (within 60 min), non-significant (90 min)	
Huang et al[47]	Compression	RT-PCR, ELISA	hPDLCs	Upregulated significantly (30, 60, 90 min)	
Nemoto et al[26]	Tension	RT-PCR, WB	hPDLCs	Upregulated significantly (2–7 d)	
Kook et al[24]	Tension	RT-PCR	hPDLCs	Upregulated significantly (within 12 h)	
Hacopian et al[22]	Compression	RT-PCR	hPDLCs	Upregulated significantly (60 min)	
Saminathan et al[32]	Tension	RT-PCR	hPDLCs	NR	
Zheng et al[49]	Fluid shear stress	RT-PCR, ELISA	hPDLCs	Upregulated significantly (within 12 h)	
Lisboa et al[48]	Compression	ELISA, WB	hPDLCs	Upregulated significantly (30 min)	
Lisboa et al[53]	Compression	Zimography	hPDLCs	NR	
Ma et al[33]	Tension	RT-PCR	hPDLCs	NR	
Nettelhoff et al[43]	Compression	RT-PCR, ELISA	hPDLCs	NR	
Jacobs et al[44]	Tension	RT-PCR, ELISA	hPDLCs	NR	
Tantilertanant et al46	Tension	RT-PCR, ELISA	hPDLCs	Upregulated significantly (6, 24 h)	
Bildt et al51	Compression tension	Zimography, WB	Human GCF	Upregulated significantly (4 wk), upregulated significantly (4 wk)	
Zhang et al50	Compression tension	RT-PCR	Human GCF	Non-significant (within 12 wk), non-significant (within 12 wk)	

Significance compared to unstimulated control.

ELISA, enzyme-linked immunosorbent assay,; GCF, gingival crevicular fluid; NR, not reported; RT-PCR, reverse transcription-polymerase chain reaction; WB, Western blot.

and plays an important role in mechanotransduction and cell migration.[59] Integrin receptors are composed of 18 α subunits and 8 β subunits, forming a total of 24 kinds of αβ heterodimers. According to the types of ECM ligands they bind, integrins can be divided into different subfamilies. The integrins expressed by PDLCs mainly include α1β1, α2β1, α3β1, α4β1, α5β1, α11β1, αvβ3 and αvβ5. Previous studies have explored the changes in the expression patterns of different integrin subunits in periodontitis. It has been demonstrated that changes in cell adhesion and migration mediated by integrins play a crucial role in the formation of the periodontal microenvironment; however, the impact of mechanical force on integrins is still unclear.

Ma et al[33] found that *ITGA5* and *ITGAL* were significantly upregulated in PDLCs after applying tension. Kawamura et al[59] revealed that the expression of ITGA3 and ITGA5 was upregulated in migrated PDLCs and demonstrated the important role of ITGA5 in the migration behaviour of PDLCs. Thus, the upregulation of integrins under mechanical force may indicate an enhanced migration ability of PDLCs. Saminathan et al[32] and Ma et al[33] reported an increase in the expression of ITGA6, ITGB1 and ITGB2 after applying tension. Integrin α6β1 binds to laminin, the downregulation of which may be related to the separation of PDLCs and ECM under mechanical force.

Other adhesion molecules

Osteonectin is a type of calcium binding matrix cell glycoprotein that has anti-adhesion properties and can disrupt cell matrix interactions. Ma et al[33] and Liu et al[60] observed that osteonectin was upregulated in PDLCs after applying cyclic stretch. Ma et al[33] found that tenascin C, which can weaken cell adhesion and upregulate MMP expression and activation, was upregulated in PDLCs under tension. TNC encodes tenascin C, which

	MMP-2	MMP-8	MMP-9	Other MMPs
	Upregulated significantly (12 h)	NR	NR	NR
	NR	NR	NR	NR
	NR	NR	NR	NR
	NR	NR	NR	MMP-3 downregulated significantly (1–7 d)
	NR	NR	NR	NR
	NR	NR	NR	NR
	NR	Downregulated significantly (24 h)	NR	MMP11 downregulated significantly (12 h)
	Upregulated significantly (within 12 h)	NR	NR	NR
	NR	NR	NR	MMP-3 downregulated significantly (30 min), MMP-10 upregulated significantly (30 min)
	Downregulated significantly (30 min)	NR	NR	NR
	NR	Downregulated significantly (6 h)	NR	NR
	NR	Upregulated significantly (12 h)	NR	NR
	NR	Upregulated significantly (12 h)	NR	NR
	Upregulated significantly (6 h)	Non-significant (6, 24 h)	NR	MMP-14 upregulated significantly (6 h)
	Upregulated significantly (4 wk), upregulated significantly (4 wk)	NR	Upregulated significantly (4 wk), non-significant (4 wk)	NR
	Upregulated significantly (12 h–4 k), upregulated significantly (12 h–4 wk)	Non-significant (within 12 wk), non-significant (within 12 wk)	Upregulated significantly (12 h–4 wk), upregulated significantly (12 h–4 wk)	MMP-14 Upregulated significantly (12 h–4 wk), upregulated significantly (12 h–4 wk)

can weaken cell adhesion and upregulate MMP expression and activation. ICAM1 is an intercellular adhesion molecule. Some studies reported that *ICAM1* was upregulated in PDLCs after stretching.[25,32,33] This may be related to the enhanced adhesion between PDLCs under mechanical force. In addition, studies have found changes in the expression of other adhesion-related molecular genes, such as *CTGF*, *CTNND2*, *TGFBI* and *CLEC3B* in PDLCs under mechanical force, but their specific functions and mechanisms still need to be explored.

Conclusion

This article reviewed the effects of mechanical forces on ECM remodelling in the PDL. Exploring the process of PDL remodelling under mechanical force can facilitate understanding of the mechanism of orthodontic tooth movement. By regulating some of these targets, it is able to accelerate tooth movement and avoid periodontal damage caused by excessive inflammatory reactions during orthodontic tooth movement.

Conflicts of interest

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

Author contribution

Dr Qian Yao YU contributed to the literatures collection and manuscript draft; Dr Yi Ping HUANG contributed to manuscript revision; Dr Wei Ran LI contributed to the study design. All the authors approved the final manuscript.

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References

1. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: A unique, multifunctional connective tissue. Periodontol 2000 1997;13:20–40.
2. Pei D, Wang M, Li W, et al. Remodeling of aligned fibrous extracellular matrix by encapsulated cells under mechanical stretching. Acta Biomater 2020;112:202–212.
3. Hynes RO. The extracellular matrix: Not just pretty fibrils. Science 2009;326:1216–1219.
4. Zhang JC, Song ZC, Xia YR, Shu R. Extracellular matrix derived from periodontal ligament cells maintains their stemness and enhances redifferentiation via the wnt pathway. J Biomed Mater Res A 2018;106:272–284.
5. Lin Z, Nica C, Sculean A, Asparuhova MB. Enhanced wound healing potential of primary human oral fibroblasts and periodontal ligament cells cultured on four different porcine-derived collagen matrices. Materials (Basel) 2020;13:3819.
6. Bissell MJ, Aggeler J. Dynamic reciprocity: How do extracellular matrix and hormones direct gene expression? Prog Clin Biol Res 1987;249:251–262.
7. Butler WT, Birkedal-Hansen H, Beegle WF, Taylor RE, Chung E. Proteins of the periodontium. Identification of collagens with the [alpha1(I)]2alpha2 and [alpha1(III)]3 structures in bovine periodontal ligament. J Biol Chem 1975;250:8907–8912.
8. Yamauchi M, Katz EP, Mechanic GL. Intermolecular crosslinking and stereospecific molecular packing in type I collagen fibrils of the periodontal ligament. Biochemistry 1986;25:4907–4913.
9. Thant L, Kaku M, Kakihara Y, et al. Extracellular matrix-oriented proteomic analysis of periodontal ligament under mechanical stress. Front Physiol 2022;13:899699.
10. Connizzo BK, Sun L, Lacin N, et al. Nonuniformity in periodontal ligament: Mechanics and matrix composition. J Dent Res 2021;100:179–186.
11. Denes BJ, Ait-Lounis A, Wehrle-Haller B, Kiliaridis S. Core matrisome protein signature during periodontal ligament maturation from pre-occlusal eruption to occlusal function. Front Physiol 2020;11:174.
12. Li Q, Luo T, Lu W, Yi X, Zhao Z, Liu J. Proteomic analysis of human periodontal ligament cells under hypoxia. Proteome Sci 2019;17:3.
13. Bielajew BJ, Hu JC, Athanasiou KA. Collagen: Quantification, biomechanics, and role of minor subtypes in cartilage. Nat Rev Mater 2020;5:730–747.
14. Sodek J. Collagen turnover in periodontal ligament. The Biology of Tooth Movement 1989:157–181.
15. Kim HJ, Kim SM, Choi MJ, Jang YJ. Golgin subfamily A member 5 is essential for production of extracellular matrix proteins during TGF-β1-induced periodontal ligament-fibroblastic differentiation. Stem Cells Int 2022;2022:3273779.
16. Bumann A, Carvalho RS, Schwarzer CL, Yen EH. Collagen synthesis from human PDL cells following orthodontic tooth movement. Eur J Orthod 1997;19:29–37.
17. Fan XP, Zhao YF, Liu WP, Zhao XQ. Expression of type III collagen mRNA in rat periodontal ligament in response to different occlusal force [in Chinese]. Sichuan Da Xue Xue Bao Yi Xue Ban 2005;36:843–846.
18. Duncan GW, Yen EH, Pritchard ET, Suga DM. Collagen and prostaglandin synthesis in force-stressed periodontal ligament in vitro. J Dent Res 1984;63:665–669.
19. Militi A, Cutroneo G, Favaloro A, et al. An immunofluorescence study on VEGF and extracellular matrix proteins in human periodontal ligament during tooth movement. Heliyon 2019;5:e02572.
20. Anastasi G, Cordasco G, Matarese G, et al. An immunohistochemical, histological, and electron-microscopic study of the human periodontal ligament during orthodontic treatment. Int J Mol Med 2008;21:545–554.
21. Redlich M, Roos H, Reichenberg E, et al. The effect of centrifugal force on mRNA levels of collagenase, collagen type-I, tissue inhibitors of metalloproteinases and beta-actin in cultured human periodontal ligament fibroblasts. J Periodontal Res 2004;39:27–32.
22. Hacopian N, Nik TH, Ghahremani MH, Rahimi HR, Ostad SN. Effects of continuous and interrupted forces on gene transcription in periodontal ligament cells in vitro. Acta Med Iran 2011;49:643–649.
23. Howard PS, Kucich U, Taliwal R, Korostoff JM. Mechanical forces alter extracellular matrix synthesis by human periodontal ligament fibroblasts. J Periodontal Res 1998;33:500–508.
24. Kook SH, Jang YS, Lee JC. Involvement of JNK-AP-1 and ERK-NF-κB signaling in tension-stimulated expression of type I collagen and MMP-1 in human periodontal ligament fibroblasts. J Appl Physiol 2011;111:1575–1583.
25. Wescott DC, Pinkerton MN, Gaffey BJ, Beggs KT, Milne TJ, Meikle MC. Osteogenic gene expression by human periodontal ligament cells under cyclic tension. J Dent Res 2007;86:1212–1216.
26. Nemoto T, Kajiya H, Tsuzuki T, Takahashi Y, Okabe K. Differential induction of collagens by mechanical stress in human periodontal ligament cells. Arch Oral Biol 2010;55:981–987.
27. Li X, Zhang L, Wang N, Feng X, Bi L. Periodontal ligament remodeling and alveolar bone resorption during orthodontic tooth movement in rats with diabetes. Diabetes Technol Ther 2010;12:65–73.
28. Li Z, Yu M, Jin S, et al. Stress distribution and collagen remodeling of periodontal ligament during orthodontic tooth movement. Front Pharmacol 2019;10:1263.
29. Wen Y, Yang H, Wu J, et al. COL4A2 in the tissue-specific extracellular matrix plays important role on osteogenic differentiation of periodontal ligament stem cells. Theranostics 2019;9:4265–4286.
30. Atkuru S, Muniraj G, Sudhaharan T, Chiam KH, Wright GD, Sriram G. Cellular ageing of oral fibroblasts differentially modulates extracellular matrix organization. J Periodontal Res 2021;56:108–120.
31. Cescon M, Gattazzo F, Chen P, Bonaldo P. Collagen VI at a glance. J Cell Sci 2015;128:3525–3531.
32. Saminathan A, Vinoth KJ, Wescott DC, et al. The effect of cyclic mechanical strain on the expression of adhesion-related genes by periodontal ligament cells in two-dimensional culture. J Periodontal Res 2012;47:212–221.
33. Ma J, Zhao D, Wu Y, Xu C, Zhang F. Cyclic stretch induced gene expression of extracellular matrix and adhesion molecules in human periodontal ligament cells. Arch Oral Biol 2015;60:447–455.
34. Shirbhate U, Bajaj P, Pandher J, Durge K. Fibronectin and its applications in dentistry and periodontics: A cell behaviour conditioner. Cureus 2022;14:e30702.
35. He Y, Macarak EJ, Korostoff JM, Howard PS. Compression and tension: Differential effects on matrix accumulation by periodontal ligament fibroblasts in vitro. Connect Tissue Res 2004;45:28–39.

36. Theilig C, Bernd A, Leyhausen G, Kaufmann R, Geurtsen W. Effects of mechanical force on primary human fibroblasts derived from the gingiva and the periodontal ligament. J Dent Res 2001;80:1777–1780.
37. Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T. Tenascin: An extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 1986;47:131–139.
38. Svoboda EL, Shiga A, Deporter DA. A stereologic analysis of collagen phagocytosis by fibroblasts in three soft connective tissues with differing rates of collagen turnover. Anat Rec 1981;199:473–480.
39. Nakamura T, Yamashita M, Ikegami K, et al. Autophagy facilitates type I collagen synthesis in periodontal ligament cells. Sci Rep 2021;11:1291.
40. Sodek J, Overall CM. Matrix degradation in hard and soft connective tissues. In: Davidovitch Z (ed). The Biological Mechanisms of Tooth Eruption and Root Resorption. Birmingham: EBSCO Media;1988:303–311.
41. Behm C, Nemec M, Weissinger F, Rausch MA, Andrukhov O, Jonke E. MMPs and TIMPs expression levels in the periodontal ligament during orthodontic tooth movement: A systematic review of in vitro and in vivo studies. Int J Mol Sci 2021;22:6967.
42. Grimm S, Wolff E, Walter C, et al. Influence of clodronate and compressive force on IL-1ß-stimulated human periodontal ligament fibroblasts. Clin Oral Investig 2020;24:343–350.
43. Nettelhoff L, Grimm S, Jacobs C, et al. Influence of mechanical compression on human periodontal ligament fibroblasts and osteoblasts. Clin Oral Investig 2016;20:621–629.
44. Jacobs C, Schramm S, Dirks I, et al. Mechanical loading increases pro-inflammatory effects of nitrogen-containing bisphosphonate in human periodontal fibroblasts. Clin Oral Investig 2018;22:901–907.
45. Bolcato-Bellemin AL, Elkaim R, Abehsera A, Fausser JL, Haikel Y, Tenenbaum H. Expression of mRNAs encoding for alpha and beta integrin subunits, MMPs, and TIMPs in stretched human periodontal ligament and gingival fibroblasts. J Dent Res 2000;79:1712–1716.
46. Tantilertanant Y, Niyompanich J, Everts V, Supaphol P, Pavasant P, Sanchavanakit N. Cyclic tensile force-upregulated IL6 increases MMP3 expression by human periodontal ligament cells. Arch Oral Biol 2019;107:104495.
47. Huang SF, Li YH, Ren YJ, Cao ZG, Long X. The effect of a single nucleotide polymorphism in the matrix metalloproteinase-1 (MMP-1) promoter on force-induced MMP-1 expression in human periodontal ligament cells. Eur J Oral Sci 2008;116:319–323.
48. Lisboa RA, Andrade MV, Cunha-Melo JR. Toll-like receptor activation and mechanical force stimulation promote the secretion of matrix metalloproteinases 1, 3 and 10 of human periodontal fibroblasts via p38, JNK and NF-kB. Arch Oral Biol 2013;58:731–739.
49. Zheng L, Huang Y, Song W, et al. Fluid shear stress regulates metalloproteinase-1 and 2 in human periodontal ligament cells: Involvement of extracellular signal-regulated kinase (ERK) and P38 signaling pathways. J Biomech 2012;45:2368–2375.
50. Zhang B, Yang L, Zheng W, Lin T. MicroRNA-34 expression in gingival crevicular fluid correlated with orthodontic tooth movement. Angle Orthod 2020;90:702–706.
51. Bildt MM, Bloemen M, Kuijpers-Jagtman AM, Von den Hoff JW. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid during orthodontic tooth movement. Eur J Orthod 2009;31:529–535.
52. Chen YJ, Jeng JH, Chang HH, Huang MY, Tsai FF, Yao CC. Differential regulation of collagen, lysyl oxidase and MMP-2 in human periodontal ligament cells by low- and high-level mechanical stretching. J Periodontal Res 2013;48:466–474.
53. Lisboa RA, Lisboa FA, de Castro Santos G, Andrade MV, Cunha-Melo JR. Matrix metalloproteinase 2 activity decreases in human periodontal ligament fibroblast cultures submitted to simulated orthodontic force. In Vitro Cell Dev Biol Anim 2009;45:614-621.
54. Cantarella G, Cantarella R, Caltabiano M, Risuglia N, Bernardini R, Leonardi R. Levels of matrix metalloproteinases 1 and 2 in human gingival crevicular fluid during initial tooth movement. Am J Orthod Dentofacial Orthop 2006;130:568.e11–e16.
55. Grant M, Wilson J, Rock P, Chapple I. Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. Eur J Orthod 2013;35:644–651.
56. Alikhani M, Chou MY, Khoo E, et al. Age-dependent biologic response to orthodontic forces. Am J Orthod Dentofacial Orthop 2018;153:632–644.
57. Surlin P, Silosi I, Rauten AM, Cojocaru M, Foia L. Involvement of TSP1 and MMP9/NGAL in angiogenesis during orthodontic periodontal remodeling. ScientificWorldJournal 2014;2014:421029.
58. Jacobs C, Walter C, Ziebart T, et al. Induction of IL-6 and MMP-8 in human periodontal fibroblasts by static tensile strain. Clin Oral Investig 2014;18:901–908.
59. Kawamura M, Yamamoto T, Yamashiro K, et al. Induction of migration of periodontal ligament cells by selective regulation of integrin subunits. J Cell Mol Med 2019;23:1211–1223.
60. Liu M, Dai J, Lin Y, et al. Effect of the cyclic stretch on the expression of osteogenesis genes in human periodontal ligament cells. Gene 2012;491:187–193.

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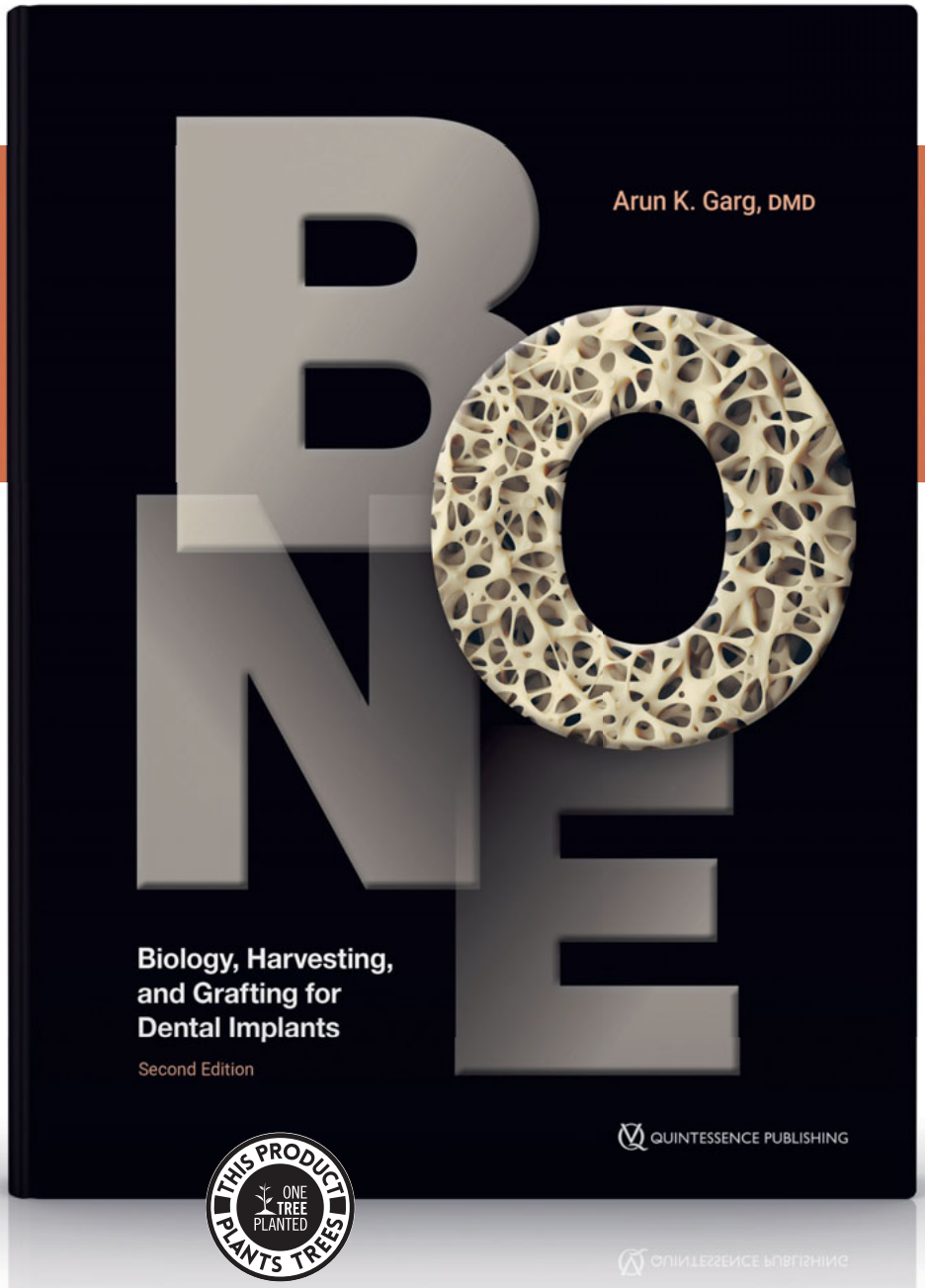

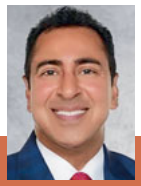

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Identification of Cuproptosis-related Gene Lipoyltransferase 1 as a Promising Biomarker in Oral Squamous Cell Carcinoma

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Objective: *To find efficient cuproptosis-related biomarkers to explore the oncogenesis and progression of oral squamous cell carcinoma (OSCC).*
Methods: *All the original data were downloaded from the Cancer Genome Atlas (TCGA) database. Univariate Cox analysis and Kaplan-Meier survival analysis were used to identify the gene related to survival. Tumor Immune Estimation Resource 2.0 (TIMER 2.0) was used to reveal the different expression of cuproptosis-related gene lipoyltransferase 1 (LIPT1) in various kinds of tumours.*
Results: *LIPT1, as a cuproptosis-related gene, was found to be differentially expressed in the OSCC group and the control group. It was also found to be related to the prognosis of OSCC. Pan cancer analysis showed LIPT1 was also involved in various kinds of tumours.*
Conclusion: *All the results demonstrate that the cuproptosis-related gene LIPT1 is highly involved in the oncogenesis and progression of OSCC. These findings give new insight for further research into the cuproptosis-related biomarkers in OSCC.*
Keywords: *biomarker, lipoyltransferase 1, oral squamous cell carcinoma*
Chin J Dent Res 2024;27(2):133–141; doi: 10.3290/j.cjdr.b5459587

Oral squamous cell carcinoma (OSCC) is a malignant tumour that has been associated with the oral microbiome.[1] Among all oral malignant and premalignant lesions, OSCC has the highest occurrence[2], and the incidence rate increases with age.[3] Despite advancements in treatments, the 5-year survival rate for OSCC remains relatively low at 64%.[4] Thus, there is an urgent need to discover new biomarkers to improve therapy for OSCC.

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Recently, immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) have been utilised in therapy; however, resistance to immunotherapy is a frequently encountered issue.[5] Therefore, exploring alternative therapies such as ferroptosis and apoptosis may provide new insights into enhancing the survival and prognosis of OSCC patients.[6-8] Cuproptosis, a distinct form of cell death, is triggered by the interaction of copper with lipoylated components of the tricarboxylic acid cycles.[9] However, the specific biomarkers associated with cuproptosis in relation to OSCC remain largely unknown. Thus, the present study aims to explore a novel therapeutic approach for OSCC based on cuproptosis. To achieve this, it is crucial to identify key cuproptosis-related genes, which will further elucidate the underlying mechanisms of OSCC.

The present authors identified 10 cuproptosis-related genes based on the existing literature (Fig 1).[9] Among these genes, lipoyltransferase 1 (LIPT1) exhibited lower expression levels in OSCC samples compared to para-tumour samples. In addition, increased expression of LIPT1 was found to be associated with a poorer

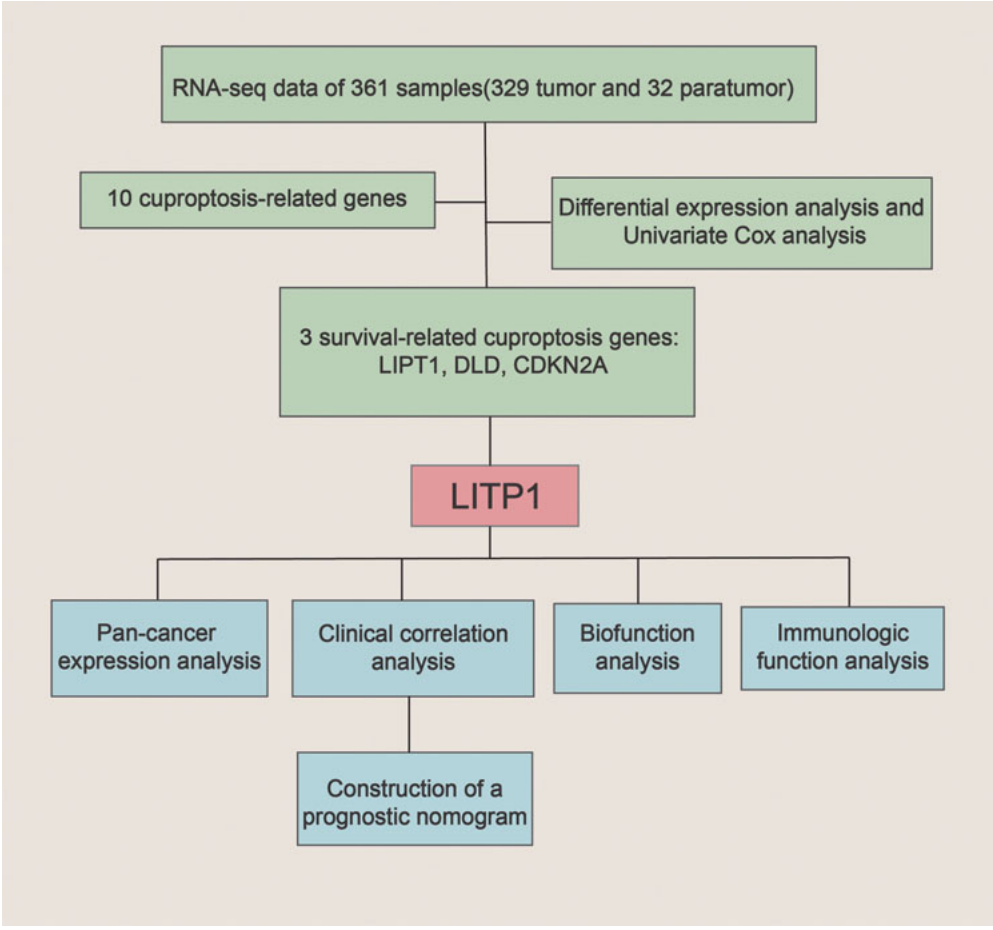

Fig 1 Study flowchart.

prognosis among individuals diagnosed with OSCC. Pan-cancer analysis was conducted to assess the role of LIPT1 across various tumour types. A nomogram incorporating clinicopathological characteristics was developed to predict the 1-, 3- and 5-year overall survival of OSCC patients. To explore the potential mechanisms involving LIPT1, the authors performed an analysis of genes associated with LIPT1. Additionally, Gene Ontology (GO)[10] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[11] enrichment analyses were conducted. The present authors' comprehensive research efforts aim to identify a potential therapeutic target for cuproptosis and provide insights into the prognosis and therapy for OSCC.

Materials and methods

Data acquisition and preprocessing

RNA sequencing data were obtained from The Cancer Genome Atlas (TCGA) and Head and Neck Squamous Cell Carcinoma (HNSC) database (https://portal.gdc.cancer.gov).[12] The data were downloaded and the expression values were converted into Log2 Fragments Per Kilobase Million (FPKM) format. A total of 361 samples with clinical data were selected, excluding samples from non-oral locations such as the hypopharynx, larynx, oropharynx and tonsils. Samples without clinical data were excluded from the analysis.

Identification of cuproptosis-related genes and survival analysis

Ten cuproptosis-related genes, namely ferredoxin 1 (FDX1, Gene ID: 2230), lipoic acid synthetase (LIAS, Gene ID: 11019), LIPT1 (Gene ID: 51601), dihydrolipoamide dehydrogenase (DLD, Gene ID: 1738), dihydrolipoamide S-acetyltransferase (DLAT, Gene ID: 1737), pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1, Gene ID: 5160), pyruvate dehydrogenase E1 subunit beta (PDHB, Gene ID: 5162), glutaminase (GLS, Gene ID: 2744), cyclin dependent kinase inhibitor 2A (CDKN2A, Gene ID: 1029) and metal regulatory transcription factor 1 (MTF1, ID: 4520), were analysed individually to determine the gene that exhibited differential expression between the para-

tumour group and tumour group samples. Univariate Cox analysis and Kaplan-Meier survival analysis were performed using R survival and survminer software (R Core Team, Vienna, Austria) to identify genes associated with overall survival.[13] The univariate Cox analysis involved fitting a Cox proportional hazards model to the gene expression data using the survival package in R.[14] This model calculates hazard ratios and their associated *P* values, providing a measure of the gene's impact on overall survival. For the Kaplan-Meier survival analysis, the survminer package was used to estimate the probability of survival over time for different gene expression groups. Log-rank tests were performed to compare the survival curves between these groups, determining if there were significant differences in survival outcomes based on gene expression levels. These methods were chosen for their ability to effectively assess the association between gene expression and overall survival in this specific dataset, providing robust statistical analysis for the present study.

Gene expression and clinical prognosis of LIPT1

Expression analysis and clinical characteristics

To assess the expression pattern of LIPT1 across different cancers, the present authors utilised TIMER 2.0 (Tumor Immune Estimation Resource 2.0, http://timer.cistrome.org) to show the expression situation of LIPT1 in different cancers.[15] A Wilcoxon rank-sum test and Dunn test were employed to examine the differential expression of LIPT1 in relation to clinicopathological characteristics such as age, sex and histological grade. These analyses were conducted using the R package ggplot2.[16] Additionally, a nomogram incorporating clinicopathological characteristics was developed using the R packages rms and survival to predict the prognosis of OSCC patients. Furthermore, univariate and multivariate Cox regression analyses were performed to demonstrate that LIPT1 serves as an independent factor for assessing the overall survival of OSCC patients.

Correlation analysis of LIPT1 in OSCC

Pearson correlation analysis was conducted to evaluate the correlation of LIPT1 with other genes, considering a correlation coefficient with an absolute value greater than 0.5 and a significance threshold of $P < 0.05$. The correlation heatmap was generated using the R package ggplot2.

Differential expression analysis and functional enrichment analysis

To identify differentially expressed genes (DEGs), samples were divided into two groups based on the median expression of LIPT1, and DEGs were determined using the criteria $|LogFC| > 2$ and $P < 0.05$. Functional enrichment analyses, including GO and KEGG analysis, were performed to elucidate the molecular mechanisms and associated signalling pathways of the DEGs. These analyses were conducted using the R package clusterProfiler.[17]

Immune checkpoint and infiltration assay

To explore the relationship between LIPT1 and immune cell infiltration, the present authors utilised the R package GSVA and conducted single-sample Gene Set Enrichment Analysis (ssGSEA). Spearman correlation analysis was conducted to assess the relationship between LIPT1 and immune checkpoint genes, and a correlation heatmap was generated to visualise the results.

Results

Deregulation of LIPT1 in OSCC

In this study, a total of 361 RNA sequencing data samples, consisting of 329 tumour and 32 paratumour samples, were obtained from the TCGA database. Among the ten cuproptosis-related genes analysed, FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1 and PDHB showed higher expression in the paratumour group, whereas GLS and CDKN2A exhibited lower expression in the paratumour group. The gene MTF1 did not show significant differential expression between the tumour and paratumour groups.

Based on univariate Cox analysis (Fig 2a, $P < 0.05$), we identified LIPT1, DLD, and CDKN2A as prognostic genes among the differentially expressed genes. Subsequently, K-M analysis revealed that LIPT1 was specifically associated with overall survival and was selected for further analysis (Fig 2b, $P < 0.05$). Moreover, it has been reported that LIPT1 is involved in various other tumorigenesis, including liver hepatocellular carcinoma, melanoma and prostate cancer.[18-20] The area under the curves (AUCs) for LIPT1 in relation to overall survival was calculated as 0.550, 0.631 and 0.635 at 1, 3 and 5 years, respectively (Fig 2c).[21] Moreover, the authors observed a decrease in the expression level

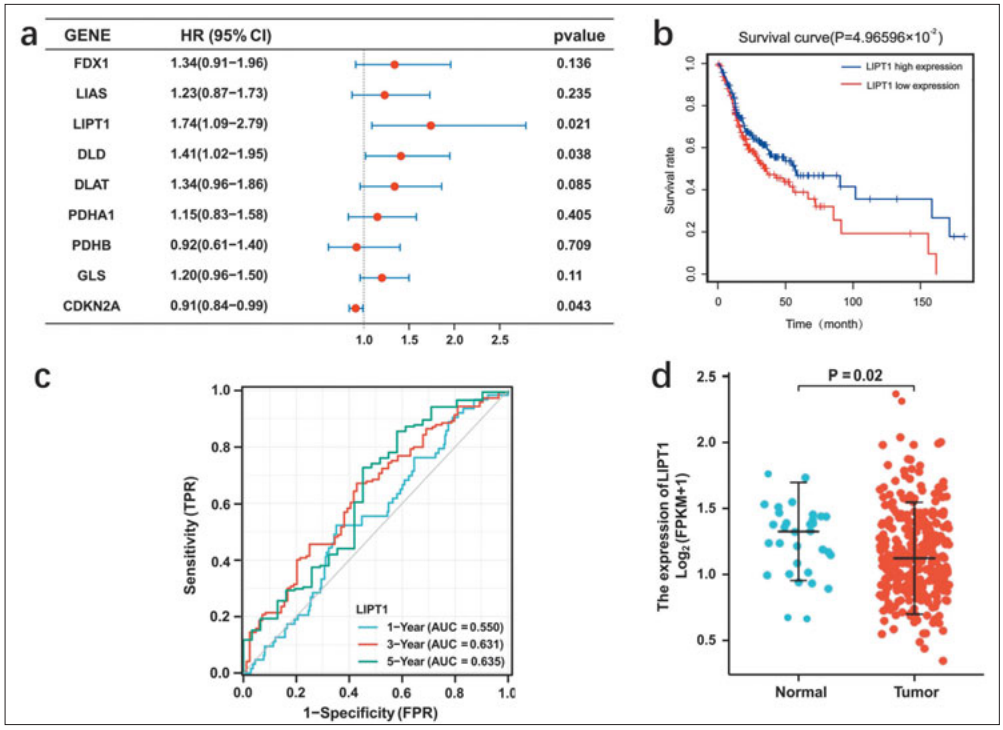

Fig 2 Identification of prognostic genes associated with cuproptosis in OSCC. Univariate Cox regression analysis results presented in a forest plot **(a)**. Kaplan-Meier survival curves illustrating overall survival in OSCC patients based on LIPT1 tumour expression **(b)**. Time-dependent receiver operating characteristic curves showcasing the predictive value of LIPT1 for overall survival at 1, 3 and 5 years **(c)**. Expression of LIPT1 was found to be lower in OSCC samples compared to normal samples **(d)**.

of LIPT1 in the tumour group compared to the paratumour group (Fig 2d).

Pan-cancer expression analysis of LIPT1

A pan-cancer expression analysis of LIPT1 was performed (Fig 3, $P < 0.05$). In CHOL, COAD, ESCA, GBM, LIHC and STAD, LIPT1 demonstrated elevated expression in the tumor group when compared to the normal group. Conversely, in BRCA, CESC, KICH, KIRC, KIRP, THCA and UCEC, LIPT1 exhibited decreased expression in the tumor group compared to the normal group. Additionally, LIPT1 expression was higher in the HNSC-HPV+ group compared to the HNSC-HPV- group, as well as in the SKCM metastasis group compared to the SKCM group.

Differential expression of LIPT1 in clinicopathological characteristics of OSCC and predictive nomogram

Significantly higher expression of LIPT1 was observed in the male group compared to the female group (Fig 4a, $P < 0.05$). In terms of histological grade, LIPT1 exhibited higher expression in grades 3 and 4 compared to grade 1, and higher expression in grades 3 and 4 compared to grade 2 (Fig 4b, $P < 0.05$). A prognostic nomogram was constructed based on clinicopathological characteristics including age, sex, race, smoking history, T stage, alcohol history, histological grade, N stage and LIPT1 expression (Fig 4c). The calibration curves demonstrated that the model fit well with the optimal one, reflecting 1-, 3- and 5-year overall survival (Fig 4d). Table 1 was utilised to conduct univariate and multivariate Cox regression analyses, aiming to determine whether clinicopathological characteristics could act as independent predictors. The results indicated that LIPT1 expression was an independent predictor associated with overall survival.

Genes correlated with LIPT1 and differentially expressed genes in OSCC

Using a Pearson correlation analysis, we identified 300 genes that exhibited a correlation coefficient > 0.5 and a significant P value (< 0.05) with LIPT1 (Table S1). The top 9 genes showing the strongest correlation with LIPT1 were selected to generate a correlation heatmap (Fig 5a). Furthermore, the authors identified 141 genes that showed differential expression between the LIPT1 high expression group and the low expression group, meeting the criteria of $|\log2FoldChange| > 2$ and $P < 0.05$ (data provided on request). Out of these genes, 8 were found to be downregulated whereas 132 were upregulated in the LIPT1 high expression group. GO analysis

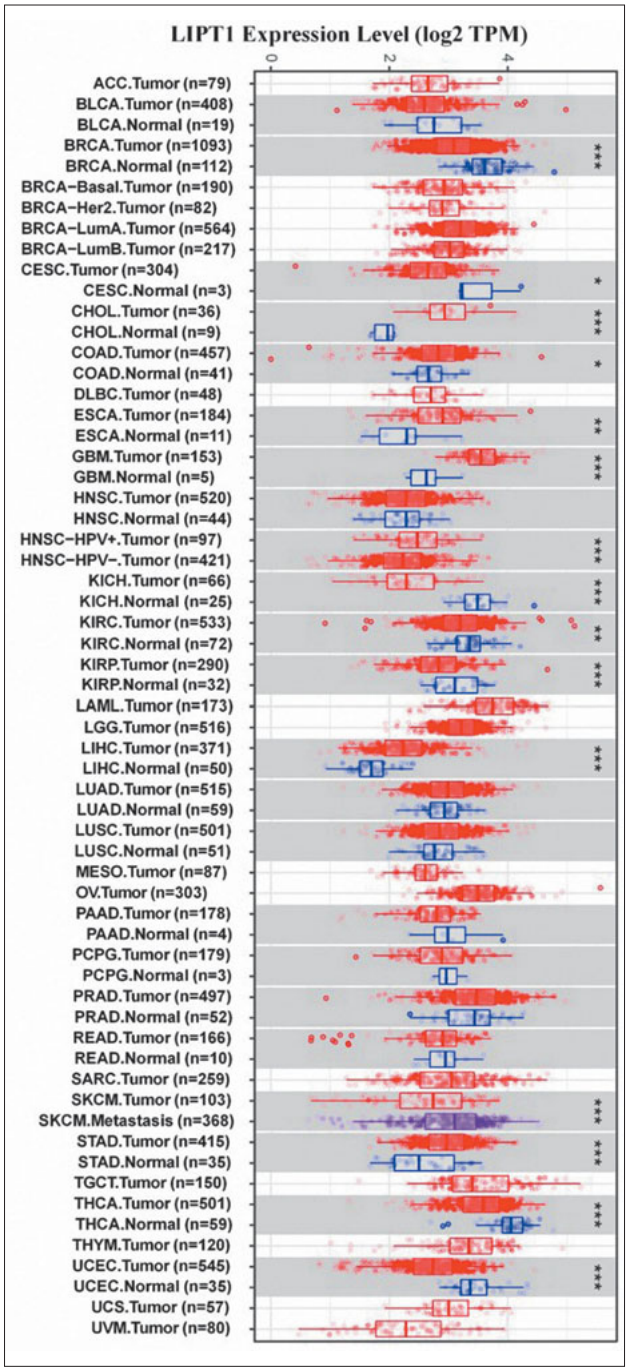

Fig 3 Pan-cancer expression analysis of LIPT1 in normal and tumour samples according to TIMER 2.0. BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; ESCA, oesophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LUSC, lung squamous cell carcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma (*P < 0.05, **P < 0.01, ***P < 0.001).

highlighted GO:0001523 (retinoid metabolic process) as the most significantly associated biological process for these genes (Fig 5b). Furthermore, KEGG analysis indicated that the most important pathway involved was hsa00830 (retinol metabolism) (Fig 5c).

Immune cell infiltration and immune checkpoint genes

Using ssGSEA, the relationship between LIPT1 and immune cells was analysed (Fig 6a). It was observed that dendritic cells (DCs), immature DCs (iDCs), mast cells, neutrophils, NK CD56dim cells, central memory T cells (Tcm), Th1 cells, regulatory T cells (Tregs) and eosinophils showed a negative correlation with LIPT1. Conversely, NK CD56bright cells and T helper cells exhibited a positive correlation with LIPT1. Furthermore, the correlation between LIPT1 and immune checkpoint genes was explored, and the results were presented in a correlation heatmap (Fig 6b). LIPT1 showed a positive correlation with TNFRSF25, TNFSF15, TNFSF4, TNFRSF18, TNFRSF4, CD200R1, CTLA4 and TNFRSF14, and a negative correlation with PDCDILG2 (P < 0.05).

Discussion

LIPT1, a pro-cuproptosis gene, is responsible for lipoate transfer and is typically considered downstream of LIPT2.[22] LIPT1 deficiency can manifest as early infantile epileptic encephalopathy, Leigh disease and secondary pyruvate dehydrogenase complex deficiency.[23] LIPT1 also plays a crucial role in the transfer of the lipoyl group from the H-protein of the glycine cleavage system to the E2 subunits of mitochondrial 2-ketoacid dehydrogenase complexes. This process is essential for the proper functioning of these complexes, which are involved in key metabolic pathways such as the tricarboxylic acid (TCA) cycle.[24] Dysfunction of LIPT1 may lead to alterations in TCA cycle metabolism.[25,26] Despite advancements in various treatment modalities, the prognosis for OSCC patients remains poor.[27-29] Cuproptosis has shown potential in overcoming resistance that may arise during chemotherapy treatment of malignant cells; however, the mechanisms underlying cuproptosis, particularly in the context of OSCC, are still largely unknown. Thus, the present authors' research aims to explore potential prognostic biomarkers associated with cuproptosis in OSCC.

A study integrated genomics and metabolomics to identify the cause of lactic acidosis and epilepsy, and revealed that LIPT1 mutations lead to metabolic defects and broader aspects of inborn errors of metabolism

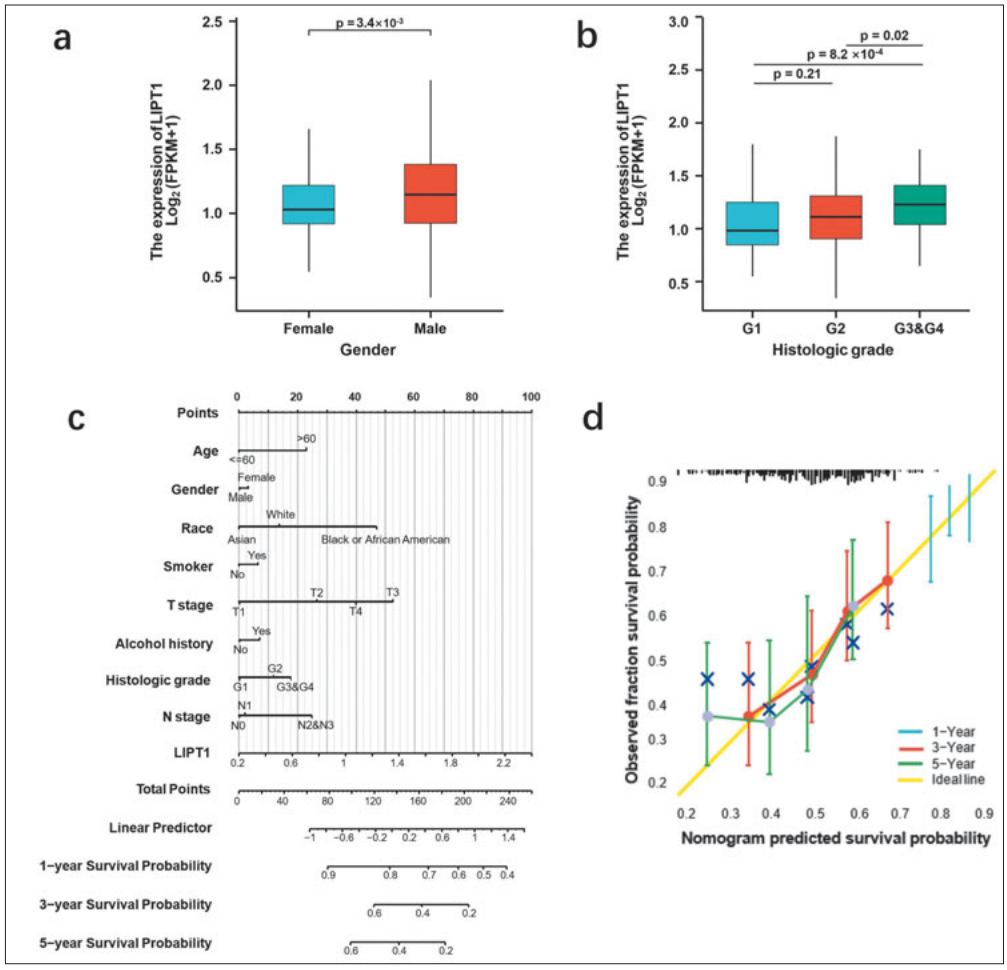

Fig 4 Correlation between LIPT1 and clinical characteristics. Sex distribution in the high and low LIPT1 expression groups **(a)**. Histological grade distribution in the high and low LIPT1 expression groups. G1-4, histologic grade 1-4, based on clinical characteristics **(b)**. Nomogram for predicting overall survival, incorporating age, sex, race, smoking history, T stage, alcohol history, histological grade, N stage and LIPT1 expression as parameters **(c)**. Calibration curves of the nomogram for predicting 1-, 3-, 5- and 10-year overall survival **(d)**.

Table 1 Univariate and multivariate Cox regression analysis of clinical characteristics for overall survival prediction.

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age	1.320 (0.954–1.826)	0.094	1.413 (1.011–1.975)	0.043
Sex	0.908 (0.648–1.273)	0.576	NA	NA
Race	1.109 (0.353–3.485)	0.860	NA	NA
Smoking	1.248 (0.845–1.844)	0.265	NA	NA
T stage	1.289 (0.603–2.755)	0.513	NA	NA
Alcohol history	1.045 (0.740–1.475)	0.804	NA	NA
Histological grade	1.523 (0.950–2.442)	0.081	1.314 (0.798–2.163)	0.283
N stage	1.320 (0.955–1.824)	0.092	1.401 (1.008–1.948)	0.045
LIPT1	1.742 (1.088–2.789)	0.021	1.845 (1.126–3.021)	0.015

NA, not available.

(IEM) pathophysiology.[25] LIPT1 is an essential enzyme for the activation of mitochondrial 2-ketoacid dehydrogenase, which is involved in fatty acylation. LIPT1 deficiency leads to impaired lipoylation and activity of 2-ketoacid dehydrogenase, resulting in increased 2-HG and depletion of structural lipids in plasma. While LIPT1 deficiency impedes lipogenesis, it increases fatty acid oxidation and regulates the balance between oxidative and reductive glutamine metabolism.[25] Pathogenic variants in the LIPT1 gene have been linked to severe lactic acidosis and poor neurocognitive outcomes, leading to neonatal death. A study presented the

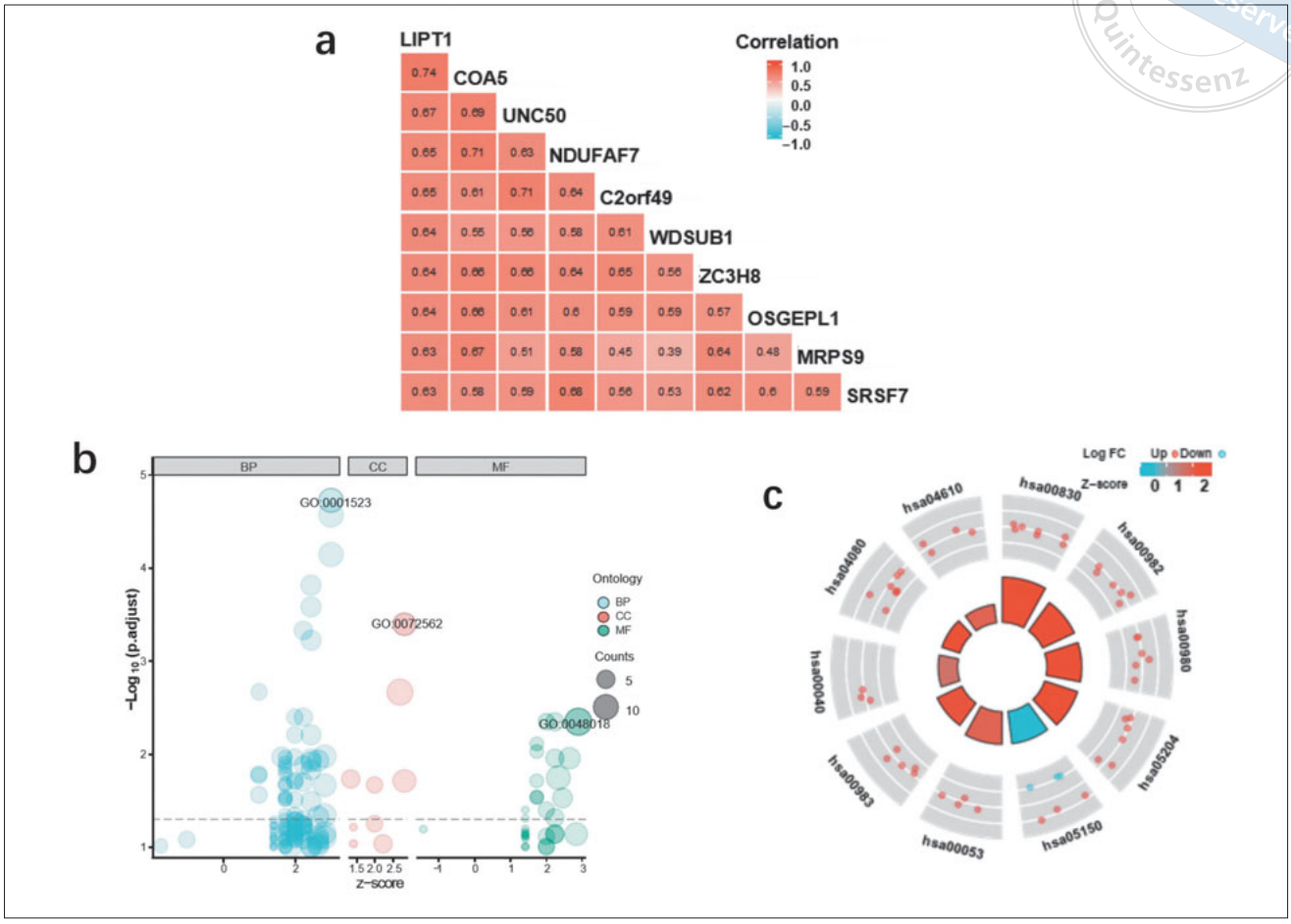

Fig 5 Identification of related genes, pathways and cellular functions of LIPT1. Heatmap showing the correlation of LIPT1 with 9 genes in OSCC samples **(a)**. GO analysis results: GO:0001523 (retinoid metabolic process), GO:0072562 (blood microparticle), GO:0048018 (receptor ligand activity) **(b)**. KEGG enrichment analysis results: hsa00830 (retinol metabolism), hsa00982 (drug metabolism - cytochrome P450), hsa00980 (metabolism of xenobiotics by cytochrome P450), hsa05204 (chemical carcinogenesis), hsa05150 (*Staphylococcus aureus* infection), hsa00053 (ascorbate and aldarate metabolism), hsa00983 (drug metabolism – other enzymes), hsa00040 (pentose and glucuronate interconversions), hsa04080 (neuroactive ligand-receptor interaction), hsa04610 (complement and coagulation cascades) **(c)**.

case of a 2-month-old boy with LIPT1 deficiency and its progression to early infantile epileptic encephalopathy, highlighting the need for exome sequencing to diagnose LIPT1 deficiency and its overlap with other metabolic disorders.[23] LIPT1 has been identified as a novel prognostic target in liver hepatocellular carcinoma and skin cutaneous melanoma.[19,20] In the present study, it was also identified as a prognosis-related cuproptosis gene. The expression of LIPT1 was significantly lower in the OSCC group compared to the paratumour group. However, during the prognostic analysis, the authors discovered that lower expression of LIPT1 correlated with a better prognosis. Cuproptosis may exhibit different effects in distinct situations. On one hand, the upregulation of LIPT1 in the paratumour group suggests that an LIPT1 deficiency might reduce cuproptosis, which plays a role in targeting tumour cell death in OSCC initiation. On the other hand, as the tumour progresses over time, the expression of LIPT1 increases, but the prognosis of OSCC patients worsens. This finding aligns with the observations made by Chen[30] in uterine corpus endometrial carcinoma. Furthermore, in pan-cancer analysis, LIPT1 was found to be downregulated in BRCA, CESC, KICH, KIRC, KIRP and THCA, indicating that LIPT1, as a pro-cuproptosis gene, may contribute to molecular mechanisms underlying cancer initiation and progression. However, the specific mechanisms by which LIPT1 affects the occurrence and progression of OSCC remain unknown.

The findings from the GO and KEGG analyses demonstrated that the DEGs were predominantly linked to metabolic processes, aligning with the observa-

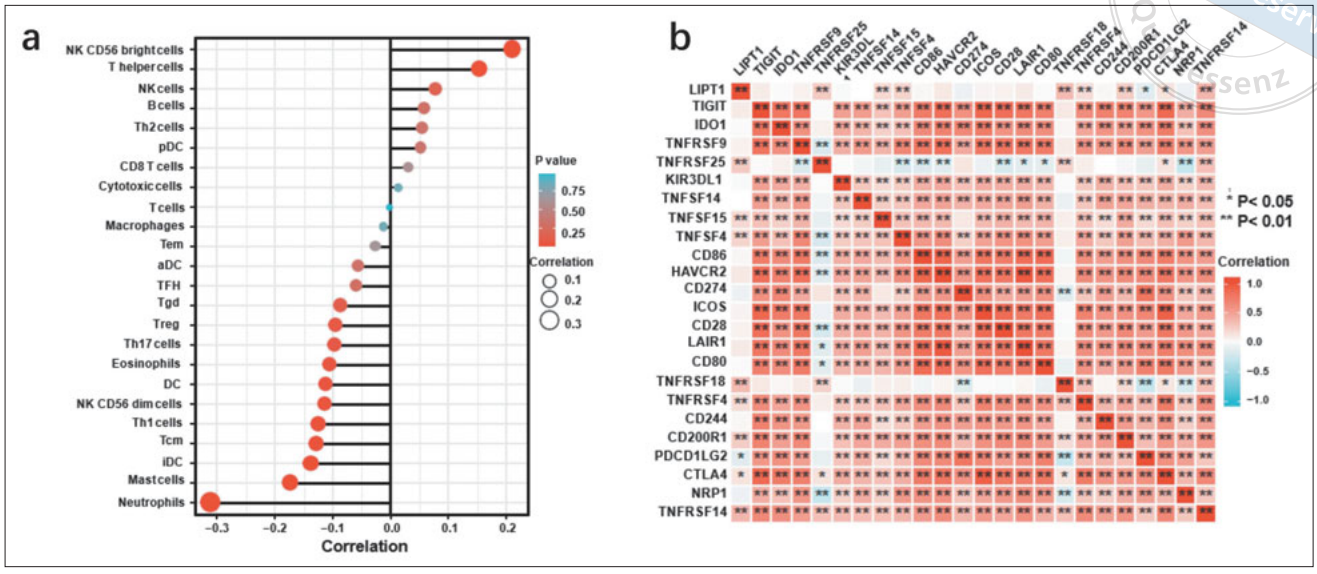

Fig 6 Correlation between LIPT1 and immune infiltration. Lollipop graph depicting the correlation between LIPT1 expression and immune infiltration, as determined by ssGSEA **(a)**. Correlation between LIPT1 and immune checkpoint-related genes **(b)**.

tions made by Zheng et al[31]. Their study revealed that cuproptosis can hinder cancer progression by targeting mitochondrial metabolism.[31] This consistency further supports the present authors' observations. In cancer patients, the tumour microenvironment plays a crucial role in influencing therapy response and clinical outcomes.[32] In the immune cell infiltration analysis, the present authors found a positive correlation between LIPT1 and certain immune cells, including Tregs and neutrophils. Lv et al[19] also reported higher levels of regulatory Tregs and neutrophils in the LIPT1 low expression group in melanoma; however, Jiang et al[33] found a positive correlation between neutrophils and LIPT1 expression in breast cancer. Moreover, mutations in the patient's LIPT1 gene result in a fatal disease characterised by a specific lipoylation defect of the 2-ketoacid dehydrogenase complexes. This patient presented with early onset fatal lactic acidosis and a combined defect of pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase activities, indicating a deficiency in lipoic acid metabolism. Immunostaining analysis revealed reduced lipoylated E2-PDH and E2-KGDH, suggesting a defect in lipoic acid transfer to specific proteins. Sequence analysis identified two heterozygous missense mutations in LIPT1, which were shown to be disease-causing and essential for the activation of 2-ketoacid dehydrogenases in humans.[34]

However, there are certain limitations to consider. Firstly, all the data used in this study were obtained from public databases, and further validation is necessary. Additionally, the 1-year AUC value for overall survival prediction was below 0.6, despite good fit in the calibration curves of the nomogram. Furthermore, DLD and CDKN2A were both found to be associated with overall survival, indicating the potential for developing a prognostic model incorporating these genes and other clinical characteristics in the future.

Conclusion

This study identified LIPT1 as a novel potential molecular biomarker associated with cuproptosis in OSCC.

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Conflicts of interest

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

Author contribution

Dr Kuang Min SHEN contributed to the study design, methodology and manuscript draft; Dr Yu Meng ZHOU contributed to the analysis and manuscript draft; Dr Mu Chun LIANG contributed to the data analysis; Dr De Mao ZHANG contributed to the supervision, review

and editing of the manuscript; Dr Qiang WEI contributed to conception, methodology and supervision; Dr Yi Lin PING contributed to conception, methodology and administration. All authors approved the final version of the manuscript.

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References

1. Irfan M, Delgado RZR, Frias-Lopez J. The oral microbiome and cancer. Front Immunol 2020;11:591088.
2. de Menezes SAF, Miranda YMS, da Silva YM, et al. Prevalence and genotyping of HPV in oral squamous cell carcinoma in Northern Brazil. Pathogens 2022;11:1106.
3. Ghazawi FM, Lu J, Savin E, et al. Epidemiology and patient distribution of oral cavity and oropharyngeal SCC in Canada. J Cutan Med Surg 2020;24:340–349.
4. Bai XX, Zhang J, Wei L. Analysis of primary oral and oropharyngeal squamous cell carcinoma in inhabitants of Beijing, China-A 10-year continuous single-center study. BMC Oral Health 2020;20:208.
5. Moskovitz J, Moy J, Ferris RL. Immunotherapy for head and neck squamous cell carcinoma. Curr Oncol Rep 2018;20:22.
6. Gu W, Kim M, Wang L, Yang Z, Nakajima T, Tsushima Y. Multi-omics analysis of ferroptosis regulation patterns and characterization of tumor microenvironment in patients with oral squamous cell carcinoma. Int J Biol Sci 2021;17:3476–3492.
7. Yang YT, Hsieh MJ, Chuang YC, et al. Anticancer effects of picrasidine I on oral squamous cell carcinoma. Environ Toxicol 2022;37:627–636.
8. Zhu W, Ye Z, Chen L, Liang H, Cai Q. A pyroptosis-related lncRNA signature predicts prognosis and immune microenvironment in head and neck squamous cell carcinoma. Int Immunopharmacol 2021;101(Pt B):108268.
9. Tsvetkov P, Coy S, Petrova B, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. Science 2022;375:1254–1261.
10. Gene Ontology Consortium. Gene Ontology Consortium: Going forward. Nucleic Acids Res 2015;43(Database issue):D1049–D1056.
11. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28:27–30.
12. Wang Z, Jensen MA, Zenklusen JC. A practical guide to The Cancer Genome Atlas (TCGA). Methods Mol Biol 2016;1418:111–141.
13. Ranstam J, Cook JA. Kaplan-Meier curve. Br J Surg 2017;104:442.
14. Emura T, Matsui S, Chen HY. Compound.Cox: Univariate feature selection and compound covariate for predicting survival. Comput Methods Programs Biomed 2019;168:21–37.
15. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020;48(W1):W509–W514.
16. Ito K, Murphy D. Application of ggplot2 to pharmacometric graphics. CPT Pharmacometrics Syst Pharmacol 2013;2:e79.
17. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innovation (Camb) 2021;2:100141.
18. Cheng B, Tang C, Xie J, et al. Cuproptosis illustrates tumor micro-environment features and predicts prostate cancer therapeutic sensitivity and prognosis. Life Sci 2023;325:121659.
19. Lv H, Liu X, Zeng X, et al. Comprehensive analysis of cuproptosis-related genes in immune infiltration and prognosis in melanoma. Front Pharmacol 2022;13:930041.
20. Yan C, Niu Y, Ma L, Tian L, Ma J. System analysis based on the cuproptosis-related genes identifies LIPT1 as a novel therapy target for liver hepatocellular carcinoma. J Transl Med 2022;20:452.
21. Park SH, Goo JM, Jo CH. Receiver operating characteristic (ROC) curve: Practical review for radiologists. Korean J Radiol 2004;5:11–18.
22. Mayr JA, Feichtinger RG, Tort F, Ribes A, Sperl W. Lipoic acid biosynthesis defects. J Inherit Metab Dis 2014;37:553-563.
23. Stowe RC, Sun Q, Elsea SH, Scaglia F. LIPT1 deficiency presenting as early infantile epileptic encephalopathy, Leigh disease, and secondary pyruvate dehydrogenase complex deficiency. Am J Med Genet A 2018;176:1184–1189.
24. Solmonson A, DeBerardinis RJ. Lipoic acid metabolism and mitochondrial redox regulation. J Biol Chem 2018;293:7522–7530.
25. Ni M, Solmonson A, Pan C, et al. Functional assessment of lipoyltransferase-1 deficiency in cells, mice, and humans. Cell Rep 2019;27:1376–1386.e6.
26. Solmonson A, Faubert B, Gu W, et al. Compartmentalized metabolism supports midgestation mammalian development. Nature 2022;604:349–353.
27. Zanoni DK, Montero PH, Migliacci JC, et al. Survival outcomes after treatment of cancer of the oral cavity (1985-2015). Oral Oncol 2019;90:115–121.
28. Garzino-Demo P, Zavattero E, Franco P, et al. Parameters and outcomes in 525 patients operated on for oral squamous cell carcinoma. J Craniomaxillofac Surg 2016;44:1414–1421.
29. Wang B, Zhang S, Yue K, Wang XD. The recurrence and survival of oral squamous cell carcinoma: A report of 275 cases. Chin J Cancer 2013;32:614–618.
30. Chen Y. Identification and validation of cuproptosis-related prognostic signature and associated regulatory axis in uterine corpus endometrial carcinoma. Front Genet 2022;13:912037.
31. Zheng P, Zhou C, Lu L, Liu B, Ding Y. Elesclomol: A copper ionophore targeting mitochondrial metabolism for cancer therapy. J Exp Clin Cancer Res 2022;41:271.
32. Wu T, Dai Y. Tumor microenvironment and therapeutic response. Cancer Lett 2017;387:61–68.
33. Jiang B, Zhu H, Feng W, et al. Database mining detected a cuproptosis-related prognostic signature and a related regulatory axis in breast cancer. Dis Markers 2022;2022:9004830.
34. Tort F, Ferrer-Cortès X, Thió M, et al. Mutations in the lipoyltransferase LIPT1 gene cause a fatal disease associated with a specific lipoylation defect of the 2-ketoacid dehydrogenase complexes. Hum Mol Genet 2014;23:1907–1915.

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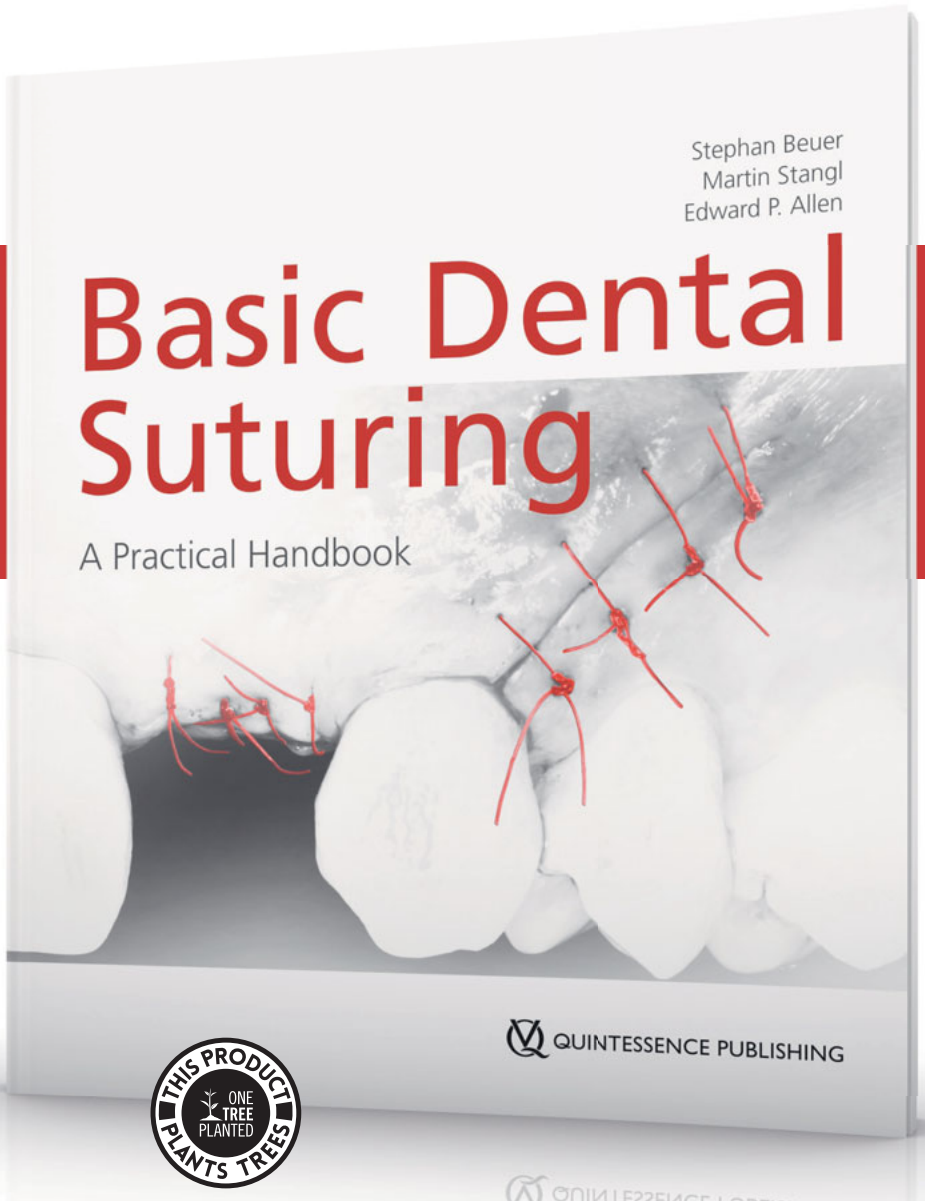

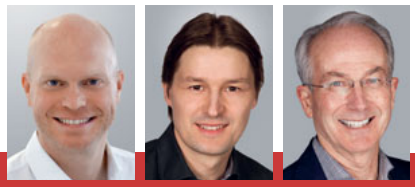

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Effect of Dental Implant System–Assisted Tooth Intentional Replantation in the Treatment of Anterior Teeth with Pathological Tooth Flaring, Drifting and Elongation in Patients with Stage III/IV Periodontitis: a Case Series

Ya Hui QIAO[1,#], Xin Yu ZHANG[1,#], Rui Qi BAI[1,2], Jing Wen CAI[1], Lin Lin ZHANG[1], Bin Jie LIU[1], Jun CHEN[1]

Objective: *To investigate the clinical effect of implant-assisted dental intentional replantation (IR) for the treatment of "drifted" anterior periodontally hopeless teeth (PHT).*
Methods: *The present authors recruited 22 patients with stage III/IV periodontitis who suffered drifting of the maxillary anterior teeth, with a total of 25 teeth. The PHT were extracted for in vitro root canal treatment (RCT). The root surface was smoothed and the shape was trimmed, and the alveolar socket was scratched. The dental implant system was used to prepare the alveolar socket according to the direction, depth and shape of the tooth implantation. The PHT were reimplanted into the prepared alveolar socket. The periodontal indicators were analysed statistically before and after surgery.*
Result: *Twenty-two patients who completed the full course of treatment, with a total of 25 PHT, had a successful retention rate of 88%. Mean periodontal probing depth (PPD) decreased by 2.880 ± 0.556 mm and 3.390 ± 0.634 mm at 6 months and 1 year, respectively, and clinical attachment loss (CAL) decreased by 2.600 ± 0.622 mm and 2.959 ± 0.731 mm at the same time points, respectively, showing significant improvement ($P < 0.05$).*
Conclusion: *Dental implant system–assisted IR can effectively preserve "drifted" natural PHT in patients with stage III/IV periodontitis.*
Keywords: *dental implant system–assisted, intentional replantation, periodontally hopeless teeth, stage III/IV periodontitis*
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Patients with stage III/IV periodontitis often have anterior teeth with pathological tooth flaring, drifting and elongation. Extraction is frequently the result of periodontally hopeless teeth (PHT)[1]; however, patients with stage III/IV periodontitis often have a strong claim to retain their natural teeth, especially young patients. Since Grossman[2] proposed the concept of intentional replantation (IR) in 1982, it has gradually become the final treatment option for refractory PHT. IR is a minimally invasive method in which PHT are extracted and reimplanted into the primary alveolar socket after root evaluation and removal of inflammatory lesions. A study reported that 48 PHT in 48 patients underwent IR and were followed up for 18 months.[3] The overall survival rate at the ninth month was 95.8% and declined to 91.7% at the eighteenth month. The improved rate of mobility was 89.1% at the ninth month and the ankylosis percentage was 77.3% at the eighteenth month. The

survival rate and improved rate of mobility of anterior teeth were both better than those of posterior teeth.[3,4] However, previous studies have focused on cleaning inflammatory granulation tissue in the alveolar sockets and have not targeted modifications to the shape of the alveolar sockets for reimplantation.[3,5] This may result in secondary occlusal trauma. The present study is the first to report the clinical effect of dental implant system–assisted IR. The authors evaluated this by recruiting 22 patients with stage III/IV periodontitis (a total of 25 teeth) over the past 7 years to provide new insights into treating PHT of patients with stage III/IV periodontitis.

Materials and methods

Subjects

Patients with stage III/IV periodontitis who visited the present authors' department from June 2015 to June 2022 and who had severe periodontal bone destruction, severe loosening and difficulty achieving preservation with conventional treatment were considered for the study. A total of 22 patients with 25 PHT were included. The inclusion criteria were as follows:

- healthy adults without systemic diseases;
- no history of smoking or areca nut chewing;
- no pregnancy or no breast feeding;
- periodontal pockets were deep to the level of the apical region, and the tooth showed grade III movability. Radiographs showed that periodontal bone destruction was severe and could not be preserved by conventional treatment;
- patient strongly in favour of retention of the PHT while accepting the risk of having to remove them if the dental implant system–assisted IR treatment fails;
- adjacent teeth could help to fix the replanted teeth (i.e., the movability of the adjacent teeth was less then grade II and the distance was within 2 mm);
- the width of the remaining alveolar bone is greater than that of the root.

The exclusion criteria were as follows:

- PHT is a residual root, residual crown or full-crown restoration;
- PHT has significant occlusal trauma or crowding;
- patient refused to receive treatment or has displayed compliance.

The study was approved by the Ethics Committee of Xiangya Stomatological Hospital, Central South University (ethics no. 20140004). All included subjects were given full details regarding the treatment procedures and signed informed consent forms.

Treatment procedure

Preoperative preparation

All included PHT patients received non-surgical periodontal therapy and oral hygiene promotion before surgery. Dental implant system-assisted IR was performed 2 months after initial periodontal therapy (Fig 1a).

Surgical procedure

PHT were extracted using a minimally invasive approach after local anaesthesia (Fig 1b and c). One dental practitioner performed subgingival scaling of the adjacent tooth and scratched the extraction pit until the bone surface was exposed (Fig 1d). Another dental practitioner performed root canal therapy (RCT) for the extracted tooth through retrograde filling in vitro and used Iroot SP (Innovative Biocreamix, Vancouver, Canada) to seal the apex of the tooth (Fig 1e). The dental implant system was employed to determine the direction of implantation (Fig 1f). The root surface of the isolated PHT was scaled to remove calculus and inflammatory granulation tissue and the root profile was trimmed to better match the prepared socket (Fig 1g). The operative area was rinsed with gentamicin (Fig 1h). The prepared implant cavity was selectively filled with an appropriate amount of Bio-Oss spongious bone substitute (Geistlich, Wolhusen, Switzerland) (Fig 1i). The in vitro prepared PHT was re-implanted into the socket and covered the Bio-Gide membrane (Geistlich) (Fig 1j). A periodontal splint was used to fix the reimplanted PHT (Fig 1k), and the occlusion was adjusted to uniform light contact (Fig 1).

Postoperative management

Amoxicillin (0.5 g, tid) and ornidazole (0.5 g, bid, after meals) were taken orally for around 3 to 5 days. Meanwhile, the patient was prescribed with 0.12% chlorhexidine mouthrinse to be used three times a day for 2 weeks. The sutures were removed after 2 weeks. Follow-up visits were conducted at 6 and 12 months after surgery, and annually thereafter.

Follow-up treatment

At each follow-up visit, the patient was given oral hygiene instruction (OHI) and supportive periodontal

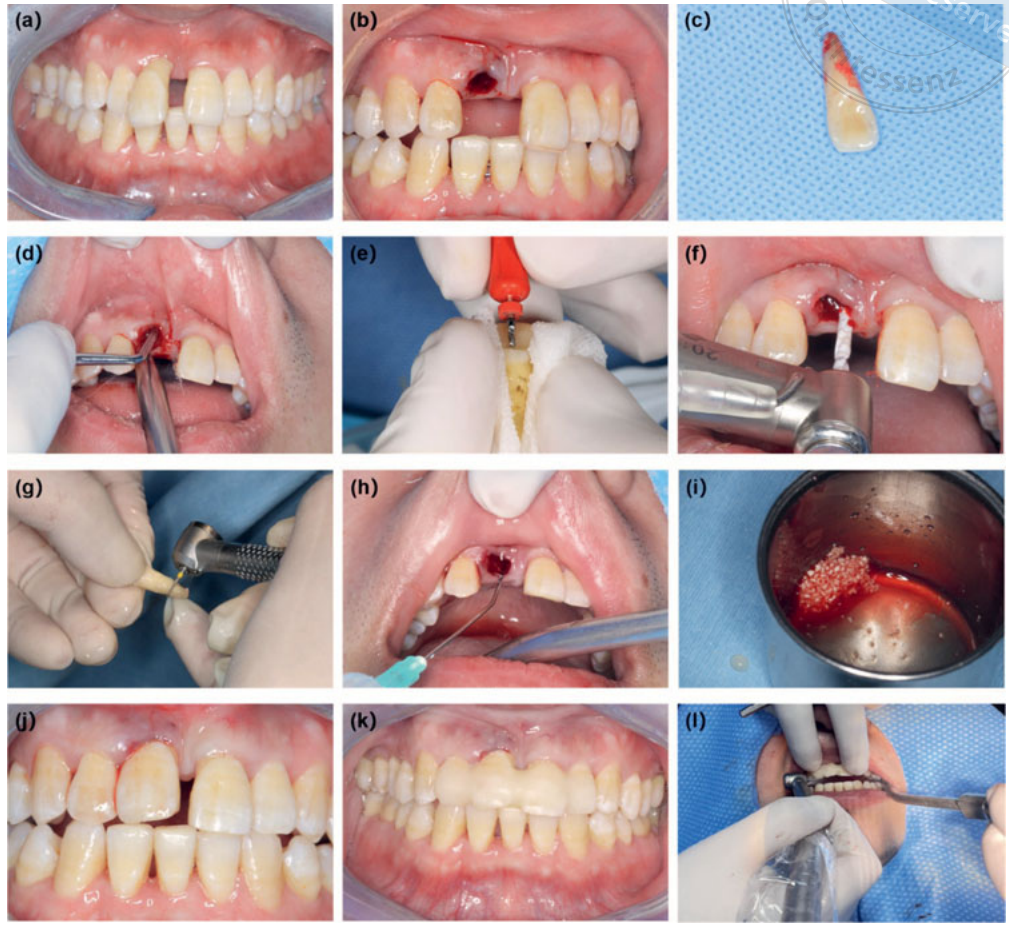

Fig 1 Surgical procedure. Two months after non-surgical periodontal therapy **(a)**. Isolation of the affected PHT **(b and c)**. Scratching the alveolar socket thoroughly **(d)**. RCT for the PHT in vitro **(e)**. Dental implant system–assisted preparation of the alveolar socket **(f)**. High-speed dental handpiece for tooth root shape trimming **(g)**. The alveolar socket was rinsed using gentamicin **(h)**. Implantation of bone powder **(i)**. The PHT prepared in vitro was reimplanted into the socket **(j)**. The PHT was fixed using the periodontal splint **(k)**. Occlusal adjustment to achieve light contact **(l)**.

therapy (SPT). Their occlusal relationship was checked to ensure that the relationship in the operated area was light or no contact. The principle of occlusal adjustment involves a small amount and multiple times.

Observation indicators

The periodontal indicators, including tooth movement, periodontal probing depth (PPD), gingival recession (GR), clinical attachment loss (CAL), Bleeding Index (BI) and horizontal overlap coverage were examined and recorded by the same dental practitioner before surgery, 6 months after surgery and 1 year after surgery. During follow-up, CBCT was used to monitor root resorption and measure the distance from the top of the labial and palatal alveolar ridge to the bone boundary of the enamel. The success criteria were as follows[6,7]:

- improvement of periodontal clinical indexes (shallow PPD, no bleeding on probing [BOP], no increase in CAL), no fistula, repeated pus overflow, percussion pain, no inflammation or other adverse reactions, and good masticatory function;
- alveolar bone height and density either increased or remained unchanged based on the imaging examination.

The criteria for failure were as follows:

- no improvement of the periodontal clinical indexes, fistula, overflowing pus, percussion pain, etc., and inability to retain the reimplanted PHT;
- imaging examination shows progressive destruction of the alveolar bone;
- poor compliance, repeated fracture of fixation, failure to follow up on schedule, poor inflammation control.

Statistical analysis

SPSS 26.0 software (IBM, Armonk, NY, USA) was used for the statistical analysis. The measurement data were expressed as mean ± standard deviation, and repeated measurement data were used for repeated measures data. The level of statistical significance was set at $P < 0.05$.

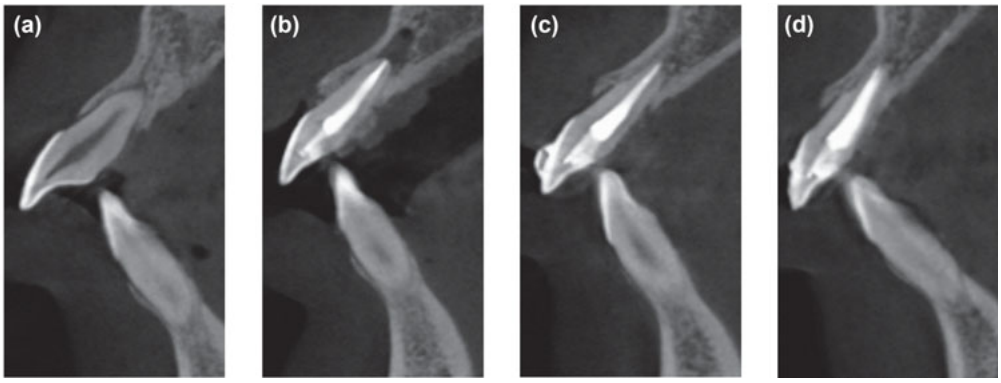

Fig 2 CBCT images before and after dental implant system–assisted IR. Preoperative imaging data **(a)**. Immediate postoperative imaging data **(b)**. Imaging data 6 months after dental implant system–assisted IR **(c)**. Imaging data 1 year after dental implant system–assisted IR **(d)**.

Results

General information

A total of 22 patients (13 men and 9 women) aged 18 to 79 years (mean age 41 years) and 25 PHT with stage III/IV periodontitis met the inclusion. The postoperative follow-up results showed that 22 reimplanted PHT were retained successfully, with a success rate of 88%.

Imaging analysis

Preoperative CBCT showed significant alveolar bone resorption in all PHT, ranging in height from half of the root to one-third of the root apex. Six months after IR, there was no obvious shadow in the root apexes of the 22 reimplanted PHT, the density was uniform and the alveolar bone height had recovered to a certain extent. The main mode of healing was tooth ankylosis (Fig 2). A reduced density projection appeared in the apical area 6 months after the IR of one reimplanted PHT. One reimplanted PHT loosened and fell out 4 months after IR, and another loosened and fell out 6 months after surgery.

Periodontal index analysis

All PHT had severe attachment loss before surgery. The looseness of all PHT ranged from degree II to III. A total of 150 sites were found in the 25 PHT, with PPD ranging from 3 to 10 mm. Six to twelve months after surgery, 22 reimplanted PHT had stable periodontal splints, without significant loosening. The periodontal splint of one reimplanted PHT was loose and the PPD was 6 mm, and the dental implant was placed after extraction. Two reimplanted PHT fell out at 4 and 6 months after surgery, respectively. The remaining reimplanted PHT showed significant improvements in PPD, CAL, GR, BI, labial and palatal alveolar bone and coverage at 6 months and 1 year after IR compared with those before IR ($P < 0.05$); however, there was no significant difference between 6 months and 1 year after surgery ($P > 0.05$) (Table 1 and Fig 3).

Discussion

As the economy, society and dental technology have developed, preserving as many natural teeth as possible has become a common goal for dental practitioners and patients. Conventional IR is applied to non-surgical RCT failure, apical microsurgery failure, external root resorption, coronal root fracture, tooth dislocation due to trauma, and anatomical factors such as a malformed lingual sulcus.[8,9] In the past, PHT caused by severe periodontitis were considered a contraindication to conventional IR[10]; however, in recent years, an increasing number of successful cases have been reported. Thus, IR has become the final possibility for preserving PHT. Studies have shown that IR combined with regenerative surgery reduces PPD and BI significantly and improves the preservation rate of PHT[3,5,8,11,12]; however, these studies focused mainly on the removal of inflammatory granulation tissue in the alveolar socket, and did not make specific modifications to the shape of the alveolar sockets for reimplantation. Stage III/IV periodontitis usually causes PHT to "drift" and "elongate", with the anterior teeth presenting a deep vertical and horizontal overlap in a false occlusal relationship. If the shape and orientation of the PHT in the alveolar fossa are not altered, this may result in secondary occlusal trauma.

The present study is the first to report the clinical effect of dental implant system–assisted IR. The results showed that the success rate of implant-assisted IR therapy in the treatment for anterior teeth with pathological tooth drifting and elongation in patients with stage III/IV periodontitis was 88%. Most of the reimplanted PHT showed a significant improvement in peri-

Table 1 Statistical analysis of periodontal indexes before and after dental implant system assisted tooth intentional replantation (mean ± standard deviation).

Index	Preoperative	6 months after surgery	1 year after surgery
PD (mm)	6.80 ± 1.56	3.92 ± 1.67	3.41 ± 1.30
CAL (mm)	6.68 ± 1.91	4.08 ± 1.53	3.73 ± 1.20
GR (mm)	3.20 ± 1.00	0.38 ± 0.71	0.18 ± 0.40
BI	4.24	1.13	0.27
Labial alveolar bone loss (mm)	6.58 ± 2.10	4.88 ± 2.02	4.35 ± 1.54
Palatal alveolar bone loss (mm)	8.64 ± 1.93	5.88 ± 1.39	5.56 ± 1.24
Horizontal overlap (mm)	6.92 ± 3.20	3.41 ± 1.30	3.38 ± 1.30

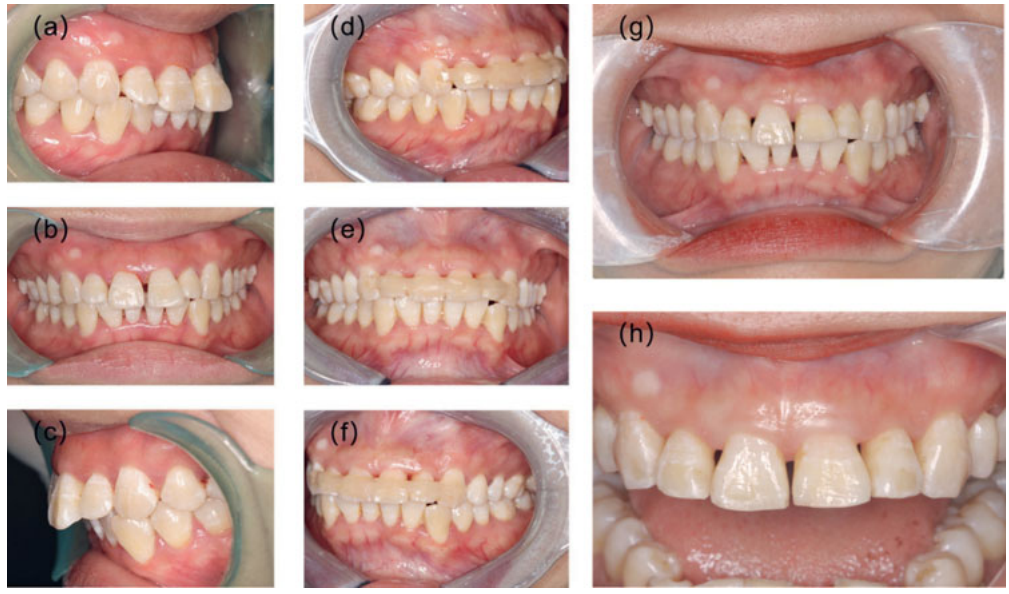

Fig 3 Pre- and postoperative intraoral photographs. Preoperative intraoral photographs **(a to c)**. Intraoral photohraphs taken 6 months postoperatively (**d to f)**. Intraoral photographs of periodontal splints removed 5 years after surgery **(g and h)**.

odontal indexes after treatment. Imaging data showed a significant increase in alveolar bone height and density. The present study suggested that dental implant system–assisted IR is an effective solution for preserving natural teeth in situations with PHT that have loosened and drifted as a result of stage III/IV periodontitis.

Dental implant system–assisted IR is a multidisciplinary combination of implantation, guided tissue regeneration, RCT and conventional IR techniques. The main advantages of IR are the absence of immune reaction that might lead to rejection of implants, the lack of foreign body sensation, and the fact there is no tooth loss stage. In addition, it can facilitate direct observation of inaccessible areas of the tooth surface, enabling repairs to be performed without damaging adjacent periodontal tissue. It also allows for more visual examination of calculus and inflamed granulation tissue on the surfaces of teeth with stage III/IV periodontitis, facilitating more thorough scaling in vitro.[8] The application of dental implant system–assisted IR has various advantages. First, stage III/IV periodontitis causes the PHT to drift and elongate, which leads to occlusal trauma. To ensure that the occlusal relationship achieved after IR is light or no contact, the surgeon prepares the cavity with an implant drill based on the alveolar height on the preoperative CBCT scan to more precisely determine the position and orientation of the reimplanted tooth. Second, the dental implant system can re-prepare the shape of the socket according to the shape of the roots so that the reimplanted tooth can better adapt to the alveolar contour, thus improving its stability in the alveolar sockets. Third, bone burns are avoided due to the good cutting performance, hardness, corrosion resistance, and low heat production of the implant drill. Nevertheless, there are still some shortcomings. The choice of implant system is mainly determined by the shape of the patient's teeth and the height of the alveolar bone in order to promote the widespread adoption of implant-assisted IR as a technique for preserving teeth deemed hopeless due to

periodontitis, so it is necessary to establish a replicable and standardised procedure. Although 3D printed surgical guides may be a viable option for achieving this, the present study did not explore this aspect, so it will be examined in future studies.

Conventional IR is mainly for periodontal healthy teeth, requiring the physician to avoid touching the root surface and scraping the socket in order not to damage the periodontal membrane and affect the prognosis[13]; however, for PHT, thoroughly removing the infected periodontium and the inflammatory granulation tissue in the tooth socket to prevent root resorption for replanted teeth is advisable.[14] In the present study, the use of a dental implant system that helped with implant positioning combined with manual scaling was able to completely remove the inflammatory granulation tissue of the alveolar socket to reduce postoperative complications such as root resorption. Furthermore, the conventional IR of tooth displacement caused by other reasons, such as trauma, usually involves a healthy periodontal condition, requiring only 2 to 4 weeks of periodontal fixation to reduce displacement.[15] In vitro studies have shown that the effect of force on periodontal ligament (PDL) fibroblasts (PDLFs) depends on the conditions under which mechanical stimulation is applied. Mechanical stretching increases the proliferation of PDLFs. Thus, mechanical stimulation is required to promote normal healing of PDL tissue and prevent tooth straightening during a certain period of the healing phase[16]; however, in the present study, periodontal destruction of the reimplanted PHT was severe. Without fixation, progressive movement is not conducive to periodontal tissue healing and infection control. As such, periodontal splinting was performed on all PHT and the splints were removed after 1 year. When the degree of tooth mobility exceeds Grade I, it is advisable to re-stabilise the loose tooth with adjacent teeth using fibre strips for an additional 3 to 6 months.

Occlusal trauma is a significant promoter of periodontitis.[17] The present analysis of surgical failure suggested that trauma is also one of the major factors that affects the prognosis of dental implant system–assisted IR. Excessive occlusal forces will make it difficult to rebuild the periodontal tissue of the affected PHT, leading to inflammation of the apical area and treatment failure. Thus, dental implant system–assisted IR requires strict and rigorous fixation of loose teeth to ensure a normal occlusal relationship.

There are four main forms of periodontal prognosis for IR in permanent teeth: periodontal healing, surface resorption, replacement resorption and inflammatory resorption.[15] In the present study, the root surface and alveolar sockets of the PHT were thoroughly debrided intraoperatively. There was almost no normal PDL. Based on the literature and clinical experience, the present authors suspected that implant-assisted IR of PHT may heal in the following way[3]:

1. Surface resorption: Inflammatory reaction and osteoclast activation may lead to localised dentine resorption. If the resorption is limited to the dentine and does not penetrate the dentinal tubules, then it is self-limiting and can be restored by reattaching new dentine and PDL.[18,19]
2. Tooth ankylosis and replacement resorption: The periodontium disappears, and the dentine on the root surface fuses with the alveolar bone to replace the root with bone, which is known as tooth ankylosis.[20] In severe cases, the root is completely absorbed and replaced by bone tissue in a manner known as replacement resorption, a form of pathological healing that can last from 5 to 20 years, meaning that the reimplanted PHT can remain in the mouth for a considerable period, which may be a special form of site preservation.[16,21]
3. Inflammatory resorption: If the inflammation is further aggravated after IR, absorption may reach the dentine. The dentine is weak and cannot resist resorption.[22] Given that significant periapical inflammation was observed in failed cases, inflammatory resorption may lead to failure of dental implant system–assisted IR. In vivo studies have shown that there are two methods to prevent tooth ankylosis and root resorption in dental IR: the non-cellular method, in which an exogenous substance (such as platelet-derived growth factor) is applied on the root surface or in the alveolar socket, and the cellular method, in which cells (such as PDL-derived cells) are planted on the root surface or placed in the alveolar socket.[16] The specific form of periodontal healing of reimplanted PHT in the present study still needs to be verified by subsequent animal experiments, and this is the focus of the present authors' follow-up research.

Conclusion

The present study preliminarily demonstrated that dental implant system–assisted IR is an effective solution for the retention of anterior teeth with pathological tooth flaring, drifting and elongation in patients with stage III/IV periodontitis. Further animal studies are needed to investigate the healing pattern of the reimplanted PHT and how to promote periodontal ligament healing. Meanwhile, to improve the success rate and predictability of PHT preservation, more potential influencing

factors should be explored, and surgical indications and the prognosis evaluation system should be improved to provide more treatment options for preserving natural teeth.

Conflicts of interest

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

Author contribution

Drs Ya Hui QIAO, Xin Yu ZHANG, Bin Jie LIU and Jing Wen CAI designed the study; Drs Ya Hui QIAO, Lin Lin ZHANG and Jun CHEN collected and analysed the data and drafted the manuscript. All authors revised the manuscript and approved the manuscript.

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References

1. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Clin Periodontol 2018;45(suppl 20):S1–S8.
2. Grossman LI. Intentional replantation of teeth: A clinical evaluation. J Am Dent Assoc 1982;104:633–639.
3. Zhang J, Luo N, Miao D, Ying X, Chen Y. Intentional replantation of periodontally involved hopeless teeth: A case series study. Clin Oral Investig 2020;24:1769–1777.
4. Santos JM, Marques JA, Esteves M, Sousa V, Palma PJ, Matos S. Intentional replantation as a starting approach for a multidisciplinary treatment of a mandibular second molar: A case report. J Clin Med 2022;11:5111.
5. Ong TK, Lim D, Singh M, Fial AV. Factors influencing the treatment outcome of intentional replantation on teeth with periapical periodontitis: A systematic review and meta-analysis. J Evid Based Dent Pract 2022;22:101722.
6. Peer M. Intentional replantation - A 'last resort' treatment or a conventional treatment procedure? Nine case reports. Dent Traumatol 2004;20:48–55.
7. Zhang JL, Wang XF, Wang X, et al. Application of intentional replantation in the preservation of teeth to be extracted in severe periodontitis [in Chinese]. Ningxia Yi Ke Da Xue Xue Bao 2020;42:1042–1046.
8. Plotino G, Abella Sans F, Duggal MS, et al. Present status and future directions: Surgical extrusion, intentional replantation and tooth autotransplantation. Int Endod J 2022;55(suppl 3):827–842.
9. Chiu YL, Chiang CP, Lee MS, Ho CJ. Intentional replantation - A case report with 20-year follow-up. J Dent Sci 2023;18:453–455.
10. Nagappa G, Aspalli S, Devanoorkar A, Shetty S, Parab P. Intentional replantation of periodontally compromised hopeless tooth. J Indian Soc Periodontol 2013;17:665–669.
11. Keceli HG, Hendek MK. Fifteen months follow-up of a hopeless tooth treated with two-step procedure involving intentional replantation and free gingival graft. Eur J Dent 2014;8:559–562.
12. Cho SY, Lee SJ, Kim E. Clinical outcomes after intentional replantation of periodontally involved teeth. J Endod 2017;43:550–555.
13. Pisano M, Di Spirito F, Martina S, Sangiovanni G, D'Ambrosio F, Iandolo A. Intentional replantation of single-rooted and multi-rooted teeth: A systematic review. Healthcare (Basel) 2022;11:11.
14. Lindskog S, Pierce AM, Blomlof L, Hammarstrom L. The role of the necrotic periodontal membrane in cementum resorption and ankylosis. Endod Dent Traumatol 1985;1:96–101.
15. Fouad AF, Abbott PV, Tsilingaridis G, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol 2020;36:331–342.
16. Ideno H, Komatsu K, Nakashima K, Nifuji A. Tooth transplantation and replantation: Biological insights towards therapeutic improvements. Genesis 2022;60:e23496.
17. Dommisch H, Walter C, Difloe-Geisert JC, Gintaute A, Jepsen S, Zitzmann NU. Efficacy of tooth splinting and occlusal adjustment in patients with periodontitis exhibiting masticatory dysfunction: A systematic review. J Clin Periodontol 2022;49(suppl 24):149–166.
18. Andreasen JO. Periodontal healing after replantation and autotransplantation of incisors in monkeys. Int J Oral Surg 1981;10:54–61.
19. Kristerson L, Andreasen JO. Influence of root development on periodontal and pulpal healing after replantation of incisors in monkeys. Int J Oral Surg 1984;13:313–323.
20. Kotsanos IN, Tzika E, Economides N, Kotsanos N. Intentional replantation and management of avulsion related ankylosis and external cervical resorption. A 10-year follow up case report. Dent Traumatol 2023;39:392–398.
21. Sapir S, Shapira J. Decoronation for the management of an ankylosed young permanent tooth. Dent Traumatol 2008;24:131–135.
22. Rumpler M, Würger T, Roschger P, et al. Osteoclasts on bone and dentin in vitro: Mechanism of trail formation and comparison of resorption behavior. Calcif Tissue Int 2013;93:526–539.

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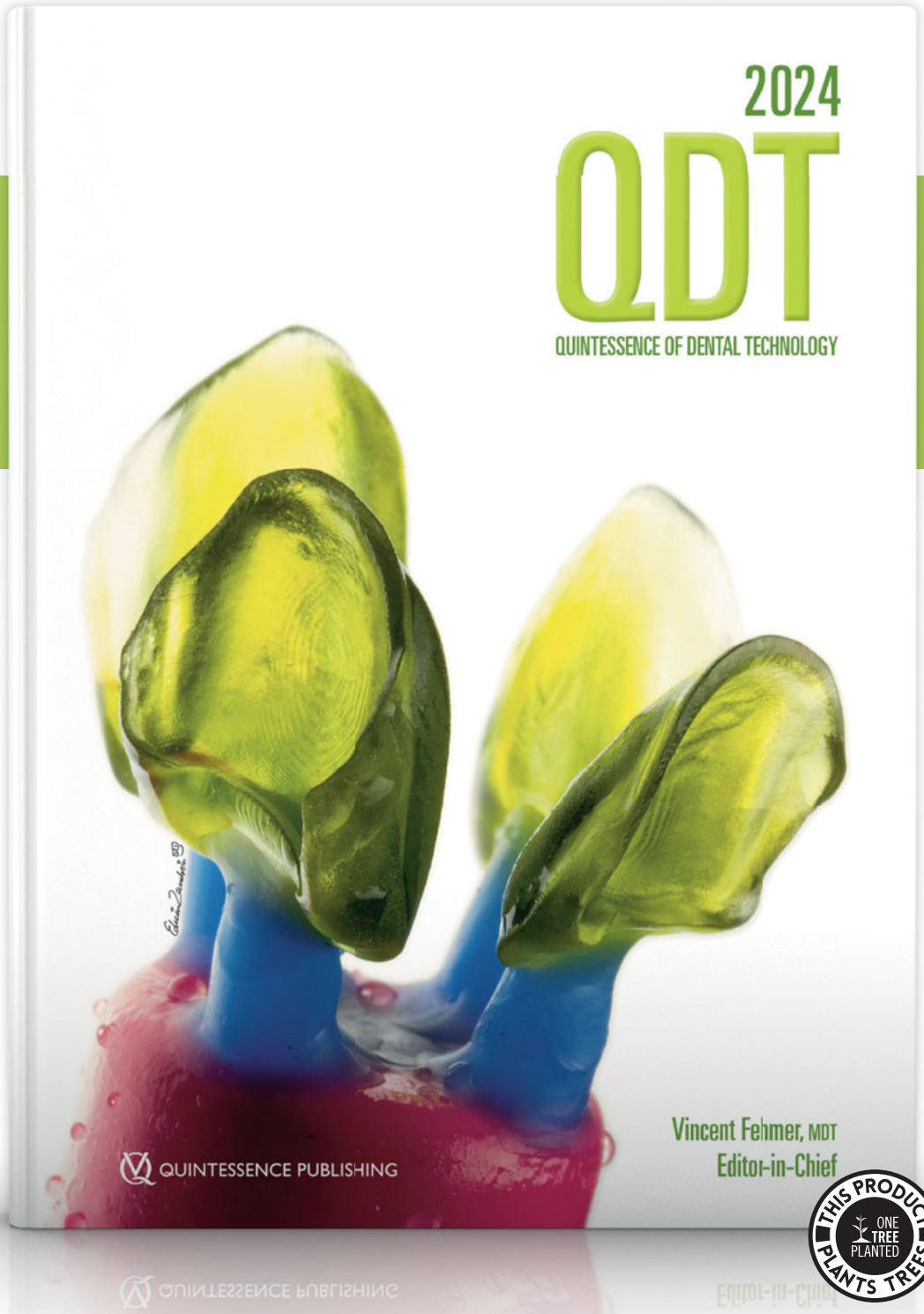

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Dental Fear and Caries in 6- to 12-Year-Old Children: a Systematic Review and Meta-analysis

Narjes AMROLLAHI[1], Sayed Ali SHAHSHAHAN[2], Firoozeh NILCHIAN[3], Mohammad Javad TARRAHI[4]

Objective: *To investigate the relationship between dental fear and dental caries in children aged 6 to 12 years in a systematic review and meta-analysis.*
Methods: *Systematic review search terms were selected according to medical subject headings (MeSH) or non-MeSH. An electronic search of studies published in English assessing the relationship between dental fear (children's fear survey schedule–dental subscale) and dental caries (DMFT or dmft index) was carried out of the Scopus, Web of Science, PubMed, Embase, Cochrane and Proquest databases up to March 2022. Of 5,759 articles retrieved initially, 16 were eligible for inclusion in the study, and 5 of these were included in the quantitative analysis. The quality of studies was evaluated based on the Newcastle-Ottawa scale. Begg tests were employed to assess the publication bias.*
Results: *According to the meta-analysis, the results revealed no statistically significant difference in mean of DMFT score in low and high fear score groups, with a mean difference of 1.28 (95% confidence interval −0.132 to 2.693) (P = 0.076). A statistically significant difference was found in the mean dmft score for the low and high fear score groups, with a mean difference of 0.227 (95% confidence interval 0.058 to 0.395) (P = 0.008). The mean dmft was significantly higher in the high fear score group.*
Conclusion: *Dental fear has a significant relationship with caries in primary teeth, but not in permanent teeth.*
Keywords: *behaviour control, child, dental anxiety, dental caries, dental fear*
Chin J Dent Res 2024;27(2):151–159; doi: 10.3290/j.cjdr.b5459595

Dental fear or anxiety in children is one of the most common problems around the world. It is defined as the abnormal fear of visiting the dental practitioner for preventive care or therapy and unwarranted anxiety over dental procedures. The relationship between dental fear and a lack of cooperation in dental situations is not straightforward. Dental fear has been observed in most but not all children with behaviour management problems.[1,2] It may create some barriers in receiving dental care and cause problems for both dental practitioners and parents.[3]

According to studies, fear due to dental treatments can continue until adolescence, leading to avoidance of regular dental visits.[4] Fear can reduce a child's cooperation and cause dental treatment to be delayed. As a result, dental caries and abscesses occur more frequently, and this poor oral health increases the child's anxiety and fear, creating a vicious cycle. Finding the causes of this cycle and stopping it as soon as possible will benefit the child. Thus, it is crucial to identify children who experience dental fear and make specific arrangements for them.[5,6]

Various tools have been provided to evaluate dental fear in children. The children's fear survey sched-

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ule–dental subscale (CFSS-DS) is a well-known psychometric scale that was developed in 1982 to evaluate children's fear of dentistry. The validity and reliability of the tool have been reported to be very good.[7-9] The tool has a better performance compared to other scales such as Veenha Picture Test and Dental Anxiety Test in some studies3,7,10.

The indexes approved by the World Health Organization to measure dental caries in permanent and primary teeth, respectively, are the Decayed, Missing and Filled Teeth (DMFT) and dmft. Individual DMFT and dmft values are the sum of the number of decayed, missing due to caries and filled) teeth in the permanent and primary teeth, respectively.[3]

The relationship between dental fear and dental caries in children remains controversial. Some studies have confirmed the existence of a relationship between dental fear and dental caries[3,7,11], whereas others found no relationship.[12-14] The age of the child plays an important role in controlling anxiety and accepting treatment, and long-term studies have shown that as the child's age increases, their fear and anxiety decrease.[15,16] Since most of the studies conducted in this field involved children aged from 6 to 12 years, the present study was conducted with the aim of summarising the results of these previous studies and investigating the relationship between dental fear and dental caries in children aged from 6 to 12 years in a systematic review and meta-analysis.

Materials and methods

This systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines.

Search strategy

According to the Cochrane guidance, in this systematic review, studies were included that assessed dental caries (outcome) in children aged 6 to 12 years (population) who had dental fear (exposure) compared to children without dental fear (comparison).

The key terms were selected based on the medical subject headings (MeSH) and non-MeSH terms in simple terms or combinations. The searched databases included Proquest, Cochrane, Embase, Scopus, Web of Science and PubMed. Electronic database searches were conducted using the following combination of key search terms: (dental fear or dental anxiety or odontophobia or dental phobia) And (child or children or primary teeth or deciduous teeth or pediatric dentistry or pediatric or dentistry for children or pedodontics) And (dental caries or dental decay or carious lesion or carious dentin or dental white spot or early childhood caries or tooth decay or severe early childhood caries or caries or baby bottle caries or decay).

The inclusion criteria were studies published in English that assessed the relationship between dental caries and dental fear in children according to the PECO question, and cross-sectional or case-control studies with full texts available and that were published up to March 2022.

The exclusion criteria were other types of studies, such as case reports, letters to the editor, pilot studies, cohort studies, historical reviews and studies published in languages other than English, as well as studies in which the children had any systemic or developmental disease such as molar incisor hypo-mineralisation (MIH), in order to minimise the effect of confounding factors.

Resource selection

First, article topics were reviewed independently by two authors (NA and SAS) according to the developed search strategy. The abstract and full texts of the articles were reviewed. Finally, hand searching was performed by checking the references of articles included in the studies and hand-searching key journals. The correlation coefficients between the two researchers' data were 0.93 and 1.00 in the abstracts and full texts, respectively. Any disagreement between the two researchers was resolved by a third researcher (FN).

Data extraction

Two researchers (NA and SAS) independently extracted data from different studies. The variables included the author's name, publication year, the age and sex of children, sample size, type of study, prevalence of dental caries (according to the DMFT or dmft index or the International Caries Detection & Assessment System [ICDAS]), dental fear assessment tools, prevalence of dental fear and a brief conclusion of each study. Different tools were used to assess dental fear in children such as CFSS-DS[17-19], dental fear visual analogue scale (DFVAS)[20], dental fear scale (DFS)[13] and questionnaires[21], which were reported in the evidence table (Table 1).

Risk of bias assessment

The Newcastle-Ottawa scale (NOS) was used to assess cross-sectional and case control studies. Each study was

Table 1 Main characteristics of studies included in the systematic review.

Study	Sample	Age, y	Sex (M/F)	DMFT		dmft	Dental fear instruments	Dental fear	Conclusion
				%	Mean				
Buldur[27]*	583	3–13	286/297	NR	6.00	NR	CFSS-DS/Frankl behaviour score	Mean Frankl behaviour score 3.0 (SD 1.0)	Dental anxiety was associated with dental caries
Yahyaoglu et al[23]*	810	6–12	408/402	NR	1.57	4.64	CFSS-DS	Mean CFSS-DS value 24.00 ± 7.99	Anxiety and dental caries were associated
Beena[26]*	444	6–12	220/224	NR	0.58	4.4	CFSS-DS	Overall mean CFSS-DS score 37.0 ± 8.89	No significant correlation
Son et al[25]*	132	7	77/55	NR	NR	6.71	CFSS-DS	Prevalence of dental fear 34.85%.	No correlation
Patcharaphol[24]*	212	12	146/66	NR	2.14	NR	CFSS-DS	Mean CFSS-DS value 31.38 ± 10.45	Patients with dental anxiety had more decayed teeth
Alsadat et al[3]	1546	6–12	798/748	51.8	NR	NR	CFSS-DS	23.50%: CFSS-DS score ≥ 32 12.50%: score > 38	Dental fear has a direct relationship with decayed permanent teeth
Nguyen et al[7]	900	8–10	427/473	89.8	NR	NR	CFSS-DS	Mean CFSS-DS score 20.8 ± 9.1	Dental fear is related to the oral health care system
Laureano et al[12]	466	8–10	207/259	88.84 (ICDAS > 0)	NR	NR	CFSS-DS	Mean total CFSS-DS score 29.97	No significant correlation
Pichot et al[20]	2734	6–12	1,419/1,315	53.59	NR	NR	DF-VAS	5% of 9- to 12-year-olds had high scores (DF-VAS > 6)	Children with lower levels of dental fear had less experience of caries
Folayan et al[18]	450	6–12	222/228	4.9	NR	NR	CFSS-DS	Mean CFSS-DS score 38.6 ± 14.4	Children who had caries had higher anxiety
Olak et al[19]	344	8–10	188/156	93	NR	NR	CFSS-DS	General fear of dentistry 6.1%	Dental fear is significant predictor of dental caries
Saber and Awad[17]	250	6–12	122/128	NR	1.46	6.62	CFSS-DS	Mean CFSS-DS score 20.9 ± 11.84	Fear may have an impact on scores for caries
Prathima et al[13]	400	6–12	200/200	NR	1.89	NR	Dental fear scale (DFS)	Low to moderate dental fear 46%; high dental fear 72%	No association
Panda et al[11]	798	8–10	238/560	NR	1.58	NR	CFSS-DS	Mean fear score 36	Children with dental fear were 1.8 times more likely to have an untreated carious tooth
Patır Münevveroğlu et al[21]	200	6–12	102/98	NR	NR	3.52	Questionnaire	Fears were related to injection (60%), tooth extraction (50%), restorations (3.6%) and the sight of dental instruments (40%)	Dental fear likely predicts dental caries
Varmazyar et al[14]	185	6–12	83/102	NR	NR	(dmft/DMFT) 3.93	CFSS-DS	CFSS-DS > 38: 26.48%	No correlation

*Studies included in the meta-analysis.

NR, not reported.

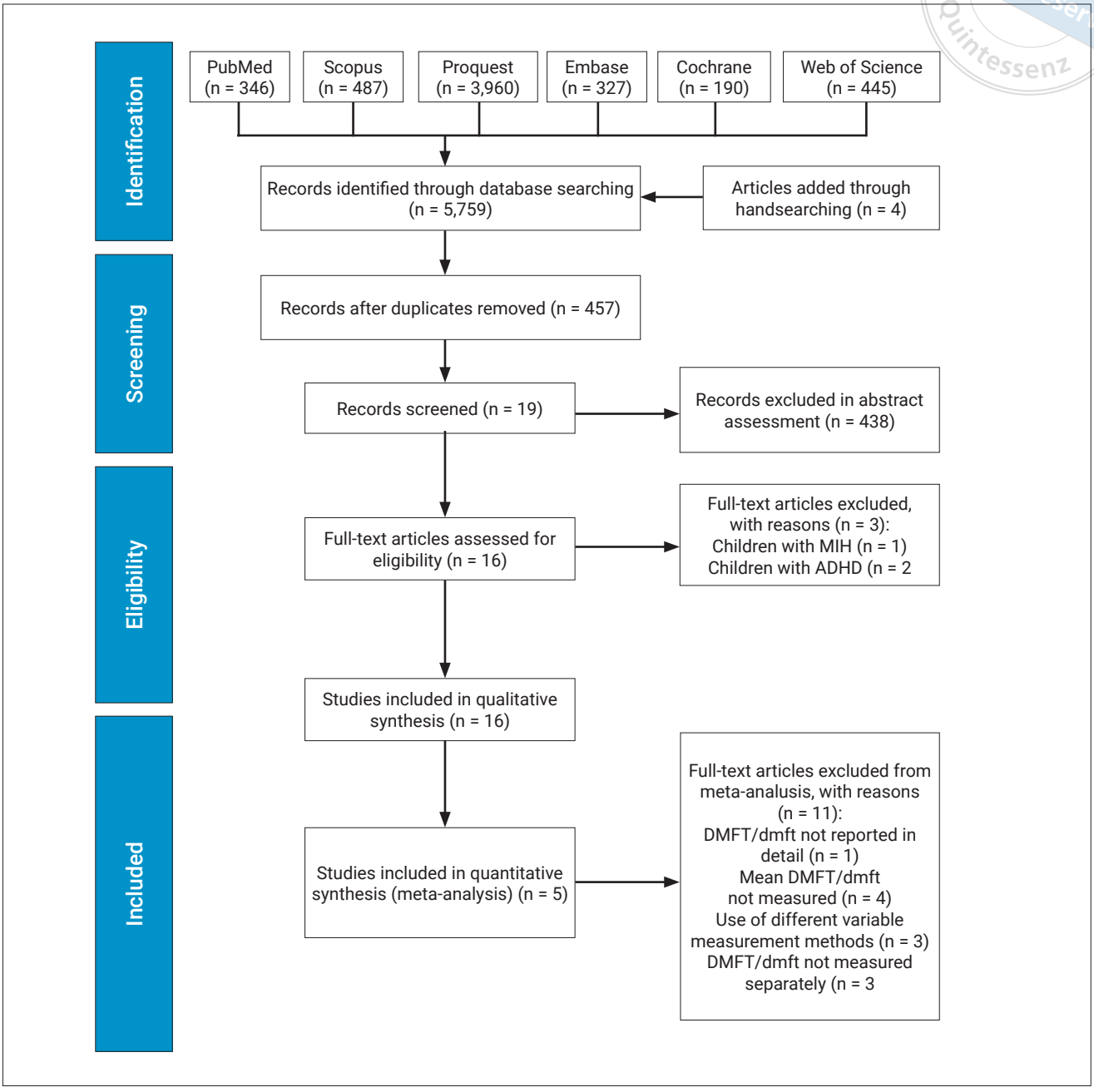

Fig 1 Flow diagram of the search strategy.

evaluated for inner-methodological risk of bias. The quality of the articles was scored by two researchers (NA and AS). In the event of disagreement between the two researchers, a third person provided their opinion (FN). Studies were rated as being of low, moderate and high quality according to the NOS scores < 5, 5 to 7 and > 7, respectively.

Statistical analysis

A meta-analysis was carried out to determine the existence of dental fear in children aged 6 to 12 years and its relationship with dental caries using Comprehensive Meta-Analysis software (version 2, BioStat, Orlando, FL, USA). For statistical analysis, the standard mean difference of the continuous data was determined at a 95%

confidence interval. The *P* value and I^2 statistic were used to analyse heterogeneity in the studies. Thus, $P < 0.05$ or $I^2 > 50\%$ showed heterogeneity. If there was no heterogeneity, the fix model was used; otherwise, the random model was utilised.

To estimate the mean difference and depict the results of the meta-analysis, a forest plot was employed. Publication bias was assessed using a Begg test.[22]

Results

There are many articles available regarding dental fear in children and its relationship with dental caries; however, due to not meeting the inclusion criteria, many of them were excluded from the present study.

Study selection

A total of 5,759 studies were obtained through searching six databases (PubMed, Scopus, Web of Science, Embase, Cochrane and Proquest) and manual searching was performed using other sources available online. After exclusion of duplicate and irrelevant studies through title screening, the abstracts of 457 articles were analysed. Finally, 19 articles underwent full-text analysis, and 3 of these were excluded due to their inclusion of hyperactive children and children with molar incisor hypomineralisation (Fig 1).

Hence, 16 studies were included in the systematic review that met the inclusion criteria and obtained scores higher than 5 in the quality assessment. Five of these were included for meta-analysis due to their similarity in the method of measuring dental fear and dental caries variables (Table 1).

Risk of bias

The NOS assessment scale is a star rating system with eight items that assigns a maximum of nine stars to three domains, namely selection (four stars), comparability (two stars) and exposure in cross-sectional studies (three stars). Studies are rated from 0 to 9, with 0 to 5 corresponding to poor quality, 5 to 7 to moderate quality, and > 7 equating to good/high quality. The studies differed in quality; four were of moderate quality, and twelve were of high quality (Table 2).

Study characteristics

Of the 16 articles included in the systematic review, 11 could not be included in the meta-analysis. In the study conducted by Saber et al[17], the mean of DMFT/dmft was not reported in detail (the standard deviation [SD] was not reported). The studies by Alsadat et al[3], Panda et al[11] and Folayan et al[18] did not report the mean values of DMFT or dmft indexes. Nguyen et al[7], Olak et al[19] and Varmazyar et al[14] reported caries of primary and permanent teeth together as a dmft/DMFT index, and these studies were therefore also not included in the meta-analysis, and neither was the study by Laureano et al[12], in which the International Caries Detection and Assessment (ICDAS) index was used to assess dental caries and severity.

Prathima et al[13], Pichot et al[20] and Patir et al[21] employed different criteria for measuring the variables of dental fear or dental caries, and were therefore also excluded from the meta-analysis. Parthima et al[13] and Pichot et al[20] used the dental fear scale (DFS) and dental fear-visual analogue scale (DF-VAS), respectively, to assess children's dental fear, whereas Patir et al[21] investigated dental fear using a questionnaire.

Finally, five studies were included in the meta-analysis for quantitative analysis.[23-27] In these studies, the CFSS-DS index was used to assess the severity of children's dental fear, which was categorised as low or high. Dental caries was reported as mean DMFT and dmft.

Quantitative analysis

Relationship between dental fear and DMFT

Four studies with a similar methodology evaluated the relationship between dental caries in permanent teeth and children's dental fear.[23,24,26,27] They used the CFSS-DS index to assess the severity of children's level of dental fear, which was categorised as low or high.[23,24,26,27] Dental caries was reported as mean DMFT and SD. The heterogeneity showed $P > 0.001$ and $I^2 = 98.886\%$, and a random-effects model was used for meta-analysis.[23,24,26,27] The results revealed no statistically significant difference in mean DMFT score in the low and high fear groups, with a mean difference of 1.28 (95% confidence interval [CI] −0.13 to 2.69) ($P = 0.08$); thus, the mean DMFT was not statistically different in the high and low fear groups.[23,24,26,27] Figure 2 shows the forest plot for DMFT. According to the Begg test, there was no publication bias in the evaluation of the relationship between DMFT and dental fear ($P = 0.24$).

Relationship between dental fear and dmft

Three studies with a similar methodology evaluated the relationship between dental caries in primary teeth and children's dental fear.[23,25,26] In these studies, the CFSS-

Table 2 Quality of evidence of included studies based on the Newcastle-Ottawa scale (NOS). A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Category		Yahyaoglu et al[23]	Beena[26]	Son et al[25]	Buldur[27]	Patcharaphol[24]	Alsadat et al[3]	Nguyen et al[7]	
Selection	Case definition	*	*	*	*	*	*	*	
	Representativeness of the cases	*	-	*	-	*	*	*	
	Selection of Controls	*	*	*	*	*	*	*	
	Definition of Controls	*	*	*	*	*	-	*	
Comparability	Comparability of cases and controls	**	*	**	*	*	-	-	
Exposure	Non-response rate	-	*	*	*	*	*	*	
	Ascertainment of the exposure	*	*	*	*	*	*	*	
	Same method for cases and controls	*	*	*	*	*	*	*	
Final judgment	8*	7*	9*	7*	8*	6*	7*	6*	

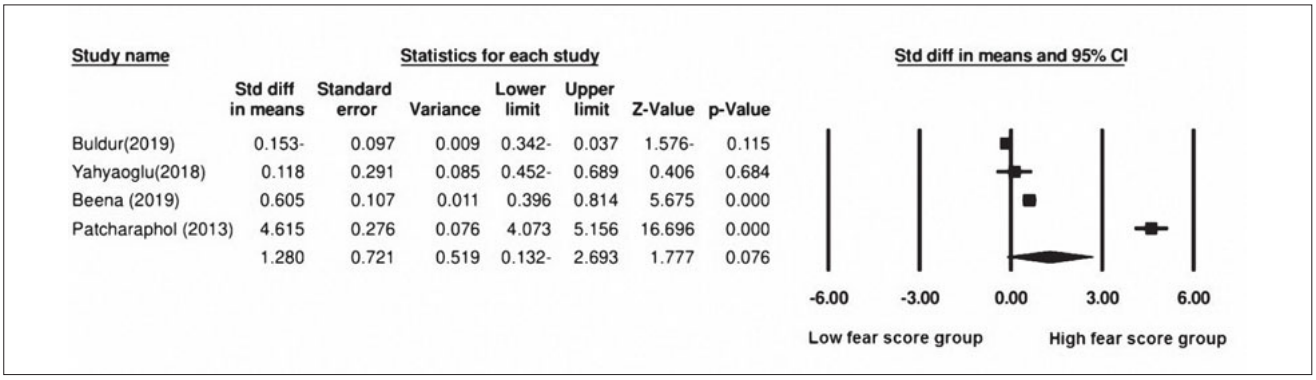

Fig 2 Forest plot of the DMFT index.

DS index was used to assess the severity of children's fear, which was categorised as low or high, and dental caries was reported as mean dmft and SD.[23,25,26] The heterogeneity showed $P = 0.418$ and $I^2 = 0.000$, so a fixed model was used for meta-analysis.[23,25,26] The results revealed a statistically significant difference in mean dmft score in the low and high fear groups, with a mean difference of 0.23 (95% CI 0.06 to 0.39) (P = 0.008); thus, the mean dmft was significantly higher in the high fear group.[23,25,26] Figure 3 shows the forest plot for dmft. According to the Begg test, there was no publication bias in the evaluation of the relationship between dmft and dental fear ($P = 0.30$).

Discussion

The several determinant variables and risk factors for dental caries among children can be categorised as individual, parental and environmental factors. Dental fear and anxiety are considered one of the most important individual factors, and are one of the reasons for avoiding attending regular dental visits. Negative behaviours during dental visits may cause dental treatment to be delayed. This in turn may lead to the progression of dental caries and its subsequent consequences, such as severe dental pain and dental abscesses. This causes children who have a history of toothache to refer to the dental practitioner and increases the fear of dentistry in children, thus creating a vicious cycle whereby the child's oral health will worsen and their dental fear will increase. As such, it can be very helpful to consider preventive measures and plans in this regard.[28,29]

The present study aimed to review the results of studies conducted on the relationship between fear and dental caries to achieve a conclusion on this topic. Of the 16 studies included in the systematic review, 5 were summarised through meta-analysis in quantitative manner.[23-27] The results of this meta-analysis showed that the relationship between dental fear (CFSS-DS) and caries in primary teeth (dmft) was significant; however, the relationship was not significant in permanent teeth (DMFT). In early childhood, children do not have enough understanding in the dental environment and usually feel afraid. This makes them avoid attending

	Olak et al[19]	Saber and Awad[17]	Prathima et al[13]	Laureano et al[12]	Pichot et al [20]	Folayan et al[18]	Varmazyar et al[14]	Patır Münevveroğlu et al[21]	Panda et al[11]
	*	*	*	*	*	*	*	*	*
	*	*	*	*	*	-	-	*	*
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	5*	8*	7*	8*	6*	7*	8*	7*	

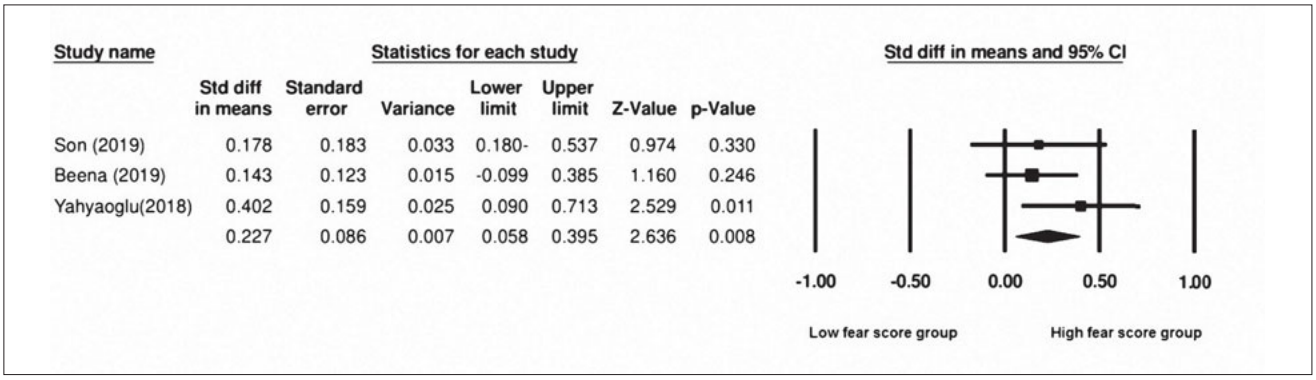

Fig 3 Forest plot of the dmft index.

the practice on a regular basis, and thus endangers their oral health. As a result, dental fear has a significant relationship with caries in primary teeth (dmft).

Permanent teeth erupt as children get older, usually when they reach the age of around 6 to 7 years. Thus, permanent teeth have been exposed to cariogenic substances for less time than primary teeth. On the other hand, as children get older, their level of cooperation in the dental environment increases, meaning their dental visits will be more regular. Oral hygiene is also better in older children. As a result, no significant relationship can be found between dental fear and dental caries in permanent teeth (DMFT).

Contradictory results have been shown by different studies in the field of dental fear and caries. Many studies have confirmed the relationship[3,7,11,17-21,23,27], whereas others did not find any relationship between dental fear and caries.[12-14,25,26]

Saber et al[17] reported a significant relationship between fear (CFSS-DS index) and caries in primary teeth (dmft index); however, no significant relationship was found between dental fear (CFSS-DS) and caries in permanent teeth in children (DMFT). This may be due to parents neglecting their child's oral and dental hygiene.

Olak et al[19] found that dental fear in children is associated with their previous dental experience and their parents' dental fear. Their results also revealed a relationship between dental caries (DMFT/dmft) in children and dental fear.[19] In this study, caries affecting the primary and permanent teeth were related to the first dental visit, which is extremely important and should be done as a simple and regular examination to familiarise the child with the dental environment.[19]

In the study by Patır et al[21], a significant difference was observed between primary and permanent dental caries and dental fear, and the age of children who were afraid of attending the dental practice was significantly lower. Similarly, in the present study, the relationship between dental fear and caries in primary teeth was significant, which indicates that children with dental fear are often younger.

In some studies, unlike in the present study, no significant relationship was found between dental fear and caries (DMFT/dmft) in children.[12,14] This seems to

be due to caries in primary and permanent teeth being reported together as a dmft/DMFT index in the study conducted by Varmazyar et al[14]. Moreover, Laureano et al[12] used the ICDAS index to assess dental caries. This is a more recent visual method and seeks to detect caries lesions in their earliest stages. The ICDAS criteria are a clinical scoring system for use in dental education, clinical practice, research and epidemiology. These criteria support and enable personalised total caries management to improve long-term health outcomes.[30] The ICDAS index in the study conducted by Laureano et al12 did not report caries affecting primary and permanent teeth separately. If the primary and permanent teeth caries index was considered separately, it would be associated with dental fear.

In two other studies, unlike the present study, no significant relationship was reported between dental fear and caries in children. [25,26] This may be due to the inclusion criteria for these two studies. The children included in these studies referred to their dental practitioner for regular dental examinations without any history of toothache.[25,26] This regular examination led to low dental fear and low dental caries in children.

A lack of effective communication between the child and the dental practitioner in the first years of the child's life can cause dental fear in children. This fear can prevent them from attending regular dental examinations, and this in turn may lead to the progression of dental caries, pain and abscesses.[31]

The relationship between dental fear and dental caries in younger children is more obvious, perhaps due to the fact that younger children are less socially developed. Thus, the effect of dental fear is more obvious in primary teeth than permanent teeth which erupt at older ages.

One of the limitations of the present study is the inconsistency of the indexes used in different studies, which meant it was not possible to consider many studies in the meta-analysis. As such, it is necessary to conduct more studies in this field to obtain a more definitive result regarding the relationship between caries and dental fear.

Conclusion

Dental fear has a significant relationship to caries in primary teeth, but not to those in permanent teeth. In the qualitative review of studies, the effect of fear on caries in primary teeth was more obvious than that for permanent teeth.

Conflicts of interest

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

Author contribution

Dr Narjes Amrollahi contributed to the data collection, analysis and manuscript draft; Dr Sayed Ali Shahshahan contributed to the data interpretation and manuscript revision; Dr Firoozeh Nilchian contributed to the study design and supervision; Dr Mohammad Javad Tarrahi contributed to the study conception and supervision, data interpretation and statistical analysis.

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References

1. Silveira ER, Costa VPP, Goettems ML, et al. The impact of cyberbullying on schoolchildren's dental anxiety in Brazil: A cross-sectional multi-level study. Community Dent Oral Epidemiol 2020;48:440–446.
2. Taani DQ, El-Qaderi SS, Abu Alhaija ES. Dental anxiety in children and its relationship to dental caries and gingival condition. Int J Dent Hyg 2005;3:83–87.
3. Alsadat FA, El-Housseiny AA, Alamoudi NM, Elderwi DA, Ainosa AM, Dardeer FM. Dental fear in primary school children and its relation to dental caries. Niger J Clin Pract 2018;21:1454–1460.
4. Klaassen MA, Veerkamp JS, Aartman IH, Hoogstraten J. Stressful situations for toddlers: Indications for dental anxiety? ASDC J Dent Child 2002;69:306–335.
5. Yamada MK, Tanabe Y, Sano T, Noda T. Cooperation during dental treatment: the Children's Fear Survey Schedule in Japanese children. Int J Paediatr Dent 2002;12:404–409.
6. Holmes RD, Girdler NM. A study to assess the validity of clinical judgement in determining paediatric dental anxiety and related outcomes of management. Int J Paediatr Dent 2005;15:169–176.
7. Nguyen MS, Nguyen TT, Nguyen BBT, Saag M, Olak J. Dental fear and anxiety in Estonian and Vietnamese schoolchildren: A comparative study of two populations. Clin Exp Dent Res 2018;4:182–188.
8. Laporte PP, Pan PM, Hoffmann MS, et al. Specific and social fears in children and adolescents: separating normative fears from problem indicators and phobias. Braz J Psychiatry 2017;39:118–125.
9. Nakai Y, Hirakawa T, Milgrom P, et al. The Children's Fear Survey Schedule-Dental Subscale in Japan. Community Dent Oral Epidemiol 2005;33:196–204.
10. Yon MJY, Chen KJ, Gao SS, Duangthip D, Lo ECM, Chu CH. An introduction to assessing dental fear and anxiety in children. Healthcare (Basel) 2020;8:86.
11. Panda S, Quadri MFA, Hadi IH, Jably RM, Hamzi AM, Jafer MA. Does dental fear in children predict untreated dental caries? An analytical cross-sectional study. Children (Basel) 2021;8:382.

12. Laureano ICC, Farias L, Fernandes LHF, et al. Dental fear in children: Association with dental caries and molar incisor hypomineralization. Braz Dent J 2020;31:673–679.
13. Prathima GS, Varghese AS, Sajeev R, Kayalvizhi G, Sanguida A, Balaji AS. Prevalence of dental fear and its relationship to dental caries and gingival disease among school children in Puducherry: A cross-sectional study. J Adv Clin Res Insights 2017;4:88–91.
14. Varmazyar A, Taram S, Rohani ZR. Dental fear of children and its relationship with caries experience. Journal Dental School 2022;39:28–32.
15. Dahlander A, Soares F, Grindefjord M, Dahllöf G. Factors associated with dental fear and anxiety in children aged 7 to 9 years. Dent J (Basel) 2019;7:68.
16. Yon MJ, Chen KJ, Gao SS, Duangthip D, Lo ECM, Chu CH. Dental fear and anxiety of kindergarten children in Hong Kong: A cross-sectional study. Int J Environ Res Public Health 2020;17:2827.
17. Saber HM, Awad SM. Is dental fear related to clinical consequences of untreated dental caries in children? Egypt Dent J 2018;64:1941–1947.
18. Folayan MO, Kolawole KA, Onyejaka NK, Agbaje HO, Chukwumah NM, Oyedele TA. General anxiety, dental anxiety, digit sucking, caries and oral hygiene status of children resident in a semi-urban population in Nigeria. BMC Oral Health 2018;18:66.
19. Olak J, Saag M, Honkala S, et al. Children's dental fear in relation to dental health and parental dental fear. Stomatologija 2013;15:26–31.
20. Pichot H, Hennequin M, Rouchon B, Pereira B, Tubert-Jeannin S. Dental status of new aledonian children: Is there a need for a new oral health promotion programme? PloS One 2014;9:e112452.
21. Patır Münevveroğlu A, Ballı Akgöl B, Erol T. Assessment of the feelings and attitudes of children towards their dentist and their association with oral health. ISRN Dent 2014;2014:867234.
22. Lin L, Chu H. Quantifying publication bias in meta-analysis. Biometrics 2018;74:785–794.
23. Yahyaoglu O, Baygin O, Yahyaoglu G, Tuzuner T. Effect of dentists' appearance related with dental fear and caries status in 6-12 years old children. J Clin Pediatr Dent 2018;42:262–268.
24. Patcharaphol S. Prevalence of dental fear and its relationship with oral health in children. Int J Clin Prev Dent 2013;9:1–5.
25. Son TM, Ngoc VT, Tran PT, et al. Prevalence of dental fear and its relationship with primary dental caries in 7-year-old-children. Pediatr Dent 2019;29:84–89.
26. Beena JP. Dental subscale of children`s fear survey schedule and dental caries prevalence. Eur J Dent 2013;7:181–185.
27. Buldur B. Pathways between parental and individual determinants of dental caries and dental visit behaviours among children: Validation of a new conceptual model. Community Dent Oral Epidemiol 2020;48:280–287.
28. Goettems ML, Shqair AQ, Bergmann VF, Cadermatori MG, Correa MB, Demarco FF. Oral health self-perception, dental caries, and pain: The role of dental fear underlying this association. Int J Paediatr Dent 2018;28:319–325.
29. Torriani DD, Ferro RL, Bonow ML, et al. Dental caries is associated with dental fear in childhood: Findings from a birth cohort study. Caries Res 2014;48:263–270.
30. Gugnani N, Pandit IK, Srivastava N, Gupta M, Sharma M. International Caries Detection and Assessment System (ICDAS): A new concept. Int J Clin Pediatr Dent 2011;4:93–100.
31. Inglehart MR. Motivational communication in dental practices: Prevention and management of caries over the life course. Dent Clin North Am 2019;63:607–620.

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Effect of Different Adhesive Resin and Composite Veneering Materials on Adhesion to Polyetheretherketone

Sezgi CINEL SAHIN[1], Lamia MUTLU-SAGESEN[1], Isil KARAOKUTAN[1], Mutlu OZCAN[2]

Objective: *To evaluate the effect of different adhesives and veneering resins on the shear bond strength (SBS) of polyetheretherketone (PEEK).*
Methods: *A total of 138 PEEK specimens were randomly divided into 6 groups according to adhesive material application: Control (C, no application), Adhese Universal (A) (Ivoclar Vivadent, Schaan, Liechtenstein), Gluma Bond Universal (G) (Heraeus Kulzer, South Bend, IN, USA), G-PremioBOND (P) (GC Corporation, Tokyo, Japan), Single Bond Universal (S) (3M, Saint Paul, MN, USA) and visio.link (V) (Bredent, Senden, Germany). Each adhesive group was divided into two subgroups according to the type of veneering material: Estenia direct composite (D) and Gradia Plus indirect composite (IN) (both GC Corporation). After the veneering process, the specimens were aged by thermal cycling. Kruskal-Wallis and Mann-Whitney U tests were used for SBS analysis (P < 0.05).*
Results: *The highest SBS results were obtained in the V_{IN} group, followed by the V_D, P_D, G_{IN}, A_{IN}, A_D, S_{IN}, S_D, P_{IN}, G_D, C_{IN} and C_D groups, respectively (P = 0.001). There were no significant differences in terms of the type of veneering composite when the same adhesive was applied (P > 0.05), except for Gluma Bond Universal (P = 0.009). All the adhesives tested showed clinically acceptable SBS results.*
Conclusion: *Visio.link offered the highest adhesion to PEEK, whereas the tested universal adhesives may be used as an alternative to visio.link in clinical settings. It was determined that changing the veneer type has no statistical difference when the same adhesive material is used.*
Keywords: *composite resin, polyetheretherketone, shear bond strength, universal adhesives*
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Polyetheretherketone (PEEK) is a thermoplastic polymer that is frequently used in dentistry for implant restorations and removable and fixed prostheses because of its superior chemical, thermal and mechanical properties and its biocompatibility.[1-4] Compared to other thermoplastic polymers, it offers many advantages such as less water absorption, high dimensional stability, high polishing properties, low plaque affinity, good wear resistance and low elastic modulus. It acts as a stress breaker, eliminating the possibility of an allergic reaction.[2,5]

Despite their advantages, PEEK materials have a greyish-brown or pearly white opaque colour that may not meet clients' aesthetic expectations.[6-8] To solve this problem, PEEK restoration surfaces can be veneered with composite resins using direct and indirect methods.[7,8] Indirect composite resins are frequently employed in veneering applications due to their low elastic modulus (8 to 10 GPa), acting as a stress breaker and reducing occlusal stresses, as well as their superior bonding performance, mechanical properties, wide colour range, easy manipulation and repairability.[9,10] However, in cases where aesthetic expectations cannot be met with indirect veneering or in cases requiring intraoral repair (especially in restorations that cannot be removed from the tooth or implant surface), direct composites are also used instead of indirect composites. Micro-filled direct composite resins

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offer a significant advantage in cases where aesthetic requirements are at the forefront in terms of providing a smooth surface and superior gloss, and they can replace traditional direct composite resins in terms of improving aesthetics.[11] Nevertheless, direct or indirect veneering methods do not provide sufficiently strong adhesion to the PEEK surface. This is considered the most significant clinical disadvantage of PEEK materials.[1,6,10,11] To achieve a strong bond between the resins and PEEK materials, the PEEK surface can be activated with surface treatment protocols before applying the adhesive material.[1,6,12-16]

Researchers have investigated surface treatment protocols, such as laser treatment, air-abrasion, application of piranha solution or sulfuric acid, and coating the surface with silica or plasma.[1,3,6,7,13,17] However, with all these surface treatment protocols, adhesive materials are still needed to form a strong bond between the resins and the PEEK materials.[6,13,18-21] Some studies have determined that visio.link material (Bredent, Senden, Germany) provides the highest SBS[18,22,23], but is suitable only for use in a laboratory and not for chairside application because it requires a special polymerisation furnace for application. To overcome these disadvantages, some researchers have proposed using universal adhesive materials and traditional light-emitting diode (LED) polymerisation to increase adhesion with PEEK materials.[8,20,23]

There is still insufficient information about the success achieved using adhesive materials and different veneering protocols to increase adhesion to PEEK materials.[1,3,6,8,18,22,23] Thus, the aim of the present study was to evaluate the effect of using different adhesives and resin veneers on the SBS of PEEK materials. The null hypothesis was that conditioning the PEEK surface with different adhesive materials and applying different composite resins as veneers would not affect the SBS of the PEEK materials.

Materials and methods

The study was approved by the Medical Ethics Committee of Pamukkale University, Denizli, Turkey (approval no. 60116787-020/54316 and 60116787-020/328868). The sample size for the study was calculated using G*Power software (version 3.1.9.2; Heinrich Heine Universität, Düsseldorf, Germany). It stipulated 12 independent groups according to an effect size of 0.4, 80% power and 0.05 sampling error.

A total of 138 PEEK specimens (CopraPeek Light; PEEK [≈ 80%], titanium dioxide [< 20%] and other additives [< 0.1%], Whitepeaks Dental Solutions, Wesel, Germany) were fabricated using CAD/CAM technology according to ISO standard 10477. They were 10 × 10 × 2 mm in size. All the specimens were then were embedded in chemically polymerised acrylic resin (Meliodent, Heraeus Kulzer, South Bend, IN, USA), and ground with 200-, 500-, 800- and 1000-grit silicon carbide abrasive papers (FEPA, Struers, Glasgow, UK) under continuous water cooling using an automatic polishing tool (Mecapol P 230, Press, Grenoble, France) at 180 rpm and for 1 minute at each step. Then, the surfaces of all the specimens were air-abraded from 10 mm away with 110 µm Al_2O_3 (Renfert Basic Classic, Renfert, Hilzingen, Germany) at 0.2 MPa at a 45-degree angle for 15 seconds. After the air-abrasion process, all specimens were cleaned in 70% isopropanol in an ultrasonic cleaner (Eurosonic Energy, Euronda, Vincenza, Italy) for 15 minutes, washed with distilled water for 10 minutes and dried under light pressure using an air spray.

All specimens were randomly divided into 6 groups (n = 23) according to the adhesive material applied to them: Control (C), Adhese Universal (A) (Ivoclar Vivadent, Schaan, Liechtenstein), Gluma Bond Universal (G) (Heraeus Kulzer, G-PremioBOND (P) (GC Corporation, Tokyo, Japan), Single Bond Universal (S) (3M, Saint Paul, MN, USA) and visio.link (V) (Bredent). All adhesive materials were used according to the manufacturers' instructions, and all application procedures were completed by the same researcher (SCS). The protocols for applying the adhesive materials are summarised in Table 1.

Subsequently, three specimens from each adhesive material group were randomly selected for analysis through scanning electron microscope (SEM) and elemental change analysis by energy-dispersive x-ray spectroscopy (EDS). For SEM analysis, all the surfaces of the specimens were coated with 80% gold and 20% palladium using a sputtered device (Q150R ES, Quorum Technologies, Laughton, UK). They were evaluated using the original 1000× magnification at 20 kV. The quantitative analysis of the elements on the specimens' surfaces was determined by EDS analysis at 20 kV.

For the SBS analysis, 20 specimens in each adhesive material group were randomly divided into 2 subgroups (n = 10 each) according to the type of veneer material: Estenia (GC Corporation) for the direct veneer group (D), and Gradia Plus (GC Corporation) for the indirect veneer group (IN) (Table 1). The result was twelve study groups using six different adhesive materials and two different composite resins:

- C_D/C_{IN}: Direct/indirect veneering control groups (no adhesive material application);

Table 1 Materials and equipment used in bonding and veneering protocols.

Product	Composition*	Application recommendation*	Manufacturer
Adhese Universal (VivaPen)	10-MDP, 2-HEMA, BisGMA, MCAP, D3MA, highly dispersed silica, ethanol, water, photo initiators and stabilisers. pH 2.8	Apply the product to the material surface with a brush for at least 20 s, disperse the material with oil- and moisture-free compressed air until a glossy, immobile film layer results. Then, perform light application with a light curing device (LED-C, Guilin Woodpecker, Guilin, China) for 10 s at a light intensity of 500–1,400 mW/cm^2 for polymerisation	Ivoclar Vivadent,
Gluma Bond Universal	4-META, MDP, methacrylate, acetone, water. pH 1.6–1.8	Apply the product to the material surface with a brush for 20 s gently, dry with a gentle oil-free air flow until the adhesive film no longer moves. Then perform light application for 10 s with a light curing device (LED-C) at a light intensity of > 500 mW/cm^2 for polymerisation	Heraeus Kulzer
G-Premio BOND	MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, initiator, silica, pH 1.5	After shaking the bottle, apply the product to the material surface with a brush for 10 s, then dry thoroughly for 5 s with oil-free air. Then, perform light application for 10 s using a light curing device (LED-C) at a light intensity of 700 mW/cm^2 for polymerisation	GC Corporation
Single Bond Universal	MDP monomer, dimethacrylate resins, HEMA, vitrebond copolymer, filler, ethanol, water, initiator, silane. pH 2.7	Apply the product to the material surface with a brush and rub it in for 20 s, direct a gentle stream of air over the liquid for about 5 s directed, and light-cured for 10 s with a light-curing device (LED-C)	3M
visio.link	MMA, 2-prepenoic acid reaction products with pentaerythritol, diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide	Apply the product to the material surface with a brush thinly and only once. Then immediately 90 s of light application with a dental laboratory polymerisation device (GC Labolight Duo, GC Corporation) at 370–400 nm wavelength range	Bredent
Essentia Direct Composite (micro-hybrid)	UTMA, other methacrylate monomers, inorganic filler (92.3%; SiO_2, BaO, Al_2O_3, La_2O_3)	20 s light application with 700 mW/cm^2 light intensity curing device (LED-C)	GC Corporation
Gradia Plus Laboratory Composite (nano-hybrid)	1%–5% Bis-GMA, 5%–10% TEGDMA, 1%–5% UDMA, ceramic filler	3 min light application in full-mode with a dental laboratory polymerisation unit (GC Labolight Duo)	GC Corporation

*Material contents are presented according to the manufacturer's information. Al, aluminium; BisGMA, Bisphenol-A glycidyl dimethacrylate; C, carbon; D3MA, Decandiol dimethacrylate; HEMA, hydroxyethyl methacrylate; MCAP, methacrylated carboxylic acid polymer; MDP, methacryloyloxydecyl dihydrogen phosphate; MEPS, methacryloyloxyalkyl thiophosphate methylmethacrylate, MET, methacryloyloxyethyl trimellitate; META, methacryloxyethyl trimellitate anhydride; MMA, methyl methacrylate; O, oxygen; Si, silicon; TEGDMA, triethyleneglycol-dimethacrylate; Ti, titanium; UDMA, urethane dimethacrylate; UTMA, urethane tetramethacrylate.

- A_D/A_{IN}: Adhese Universal direct/indirect veneering groups;
- G_D/G_{IN}: Gluma Bond Universal direct/indirect veneering groups;
- P_D/P_{IN}: G-Premio BOND direct/indirect veneering groups;
- S_D/S_{IN}: Single Bond Universal direct/indirect veneering groups;
- V_D/V_{IN}: visio.link direct/indirect veneering groups.

The veneering composite resins were applied by the same researcher (SCS) to the PEEK surfaces treated with different adhesive materials using a 2-mm diameter and 3-mm-high disc-shaped silicone mould. The mould was removed after each composite resin was polymerised according to the manufacturer's instructions. The calibration of the light-curing device was checked with a radiometer (Bluephase Meter II, Ivoclar Vivadent, Schaan, Liechtenstein) after every 10 samples throughout the polymerisation procedure. Information about the composition of the resins and the application protocols is summarised in Table 1. After the veneering procedure was completed, the specimens were kept in distilled water for 1 day at room temperature in a dark

Table 2 SBS data according to the different adhesive materials and veneering composite resin materials.

Group (n = 10)	SBS (MPa)		*P* value*
	Mean ± SD	Min–max (median)	
C_D	12.54 ± 4.90	3.23–17.48 (13.86)[a,c]	0.001
C_{IN}	17.29 ± 4.18	12.33–25.62 (16.43)[a,b,c]	
A_D	58.38 ± 20.05	30.47–98.5 (52.86)[b,e,f]	
A_{IN}	57.73 ± 18.82	31.63–99.41 (54.19)[b,d]	
G_D	22.63 ± 12.93	7.88–47.47 (21.89)[c]	
G_{IN}	56.23 ± 11.35	30.44–69.14 (58.56)[b,d]	
P_D	62.19 ± 15.02	37.83–80.99 (60.83)[d,f]	
P_{IN}	31.1 ± 12.94	2.71–45.29 (31.40)[a,b,c,f]	
S_D	50.76 ± 15.75	25.23–78.19 (49.96)[a,b,c,d,e]	
S_{IN}	56.91 ± 25.08	28.04–102.18 (52.02)[a,b,d,e]	
V_D	89.62 ± 30.36	63.65–164.50 (77.56)[d,f]	
V_{IN}	103.85 ± 36.33	72.6–186.05 (94.43)[d,f]	

*Kruskal-Wallis test and Mann-Whitney U test with pairwise analysis: $P < 0.05$ and $P < 0.001$. There was no statistically significant difference between the adhesive materials and veneering composite resin materials represented by the same letters, but a statistically significant difference was found between the groups with different letters.

SD, standard deviation.

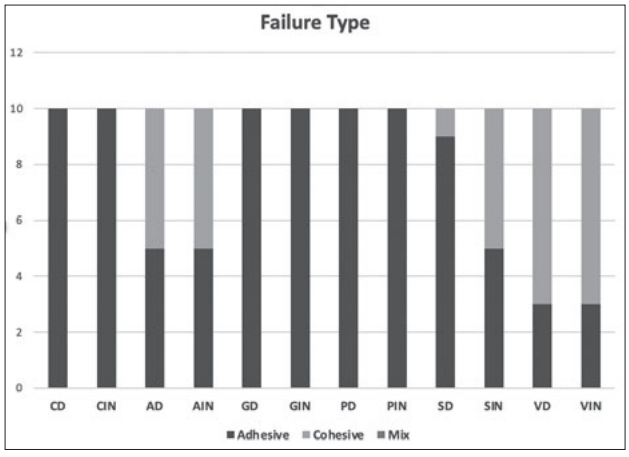

Fig 1 Failure distribution of specimens.

environment, then aged by thermal cycling for 5000 cycles between 5°C and 55°C with a dwell time of 20 seconds in distilled water in an automated thermocycling machine (Gökceler Machines, Sivas, Turkey).

A universal test machine (Autograph AGS X; Shimadzu Co, Kyoto, Japan) was used for the SBS test at a crosshead speed of 1 mm/minute. The SBS values were calculated in megapascals (MPa) by dividing failure load (N) by the area of the composite resin (a = P/A). The failure types of all the specimens were analysed using an optical microscope (MP 320; Carl Zeiss, Oberkochen, Germany) at 50× magnification. They were categorised as adhesive (failure at the interface between PEEK and the composite resin veneer), cohesive (failure in the PEEK material or the composite resin) and mixed (adhesive and cohesive failure of at least 25% of the surface).

SPSS for Windows version 21.0 (IBM, Armonk, NY, USA) was employed for statistical analysis. The normal distribution of the data was evaluated using a Kolmogorov-Smirnov test. Since the data were not normally distributed, a Kruskal-Wallis test was used to analyse the differences between SBS data according to the different adhesive materials and the composite resins. A Mann-Whitney U test was used for pairwise comparisons of groups with significant differences. Significance was evaluated as $P < 0.05$ and $P < 0.001$.

Results

The highest SBS results were obtained in the V_{IN} group, followed by the V_D, P_D, G_{IN}, A_{IN}, A_D, S_{IN}, S_D, P_{IN}, G_D, C_{IN} and C_D groups. Statistically significant differences were observed between the groups ($P = 0.001$). The types of composite resin for the same adhesive system did not have a significant impact on the results ($P > 0.05$), except for Gluma Bond Universal adhesive (Table 2). There was a statistically significant difference between the G_D and G_{IN} groups in this respect ($P = 0.009$; $P < 0.05$).

Regardless of the type of composite resin, when the control group was compared with the other groups, the C_D group had statistically lower SBS values compared to the A_{IN} ($P = 0.044$), A_D ($P = 0.039$), G_{IN} ($P = 0.04$), P_D ($P = 0.006$), V_D ($P = 0.001$) and V_{IN} ($P = 0.001$) groups. The C_{IN} group had statistically lower SBS values compared to the V_{IN} ($P = 0.001$), V_D ($P = 0.001$) and P_D ($P = 0.044$) groups. In addition, the P_{IN} group had a statistically significant difference only with the V_D ($P = 0.001$) and V_{IN} ($P = 0.002$) groups, which presented the highest SBS values (Table 2). The G_D group with the lowest SBS data

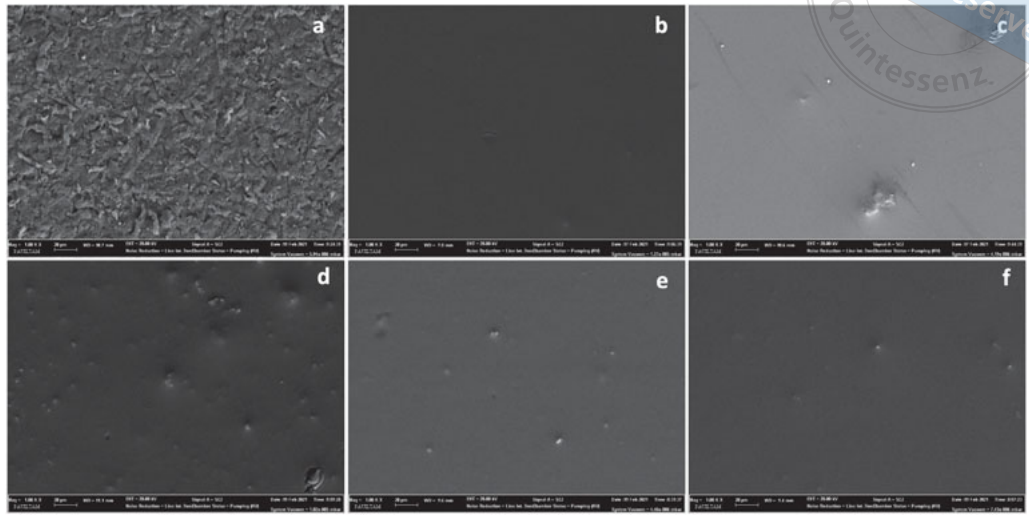

Fig 2 SEM imaging findings at 1000x magnification of PEEK materials after the adhesive material application. Control group, C **(a)**; Adhese Universal group, A **(b)**; Gluma Bond Universal group, G **(c)**; G-Premio BOND group, P **(d)**; Single Bond Universal group, S **(e)**; Visio.link group, V **(f)**.

had the highest statistical difference among the adhesive groups. Accordingly, there was a significant difference between the G_D group and the S_{IN} ($P = 0.018$), A_{IN} ($P = 0.01$), A_D ($P = 0.008$), G_{IN} ($P = 0.009$), P_D ($P = 0.001$), V_{IN} ($P = 0.001$) and V_D ($P = 0.001$) groups (Table 2).

Adhesive failure was detected in the C_{IN}, C_D, P_{IN}, P_D, G_{IN} and G_D groups, whereas both adhesive and cohesive failure were observed in the other groups. No mixed failure was found in any of the study groups (Fig 1).

SEM images of the specimens' surface topography after application of the adhesive material are shown in Fig 2. The surface of the C group had dense microporous areas, and there were differences between the groups regarding the surface covering of the adhesive material. The coverage was more homogeneous in group A (Fig 2b), and some porous areas could still be seen, especially in group P (Fig 2d).

The quantitative data of the elements detected by EDS analysis of the specimen surfaces are presented in Table 3. The elements most detected were C, O, Si and Ti. The most C was found in group A, the most O in group G, the most Ti in group C and the most Si in groups S and V. Moreover, Al was found in the C and P adhesive material groups (Table 3).

Discussion

This study evaluated the effect of different adhesives and composite resin veneers on the SBS of PEEK materials. The results showed that different adhesives and resins affected SBS. Thus, the null hypothesis, namely that the conditioning of the PEEK surface with different adhesives and the application of different composite resin veneers would not affect the SBS of the PEEK materials, was rejected.

The characteristic of the adherent surface is one of the most important parameters in adhesive applications. For this reason, a wide variety of surface pretreatments have been applied to improve the surface properties of polymeric materials and increase the adhesion of the surface area.[15] Air-abrasion is one of the simplest pretreatment methods to increase surface roughness. The adhesion surface area is expanded, organic pollutants are removed from the material surface and an active surface layer is formed.[14,24] Because of this, airborne-particle abrasion was the preferred surface pretreatment process in the present study.

Pretreatment modulates the PEEK surface to strengthen the micromechanical bond of the resin-containing materials; however, the use of adhesive systems is essential to establish a strong bond between the PEEK and the resin-containing materials. The SBS is related to the content of the adhesive materials.[6] Many studies have shown that adhesive materials containing methyl methacrylate (MMA) monomers exhibit greater SBS between resins and PEEK materials.[8,15,18,22,24] Among the adhesive materials tested in the present study, the MMA monomer was present only in the visio.link structure. Consistent with the literature, the highest SBS values were observed in the visio.link application groups. In addition, the higher SBS values of the visio.link groups may be related to the pentaerythritol structure, which is one of the main components of the material. The high capacity of pentaerythritol to modify the PEEK surface may have led to statistical differences between visio.link and other adhesive materials that contained methacrylate monomers or different forms of methacrylate.[6] It has been reported that pentaerythritol dissolves the PEEK surface, whereas MMA monomers form active reaction surfaces by swelling the dissolved surface and

Table 3 Percentage values by weight of the elements detected on specimen surfaces after adhesive application.

Element		Group (n = 3)					
		C	A	G	P	S	V
Mean weighted % (± SD)	C	52.27 ± 3.57	64.32 ± 0.52	60.39 ± 0.17	54.65 ± 1.13	53.58 ± 1.40	56.6 ± 2.49
	O	30.44 ± 2.85	32.29 ± 0.57	38.87 ± 0.16	37.51 ± 4.87	28.45 ± 1.31	36.98 ± 7.42
	Ti	7.59 ± 6.08	1.17 ± 0.19	0.73 ± 0.01	1.2 ± 0.13	0.88 ± 0.69	0.79 ± 0.07
	Si	4.59 ± 6.32	2.22 ± 0.92	NA	6.49 ± 3.66	17.08 ± 1.23	16.85 ± 0.02
	Al	6.63 ± 5.36	NA	NA	0.64	NA	NA
Element ratios	C/O	1.71	1.99	1.55	1.45	1.88	1.53

NA, not available; SD, standard deviation.

bonding to the composite resin veneers.[22] It is thought that this mechanism brings about an increase in SBS.[22] On the other hand, it has been reported that using adhesives that contain 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) has a negative effect on SBS.[22] This is because the functional group of the MDP monomer is occupied by a phosphate group that cannot chemically react with the PEEK substrate or the composite resins.[22] The present study showed that the SBS of the adhesives that contained MDP particles was lower than visio.link groups regardless of the type of composite resin.

Hydroxyethyl methacrylate (HEMA) is also a monomethacrylate known not to provide good long-term bond strength.[23] In the present study, only two adhesive systems (Adhese Universal and Single Bond Universal) containing the HEMA group were tested, and in accordance with the literature, the SBS values of these adhesive systems were lower than those of the P_D and G_{IN} groups, to which HEMA-free adhesive materials were applied.

The solvent in the adhesive material is also an important factor for crosslinking polymers. The solvent particles help the adhesive material to penetrate the polymer's structure.[17] Adhese Universal and Single Bond Universal materials contain ethanol as a solvent. It can penetrate deeper into the PEEK surface, but this may cause some adverse effects on adhesion. These may occur because of the disappearance of the adhesive material in the PEEK material structure and the absence of a reaction between the composite resins.[17,23] In the present study, the SBS values for the Adhese Universal and Single Bond Universal adhesive groups were lower than for other groups. This result may be related to the ethanol solvent in the adhesive material. These results were in contrast with those reported by Lümkemann et al[23], who tested similar universal adhesives (Adhese Universal, Scotchbond Universal [3M and G-premio BOND). Adhese Universal and Scotchbond Universal had higher tensile bond strength (TBS) values than G-Premio BOND. It is possible that the different findings reported by Lümkemann et al[23] came from combining adhesive materials with resin cement and using the TBS test.

Some of the present results may be due to the presence of substances in the universal adhesive materials that are not reported by the manufacturer but that may promote adhesion to PEEK surfaces. The data provided by the manufacturers about the composition of their materials are very limited. This not only makes it difficult to compare the results from different studies, but also negatively affects the authors' ability to reach a definitive conclusion about the effects of materials on PEEK bond strength.[15,22,23]

The EDS analysis showed that there were more elements on the surfaces of the samples in the control group than on those where adhesives were applied. The amounts of Ti and Al were much higher in the control group than in the other groups. It is thought that Ti particles are exposed through the airborne-particle abrasion process in PEEK materials, and after this process, Al particles remain on the surface due to the sand content. The SEM images of the groups where adhesive had been applied showed that the adhesive materials spread and filled the microporous areas on the materials' surface. This was different from the control group. A possible reason for this is that the diversity of elements and the ratios detected where adhesive material was applied were lower.

There is still no clear information about bonding composite resins to PEEK surfaces. The results of many studies on this subject are controversial and contradictory.[7,12,15,16,25] In the present study, when each adhesive system was evaluated within itself, it was determined that indirect composite application exhibited higher SBS values in all adhesive groups except the G-Premio BOND groups (P_D and P_{IN}). For them, the P_D group exhibited higher SBS values than the P_{IN} group, but the difference was not statistically significant ($P > 0.05$). The higher SBS values obtained from application of Essentia direct composite combined with G-Premio BOND adhesive material may be due to the fact that this adhesive is recommended in the manufacturer's instructions for the composite material. On the other hand, using different composite resins after application of the same adhesive led to a statistical difference only in the G_D and

G_{IN} groups ($P < 0.009$). The change in the type of veneer did not make a statistically significant difference in any other adhesive group ($P > 0.05$). The difference in SBS values between the G_D and G_{IN} groups could not be fully explained. In general, adhesive materials have different compositions according to their intended use, and it should not be expected that systems developed mainly for the adhesion of resin materials will guarantee adequate adhesion to PEEK in every application.[18]

Some previous studies have shown that the mechanical adhesion of composite resin veneers to the adherent surface depends on the viscosity of the material and thus the weight percent of the filler content. An increase in the particle content increases the material's viscosity, and this might negatively influence mechanical retention. Also, the chemical composition and low surface energy of PEEK can cause difficulties in bonding with composite resins. To solve this problem, it is known that in addition to micromechanical bonding, chemical bonding provided by adhesive materials is required.[24] When the direct and indirect composite that was preferred in the current study was examined in terms of filler content and viscosity, Essentia direct composite and Gradia Plus indirect composite contained 81% and 80% filler by weight, respectively. The results also showed that their viscosity was also comparable (0.34 to 0.36 kPa s for Essentia and 0.41 to 0.43 kPa s for Gradia Plus).[26] In the present study, indirect and direct applications of composites did not have a significant effect on the SBS, except for in the two adhesive groups. This can be explained by the similarity of the filler content and the viscosities of the materials. In addition, some researchers have found that the viscosity of the composite resins does not affect the quality of adhesion.[15]

In some studies focusing on the SBS values of veneered PEEK materials, results were obtained that support the present findings.[5,27,28] In a recent study comparing direct composite, indirect composite and flowable composites used in the veneering process, regardless of the PEEK material and surface treatments employed, it was determined that material viscosity was one of the most effective factors.[27] It was observed that flowable composites exhibited the highest SBS value in groups where the same main material and surface treatment were applied, and the current result was associated with the wettability of the surface. The viscosity parameter and filler ratios of the veneer material are critical in eliminating the disadvantage of the low surface energy of the PEEK material.[5,28] In the present study, the selection of composite materials that were very close to each other in terms of these two parameters may have been insufficient to reveal the possible effect of the veneering type on bond strength. It is critical to eliminate these scientific uncertainties by repeating this study with direct and indirect composites with different filler ratios and viscosities in the future.

Other studies have shown that SBS values higher than 10 MPa are clinically acceptable.[15,19] In this context, SBS values of all adhesive materials and combinations of composite resins in the present study were above this limit. These results showed that it is possible to use alternative materials when visio.link cannot be used. On the other hand, using airborne-particle abrasion as the initial surface treatment caused even the control groups (C_D and C_{IN}) to exhibit SBS results above the 10 MPa threshold. This means that airborne-particle abrasion is extremely important for adhering to the PEEK surface, as other studies have found.[14,24]

The present study has some limitations. To the best of the authors' knowledge, it is the first study to test direct and indirect veneering with composite resins and different combinations of universal adhesive materials and to compare the effectiveness of these materials on the SBS of PEEK materials. Thus, it was difficult to compare these findings with those of previous studies. In the present study, thermal cycling was used for artificial aging, and this may have affected the materials in two different ways. First, it might have increased the SBS caused by post-polymerisation in the contact area between the PEEK surface and the adhesive material and the composite resins. Second, the thermal stress from this aging method may have caused the materials to exhibit different volumetric changes and created mechanical stress in the adhesion area.[18,21,29] Therefore, to better understand the effect of the aging method, it is important to examine groups that are not subjected to the aging procedure. In addition, only 5000 cycles were used in the aging protocol in this study, but it is clinically important to investigate how the SBS values are affected after long-term aging procedures. Intraoral conditions could not be imitated fully in the present study; however, the results may provide clinicians with insight into alternative adhesive materials that can be used to achieve reliable bond strength between PEEK materials and different composite resins. Thus, more in vivo and in vitro studies are needed to evaluate the long-term clinical performance of both adhesive systems and composite resin veneers.

Conclusion

The visio.link groups (V_{IN} and V_D) showed the highest SBS results, followed by the P_D and G_{IN} groups. The type

of composite resin veneer did not make a significant difference in terms of SBS values for both visio.link and the tested universal adhesives, except for Gluma Bond Universal. All the tested universal adhesives showed clinically acceptable SBS results.

Conflicts of interest

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

Author contribution

Dr Cinel Sahin contributed to the conception, methodology, experiment and manuscript draft; Dr Mutlu-Sagesen contributed to the data analysis; Drs Karaokutan and Özcan contributed to the editing and revision of the manuscript. All the authors approved the final version of the manuscript.

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References

1. Silthampitag P, Chaijareenont P, Tattakorn K, Banjongprasert C, Takahashi H, Arksornnukit M. Effect of surface pretreatments on resin composite bonding to PEEK. Dent Mater J 2016;35:668–674.
2. Najeeb S, Zafar MS, Khurshid Z, Siddiqui F. Applications of polyetheretherketone (PEEK) in oral implantology and prosthodontics. J Prosthodont Res 2016;60:12–19.
3. Gama LT, Duque TM, Özcan M, Philippi AG, Mezzomo LAM, Gonçalves TMSV. Adhesion to high-performance polymers applied in dentistry: A systematic review. Dent Mater 2020;36:e93–e108.
4. Qin L, Yao S, Zhao J, et al. Review on development and dental applications of polyetheretherketone-based biomaterials and restorations. Materials (Basel) 2021;14:408.
5. Stawarczyk B, Beuer F, Wimmer T, et al. Polyetheretherketone-A suitable material for fixed dental prostheses? J Biomed Mater Res B Appl Biomater 2013;101:1209–1216.
6. Caglar I, Ates SM, Yesil Duymus Z. An in vitro evaluation of the effect of various adhesives and surface treatments on bond strength of resin cement to polyetheretherketone. J Prosthodont 2019;28:e342–e349.
7. Stawarczyk B, Jordan P, Schmidlin PR, et al. PEEK surface treatment effects on tensile bond strength to veneering resins. J Prosthet Dent 2014;112:1278–1288.
8. Stawarczyk B, Taufall S, Roos M, Schmidlin PR, Lümkemann N. Bonding of composite resins to PEEK: the influence of adhesive systems and air-abrasion parameters. Clin Oral Investig 2018;22:763–771.
9. Gracis S, Nicholls JI, Chalupnik JD, Yuodelis RA. Shock-absorbing behavior of five restorative materials used on implants. Int J Prosthodont 1991;4:282–291.
10. Barto A, Vandewalle KS, Lien W, Whang K. Repair of resin-veneered polyetheretherketone after veneer fracture. J Prosthet Dent 2021;125:704.e1-704.e8.
11. Lambrechts P, Ameye C, Vanherle G. Conventional and microfilled composite resins. Part II. Chip fractures. J Prosthet Dent 1982;48:527–538.
12. Kern M, Lehmann F. Influence of surface conditioning on bonding to polyetheretherketone (PEEK). Dent Mater 2012;28:1280–1283.
13. Ha SW, Kirch M, Birchler F, et al. Surface activation of polyetheretherketone (PEEK) and formation of calcium phosphate coatings by precipitation. J Mater Sci Mater Med 1997;8:683–690.
14. Zhou L, Qian Y, Zhu Y, Liu H, Gan K, Guo J. The effect of different surface treatments on the bond strength of PEEK composite materials. Dent Mater 2014;30:e209–e215.
15. Rosentritt M, Preis V, Behr M, Sereno N, Kolbeck C. Shear bond strength between veneering composite and PEEK after different surface modifications. Clin Oral Investig 2015;19:739–744.
16. Schmidlin PR, Stawarczyk B, Wieland M, Attin T, Hämmerle CH, Fischer J. Effect of different surface pre-treatments and luting materials on shear bond strength to PEEK. Dent Mater 2010;26:553–559.
17. Hallmann L, Mehl A, Sereno N, Hämmerle CHF. The improvement of adhesive properties of PEEK through different pretreatments. Applied Surface Science 2012;258:7213–7218.
18. Stawarczyk B, Keul C, Beuer F, Roos M, Schmidlin PR. Tensile bond strength of veneering resins to PEEK: Impact of different adhesives. Dent Mater J 2013;32:441–448.
19. Behr M, Proff P, Kolbeck C, et al. The bond strength of the resin-to-zirconia interface using different bonding concepts. J Mech Behav Biomed Mater 2011;4:2–8.
20. Bunz O, Benz CI, Arnold WH, Piwowarczyk A. Shear bond strength of veneering composite to high performance polymers. Dent Mater J 2021;40:304-311.
21. Bähr N, Keul C, Edelhoff D, et al. Effect of different adhesives combined with two resin composite cements on shear bond strength to polymeric CAD/CAM materials. Dent Mater J 2013;32:492–501.
22. Keul C, Liebermann A, Schmidlin PR, Roos M, Sener B, Stawarczyk B. Influence of PEEK surface modification on surface properties and bond strength to veneering resin composites. J Adhes Dent 2014;16:383–392.
23. Lümkemann N, Strickstrock M, Eichberger M, Zylla I, Stawarczyk B. Impact of air-abrasion pressure and adhesive systems on bonding parameters for polyetheretherketone dental restorations. Int J Adhes Adhes 2018;80:30–38.
24. Uhrenbacher J, Schmidlin PR, Keul C, et al. The effect of surface modification on the retention strength of polyetheretherketone crowns adhesively bonded to dentin abutments. J Prosthet Dent 2014;112:1489–1497.
25. Stawarczyk B, Bähr N, Beuer F, et al. Influence of plasma pretreatment on shear bond strength of self-adhesive resin cements to polyetheretherketone. Clin Oral Investig 2014;18:163–170.
26. Marcondes RL, Lima VP, Barbon FJ, et al. Viscosity and thermal kinetics of 10 preheated restorative resin composites and effect of ultrasound energy on film thickness. Dent Mater 2020;36:1356–1364.
27. Bötel F, Zimmermann T, Sütel M, Müller WD, Schwitalla AD. Influence of different low-pressure plasma process parameters on shear bond strength between veneering composites and PEEK materials. Dent Mater 2018;34:e246–e254.
28. Schwitalla AD, Bötel F, Zimmermann T, Sütel M, Müller WD. The impact of argon/oxygen low-pressure plasma on shear bond strength between a veneering composite and different PEEK materials. Dent Mater 2017;33:990–994.
29. Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent 1999;27:89–99.

Sustained Release of Liposomal Curcumin: Enhanced Periodontal Outcomes in Diabetic Patients

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Objective: *To evaluate the effect of entrapment of curcumin within liposomal formulation and the sustained release attitude of the formulated liposomal gel on periodontal defects in diabetic patients in clinical and biochemical terms.*

Methods: *Thirty diabetic patients with periodontitis were randomly assigned to three equal groups and ten healthy participants were assigned as the control group. Group I was subjected to scaling and root planing (SRP) with application of sustained release liposomal curcumin gel. Group II was subjected to scaling and root planning with application of curcumin gel. Group III was subjected to scaling and root planning with application of placebo gel. Group IV (control group), no intervention was done. The following parameters were evaluated before treatment and after 6 and 12 weeks: plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment level (CAL), tumour necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and total antioxidant capacity (TAC).*

Results: *All study groups showed improvement in clinical and biochemical parameters that are statistically significant. Upon comparing the results of treatment modalities, the highest improvement was achieved in group I followed by group II then group III.*

Conclusion: *Sustained release liposomal curcumin gel enhanced the antioxidant capacity, decreased the inflammatory mediators and showed more improvement in clinical outcome for treatment of periodontitis in diabetic patients.*

Keywords: *curcumin, diabetes mellitus, liposomes gel, periodontitis, sustained release*

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Periodontitis is a multifactorial inflammatory illness characterized by gradual deterioration of the tooth supporting apparatus and linked to some bacterial species of dysbiotic plaque biofilms1. Diabetes mellitus (DM) is a chronic, complex condition that necessitates ongoing treatment and multifaceted approaches to maintain glycemic control. Type 1 and type 2 DM are the two most common varieties. Type 1 diabetes is defined by insulin insufficiency produced by autoimmune destruction of pancreatic B-cells, whereas type 2 diabetes is caused by insulin resistance[1,2]. Periodontitis is considered as one of the complications in diabetic patients[3]. The periodontium is affected by diabetes in two ways: first, by modifying the host immunological, inflammatory, and wound-healing responses, encouraging the accumulation of advanced glycation end products; and second, by producing high amounts of pro-inflammatory cytokines.[4]

Curcumin is a polyphenolic dietary phytochemical, and the bioactive pigment present in the roots of Curcuma longa L. (turmeric)[5]. The major yellow bioactive component of turmeric, curcumin (diferuloylmethane), has been proven in literatures to have a wide range of biological actions such as anti-inflammatory, antioxidant antibacterial and anti-fungal properties.[6,7] Natural or chemically modified curcumin has been proposed as a possible therapy alternative for diabetes and related problems such as periodontitis due to its anti-inflammatory and antioxidant effects.[8-12]

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However, suitable dosage form should be designed to guarantee easy administration, patient compliance, enhanced permeation through membranes and maximized therapeutic effect. Many reports have revealed the impact of nano-carriers on the improvement of targeting and cellular permeation of entrapped natural product.[13-17]

Liposomes are lipid-based nanostructures which have the advantages of being biocompatible, encapsulate both hydrophilic and lipophilic components within their aqueous core and lipid bilayer, respectively, enhance the stability of entrapped cargo and maximize cellular penetration[13-17]. The thick network of gel formulation retard the release of the entrapped components allowing sustained release and prolonged time of action.[18]

Hence, the present study was carried out as a clinical trial to evaluate the effect of sustained release gel of curcumin and liposomal curcumin on periodontal therapy in diabetic patient.

Materials and methods

This study was conducted on a total of 40 participant, 30 diabetic patients (type 2 DM) with periodontitis that were divided randomly to 3 groups each having 10 patients and 10 healthy participants (no systemic disease or periodontitis) as control group. The patients were selected from the outpatient's clinic of the Oral medicine, Oral diagnosis, and Periodontology Department, Faculty of Dentistry, Minia University.

Ethical regulations

The complete treatment plan was explained to all patients including detailed steps, risks, and expected results, and their full signed consent was obtained before entry into the study. The study complied with the rules set by the International Conference on Harmonization Good Clinical Practice Guidelines, and the Declaration of Helsinki. The study was approved by the research ethics committee of the Faculty of Dentistry, Minia University (No. 663 in 2022).

Patient selection

Selected patients of both sexes were from 35-60 years with confirmed diabetes type 2, according to WHO guidelines in 1999 and its update in 2009 and all patients were screened for glycosylated hemoglobin (HbA1c) to confirm the diagnosis (6.5% or more)[19,20], and stage II periodontitis. Ten healthy participants for control group. All subjects had not received any type of periodontal therapy, prescribed antibiotics, or anti-inflammatory medication within the preceding 6 months. Pregnant, lactating females and smokers were excluded from the study.

Preparation of sustained release curcumin gel (Cu-gel) and curcumin liposomal gel (Cuc-Lip gel)

Cuc-Lip were prepared using a new modified injection method.[21] Briefly, lipoid s75 (50 mmol), cholesterol (20% w/v) and curcumin (25 mg) were dissolved in the least volume of absolute ethanol then the solution was transferred to a spraying apparatus. In a closed system, the solution of lipids and curcumin was sprayed (200 ul per five seconds) on the surface of 1 ml of an aqueous media of distilled water containing sucrose (9% w/v) stirred at 1500 rpm at 80°C. Excess ethanol was evaporated with stirring and liposomes were formed spontaneously after further evaporation of the residual ethanol. Prepared liposomes were kept at 4°C overnight to allow annealing of the lipid bilayer.[22] The liposomal suspension was then available for further processes of evaluation and in-vitro characterization. Particle size, poly dispersity index (PDI), and transmission electron microscopy (TEM). Curcumin liposomal gel (Cuc-Lip gel) was prepared using carboxy methyl cellulose (CMC). CMC solution (1 mg/mL) was injected on the surface of prepared Cuc-Lip under magnetic stirring at room temperature for 30 min. The suspension was sonicated in an ice bath.[23] Curcumin gel (Cu-gel) and curcumin liposomal gel were prepared by the injection of CMC solution (1 mg/mL) on the surface of curcumin suspension (25 mg/ml) and Cuc-Lip (25 mg/ml), respectively, with continuous stirring at room temperature. Liposomal size and size distribution were determined using laser light diffraction techniques. Briefly, the liposomal preparation was diluted using purified deionized water and was analyzed at 25°C using Mastersizer (3000E Malvern Instruments, UK). This procedure was done in triplicate for each preparation and the average values were used.[24]

Treatment protocol

Each patient was given detailed instruction on self-performed oral hygiene measures using toothbrush and interdental brush or floss. Patients then were recalled after 7 days for baseline measurements of clinical parameters and baseline gingival cervicular fluid (GCF) sample collection.

The 30 patients were grouped into three 3 equal groups (I, II and III) and subjected to non-surgical periodontal therapy in the form of full mouth supra and sub

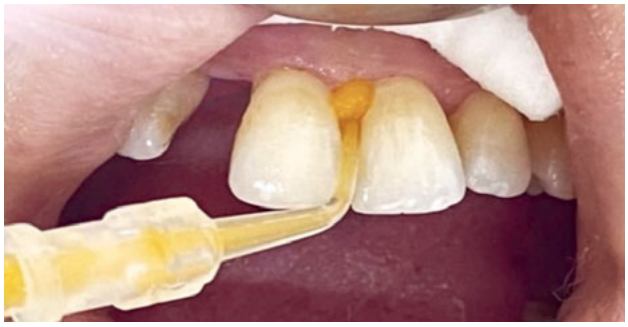

Fig 1 Application of curcumin gel.

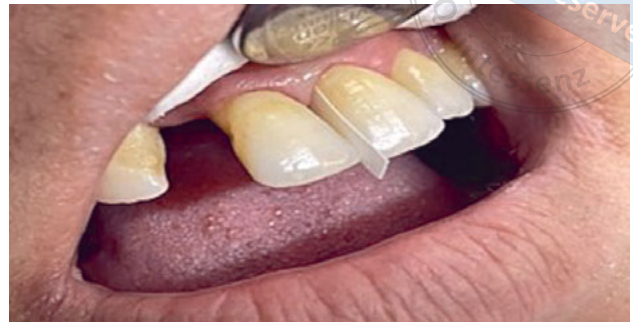

Fig 2 GCF sampling.

gingival scaling and root planning using hand instruments with supragingival debridement followed by universal curette for proper subgingival debridement. The treatment was completed in three sessions over a period of 2 weeks. Ten healthy subjects were chosen as volunteers for negative control group (IV).

After one week of SRP, Group I received curcumin liposomal gel (Cuc-Lip gel), Group II received curcumin gel (Cu-gel) and Group III received placebo gel. Group IV (negative control group) no intervention was done.

The four groups were instructed to follow maintenance program consists of brushing 2 times daily using tooth paste with soft toothbrush, Interdental cleaning twice daily using dental floss or an interdental brush.

Application of the gel

After the isolation using cotton rolls, the gel was applied by a curved blunt-ended plastic tip syringe. The needle was carefully inserted in the periodontal pocket and the gel was injected to fill the selected pocket till the gingival margin (Fig 1). Patients were instructed to follow oral hygiene measures during the study period. They were also asked not to use the toothbrush at selected site after gel application for 24 hours and not to chew hard or sticky foods at the gel placement sites.

Assessment of periodontal parameters

The 3 study groups were evaluated clinically regularly at baseline, 6 weeks and 12 weeks post-operative. Every patient was assessed by the following clinical parameters include PI, GI, PD and CAL. Control group was assessed at baseline only.

GCF sampling

GCF samples were collected at baseline, 6 weeks and 12 weeks from study groups and at baseline from control group to determine total antioxidant capacity (TAC), IL-1β and TNF-α using the absorbent filter paper from the deepest probing site. The selected tooth sites were isolated with sterile cotton rolls, the teeth were gently air-dried. The paper strips were placed carefully inside the gingival crevice for 30 seconds and then placed in Eppendorf tubes containing phosphate - buffered saline and stored at -20°C until further use (Fig 2).

Biochemical assessment

GCF samples were assessed using the total antioxidant capacity ELISA human kit (TAC ELISA kit) and The Picokine™ Human IL-1β and TNF-α ELISA Kit (Boster, Pleasanton, California, USA) for the accurate detection of human total antioxidant capacity, IL1-β and TNF-α respectively.

Statistical analysis

Data was entered and analyzed by statistical program (SPSS version 20), displayed as mean and standard deviation. Comparison was done by one-way analysis of variance (ANOVA) test, considered significant at $P < 0.05$.

Results

Preparation of CU-Lip gel

The spraying method successfully produced nanosized uniform curcumin liposomal formulation (Fig 3), with a very unnoticeable aggregation of unencapsulated curcumin during preparation, and neat macroscopic appearance of the prepared liposomes. The prepared formulation (Cuc-Lip gel) exhibited particle size of 84.5 ± 5.6 nm. The prepared liposomes were homogenously distributed with PDI 0.4.

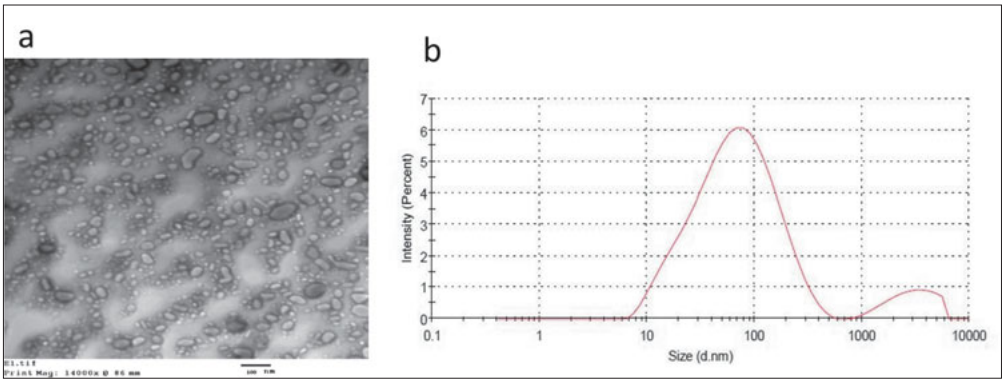

Fig 3 TEM and size distribution of Cuc-lip gel.

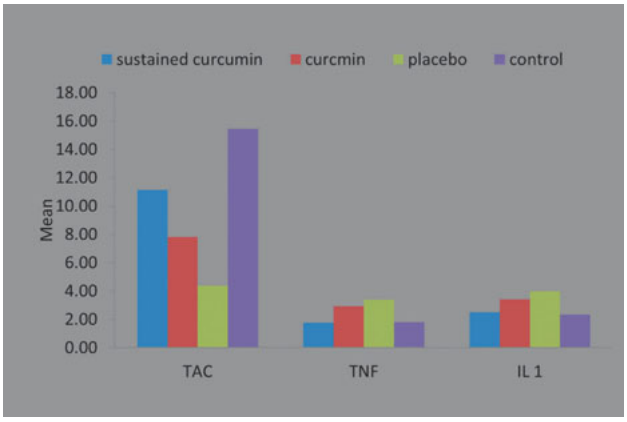

Fig 4 comparison of biochemical parameters of different groups 12 weeks post-treatment.

Assessment of periodontal and biochemical parameters

The mean PI, GI, PD, CAL, IL-1β, TNF-α and TAC at baseline, 6 weeks and 12 weeks after treatment are presented in Table 1.

Results of all treatments used in this study showed that all study groups showed statistically significant improvement after 12 weeks of treatment regarding PI, GI, PD, CAL, IL-1β, TNF-α and TAC. Upon comparison of all groups, the highest improvement in clinical and biochemical parameters was in group I (sustained liposomal curcumin gel), followed by group II (curcumin gel) and least improvement was in group III (placebo).

Discussion

Periodontitis and diabetes mellitus have a bidirectional link that exists irrespective of associated risk factors, and the two diseases affect each other. Oxidative stress processes are increasingly implicated in the pathobiology of periodontitis and diabetes mellitus.[25]

Antioxidants have been shown in epidemiological research to limit the effects of reactive oxygen species activity and reduce illness incidence, making natural-based antioxidants one of the most useful therapeutic agents for reducing oxidative stress-related disorders. Considering that total antioxidant level influences metabolic regulation and tissue damage, antioxidant supplementation as an adjunct to SPR in type 2 diabetic patients may be beneficial11. Therefore, the current study was conducted to evaluate the potential of curcumin as a potent antioxidant and anti-inflammatory agent in periodontitis in diabetic patients.

Curcumin stimulates the activity of antioxidant enzymes resulting in high antioxidant activity. Curcumin's ability to scavenge free radicals is another mechanism maintained in the antioxidant activity of the drug. A previous study has reported the impact of reduction of levels of free radicles in human by increasing the activity of antioxidant enzymes.[11]

This study evaluated the effect of curcumin on the improvement of clinical and biochemical parameters in diabetic patients with periodontitis. Moreover, the impact of encapsulating curcumin within liposomal gel of sustained release behavior with scaling and root planning was evaluated.

Bibi et al[12] reported that the typical operating method for GCF specimens includes using perio-paper as the best transportation source. This method has a number of advantages, including ease of use and ability to collect data from discrete spots.[4] As a result, it was chosen for this study.

All groups demonstrated statistically significant improvements in clinical and biochemical parameters. The improvement in group III is likely to be due to correct scaling, root planning, and reinforcement of oral hygiene measurements during the initial therapy and follow-up periods. The further clinical and biochemical improvement in group II could be attributed to the antioxidant and anti-inflammatory effect of curcumin gel.

Table 1 Comparison of clinical and biomechanical parameters for all groups.

Clinical parameter		Sustained curcumin	Curcumin	Placebo	Control	*P* value
PI	0	1.90 ± 0.21	1.94 ± 0.16	1.90 ± 0.21	0.01 ± 0.02	< 0.001*
	6 wk	0.65 ± 0.33	0.83 ± 0.25	0.80 ± 0.25	0.01 ± 0.02	0.337
	12 wk	0.05 ± 0.08	0.06 ± 0.16	0.37 ± 0.46	0.01 ± 0.02	0.034*
GI	0	1.90 ± 0.31	1.95 ± 0.15	1.95 ± 0.15	0.00 ± 0.00	< 0.001*
	6 wk	0.46 ± 0.35	0.60 ± 0.21	0.85 ± 0.24	0.00 ± 0.00	0.013*
	12 wk	0.25 ± 0.05	0.60 ± 0.15	0.50 ± 0.31	0.00 ± 0.00	< 0.001*
PD	0	2.93 ± 0.30	3.73 ± 0.66	3.65 ± 0.82	0.90 ± 0.07	< 0.001*
	6 wk	2.61 ± 0.20	2.93 ± 0.52	2.98 ± 0.65	0.90 ± 0.07	0.219
	12 wk	1.87 ± 0.34	2.44 ± 0.55	2.88 ± 0.55	0.90 ± 0.07	< 0.001*
CAL	0	0.98 ± 0.27	1.14 ± 0.50	1.01 ± 0.58	0.00 ± 0.00	< 0.001*
	6 wk	0.33 ± 0.20	0.53 ± 0.30	0.50 ± 0.29	0.00 ± 0.00	0.232
	12 wk	0.09 ± 0.15	0.26 ± 0.22	0.33 ± 0.39	0.00 ± 0.00	0.170
TAC	0	5.44 ± 0.71	3.62 ± 1.00	2.37 ± 1.31	15.45 ± 1.48	< 0.001*
	6 wk	7.79 ± 0.53	5.48 ± 1.04	3.66 ± 1.22	15.45 ± 1.48	< 0.001*
	12 wk	11.15 ± 0.75	7.82 ± 0.76	4.39 ± 1.03	15.45 ± 1.48	< 0.001*
IL-1β	0	4.33 ± 0.15	6.49 ± 1.98	8.20 ± 0.21	2.35 ± 0.10	< 0.001*
	6 wk	3.52 ± 0.10	5.35 ± 0.19	6.18 ± 0.28	2.35 ± 0.10	< 0.001*
	12 wk	2.52 ± 0.09	3.42 ± 0.13	3.97 ± 0.16	2.35 ± 0.10	< 0.001*
TNF-α	0	3.46 ± 0.29	5.55 ± 0.10	6.62 ± 0.19	1.79 ± 0.11	< 0.001*
	6 wk	2.51 ± 0.20	4.81 ± 0.07	5.22 ± 0.10	1.79 ± 0.11	< 0.001*
	12 wk	1.78 ± 0.18	2.92 ± 0.11	3.39 ± 0.08	1.79 ± 0.11	< 0.001*

*Statistically significant.

Those findings come in accordance with Mohammad et al[26] who revealed that curcumin gel had a significant effect on the reduction of IL-1β, TNF-α, copper, and clinical parameters and increase of zinc and magnesium levels.

The obvious significant enhancement in all clinical and biochemical parameters in Group I, receiving the sustained release liposomal gel, compared to the group receiving curcumin gel (group II) can be attributed to the impact of liposomal formulation in entrapping both the hydrophilic and the lipophilic components of the curcumin within the formed vesicles. Moreover, the small particle size and the homogenous distribution of the formulated vesicles enhance the cellular penetration of the nano-structured vesicles along with the entrapped cargo.[27] In addition, the formulation of curcumin liposomes within a sustained release gel formulation permits the prolonged action of the administered dose of curcumin.[28] This is significantly apparent in the remarkable reduction in IL-1β and TNF-α and substantial enhancement of PT, GI. CAL and TAC after 3 weeks of application of the curcumin liposomal gel (Group I) compared to application of curcumin gel (group II).

This study also revealed the antioxidant and anti-inflammatory effect of curcumin in treatment of periodontitis in diabetic patient thus support its employment as a therapeutic strategy in treatment of periodontitis in diabetic circumstances. More studies with larger numbers of subjects are recommended to ensure the results of IL-1β and TNF-α since the base line of those tests are significantly different.

Conclusion

Sustained liposomal curcumin gel enhanced the antioxidant capacity, decreased the inflammatory mediators (IL-1β, TNF-α) and showed more improvement in clinical outcome in treatment of periodontitis diabetic patients by entrapped curcumin.

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Conflicts of interest

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

Author contribution

Both authors contributed to methodology, interpretation of data, editing and revising the manuscript.

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References

1. Tasdemir Z, Oskaybas MN, Alkan AB, Cakmak O. The effects of ozone therapy on periodontal therapy: A randomized placebo-controlled clinical trial. Oral Dis 2019;25:1195-1202.
2. Souto MLS, Rovai ES, Ganhito JA, Holzhausen M, Chambrone L, Pannuti CM. Efficacy of systemic antibiotics in nonsurgical periodontal therapy for diabetic subjects: a systematic review and meta-analysis. Int Dent J 2018;68:207-220.
3. Chandra S, Shashikumar P. Diode Laser - A Novel Therapeutic Approach in the Treatment of Chronic Periodontitis in Type 2 Diabetes Mellitus Patients: A Prospective Randomized Controlled Clinical Trial. J Lasers Med Sci 2019;10:56-63.
4. Mauri-Obradors E, Merlos A, Estrugo-Devesa A, Jané-Salas E, López-López J, Viñas M. Benefits of non-surgical periodontal treatment in patients with type 2 diabetes mellitus and chronic periodontitis: A randomized controlled trial. J Clin Periodontol 2018;45:345-353.
5. Abdollahi E, Momtazi AA, Johnston TP, Sahebkar A. Therapeutic effects of curcumin in inflammatory and immune-mediated diseases: A nature-made jack-of-all-trades? J Cell Physiol 2018;233:830-848.
6. Bhatia M, Urolagin SS, Pentyala KB, Urolagin SB, K B M, Bhoi S. Novel therapeutic approach for the treatment of periodontitis by curcumin. J Clin Diagn Res 2014;8:ZC65-ZC69.
7. Sha AM, Garib BT. Antibacterial Effect of Curcumin against Clinically Isolated Porphyromonas gingivalis and Connective Tissue Reactions to Curcumin Gel in the Subcutaneous Tissue of Rats. Biomed Res Int 2019;2019:6810936.
8. Pimentel SP, Casati MZ, Ribeiro FV, et al. Impact of natural curcumin on the progression of experimental periodontitis in diabetic rats. J Periodontal Res 2020;55:41-50.
9. Altıngöz SM, Kurgan Ş, Önder C, et al. Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: A cross-sectional study. J Periodontol 2021;92:1274-1285.
10. Araújo EG, Oliveira DMSL, Martins CC, Stefani CM. Efficacy of Antioxidant Supplementation to Non-Surgical Periodontal Therapy on Metabolic Control in Type 2 Diabetes Patients: A Network Meta-Analysis. Antioxidants (Basel) 2022;11:621.
11. Park JH, Lee BM, Kim HS. Potential protective roles of curcumin against cadmium-induced toxicity and oxidative stress. J Toxicol Environ Health B Crit Rev 2021;24:95-118.
12. Bibi T, Khurshid Z, Rehman A, Imran E, Srivastava KC, Shrivastava D. Gingival crevicular fluid (GCF): a diagnostic tool for the detection of periodontal health and diseases. Molecules 2021;26:1208.
13. Mohamed M, Abu Lila AS, Shimizu T, et al. PEGylated liposomes: immunological responses. Sci Technol Adv Mater 2019;20:710-724.
14. Refaat H, Mady FM, Sarhan HA, Rateb HS, Alaaeldin E. Optimization and evaluation of propolis liposomes as a promising therapeutic approach for COVID-19. Int J Pharm 2021;592:120028.
15. El Sayed MM, Takata H, Shimizu T, et al. Hepatosplenic phagocytic cells indirectly contribute to anti-PEG IgM production in the accelerated blood clearance (ABC) phenomenon against PEGylated liposomes: Appearance of an unexplained mechanism in the ABC phenomenon. J Control Release 2020;323:102-109.
16. Mohamed M, Alaaeldin E, Hussein A, SarhaHA. Liposomes and PEGylated liposomes as drug delivery system. Journal of advanced Biomedical and Pharmaceutical Sciences 2020;3:80-88.
17. Owis AI, El-Hawary MS, El Amir D, et al. Flavonoids of Salvadora persica L. (meswak) and its liposomal formulation as a potential inhibitor of SARS-CoV-2. RSC Adv 2021;11:13537-13544.
18. Steinberg D, Friedman M. Dental drug-delivery devices: local and sustained-release applications. Crit Rev Ther Drug Carrier Syst 1999;16:425-459.
19. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee; Goldenberg R, Punthakee Z. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Can J Diabetes 2013;37 Suppl 1:S8-S11.
20. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-553.
21. Refaat H, Naguib YW, Elsayed MMA, Sarhan HAA, Alaaeldin E. Modified Spraying Technique and Response Surface Methodology for the Preparation and Optimization of Propolis Liposomes of Enhanced Anti-Proliferative Activity against Human Melanoma Cell Line A375. Pharmaceutics 2019;11:558.
22. Füldner HH. Characterization of a third phase transition in multilamellar dipalmitoyllecithin liposomes. Biochemistry 1981;20:5707-5710.
23. Tian MP, Song RX, Wang T, Sun MJ, Liu Y, Chen XG. Inducing sustained release and improving oral bioavailability of curcumin via chitosan derivatives-coated liposomes. Int J Biol Macromol 2018;120(Pt A):702-710.
24. Mostafa M, Alaaeldin E, Aly UF, Sarhan HA. Optimization and Characterization of Thymoquinone-Loaded Liposomes with Enhanced Topical Anti-inflammatory Activity. AAPS PharmSciTech 2018;19:3490-3500.
25. Barutta F, Bellini S, Durazzo M, Gruden G. Novel Insight into the Mechanisms of the Bidirectional Relationship between Diabetes and Periodontitis. Biomedicines 2022;10:178.
26. Mohammad CA. Efficacy of Curcumin Gel on Zinc, Magnesium, Copper, IL-1β, and TNF-α in Chronic Periodontitis Patients. Biomed Res Int 2020;2020:8850926.
27. Alaaeldin E, Mostafa M, Mansour HF, Soliman GM. Spanlastics as an efficient delivery system for the enhancement of thymoquinone anticancer efficacy: Fabrication and cytotoxic studies against breast cancer cell lines. Journal of Drug Delivery Science and Technology 2021;65:102725.
28. Alaaeldin E, Gomaa MD, Eltewacy NK, Ali A, Khalil AA. Formulation and characterization of thymoquinone bioadhesive gel for treatment of chronic gum inflammation. Int J Sci Basic Appl Res 2017;31:321-330.

Preservation of Pulp Vitality in Type IIIA Dens Invaginatus with an Extensive Peri-invagination Lesion: a case Report with 5-year Follow-up

Fei LIN[1], Lin YUE[1]

Dens invaginatus may be associated with peri-invagination lesions and vital pulp concurrently. This case report examines the successful preservation of vital pulp and minimally invasive treatment of invagination for Oehlers type IIIA dens invaginatus with an extensive peri-invagination lesion. A healthy 19-year-old man presented with occasional swelling of the left maxillary anterior region. Pulp vitality tests revealed vital and healthy tooth pulp. CBCT indicated Oehlers type IIIA dens invaginatus with an invagination parallel to the pulp cavity. The diagnosis was type IIIA dens invaginatus with a peri-invagination lesion. The treatment plans involved preservation of the vital pulp and minimally invasive treatment of the invagination. A 5-year follow-up revealed that both healing of the peri-invagination lesion and preservation of the vital pulp had been successful. Pulp vitality can be preserved in type IIIA dens invaginatus associated with a peri-invagination lesion through minimally invasive treatment of the invagination.

Keywords: *dens invaginatus, minimally invasive, peri-invagination lesion, vital pulp preservation*

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Dens invaginatus is a developmental malformation of teeth, induced by an invagination of the enamel organ into the dental papilla during tooth development.[1,2] The invagination may also result from the infolding of the Hertwig sheath and lead to radicular dens invaginatus.[3,4] According to the classification proposed by Oehlers[5], there are three types of dens invaginatus:

- Type I: the enamel-lined invagination is confined within the crown of the tooth and does not extend beyond the cementoenamel junction.
- Type II: the invagination invades into the root but remains confined within it as a blind sac.
- Type III: the invagination penetrates through the root and communicates with the periodontal ligament laterally (IIIA) or apically (IIIB) as a pseudoforamen.

Among the above types, type III was reported as the least common, with an incidence of 4.6~5%, followed by type II (15~29.5%) and type I (65.9~79%).[6,7]

In teeth with type III dens invaginatus, the variable anatomy of invagination could be associated with the anatomy of the pulp chamber, leading to several morphological changes of the root canal system, such as wave-like constrictions, dilatation or tear-shaped structures.[1] The morphological complexities of the invagination and pulp and the possible connections between them sometimes make it difficult for dental practitioners to determine whether the pulp is under pathosis or if endodontic treatment should be performed.[1,7] Thus, there are conflicting opinions on conserving vital pulp or treating invagination together with the pulp.[8,9]

One option is to perform endodontic treatment as well as treating invagination, especially in dens invaginatus associated with pulpal diseases. It has been

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reported that the dentine surrounding the invagination may contain connective tissue inclusions or communications to the pulp tissue.[10] The lined enamel within the invagination could also be associated with possible defects or dehiscence, or hypomineralised.[1] Thus, in many case reports, pulpal pathosis was found in teeth with type III dens invaginatus (Table 1).[1,7,11-14] To treat these teeth, endodontic treatment, in addition to sealing or removal of the invagination, or combined with a surgical procedure, should be considered.[8,15,16]

There is another clinical scenario where the invagination may have no communications with the pulp tissue in teeth associated with type III dens invaginatus.[5,17,18] In such cases, the invagination may be affected by microbial contamination through the coronal path in the oral cavity and subsequently induce peri-invagination periodontitis.[1] Meanwhile, the pulp tissue could remain vital and healthy. The treatment protocol for this type of dens invaginatus with vital pulp is to treat the invagination in isolation from the root canal system (Table 1)[18,19]; however, due to the aberrant anatomy of the invagination and the irregular root canal morphology associated with it, the complication of pulp exposure might be increased.[8] Thus, creating a minimally invasive path to the invagination and minimising damage to healthy dentine is critical for preserving vital pulp. Nevertheless, to the best of the present authors' knowledge, minimally invasive treatment was rarely mentioned in the reported cases associated with type III dens invaginatus. Moreover, most cases published in the literature had a follow-up period of 2 years or less, indicating a lack of long-term prognosis reported.

The purpose of the present case report was to examine the preservation of pulp vitality and the successful management of a vital type III dens invaginatus associated with an extensive periradicular lesion through minimally invasive treatment of the invagination. After a 5-year follow-up period, a satisfactory outcome was reported.

Case report

A 19-year-old man reported to the dental hospital with the chief complaint of recurrent swelling in the left maxillary anterior region for the last 12 months. At the initial visit, the patient was asymptomatic. His medical and dental history were not significant. Extraoral examination revealed no asymmetry, and intraoral examination revealed that the maxillary left lateral incisor had a relatively short and small crown (Fig 1a). A pit associated with caries was found on the palatal surface of the tooth (Fig 1b). There was no pain on percussion, palpation or biting. The periodontal conditions were within the normal limit (2~3 mm probing depth, no mobility or sinus tract). The maxillary left lateral incisor responded normally to both cold and hot thermal tests and the electric pulp vitality test, similar to the contralateral tooth.

A parallel periapical radiograph was taken and the radiographic interpretation revealed an invagination within the maxillary left lateral incisor (Fig 1c). The invagination had a hollow strip-shaped structure with high density, starting from the crown and extending to the middle third of the root. A large periradicular radiolucency was seen around the apex of the tooth. The lesion centred slightly at the mesial side of the tooth with its mesial margin adjacent to the periradicular region of the maxillary left central incisor.

A CBCT scan (NewTom VGI, NewTom, Verona, Italy) was taken of the maxillary left lateral incisor as a complementary examination. The CBCT exposure parameters were 110 kVp and 5.2 mA with a 6 × 6 cm field of view and 90-mm voxel size. The CBCT images revealed Oehlers IIIA dens invaginatus of the maxillary left lateral incisor (Fig 1d to f). The invagination started from the palatal surface of the crown and extended to the middle third of the root with an apical opening at the palatal side of the root (Fig 1f). There were no communications between the invagination and the pulp chamber or root canal system. The root canal was visualised and located on the labial side of the invagination. The periradicular radiolucency of the maxillary left lateral incisor was well-defined, centred on the apical opening of the invagination at the palatal side of the root, with a volume of 13 × 8 × 8 mm. Thus, a diagnosis was made of Oehlers type III dens invaginatus of the maxillary left lateral incisor with a peri-invagination lesion and normal pulp.

The treatment plan for the maxillary left lateral incisor was to preserve the vital pulp, clean the contaminated dentine or potential caries and seal the invagination. Informed consent was obtained from the patient before the treatment started. All the procedures were performed under a dental operating microscope (OPMI Pico, Carl Zeiss, Göttingen, Germany). Under rubber dam isolation (Coltène, Altstatten, Switzerland), an access opening to the invagination was prepared from the pit of the palatal surface of the maxillary left lateral incisor using a fine diamond bur (TC-11, MANI, Tochigi, Japan). The path was prepared parallel to the normal endodontic access path since the CBCT images showed that the invagination was on the palatal side and parallel to the pulp cavity (Fig 1f). After gaining access, the invagination was exposed as

a narrow path associated with carious tissue and soft necrotic debris (Fig 2a). No bleeding or purulent exudate was observed within the invagination. A size #8 C-file (VDW, Munich, Germany) was used to achieve the glide path of the invagination. The working length of the invagination was measured using an electronic apex locator (DentaPort Root ZX, J. Morita, Kyoto, Japan) and confirmed by radiographs. The invagination was prepared using ultrasonic instruments ET 40 and ET 20D (Acteon, Merignac, France) to remove the enamel layer, then ProTaper Next rotary files (Dentsply Sirona, Charlotte, NC, USA) were used to prepare the invaginated path up to size X2. Subsequently, size #30 and #35 stainless-steel K-files (VDW) were used to further prepare the invagination. During instrumentation, 5.25% sodium hypochlorite (Peking University Hospital of Stomatology, Beijing, China), 17% ethylenediaminetetraacetic acid (EDTA, Peking University Hospital of Stomatology) and passive ultrasonics (Acteon) were used to irrigate and disinfect the invagination (Fig 2b). Mineral trioxide aggregate (MTA) (Dentsply Sirona) was used to fill the entire invagination using an endodontic plugger (Fig 2c). A moist cotton pellet was placed over the MTA and the access opening was sealed with temporary cement (Ceivitron, DongQuan, Taiwan, China). After 1 week, the patient returned asymptomatic. The access cavity was permanently restored with resin composite (Filtek Z 350 XT, 3M, St Paul, MN, USA). Cold thermal and electric pulp vitality tests were conducted again to verify the vitality of the pulp. The maxillary left lateral incisor responded normally to both tests.

At the 1-, 2-, 3- and 5-year follow-up visits, the patient was free of symptoms. The tooth responded normally to the cold thermal test and electric pulp vitality test. The periodontal condition was normal with no sinus tract. The radiograph showed that the periradicular radiolucency reduced continuously after the first year of treatment (Fig 3a to d). At 5 years, the radiolucency completely disappeared, indicating a complete healing of the apical pathosis (Fig 3e and 4a to f).

Discussion

Determining whether an apical lesion of dens invaginatus is associated with the root canal system, the invagination or both is important for diagnosing and making treatment plans.[19] Thus, preoperative CBCT imaging is necessary to understand the position of the apical pathosis, the type of dens invaginatus, and its relationship with the root canal system.[12,20] In the present case, the interpretation of the CBCT imaging revealed a parallel invaginated path to the pulp cavity with no connections between each other. Since the apical lesion was distributed around the opening of the invagination other than around the apex, it was suspected that the pulp could possibly be vital. Moreover, both thermal and electric pulpal vitality tests revealed vital pulp. Thus, the lesion in the present case was regarded as a peri-invagination lesion that was induced solely by the infected invagination.

The challenge of vital pulp preservation in teeth affected by dens invaginatus is to accurately address the necrotic invaginated path without impacting the remaining pulp tissue vitality.[14] Due to the anatomical complexity of the invagination, the dental operating microscope and ultrasound instruments were used in the present case to acquire conservative access to the invagination under the minimally invasive principle. To clean the invagination, diamond ultrasound tips and stainless-steel K-files were utilised in addition to regular rotary NiTi instruments and chemical disinfection approaches to thoroughly instrument the invaginated path that was lined by hard enamel-like tissues.[21] The invagination was obturated by MTA in the present case. The high biocompatibility and superior sealing property of the material could better prevent bacteria from penetrating into the invagination, safeguard the fracture resistance of the tooth, promote apical healing of lesions and enhance the effects of pulp preservation, even if there are potential connections with the pulp tissue.[17,19,22,23] The outcome of the present case was favourable over a 5-year follow-up period.

To the best of the present authors' knowledge, there were 18 reported Oehlers type III dens invaginatus cases in permanent teeth in the recent 20 years, with variations in pulp vitality, treatment plan and outcome (Table 1). Of these cases, seven revealed vital pulp in preoperative examinations, as in the present study. Five of these treated the invagination and tried to preserve pulp vitality[18,24-27], whereas the other two also performed non-surgical endodontic treatment for the root canals.[28,29] Apical surgery was conducted in one of the cases to ensure the cleaning and sealing effects of the complex invagination.[24] For sealing the invagination, gutta-percha and sealer were used in six cases to obturate the path.[18,24-27,29] The other one used MTA to seal the apical foramen prior to gutta-percha obturation.[28] The outcomes revealed that the teeth responded normally to pulp vitality tests in all cases except the one in which endodontic treatment was performed. The follow-up duration ranged from 4 months to 10 years. Four cases with follow-up durations longer than 18 months reported complete healing of the radiolucency[25-27,29], whereas the other three cases (with 4-month, 6-month

Table 1 Reported cases of the pulpal status and treatment approaches of Oehlers type III dens invaginatus in permanent teeth in the last 20 years.

Study	Year of publication	Oehlers classification of invagination	Pulp vitality	Periapical lesion	Endodontic treatment	
Li et al[32]	2022	IIIB	No response to the electric pulp vitality test	Yes	Yes	
Hernández et al[29]	2022	IIIA	One of the two cases had necrotic pulp, the other had vital pulp	Yes	Yes	
Zhang et al[26]	2022	IIIB	One of the teeth with dens invaginatus was vital whereas the other was non-vital	Yes	Root canal therapy performed only on the non-vital tooth	
Ali et al[33]	2022	IIIA	No response to the electric pulp vitality test	Yes	Yes	
Kalogeropoulos et al[27]	2022	IIIB	Normal	Yes	No	
Kamio et al[18]	2021	IIIA and IIIB	Normal	Yes (peri-invagination lesion)	No	
Lee et al[17]	2020	IIIA	No response to the electric pulp vitality test	Yes (peri-invagination lesion)	No	
Zhang and Wei[12]	2017	IIIB	Necrosis	Yes	Yes	
Agrawal et al[13]	2016	IIIB	Necrosis	Yes	Apexification using MTA	
Zoya et al[31]	2015	IIIB and II	No response to the electric pulp vitality test	Yes	Yes	
Nosrat and Schneider[14]	2015	IIIB	Necrosis	Yes	Yes	
Wayama et al[35]	2014	IIIA	Not mentioned	Yes	Yes	
Narayana et al[34]	2012	IIIB	No response to the thermal pulp vitality test	Yes	Performed the pulp revascularisation procedure	
Lichota et al[30]	2008	III	No response to cold and electric pulp testing	Yes	Yes	
Štamfelj et al[36]	2007	III	Pulp sensitivity testing not performed	Yes	No	
Steffen and Splieth[28]	2005	III	The tooth responded to thermal and electrical stimuli	Yes	Yes	
Nallapati[24]	2004	III	Normal response to cold	Yes (peri-invagination lesion)	No	
Gound and Maixner[25]	2004	III	Normal	Yes	No	

	Treatment of invagination	Apical surgery	Follow-up duration	Outcome
	Prepared the invagination, then sealed it with iRoot BP	Yes, with intentional replantation	4~39 mo	Eight of ten cases healed with no lesion or other adverse effects
	Prepared the invagination, then sealed it with gutta-percha and sealer	No	6~120 mo	Healing of the periapical lesion in both cases
	Prepared the invagination, then sealed it with gutta-percha and iRoot SP sealer	No	18 mo	Complete healing of the periapical lesions of both teeth
	Prepared the invagination, then sealed it with MTA	Yes	1 y	Complete healing of the periapical lesion
	Prepared the invagination, then sealed it with gutta-percha and bioceramic sealer	No	2 y	The pulp was normal. There is complete healing of the periapical lesion
	Prepared the invagination, then sealed it with gutta-percha	No	6 mo	The pulp was normal. The lesion decreased
	Prepared the invagination, then sealed it with MTA	No	2 y	The pulp responded normally to pulp vitality testing. Significant progression of periapical healing was evident
	Removed the invaginated tissue	Yes	6 mo	Healing on the soft tissue and on the periapical lesion
	Prepared the invagination, then sealed it with gutta-percha	No	2 y	Significant osseous healing of the periapical lesion
	Prepared and sealed the invagination with MTA	Yes	1 y	Successful treatment outcome
	Prepared the invagination, then sealed it with gutta-percha	No	6 mo	Significant osseous healing of the preoperative lesion
	Retrograde preparation and seal of the invagination with gutta-percha and sealer	Yes	18 y	The tooth associated with crown fracture, but a satisfied healing of periapical lesion was revealed from radiograph
	Removed the invaginated mass	No	12 mo	The periapical lesion appeared to have normal trabeculation with the appearance of bridge formation
	Prepared the invagination, then sealed it with gutta-percha	No	24 mo	Satisfactory bone healing was revealed
	No	No	Extraction	-
	Prepared the invagination, then sealed it with gutta-percha and MTA	No	1 y	Apical repair was observed
	The invagination was prepared and sealed with gutta-percha and dual-cured paste	Yes	4 mo	Progressive healing of the lesion
	Prepared and sealed with gutta-percha	No	6 y	The radiolucent defect had healed completely

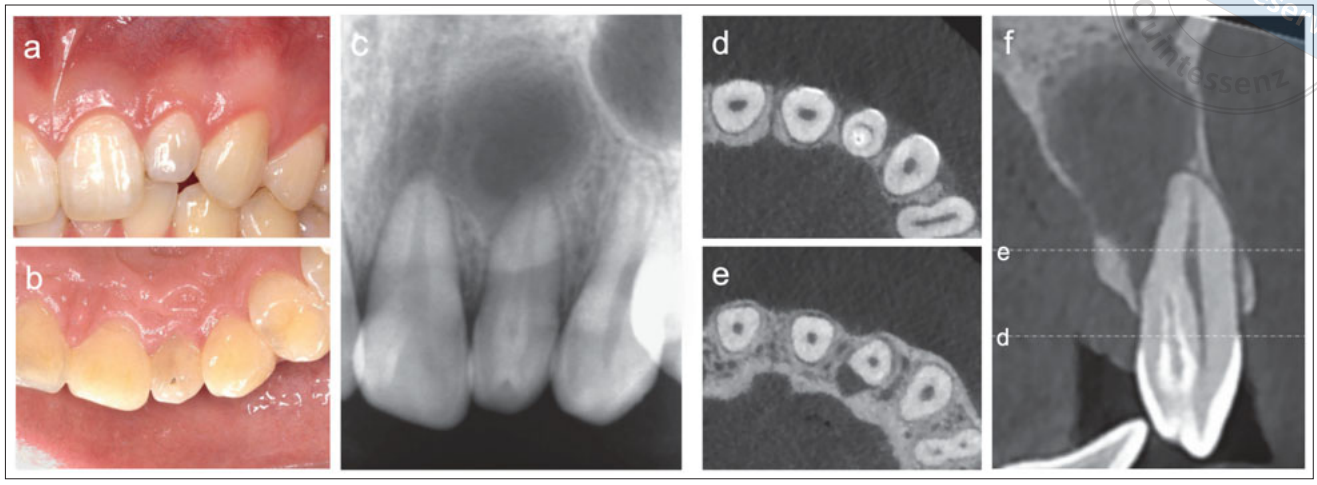

Fig 1 Labial view of the preoperative intraoral examination of the maxillary left lateral incisor with Oehlers type IIIA dens invaginatus **(a)**. The palatal view shows a pit associated with caries on the palatal side of the maxillary left lateral incisor **(b)**. The preoperative radiograph shows a hollow strip-shaped high-density invagination that starts from the crown and propagates into the middle third of the root, inside the maxillary left lateral incisor. A large apical radiolucency is shown around the apex of this tooth **(c)**. Axial sections of CBCT images **(d and e)**. The sagittal section of the CBCT images shows an Oehlers type IIIA dens invaginatus inside the maxillary left lateral incisor **(f)**. The dashed lines in **(f)** indicate the cutting sections of **(d and e)**.

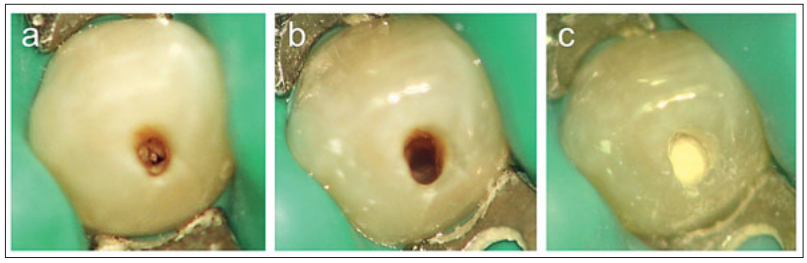

Fig 2 Images captured under the dental operating microscope during the treatment of dens invaginatus. The coronal access to the invagination with a minimally invasive cavity, with decayed tissue found along the invaginatus path **(a)**. Completion of cleaning and preparation of the invagination without damaging the pulp **(b)**. Obturation of the invagination with MTA under the dental microscope **(c)**.

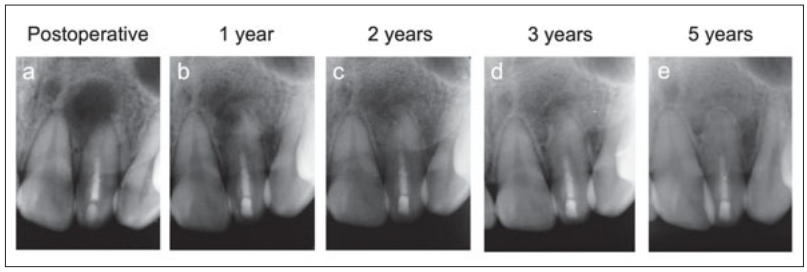

Fig 3 Postoperative radiograph after sealing the invagination using MTA **(a)**. The 1- **(b)**, 2- **(c)** and 3-year **(d)** follow-ups indicate a continuous decrease of the peri-invagination lesion. The 5-year follow-up shows complete healing of the peri-invagination lesion **(e)**.

and 1-year follow-ups) reported partial but progressive healing of the radiolucency.[18,24,28] The present case showed complete healing at the 5-year follow-up. Through these cases, it could be concluded that vital pulp is not commonly seen in permanent teeth associated with type III dens invaginatus; however, if this is the case, addressing only the invagination could possibly preserve vital pulp. A long follow-up duration might be required for complete healing of the lesion, as shown in the present case and the other cases reported.

Non-vital pulp was revealed in 9 of the 18 type III dens invaginatus cases[12-14,30-35], whereas pulp vitality was not mentioned in 2 of the 18 cases.[35,36] Non-surgical endodontic treatments only were performed in 5 of these 11 cases[13,14,30,32,33], whereas apical surgery was also carried out in 4 of them.[12,31,32,35] In one case, the invaginated mass was removed and a pulp revascularisation procedure was conducted.[34] In the last of the 18 cases, tooth extraction was performed.[36] It could be seen that pulp vitality was affected in over half of the reported type III dens invaginatus cases. To treat these cases, surgical, non-surgical or regenerative endodontic approaches could be utilised separately or together to control the infection of the root canal system and the invagination.

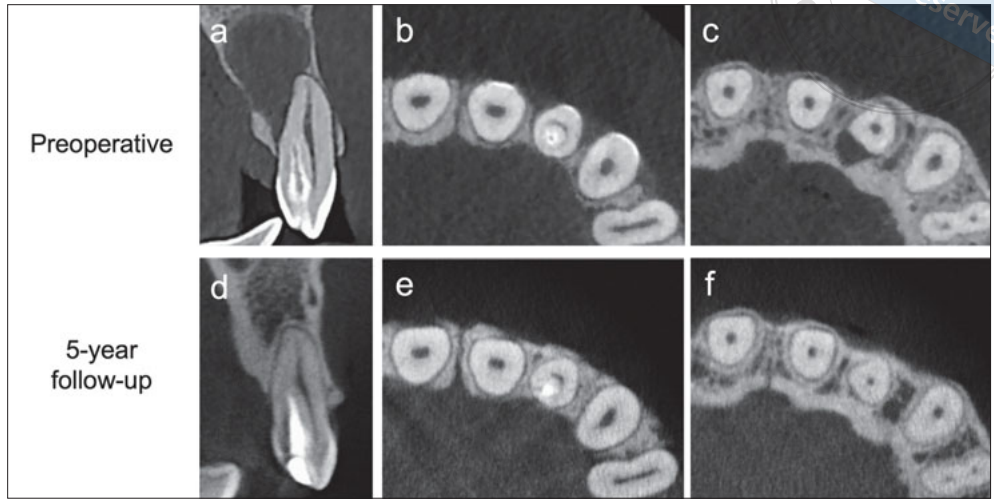

Fig 4 Preoperative CBCT images of the maxillary left lateral incisor **(a to c)**. The 5-year follow-up CBCT images show complete healing of the radiolucency **(d to f)**.

Conclusion

This case report demonstrates successful vital pulp preservation of type IIIA dens invaginatus with a large peri-invagination lesion. In summary, type IIIA dens invaginatus could be associated with vital pulp. Clinicians should be aware of this when examining and diagnosing such cases. Both preoperative pulp vitality tests and CBCT examination are important to reveal the pulp status, anatomical structure of the invagination and the relationship between the invagination and the pulp cavity. Vital pulp could be preserved successfully through minimally invasive treatment of the invagination combined with a series of effective infection control strategies.

Conflicts of interest

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

Author contribution

Dr Fei LIN contributed to the diagnosis and treatment of the clinical case, literature review and manuscript draft; Dr Lin YUE supervised the treatment of the clinical case and revised and approved the manuscript.

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References

1. Alani A, Bishop K. Dens invaginatus. Part 1: Classification, prevalence and aetiology. Int Endod J 2008;41:1123–1136.
2. Hülsmann M. Dens invaginatus: Aetiology, classification, prevalence, diagnosis, and treatment considerations. Int Endod J 1997;30:79–90.
3. Oehlers FA. The radicular variety of dens invaginatus. Oral Surg Oral Med Oral Pathol 1958;11:1251–1260.
4. Lin F, Yue L. Radicular invaginatus caused by the developmental abnormalities of epithelial root sheath. Chin J Stomatol 2023;58:3–10.
5. Oehlers FA. Dens invaginatus (dilated composite odontome). II. Associated posterior crown forms and pathogenesis. Oral Surg Oral Med Oral Pathol 1957;10:1302–1316.
6. Capar ID, Ertas H, Arslan H, Tarim Ertas E. A retrospective comparative study of cone-beam computed tomography versus rendered panoramic images in identifying the presence, types, and characteristics of dens invaginatus in a Turkish population. J Endod 2015;41:473–478.
7. Ridell K, Mejàre I, Matsson L. Dens invaginatus: A retrospective study of prophylactic invagination treatment. Int J Paediatr Dent 2001;11:92–97.
8. Bishop K, Alani A. Dens invaginatus. Part 2: Clinical, radiographic features and management options. Int Endod J 2008;41:1137–1154.
9. Chen YH, Tseng CC, Harn WM. Dens invaginatus: Review of formation and morphology with 2 case reports. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;86:347–352.
10. Piattelli A, Tris P. Dens invaginatus: A histological study of undemineralized material. Dent Traumatol 1993;9:191–195.
11. Chung SH, Hwang YJ, You SY, Hwang YH, Oh S. A case report of multiple bilateral dens invaginatus in maxillary anteriors. Restor Dent Endod 2019;44:e39.
12. Zhang P, Wei X. Combined therapy for a rare case of Type III dens invaginatus in a mandibular central incisor with a periapical lesion: A case report. J Endod 2017;43:1378–1382.
13. Agrawal PK, Wankhade J, Warhadpande M. A rare case of type III dens invaginatus in a mandibular second premolar and its nonsurgical endodontic management by using cone-beam computed tomography: A case report. J Endod 2016;42:669–672.
14. Nosrat A, Schneider SC. Endodontic management of a maxillary lateral incisor with 4 root canals and a dens invaginatus tract. J Endod 2015;41:1167–1171.
15. Siqueira JF Jr, Rôças IN, Hernández SR, et al. Dens invaginatus: Clinical implications and antimicrobial endodontic treatment considerations. J Endod 2022;48:161–170.

16. Gallacher A, Ali R, Bhakta S. Dens invaginatus: Diagnosis and management strategies. Br Dent J 2016;221:383–387.
17. Lee JK, Hwang JJ, Kim HC. Treatment of peri-invagination lesion and vitality preservation in an immature type III dens invaginatus: A case report. BMC Oral Health 2020;20:29.
18. Kamio N, Gomyo N, Matsushima K. Successful pulp-preserving treatment for peri-invagination periodontitis of double dens invaginatus with Oehlers type IIIA and IIIB: A case report. J Endod 2021;47:1515–1520.
19. Parirokh M, Torabinejad M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: An updated overview - Part I: Vital pulp therapy. Int Endod J 2018;51:177–205.
20. Teixidó M, Abella F, Duran-Sindreu F, Moscoso S, Roig M. The use of cone-beam computed tomography in the preservation of pulp vitality in a maxillary canine with type 3 dens invaginatus and an associated periradicular lesion. J Endod 2014;40:1501–1504.
21. Ricucci D, Milovidova I, Siqueira JF Jr. Unusual location of dens invaginatus causing a difficult-to-diagnose pulpal involvement. J Endod 2020;46:1522–1529.
22. Aktemur Türker S, Uzunoğlu E, Deniz Sungur D, Tek V. Fracture resistance of teeth with simulated perforating internal resorption cavities repaired with different calcium silicate–based cements and backfilling materials. J Endod 2018;44:860–863.
23. Bortoluzzi EA, Souza EM, Reis JM, Esberard RM, Tanomaru-Filho M. Fracture strength of bovine incisors after intra-radicular treatment with MTA in an experimental immature tooth model. Int Endod J 2007;40:684–691.
24. Nallapati S. Clinical management of a maxillary lateral incisor with vital pulp and type 3 dens invaginatus: A case report. J Endod 2004;30:726–731.
25. Gound TG, Maixner D. Nonsurgical management of a dilacerated maxillary lateral incisor with type III dens invaginatus: A case report. J Endod 2004;30:448–451.
26. Zhang J, Wang Y, Xu L, Wu Z, Tu Y. Treatment of type III dens invaginatus in bilateral immature mandibular central incisors: A case report. BMC Oral Health 2022;22:28.
27. Kalogeropoulos K, Solomonidou S, Xiropotamou A, Eyuboglu TF. Endodontic management of a double-type IIIB dens invaginatus in a vital maxillary central incisor aided by CBCT: A case report. Aust Endod J 2023;49:365–372.
28. Steffen H, Splieth C. Conventional treatment of dens invaginatus in maxillary lateral incisor with sinus tract: One year follow-up. J Endod 2005;31:130–133.
29. Hernández SR, Pérez AR, Baasch AC, et al. Management of teeth with dens invaginatus and apical periodontitis: A case series. J Am Dent Assoc 2022;153:470–478.
30. Lichota D, Lipski M, Woźniak K, Buczkowska-Radlińska J. Endodontic treatment of a maxillary canine with type 3 dens invaginatus and large periradicular lesion: A case report. J Endod 2008;34:756–758.
31. Zoya A, Ali S, Alam S, et al. Double dens invaginatus with multiple canals in a maxillary central incisor: Retreatment and managing complications. J Endod 2015;41:1927–1932.
32. Li N, Xu H, Kan C, Zhang J, Li S. Retrospective study of intentional replantation for type IIIb dens invaginatus with periapical lesions. J Endod 2022;48:329–336.
33. Ali A, Zoya A, Ali S, Arslan H. Rare variant of dens invaginatus with accessory root and labial talon cusp in maxillary lateral incisor-Case report. Aust Endod J 2023;49:192–201.
34. Narayana P, Hartwell GR, Wallace R, Nair UP. Endodontic clinical management of a dens invaginatus case by using a unique treatment approach: A case report. J Endod 2012;38:1145–1148.
35. Wayama MT, Valentim D, Gomes-Filho JE, Cintra LTA, Dezan E. 18-year follow-up of dens invaginatus: Retrograde endodontic treatment. J Endod 2014;40:1688–1690.
36. Štamfelj I, Kansky AA, Gašperšič D. Unusual variant of type 3 dens invaginatus in a maxillary canine: A rare case report. J Endod 2007;33:64–68.

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