Restoring endodontically treated teeth is undoubtedly a complex procedure. A sound knowledge of biomechanical and clinical principles, careful planning, and the selection of appropriate materials and restorative techniques is essential to achieve the best functional and esthetic outcomes. This concise and exquisitely illustrated book, based on the philosophy and clinical experience of its authors and on sound scientific evidence, is divided into 10 chapters in a didactic sequence to make it easier for dentists to apply the techniques in everyday practice. The focus is on preserving the healthy tooth structure as much as possible using both conventional and digital methodologies.
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Extracellular Matrix Remodelling of the Periodontium under Orthodontic Force

Qian Yao YU1, Yi Ping HUANG1,2, Wei Ran LI1,2

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

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
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, Malassez 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. 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. Previous studies have shown that collagens I, III and V make up 75%, 20% and 5% of PDL, respectively. In recent years, Thant et al 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, which has been proven to be upregulated under cyclic tension stretch and hypoxic conditions. Collagen II allows the ECM to accommodate more water molecules to disperse stress, 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. 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. 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. 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 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. 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 orthodont-
tic force application. As early as 1984, Duncan et al.\textsuperscript{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.\textsuperscript{16}, Militi et al.\textsuperscript{19} and Anastasi et al.\textsuperscript{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.\textsuperscript{21} and Hacopian et al.\textsuperscript{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.\textsuperscript{16} Regarding tensional force, Pei et al.\textsuperscript{2} demonstrated that tension promoted collagen remodelling in the ECM and increased the secretion of collagen I in PDLCs. Howard et al.\textsuperscript{23} and Kook et al.\textsuperscript{24} also found that the expression of collagen I and III in PDLCs was upregulated under tension. For in vivo experiments, Militi et al.\textsuperscript{19} and Anastasi et al.\textsuperscript{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.\textsuperscript{25} and Nemoto et al.\textsuperscript{26} reported downregulation of the short-chain collagen genes \textit{COL3A1} and \textit{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 increased and the ratio of collagen III to I continued to increase and did not show a downward trend, suggesting that diabetes causes abnormal collagen remodelling during orthodontic tooth movement.\textsuperscript{27} In another study, Li et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{30} In previous studies, the expression of IV collagen in the PDL mainly showed a downward trend under mechanical force. Anastasi et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{10} Saminathan et al.\textsuperscript{32} found a significant downregulation of the expression of the short-chain collagen gene \textit{COL6A1} after applying cyclic tension. Thant et al.\textsuperscript{9} demonstrated that the proportion of collagen VI in the PDL decreased on the compression side in orthodontically treated mice. Bumann et al.\textsuperscript{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.\textsuperscript{16} found that the secretion of collagen V increased under compression. Ma et al.\textsuperscript{23} also reported that stretching force upregulated the expression of \textit{COL5A1} in PDLCs. In the study conducted by Saminathan et al.\textsuperscript{32}, the expression of \textit{COL8A1} was downregulated in PDLCs under tension. Regarding collagen XI, studies demonstrated consistent results that stretching force downregulates \textit{COL11A1} in PDLCs.\textsuperscript{25,32,33} Thant et al.\textsuperscript{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>
<thead>
<tr>
<th>Study</th>
<th>Force type</th>
<th>Methods</th>
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<th>Outcome</th>
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<tr>
<td>Howard et al(^{23})</td>
<td>Tension</td>
<td>ELISA</td>
<td>hPDLCs</td>
<td>Upregulated significantly (5%, 24 h), non-significant (10%, 24 h)</td>
</tr>
<tr>
<td>Redlich et al(^{21})</td>
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<td>RT-PCR</td>
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</tr>
<tr>
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<td>RT-PCR</td>
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</tr>
<tr>
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</tr>
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<td>Ma et al(^{33})</td>
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<td>RT-PCR</td>
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<td>Upregulated significantly (within 15 d)</td>
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<tr>
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<td>Duncan et al(^{18})</td>
<td>NR</td>
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</tr>
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<td>Compression tension</td>
<td>ELISA</td>
<td>Extracted human pre-molars</td>
<td>Upregulated significantly (14 d), non-significant (14 d)</td>
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<td>Anastasi et al(^{20})</td>
<td>Compression tension</td>
<td>IHC</td>
<td>Extracted human pre-molars</td>
<td>Upregulated significantly (within 14 d), upregulated significantly (3, 72 h), downregulated significantly (7, 14 d)</td>
</tr>
<tr>
<td>Militi et al(^{19})</td>
<td>Compression tension</td>
<td>IHC</td>
<td>Extracted human pre-molars</td>
<td>Upregulated significantly (within 30 d), upregulated significantly (1 d), downregulated significantly (7, 14 d), non-significant (30 d)</td>
</tr>
</tbody>
</table>

Significance compared to unstimulated control.

ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; NR, not reported.
<table>
<thead>
<tr>
<th>COL-III</th>
<th>COL-IV</th>
<th>COL-VI</th>
<th>Other collagens</th>
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<tr>
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<td>NR</td>
<td>NR</td>
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<tr>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Downregulated significantly (12 h)</td>
<td>NR</td>
<td>NR</td>
<td>COL2A1 upregulated significantly (12 h)</td>
</tr>
<tr>
<td>NR</td>
<td>Upregulated significantly (within 7 d)</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>COL6A1 downregulated significantly (12 h), COL8A1 downregulated significantly (12 h), COL11A1 downregulated significantly (24 h)</td>
</tr>
<tr>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>COL5A1 upregulated significantly (24 h), COL11A1 downregulated significantly (6 h)</td>
</tr>
<tr>
<td>Upregulated significantly (3 d)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>NR</td>
<td>Upregulated significantly (3, 7 d), non-significant (14 d)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Upregulated significantly (7 d), upregulated significantly (7 d)</td>
<td>NR</td>
<td>NR</td>
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</tr>
<tr>
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<td>NR</td>
<td>Upregulated significantly (14 d), non-significant (14 d)</td>
<td>COL-II non-significant (14 d), COL-XII upregulated (14 d), COL-II non-significant (14 d), COL-XII non-significant (14 d)</td>
</tr>
<tr>
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<td>NR</td>
<td>Upregulated significantly (14 d), non-significant (14 d)</td>
<td>COL-V upregulated significantly (14 d), non-significant (14 d)</td>
</tr>
<tr>
<td>NR</td>
<td>Downregulated significantly (3, 72 h), upregulated significantly (7, 14 d), downregulated significantly (3, 72 h), upregulated significantly (7, 14 d)</td>
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<td>NR</td>
</tr>
<tr>
<td>NR</td>
<td>Downregulated significantly (1, 7, 14 d), upregulated significantly (21, 30 d), downregulated significantly (within 30 d)</td>
<td>NR</td>
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</tr>
</tbody>
</table>
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. 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 first reported the upregulation of fibronectin in PDLCs under stretching force. Subsequently, Militi et al 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 also found that fibronectin increased on the compression side and decreased on the tension side of the moved teeth; however, He et al reported that compression caused a downregulation of fibronectin expression. After applying centrifugal force to PDLCs, Theilig et al also observed a decrease in fibronectin expression accompanied by a decrease in tenasin and laminin. It is known that tenasin can inhibit cell adhesion to the ECM by binding fibronectin. 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. 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. Svoboda et al demonstrated that the turnover rate of collagen in periodontal tissue was related to the amount of collagen ingested by fibroblasts. Nakamura et al 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. 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). 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 and Nettelhoff et al found that MMP-8 was upregulated in PDLCs under compressive force; however, the results are diverse under tensional force. Ma et al and Saminathan et al found that the expression of the MMP-8 gene was significantly downregulated under tension, whereas Jacobs et al 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. Under compression, others found simi-
lar results that the expression of MMP-1 increases in PDLCs. In addition to traditional compression and tension, Zheng et al. found that the expression of MMP-1 was significantly upregulated in PDLCs under shear force. Regarding experiments in vivo, Zhang et al. and Bildt et al. 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., Tantilertanant et al. and Chen et al. found that the expression of MMP-2 in PDLCs was increased under tension, and the results recorded by Lisboa et al. showed a downregulation of MMP-2 expression under compression. Zheng et al. 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. 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. 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.

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Other enzymes

The ADAMTS family is involved in the maturation of collagens and the hydrolysis of ECM. Saminathan et al. and Ma et al. 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. 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 tenasin 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.
and plays an important role in mechanotransduction and cell migration.\textsuperscript{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\textsuperscript{33} found that ITGA5 and ITGAL were significantly upregulated in PDLCs after applying tension. Kawamura et al\textsuperscript{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\textsuperscript{32} and Ma et al\textsuperscript{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\textsuperscript{33} and Liu et al\textsuperscript{60} observed that osteonectin was upregulated in PDLCs after applying cyclic stretch. Ma et al\textsuperscript{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

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### Table 2  MMP outcomes from all included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Force type</th>
<th>Methods</th>
<th>Object</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolcato-Bellemin et al\textsuperscript{45}</td>
<td>Tension</td>
<td>RT-PCR</td>
<td>hPDLCs</td>
<td>Upregulated significantly (12 h)</td>
</tr>
<tr>
<td>Redlich et al\textsuperscript{21}</td>
<td>Compression</td>
<td>RT-PCR</td>
<td>hPDLCs</td>
<td>Upregulated significantly (within 60 min), non-significant (90 min)</td>
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<tr>
<td>Huang et al\textsuperscript{47}</td>
<td>Compression</td>
<td>RT-PCR, ELISA</td>
<td>hPDLCs</td>
<td>Upregulated significantly (30, 60, 90 min)</td>
</tr>
<tr>
<td>Nemoto et al\textsuperscript{26}</td>
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<td>RT-PCR, WB</td>
<td>hPDLCs</td>
<td>Upregulated significantly (2–7 d)</td>
</tr>
<tr>
<td>Kook et al\textsuperscript{24}</td>
<td>Tension</td>
<td>RT-PCR</td>
<td>hPDLCs</td>
<td>Upregulated significantly (within 12 h)</td>
</tr>
<tr>
<td>Hacopian et al\textsuperscript{22}</td>
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<td>RT-PCR</td>
<td>hPDLCs</td>
<td>Upregulated significantly (60 min)</td>
</tr>
<tr>
<td>Saminathan et al\textsuperscript{32}</td>
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<td>RT-PCR</td>
<td>hPDLCs</td>
<td>NR</td>
</tr>
<tr>
<td>Zheng et al\textsuperscript{49}</td>
<td>Fluid shear stress</td>
<td>RT-PCR, ELISA</td>
<td>hPDLCs</td>
<td>Upregulated significantly (within 12 h)</td>
</tr>
<tr>
<td>Lisboa et al\textsuperscript{48}</td>
<td>Compression</td>
<td>ELISA, WB</td>
<td>hPDLCs</td>
<td>Upregulated significantly (30 min)</td>
</tr>
<tr>
<td>Lisboa et al\textsuperscript{53}</td>
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<td>Zimography</td>
<td>hPDLCs</td>
<td>NR</td>
</tr>
<tr>
<td>Ma et al\textsuperscript{33}</td>
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<td>RT-PCR</td>
<td>hPDLCs</td>
<td>NR</td>
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<tr>
<td>Nettelhoff et al\textsuperscript{43}</td>
<td>Compression</td>
<td>RT-PCR, ELISA</td>
<td>hPDLCs</td>
<td>NR</td>
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<tr>
<td>Jacobs et al\textsuperscript{44}</td>
<td>Tension</td>
<td>RT-PCR, ELISA</td>
<td>hPDLCs</td>
<td>NR</td>
</tr>
<tr>
<td>Tantilertanant et al\textsuperscript{46}</td>
<td>Tension</td>
<td>RT-PCR, ELISA</td>
<td>hPDLCs</td>
<td>Upregulated significantly (6, 24 h)</td>
</tr>
<tr>
<td>Bildt et al\textsuperscript{51}</td>
<td>Compression tension</td>
<td>Zimography, WB</td>
<td>Human GCF</td>
<td>Upregulated significantly (4 wk), upregulated significantly (4 wk)</td>
</tr>
<tr>
<td>Zhang et al\textsuperscript{50}</td>
<td>Compression tension</td>
<td>RT-PCR</td>
<td>Human GCF</td>
<td>Non-significant (within 12 wk), non-significant (within 12 wk)</td>
</tr>
</tbody>
</table>

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.
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


Dental implant placement often requires bone grafting to ensure sufficient bony support for the implants being placed. Depending on the biologic conditions of the patient, including the level of bone atrophy and the status of the remaining teeth in the mouth, more adjunctive procedures like bone harvesting or sinus grafting may be required. This book covers it all, from the biology of bone and how dental implants work within that framework to the many procedures for harvesting bone and using it to augment sites for implant placement. The different types of bone grafts and membranes are discussed as well as procedures to preserve the alveolar ridge following tooth extraction. Dr Garg was a pioneer in dental bone grafting, and this new edition keeps him at the forefront of the field.
Identification of Cuproptosis-related Gene Lipoyltransferase 1 as a Promising Biomarker in Oral Squamous Cell Carcinoma

Kuang Min SHEN¹, Yu Meng ZHOU²,³, Mu Chun LIANG³,⁴, De Mao ZHANG³,⁴, Qiang WEI³, Yi Lin PING²

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

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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 occurrence², 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.

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 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
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) and Kyoto Encyclopedia of Genes and Genomes (KEGG) 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). 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. The univariate Cox analysis involved fitting a Cox proportional hazards model to the gene expression data using the survival package in R. 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. 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. 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.

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 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. Moreover, the authors observed a decrease in the expression level.
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 \( |\log_{2}\text{FoldChange}| > 2 \) 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
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.
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. 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 relationship between LIPT1 and clinical characteristics. Sex distribution, histological grade distribution, and LIPT1 expression as parameters. A nomogram for predicting overall survival, incorporating age, sex, race, smoking history, T stage, alcohol history, histological grade, N stage and LIPT1 expression as parameters. Calibration curves of the nomogram for predicting 1-, 3-, 5- and 10-year overall survival.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI)</td>
<td>P value</td>
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<tr>
<td>Age</td>
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<tr>
<td>Sex</td>
<td>0.908 (0.648–1.273)</td>
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<tr>
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<tr>
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<td>LIPT1</td>
<td>1.742 (1.088–2.789)</td>
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</table>

NA, not available.
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. LIPT1 has been identified as a novel prognostic target in liver hepatocellular carcinoma and skin cutaneous melanoma. 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 in uterine corpus endometrial carcinoma. Furthermore, in pan-cancer analysis, LIPT1 was found to be down-regulated 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-

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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).
tions made by Zheng et al. Their study revealed that cuproptosis can hinder cancer progression by targeting mitochondrial metabolism. 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. 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 also reported higher levels of regulatory Tregs and neutrophils in the LIPT1 low expression group in melanoma; however, Jiang et al 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.

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.

Acknowledgements
The authors would like to express their gratitude to Dr Mi Su from the Functional Laboratory at the West China School of Basic Medical Sciences & Forensic Medicine for the technical support for this study.

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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32. Zeng X, Liu X, Shen et al
This book is a concise resource for understanding the myriad suturing options available to dentists today. It includes recently developed suturing concepts pertinent to both conventional oral surgery and oral microsurgery. The text is supplemented with step-by-step drawings and photographs of the various techniques performed on an animal model, which once mastered can be readily applied in a clinical setting. The final chapter discusses the management of the occasional complications encountered during and after surgery.
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 QIAO¹,², Xin Yu ZHANG¹,², Rui Qi BAI¹,², Jing Wen CAI¹, Lin Lin ZHANG¹, Bin Jie LIU¹, Jun CHEN¹

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

survival rate and improved rate of mobility of anterior teeth were both better than those of posterior teeth. 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. 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 reimplemented 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
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:

- 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 $P < 0.05$. 

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**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 hand-piece 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).
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 split 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; 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).

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

Fig 3 Pre- and postoperative intraoral photographs. Preoperative intraoral photographs (a to c). Intraoral photographs 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. 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; 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. 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. 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; 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. 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. 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:

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.

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. 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.

3. Inflammatory resorption: If the inflammation is further aggravated after IR, absorption may reach the dentine. The dentine is weak and cannot resist resorption. 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. 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 ZHANG 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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QDT 2024 truly lives up to its name as the quintessence of all things dental technology. The 16 articles in this year’s volume demonstrate absolute excellence in clinical and laboratory protocols with stunning visuals and clear narratives. Featuring contributions from some of the best restorative teams out there, QDT 2024 showcases complex cases and focuses on how digital technologies can help to simplify treatment. Step-by-step documentation is presented so readers can understand the intricate procedures, and the pivotal role of proper diagnosis and documentation—the cornerstone of successful dental treatment—is emphasized throughout.
Dental Fear and Caries in 6- to 12-Year-Old Children: a Systematic Review and Meta-analysis

Narjes AMROLLAHI¹, Sayed Ali SHAHSHAHAN², Firoozeh NILCHIAN³, Mohammad Javad TARRAHI⁴

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

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-
The dental fear-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. The tool has a better performance compared to other scales such as Veenha Picture Test and Dental Anxiety Test in some studies.

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.

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, whereas others found no relationship. 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. 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, dental fear visual analogue scale (DF-VAS), dental fear scale (DFS) and questionnaires, 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.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Age, y</th>
<th>Sex (M/F)</th>
<th>DMFT %</th>
<th>Mean</th>
<th>Dental fear instruments</th>
<th>Dental fear</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buldur et al27*</td>
<td>583</td>
<td>3–13</td>
<td>286/297</td>
<td>NR</td>
<td>6.00</td>
<td>CFSS-DS/Frankl behaviour score</td>
<td>Mean Frankl behaviour score 3.0 (SD 1.0)</td>
<td>Dental anxiety was associated with dental caries</td>
</tr>
<tr>
<td>Yahyaoglu et al23*</td>
<td>810</td>
<td>6–12</td>
<td>408/402</td>
<td>NR</td>
<td>1.57</td>
<td>CFSS-DS</td>
<td>Mean CFSS-DS value 24.00 ± 7.99</td>
<td>Anxiety and dental caries were associated</td>
</tr>
<tr>
<td>Beena26*</td>
<td>444</td>
<td>6–12</td>
<td>220/224</td>
<td>NR</td>
<td>0.58</td>
<td>CFSS-DS</td>
<td>Overall mean CFSS-DS score 37.0 ± 8.89</td>
<td>No significant correlation</td>
</tr>
<tr>
<td>Son et al25*</td>
<td>132</td>
<td>7</td>
<td>77/55</td>
<td>NR</td>
<td>6.71</td>
<td>CFSS-DS</td>
<td>Prevalence of dental fear 34.85%</td>
<td>No correlation</td>
</tr>
<tr>
<td>Patcharaphol24*</td>
<td>212</td>
<td>12</td>
<td>146/66</td>
<td>NR</td>
<td>2.14</td>
<td>CFSS-DS</td>
<td>Mean CFSS-DS value 31.38 ± 10.45</td>
<td>Patients with dental anxiety had more decayed teeth</td>
</tr>
<tr>
<td>Alsadat et al8</td>
<td>1546</td>
<td>6–12</td>
<td>798/748</td>
<td>51.8</td>
<td>NR</td>
<td>CFSS-DS</td>
<td>23.50%: CFSS-DS score ≥ 32 12.50%: score &gt; 38</td>
<td>Dental fear has a direct relationship with decayed permanent teeth</td>
</tr>
<tr>
<td>Nguyen et al7</td>
<td>900</td>
<td>8–10</td>
<td>427/473</td>
<td>89.8</td>
<td>NR</td>
<td>CFSS-DS</td>
<td>Mean CFSS-DS score 20.8 ± 9.1</td>
<td>Dental fear is related to the oral health care system</td>
</tr>
<tr>
<td>Laureano et al12</td>
<td>466</td>
<td>8–10</td>
<td>207/259</td>
<td>88.84</td>
<td>(ICDAS &gt; 0)</td>
<td>NR</td>
<td>NR</td>
<td>CFSS-DS</td>
</tr>
<tr>
<td>Pichot et al20</td>
<td>2734</td>
<td>6–12</td>
<td>1,419/1,315</td>
<td>53.59</td>
<td>NR</td>
<td>DF-VAS</td>
<td>5% of 9- to 12-year-olds had high scores (DF-VAS &gt; 6)</td>
<td>Children with lower levels of dental fear had less experience of caries</td>
</tr>
<tr>
<td>Folayan et al18</td>
<td>450</td>
<td>6–12</td>
<td>222/228</td>
<td>4.9</td>
<td>NR</td>
<td>CFSS-DS</td>
<td>Mean CFSS-DS score 38.6 ± 14.4</td>
<td>Children who had caries had higher anxiety</td>
</tr>
<tr>
<td>Olak et al19</td>
<td>344</td>
<td>8–10</td>
<td>188/156</td>
<td>93</td>
<td>NR</td>
<td>CFSS-DS</td>
<td>General fear of dentistry 6.1%</td>
<td>Dental fear is significant predictor of dental caries</td>
</tr>
<tr>
<td>Saber and Awad17</td>
<td>250</td>
<td>6–12</td>
<td>122/128</td>
<td>NR</td>
<td>1.46</td>
<td>6.62</td>
<td>Mean CFSS-DS score 20.9 ± 11.84</td>
<td>Fear may have an impact on scores for caries</td>
</tr>
<tr>
<td>Prathima et al13</td>
<td>400</td>
<td>6–12</td>
<td>200/200</td>
<td>NR</td>
<td>1.89</td>
<td>Dental fear scale (DFS)</td>
<td>Low to moderate dental fear 46%; high dental fear 72%</td>
<td>No association</td>
</tr>
<tr>
<td>Panda et al11</td>
<td>798</td>
<td>8–10</td>
<td>238/560</td>
<td>NR</td>
<td>1.58</td>
<td>CFSS-DS</td>
<td>Mean fear score 36</td>
<td>Children with dental fear were 1.8 times more likely to have an untreated carious tooth</td>
</tr>
<tr>
<td>Patir Müneveroğlu et al21</td>
<td>200</td>
<td>6–12</td>
<td>102/98</td>
<td>NR</td>
<td>NR</td>
<td>3.52</td>
<td>Questionnaire</td>
<td>Fears were related to injection (60%), tooth extraction (50%), restorations (3.6%) and the sight of dental instruments (40%)</td>
</tr>
<tr>
<td>Varmazyar et al14</td>
<td>185</td>
<td>6–12</td>
<td>83/102</td>
<td>NR</td>
<td>NR</td>
<td>(dmft/DMFT) 3.93</td>
<td>CFSS-DS</td>
<td>CFSS-DS &gt; 38: 26.48%</td>
</tr>
</tbody>
</table>

*Studies included in the meta-analysis.
NR, not reported.
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 al17, the mean of DMFT/dmft was not reported in detail (the standard deviation [SD] was not reported). The studies by Alsadat et al3, Panda et al11 and Folayan et al18 did not report the mean values of DMFT or dmft indexes. Nguyen et al7, Olak et al19 and Varmazyar et al14 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 al12, in which the International Caries Detection and Assessment (ICDAS) index was used to assess dental caries and severity.

Prathima et al13, Pichot et al20 and Patir et al21 employed different criteria for measuring the variables of dental fear or dental caries, and were therefore also excluded from the meta-analysis. Parthima et al13 and Pichot et al20 used the dental fear scale (DFS) and dental fear-visual analogue scale (DF-VAS), respectively, to assess children's dental fear, whereas Patir et al21 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-
fear, which was categorised as low or high, and dental caries was reported as mean dmft and SD.\textsuperscript{23,25,26} The heterogeneity showed $P = 0.418$ and $I^2 = 0.000$, so a fixed model was used for meta-analysis.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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
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 with caries in primary teeth (dmft index); however, no significant relationship was found between dental fear (CFSS-DS) and caries in primary teeth (dmft index); the relationship 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. This may be due to parents neglecting their child's oral and dental hygiene.

Olak et al 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. 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.

In the study by Patr et al, 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.
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\textsuperscript{14}. Moreover, Laureano et al\textsuperscript{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.\textsuperscript{30} The ICDAS index in the study conducted by Laureano et al\textsuperscript{12} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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**


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

Sezgi CINEL SAHIN1, Lamia MUTLU-SAGESEN1, Isil KARAOKUTAN1, Mutlu OZCAN2

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 VIN group, followed by the VD, PD, GIN, AD, SIN, SD, PIN, GD, CIN and CD 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

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.5,10 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...
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. 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. 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.

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. However, with all these surface treatment protocols, adhesive materials are still needed to form a strong bond between the resins and the PEEK materials. Some studies have determined that visio.link material (Bredent, Senden, Germany) provides the highest SBS, but is only suitable 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.

There is still insufficient information about the success achieved using adhesive materials and different veneering protocols to increase adhesion to PEEK materials. 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 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 Al2O3 (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);
A D/AIN: Adhese Universal direct/indirect veneering groups;
G D/GIN: Gluma Bond Universal direct/indirect veneering groups;
P D/PIN: G-Premio BOND direct/indirect veneering groups;
S D/SIN: Single Bond Universal direct/indirect veneering groups;
V D/VIN: 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>
<thead>
<tr>
<th>Product</th>
<th>Composition*</th>
<th>Application recommendation*</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhese Universal (VivaPen)</td>
<td>10-MDP, 2-HEMA, BisGMA, MCAP, D3MA, highly dispersed silica, ethanol, water, photo initiators and stabilisers. pH 2.8</td>
<td>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/cm² for polymerisation.</td>
<td>Ivoclar Vivadent,</td>
</tr>
<tr>
<td>Gluma Bond Universal</td>
<td>4-META, MDP, methacrylate, acetone, water. pH 1.6–1.8</td>
<td>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 &gt; 500 mW/cm² for polymerisation.</td>
<td>Heraeus Kulzer</td>
</tr>
<tr>
<td>G-Premio BOND</td>
<td>MDP, 4-MET, MEPS, methacrylate monomer, acetone, water, initiator, silica, pH 1.5</td>
<td>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/cm² for polymerisation.</td>
<td>GC Corporation</td>
</tr>
<tr>
<td>Single Bond Universal</td>
<td>MDP monomer, dimethacrylate resins, HEMA, vitrebond copolymer, filler, ethanol, water, initiator, silane. pH 2.7</td>
<td>Apply the product to the material surface with a brush and rub it in for 20 s, then immediately apply the product to the material surface with a brush thinly and only once. Then immediately 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.</td>
<td>3M</td>
</tr>
<tr>
<td>visio.link</td>
<td>MMA, 2-prepenoic acid reaction products with pentaerythritol, diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide</td>
<td>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.</td>
<td>Bredent</td>
</tr>
<tr>
<td>Essentia Direct Composite</td>
<td>UTMA, other methacrylate monomers, inorganic filler (92.3%; SiO₂, BaO, Al₂O₃, La₂O₃)</td>
<td>20 s light application with 700 mW/cm² light intensity curing device (LED-C)</td>
<td>GC Corporation</td>
</tr>
<tr>
<td>Gradia Plus Laboratory Composite</td>
<td>1%–5% Bis-GMA, 5%–10% TEGDMA, 1%–5% UDMA, ceramic filler</td>
<td>3 min light application in full-mode with a dental laboratory polymerisation unit (GC Labolight Duo)</td>
<td>GC Corporation</td>
</tr>
</tbody>
</table>

*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, methacyryloxydecyl dihydrogen phosphate; MEPS, methacryloyloxyalkyl thiophosphate methylmethacrylate, MET, methacryloyloxyethyl trimellitate; META, methacryloyethyl trimellitate anhydride; MMA, methyl methacrylate; O, oxygen; Si, silicon; TEGDMA, triethyleneglycol-dimethacrylate; Ti, titanium; UDMA, urethane dimethacrylate; UTMA, urethane tetramethacrylate.
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 Vin group, followed by the Vd, Pd, Gin, AIN, Ad, Sn, SD, PIN, GD, CIN and CD 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 GD and GIN 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 CD group had statistically lower SBS values compared to the AIN (P = 0.044), Ad (P = 0.039), Gin (P = 0.04), Pd (P = 0.006), Vd (P = 0.001) and Vin (P = 0.001) groups. The CIN group had statistically lower SBS values compared to the Vin (P = 0.001), Vd (P = 0.001) and Pn (P = 0.044) groups. In addition, the Pn group had a statistically significant difference only with the Vd (P = 0.001) and Vin (P = 0.002) groups, which presented the highest SBS values (Table 2). The GD group with the lowest SBS data

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

<table>
<thead>
<tr>
<th>Group (n = 10)</th>
<th>SBS (MPa)</th>
<th>Min–max (median)</th>
<th>P value*</th>
</tr>
</thead>
</table>
| C0            | 12.54 ± 4.90 | 3.23–17.48 (13.86)
| CIN           | 17.29 ± 4.18 | 12.33–25.62 (16.43)
| A0            | 58.38 ± 20.05 | 30.47–98.5 (52.86)
| AIN           | 57.73 ± 18.82 | 31.63–99.41 (54.19)
| G0            | 22.63 ± 12.93 | 7.88–47.47 (21.89)
| Gin           | 56.23 ± 11.35 | 30.44–69.14 (58.56)
| P0            | 62.19 ± 15.02 | 37.83–80.99 (60.83)
| PIN           | 31.1 ± 12.94  | 2.71–45.29 (31.40)
| S0            | 50.76 ± 15.75 | 25.23–78.19 (49.96)
| SIN           | 56.91 ± 25.08 | 28.04–102.18 (52.02)
| V0            | 89.62 ± 30.36 | 63.65–164.50 (77.56)
| VIN           | 103.85 ± 36.33 | 72.6–186.05 (94.43) |

*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.
Cinel Sahin et al. had the highest statistical difference among the adhesive groups. Accordingly, there was a significant difference between the GD group and the S IN (P = 0.018), AIN (P = 0.01), AD (P = 0.008), GIN (P = 0.009), PD (P = 0.001), VIN (P = 0.001) and VD (P = 0.001) groups (Table 2).

Adhesive failure was detected in the CIN, CD, PIN, PD, GIN and GD 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).

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).

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. 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. 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. Many studies have shown that adhesive materials containing methyl methacrylate (MMA) monomers exhibit greater SBS between resins and PEEK materials. 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. It has been reported that pentaerythritol dissolves the PEEK surface, whereas MMA monomers form active reaction surfaces by swelling the dissolved surface and
bonding to the composite resin veneers.\textsuperscript{22} It is thought that this mechanism brings about an increase in SBS.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 mono-methacrylate known not to provide good long-term bond strength.\textsuperscript{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 PD and G\textsubscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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.\textsuperscript{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 material's 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.\textsuperscript{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\textsubscript{D} and P\textsubscript{IN}). For them, the P\textsubscript{D} group exhibited higher SBS values than the P\textsubscript{IN} group, but the difference was not statistically significant (\textit{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\textsubscript{D} and

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

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

NA, not available; SD, standard deviation.
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
Sustained Release of Liposomal Curcumin: Enhanced Periodontal Outcomes in Diabetic Patients

Ahmed ABDALLAH KHALIL\(^1\), Eman ALAAELDIN\(^2,3\)

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-\(\alpha\)), interleukin 1 beta (IL-1\(\beta\)) 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


Periodontitis is a multifactorial inflammatory illness characterized by gradual deterioration of the tooth supporting apparatus and linked to some bacterial species of dysbiotic plaque biofilms\(^1\). 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\(^8-12\).
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.\textsuperscript{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\textsuperscript{13-17}. The thick network of gel formulation retard the release of the entrapped components allowing sustained release and prolonged time of action.\textsuperscript{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)\textsuperscript{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.\textsuperscript{21} Briefly, lipid 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 cervical 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
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 homogeneously distributed with PDI 0.4.
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. 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 beneficial. 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 radicals in human by increasing the activity of antioxidant enzymes. 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 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. 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.
Those findings come in accordance with Mohammad et al.\(^26\) who revealed that curcumin gel had a significant effect on the reduction of IL-1\(\beta\), TNF-\(\alpha\), 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-1\(\beta\) and TNF-\(\alpha\) and substantial enhancement of PI, 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-1\(\beta\) and TNF-\(\alpha\) since the base line of those tests are significantly different.

### Conclusion

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

### Acknowledgements

The authors would like to thank Deraya University for supporting the work.

### Conflicts of interest

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

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

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Sustained curcumin</th>
<th>Curcumin</th>
<th>Placebo</th>
<th>Control</th>
<th>(P) value</th>
</tr>
</thead>
</table>
| PI  
0     | 1.90 ± 0.21  
6 wk  | 0.65 ± 0.33  
12 wk | 0.05 ± 0.08  |
| GI  
0     | 1.90 ± 0.31  
6 wk  | 0.46 ± 0.35  
12 wk | 0.25 ± 0.05  |
| PD  
0     | 2.93 ± 0.30  
6 wk  | 2.61 ± 0.20  
12 wk | 1.87 ± 0.34  |
| CAL  
0     | 0.98 ± 0.27  
6 wk  | 0.33 ± 0.20  
12 wk | 0.09 ± 0.15  |
| TAC  
0     | 5.44 ± 0.71  
6 wk  | 7.79 ± 0.53  
12 wk | 11.15 ± 0.75 |
| IL-1\(\beta\)  
0     | 4.33 ± 0.15  
6 wk  | 3.52 ± 0.10  
12 wk | 2.52 ± 0.09  |
| TNF-\(\alpha\)  
0     | 3.46 ± 0.29  
6 wk  | 2.51 ± 0.20  
12 wk | 1.78 ± 0.18  |

*Statistically significant.
Author contribution

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

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References

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

Fei LIN¹, Lin YUE¹

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


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 Oehlers⁵, 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.\textsuperscript{10} The lined enamel within the invagination could also be associated with possible defects or dehiscence, or hypomineralised.\textsuperscript{1} Thus, in many case reports, pulpal pathosis was found in teeth with type III dens invaginatus (Table 1).\textsuperscript{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.\textsuperscript{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.\textsuperscript{5,17,18} In such cases, the invagination may be associated with an extensive periradicular lesion through the coronal path in the oral cavity and subsequently induce peri-invagination periodontitis.\textsuperscript{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);\textsuperscript{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.\textsuperscript{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 (Cölnèe, 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, Mérignac, 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. 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. 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. 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. 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. 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, whereas the other two also performed non-surgical endodontic treatment for the root canals. Apical surgery was conducted in one of the cases to ensure the cleaning and sealing effects of the complex invagination. For sealing the invagination, gutta-percha and sealer were used in six cases to obturate the path. The other one used MTA to seal the apical foramen prior to gutta-percha obturation. 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, 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.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year of publication</th>
<th>Oehlers classification of invagination</th>
<th>Pulp vitality</th>
<th>Periapical lesion</th>
<th>Endodontic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al(^{32})</td>
<td>2022</td>
<td>IIIB</td>
<td>No response to the electric pulp vitality test</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hernández et al(^{29})</td>
<td>2022</td>
<td>IIIA</td>
<td>One of the two cases had necrotic pulp, the other had vital pulp</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Zhang et al(^{26})</td>
<td>2022</td>
<td>IIIB</td>
<td>One of the teeth with dens invaginatus was vital whereas the other was non-vital</td>
<td>Yes</td>
<td>Root canal therapy performed only on the non-vital tooth</td>
</tr>
<tr>
<td>Ali et al(^{33})</td>
<td>2022</td>
<td>IIIA</td>
<td>No response to the electric pulp vitality test</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Kalogeropoulos et al(^{27})</td>
<td>2022</td>
<td>IIIB</td>
<td>Normal</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Kamio et al(^{18})</td>
<td>2021</td>
<td>IIIA and IIIB</td>
<td>Normal</td>
<td>Yes (peri-invagination lesion)</td>
<td>No</td>
</tr>
<tr>
<td>Lee et al(^{17})</td>
<td>2020</td>
<td>IIIA</td>
<td>No response to the electric pulp vitality test</td>
<td>Yes (peri-invagination lesion)</td>
<td>No</td>
</tr>
<tr>
<td>Zhang and Wei(^{12})</td>
<td>2017</td>
<td>IIIB</td>
<td>Necrosis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Agrawal et al(^{13})</td>
<td>2016</td>
<td>IIIB</td>
<td>Necrosis</td>
<td>Yes</td>
<td>Apexification using MTA</td>
</tr>
<tr>
<td>Zoya et al(^{31})</td>
<td>2015</td>
<td>IIIB and II</td>
<td>No response to the electric pulp vitality test</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nosrat and Schneider(^{14})</td>
<td>2015</td>
<td>IIIB</td>
<td>Necrosis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Wayama et al(^{35})</td>
<td>2014</td>
<td>IIIA</td>
<td>Not mentioned</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Narayana et al(^{34})</td>
<td>2012</td>
<td>IIIB</td>
<td>No response to the thermal pulp vitality test</td>
<td>Yes</td>
<td>Performed the pulp revascularisation procedure</td>
</tr>
<tr>
<td>Lichota et al(^{30})</td>
<td>2008</td>
<td>III</td>
<td>No response to cold and electric pulp testing</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Štamfelj et al(^{36})</td>
<td>2007</td>
<td>III</td>
<td>Pulp sensitivity testing not performed</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Steffen and Splieth(^{28})</td>
<td>2005</td>
<td>III</td>
<td>The tooth responded to thermal and electrical stimuli</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nallapati(^{24})</td>
<td>2004</td>
<td>III</td>
<td>Normal response to cold</td>
<td>Yes (peri-invagination lesion)</td>
<td>No</td>
</tr>
<tr>
<td>Gound and Maixner(^{25})</td>
<td>2004</td>
<td>III</td>
<td>Normal</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Treatment of invagination</td>
<td>Apical surgery</td>
<td>Follow-up duration</td>
<td>Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with iRoot BP</td>
<td>Yes, with intentional replantation</td>
<td>4~39 mo</td>
<td>Eight of ten cases healed with no lesion or other adverse effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha and sealer</td>
<td>No</td>
<td>6~120 mo</td>
<td>Healing of the periapical lesion in both cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha and iRoot SP sealer</td>
<td>No</td>
<td>18 mo</td>
<td>Complete healing of the periapical lesions of both teeth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with MTA</td>
<td>Yes</td>
<td>1 y</td>
<td>Complete healing of the periapical lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha and bioceramic sealer</td>
<td>No</td>
<td>2 y</td>
<td>The pulp was normal. There is complete healing of the periapical lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha</td>
<td>No</td>
<td>6 mo</td>
<td>The pulp was normal. The lesion decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with MTA</td>
<td>No</td>
<td>2 y</td>
<td>The pulp responded normally to pulp vitality testing. Significant progress of periapical healing was evident</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Removed the invaginated tissue</td>
<td>Yes</td>
<td>6 mo</td>
<td>Healing on the soft tissue and on the periapical lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha</td>
<td>No</td>
<td>2 y</td>
<td>Significant osseous healing of the periapical lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared and sealed the invagination with MTA</td>
<td>Yes</td>
<td>1 y</td>
<td>Successful treatment outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha</td>
<td>No</td>
<td>6 mo</td>
<td>Significant osseous healing of the preoperative lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrograde preparation and seal of the invagination with gutta-percha and sealer</td>
<td>Yes</td>
<td>18 y</td>
<td>The tooth associated with crown fracture, but a satisfied healing of periapical lesion was revealed from radiograph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Removed the invaginated mass</td>
<td>No</td>
<td>12 mo</td>
<td>The periapical lesion appeared to have normal trabeculation with the appearance of bridge formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha</td>
<td>No</td>
<td>24 mo</td>
<td>Satisfactory bone healing was revealed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>Extraction</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared the invagination, then sealed it with gutta-percha and MTA</td>
<td>No</td>
<td>1 y</td>
<td>Apical repair was observed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The invagination was prepared and sealed with gutta-percha and dual-cured paste</td>
<td>Yes</td>
<td>4 mo</td>
<td>Progressive healing of the lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepared and sealed with gutta-percha</td>
<td>No</td>
<td>6 y</td>
<td>The radiolucent defect had healed completely</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and 1-year follow-ups) reported partial but progressive healing of the radiolucency.\textsuperscript{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\textsuperscript{12-14,30-35}, whereas pulp vitality was not mentioned in 2 of the 18 cases.\textsuperscript{35,36} Non-surgical endodontic treatments only were performed in 5 of these 11 cases\textsuperscript{13,14,30,32,33}, whereas apical surgery was also carried out in 4 of them.\textsuperscript{12,31,32,35} In one case, the invaginated mass was removed and a pulp revascularisation procedure was conducted.\textsuperscript{34} In the last of the 18 cases, tooth extraction was performed.\textsuperscript{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 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).
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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