OPEN ACCESS
[DOI]10.12016/j.issn.2096-1456.202660043

·基础研究·

白皮杉醇对乳牙脱矿牙本质基质生物改性的研究

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【摘要】　目的　探讨不同浓度的白皮杉醇（PIC）溶液对乳牙脱矿牙本质基质生物改性的能力，为改善树脂-乳牙牙本质粘接耐久性提供依据。**方法**　本研究已获得单位医学伦理委员会批准。通过分子对接分别预测PIC与牙本质Ⅰ型胶原、基质金属蛋白酶（MMPs）的相互作用效果。临床选取因乳牙滞留拔除的牙冠完整的乳磨牙124颗，将76颗乳磨牙制成完全脱矿的60块牙本质条和24块牙本质片。将60块脱矿牙本质条平均分配用于干燥质量损失测试和膨胀率测试，每个实验30块。两实验均将牙本质条随机分为以下5组（n=6）：Control组，5%戊二醛（GA）组和2.5 mg/mL、5 mg/mL、10 mg/mL PIC组，以检测Ⅰ型胶原的抗酶解和机械性能。将24块脱矿牙本质片中的15块牙本质片分为上述5组（n=3）进行傅里叶变换红外光谱（FTIR）检测，探讨交联机制。将剩余9块脱矿牙本质片随机分为3组（n=3）：蒸馏水A组（酶解前电镜观察）、蒸馏水B组（酶解后电镜观察）、10 mg/mL PIC组（10 mg/mL PIC处理后，酶解后电镜观察），扫描电镜（SEM）观察交联剂对脱矿牙本质胶原的交联效果。剩余48颗乳磨牙制备成粘接试件，随机分为4组（n=12）：Control组（蒸馏水组），2.5mg/mL PIC组，5 mg/mL PIC组和10 mg/mL PIC组，检测即刻剪切粘接强度（SBS）和老化SBS，体视显微镜下观察各组样本的断裂模式，探究不同浓度PIC溶液对乳牙牙本质粘接耐久性的影响。**结果**　分子对接结果显示PIC与Ⅰ型胶原、MMPs的结合活性较强，可形成氢键、静电势能以及疏水作用力；FTIR结果证实了氢键的形成；干燥质量损失测试结果显示：2.5 mg/mL、5 mg/mL、10 mg/mL PIC组的质量损失率与Control组相比均降低，差异具有统计学意义（P<0.05），其中10 mg/mL PIC组交联效果最好；膨胀率测试结果显示2.5 mg/mL PIC组、5 mg/mL PIC组、10 mg/mL PIC组的脱矿牙本质基质膨胀率均降低，机械性能得到有效改善；扫描电镜下可见10 mg/mL PIC组酶解后的牙本质胶原最大程度地保留了其天然三维网络，与经酶解处理的蒸馏水B组未经交联并已崩解的牙本质胶原形成显著对比，与未经酶解处理的蒸馏水A组胶原形态相似。SBS实验结果显示：无论即刻SBS还是老化SBS，10 mg/mL PIC组均大于Control组，差异均具有统计学意义（P<0.05），10 mg/mL PIC组即刻SBS大于2.5 mg/mL PIC组，差异具有统计学意义（P<0.05），其余组间相比差异无统计学意义（P>0.05）。断裂模式分析结果显示：各组断裂模式大多为混合断裂。与Control组相比，随着PIC溶液浓度增加，粘接界面断裂模式比例减少。**结论**　10 mg/mL PIC溶液可有效交联乳牙脱矿牙本质基质，并在临床操作时间内提高其抗酶解能力和机械性能，增强粘接强度和粘接界面的稳定性，并有望提高树脂-牙本质的粘接耐久性。

【关键词】　白皮杉醇；　乳牙牙本质基质；　牙本质Ⅰ型胶原；　胶原交联；　牙本质生物改性；　交联剂；　基质金属蛋白酶；　粘接耐久性
【中图分类号】　R78　**【文献标志码】**　A　**【文章编号】**　2096-1456（2026）07-0668-12
【引用著录格式】　王曼泽，李润航，吕晶，等. 白皮杉醇对乳牙脱矿牙本质基质生物改性的研究[J]. 口腔疾病防治, 2026, 34(7): 668-679. doi:10.12016/j.issn.2096-1456.202660043.

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Study on the biomodification of demineralized dentin matrices in primary teeth with piceatannol　WANG Manze, LI Runhang, LV Jing, LV Xuechao, JIN Xing'ai.　The First Affiliated Hospital of Harbin Medical University, School of Stomatology, Harbin Medical University, Harbin 150000, China

【收稿日期】2026-01-26；　**【修回日期】**2026-04-03
【基金项目】黑龙江省自然科学基金（LH2022H042）
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【**Abstract**】 **Objective** To investigate the ability of different concentrations of piceatannol (PIC) solution to biomodify decalcified dentin matrices in primary teeth, thereby providing a theoretical basis for improving the durability of resin – dentin bonding. **Methods** This study was approved by the Medical Ethics Committee of the institution. Molecular docking was performed to predict the interaction effects of PIC with type I collagen and matrix metalloproteinases (MMPs) in dentin. A total of 124 clinically extracted intact primary molars were selected due to retention; of these, 76 were prepared into 60 completely decalcified dentin beams and 24 dentin slices. The 60 beams were equally allocated for dry mass loss and swelling ratio testing (30 beams each). For both experiments, the beams were randomly divided into five groups (n = 6): a control group, 5% glutaraldehyde group, and 2.5, 5, and 10 mg/mL PIC solution groups. This was to evaluate the anti-enzymatic hydrolytic properties and mechanical performance of type I collagen. Of the 24 slices, 15 were assigned to the aforementioned five groups (n = 3) for Fourier-transform infrared spectroscopy (FTIR) to explore the cross-linking mechanism. The remaining nine slices were randomly divided into three groups (n = 3): distilled water group A (SEM examination prior to enzymatic hydrolysis), distilled water group B (SEM examination after enzymatic hydrolysis) , and a 10 mg/mL PIC solution group (SEM examination after treatment with 10 mg/mL PIC and subsequent enzymatic hydrolysis). This was to evaluate the cross-linking effect on demineralized dentin collagen. The remaining 48 molars were fabricated into bonding specimens and randomly divided into four groups (n = 12): a control group (distilled water group) and 2.5, 5, and 10 mg/mL PIC groups. Immediate and aged shear bond strength (SBS) were measured, and failure modes were observed under a stereomicroscope to investigate the impact of different PIC concentrations on the bonding durability of primary tooth dentin. **Results** Molecular docking revealed strong binding activity between PIC and type I collagen/MMPs via hydrogen bonds, electrostatic potential, and hydrophobic forces; the FTIR results confirmed hydrogen bond formation. In the dry mass loss test, mass loss rates in the 2.5, 5, and 10 mg/mL PIC groups were significantly lower than that in the control group (P < 0.05), with the 10 mg/mL PIC group showing the most optimized cross-linking effect. Swelling tests indicated that all three PIC concentrations reduced the swelling ratio of the demineralized matrix, effectively improving its mechanical properties. SEM showed that collagen from the 10 mg/mL PIC group retained its natural three-dimensional network post-enzymolysis, contrasting sharply with the disintegrated collagen in the enzymolyzed distilled water group B but resembling the morphology of the non-enzymolyzed distilled water group A. SBS results demonstrated that the 10 mg/mL PIC group exhibited significantly higher bond strengths than the control group in both the immediate and aged tests (P < 0.05); immediate SBS in the 10 mg/mL PIC group was also significantly greater than that in the 2.5 mg/mL PIC group (P < 0.05), while no other intergroup differences were found (P > 0.05). Failure mode analysis indicated predominantly mixed fractures, with the proportion of interfacial fractures decreasing as PIC concentration increased compared with the control group. **Conclusion** A 10 mg/mL PIC solution can effectively cross-link decalcified dentin matrices in primary teeth, enhancing the anti-enzymatic hydrolytic capacity and mechanical properties during clinical operation, thereby promoting bond strength and interface stability, which promises to improve the long-term durability of resin – dentin bonding.

【**Key words**】 piceatannol; primary dentin matrix; type I dentin collagen; collagen crosslinking; dentin biomodification; crosslinking agent; matrix metalloproteinases; bonding durability

J Prev Treat Stomatol Dis, 2026, 34(7): 668-679.

乳牙龋病是影响儿童口腔健康最主要的疾病之一,树脂充填是治疗乳牙牙体缺损的常用方法。树脂-牙本质之间的粘接力主要依赖于酸蚀后脱矿牙本质表层的胶原纤维网(主要是Ⅰ型胶原)和粘接剂所组成的混合层[1]。然而,粘接剂渗透至牙本质小管的深度无法与酸蚀剂的脱矿程度一致[2],最底部未被粘接剂包裹的胶原纤维易被内源性基质金属蛋白酶(matrix metalloproteinases,MMPs)所降解,从而导致粘接界面失效[3]。牙本质生物改性是使用胶原交联剂增强牙本质胶原纤维的机械性能并控制其降解率,从分子层面主动巩固粘接界面的一种仿生修复策略[4]。

乳牙牙本质较恒牙具有更低的硬度和弹性模量[5],因此使用外源性交联剂维持乳牙牙本质胶原的自然交联状态尤为重要。近年来,植物中提取的天然多酚类化合物因其生物相容性良好、细胞

毒性低等特点受到口腔领域学者们的更多关注[6]。本课题组前期已研究证实白藜芦醇作为牙本质预处理剂可增强树脂-牙本质粘接耐久性，显著抑制MMPs的活性[7]，而白皮杉醇（piceatannol，PIC）与白藜芦醇化学结构相似，且比白藜芦醇多一个单位的酚羟基[8]，可能表现出优于白藜芦醇的生物学活性。研究显示，PIC具有抗肿瘤、抗菌抗炎、抗氧化以及保护血管和神经等多种作用[9-11]。然而，还未有研究直接评估PIC能否对乳牙脱矿牙本质基质进行生物改性，其交联机制、交联效果尚不清楚。因此，本研究旨在评价PIC对乳牙脱矿牙本质基质机械性能和抗降解能力的直接影响，以及PIC作为预处理剂是否具有提高乳牙牙本质粘接耐久性的能力，以期为提高树脂-牙本质粘接界面的稳定性提供一种高效可行的新方法。

1　材料和方法

1.1　实验材料和主要仪器

PIC粉末（Sigma Aldrich，美国）；I型胶原酶（Sigma Aldrich，美国）；85%磷酸（上海阿拉丁生化科技股份有限公司，中国）；25%戊二醛（glutaraldehyde，GA）（上海阿拉丁生化科技股份有限公司，中国）；六甲基二硅氮烷（上海阿拉丁生化科技股份有限公司，中国）；碳酸氢铵（国药集团化学试剂有限公司，中国）；无水乙醇（国药集团化学试剂有限公司，中国）；37%磷酸酸蚀剂（SPIDENT，韩国）；Single bond Universal Adhesive（3MESPE，美国）；复合树脂Z250（3MESPE，美国）；电子天平（EXP125ZH/AD，上海奥豪斯仪器有限公司，中国）；低速金刚石硬组织切割机（SYJ-160，沈阳科晶自动化设备有限公司，中国）；恒温摇床（ZQLY-380，上海知楚仪器有限公司，中国）；扫描电子显微镜（SZ61，OLYM-PUS，日本）；衰减全反射傅里叶变换红外光谱（Fourier transform infrared spectroscopy，FTIR）仪（Nicolet iS10，Thermo Scientific，美国）；电子万能试验机（3340，Instron，美国）；体式显微镜（SZ61，OLYMPUS，日本）。

1.2　实验牙收集

本研究已通过哈尔滨医科大学附属第一医院伦理委员会批准（审批号：2025589），研究实施严格遵守《赫尔辛基宣言》，获得患者书面同意，共收集124颗因滞留拔除的无龋或早期龋乳磨牙，要求牙冠无充填体和裂纹，去除色素、牙石及牙周膜后于4 ℃的生理盐水中保存。

1.3　脱矿牙本质条、牙本质片的制备

使用低速切割机在流动水冷却下去除牙釉质以及牙根，垂直于牙体长轴将牙冠切割成24块1 mm×6 mm×6 mm的牙本质片和60块1 mm×2 mm×6 mm的牙本质条。蒸馏水超声荡洗20 min去除表面杂质后冲洗晾干。用85%磷酸加蒸馏水稀释成浓度为10%磷酸溶液，将切割好的脱矿牙本质条、牙本质片置于10%磷酸溶液中，25 ℃恒温摇床震荡24 h。蒸馏水冲洗3遍，水合1 h备用。

1.4　白皮杉醇和戊二醛溶液的配制

使用电子天平精确称取PIC粉末，避光条件下将其溶于无水乙醇中，配制成浓度分别为2.5 mg/mL、5 mg/mL、10 mg/mL的PIC溶液。

1.5　分子对接

1.5.1　白皮杉醇与牙本质I型胶原的分子对接　从Protein Date Bank数据库下载牙本质I型胶原（PDB IDs：1QSU）的晶体结构，PIC的3D结构从PubChem数据库下载获得，并在MMFF94力场下进行能量最小化。使用PyMOL软件去除水分子和受体蛋白后加氢。最后使用AutoDock Vina将I型胶原与PIC进行分子对接，其结合能（kcal/mol）值即代表两者的结合能力。使用PyMOL-2.3.0从9个小分子构象中选择出最优的模型，分析PIC与I型胶原蛋白的作用关系。

1.5.2　白皮杉醇与MMPs的分子对接　对接使用的MMP-2（PDB IDs：1QIB）、MMP-9（PDB IDs：2OW0）蛋白晶体结构从Protein Date Bank数据库中获得，PIC结构获得和处理方法与上述一致。使用PyMol 2.5.5处理受体蛋白，去除水分子、盐离子及小分子，并设置对接盒子包裹整个蛋白结构。使用ADFR Suite 1.0将处理后的PIC和MMPs转换为PDBQT格式。使用AutoDock Vina 1.2.3软件进行对接，全局搜索设为32，输出最高得分的构象为结合构象，最后使用PyMol进行可视化分析。

1.6　干燥质量损失测试

电子天平精确称取0.794 g碳酸氢铵粉末溶于蒸馏水中配置浓度为0.2 mol/L的碳酸氢铵缓冲液。量取5 mg I型胶原酶与碳酸氢铵缓冲液混合得到浓度为0.1 mg/mL的I型胶原酶溶液，调pH至7.1~7.2备用。将60块脱矿牙本质条中的30块随机分为5组（n=6）：Control组（空白对照组）、2.5 mg/mL PIC组、5 mg/mL PIC组、10 mg/mL PIC组和5% GA组（阳性对照组）。Control组样本用蒸馏水处理60 s，2.5 mg/mL、5 mg/mL、10 mg/mL PIC组样本用

2.5 mg/mL、5 mg/mL、10 mg/mLPIC溶液处理60 s，5%GA组样本在5%(w/w)GA溶液中处理60 s，结束后大量蒸馏水冲洗各组样本，置于含有二氧化硅颗粒的干燥皿中干燥24 h，使用电子天平对每块脱矿牙本质条进行称重，测量3次取平均值，记为m_0。称重后重新将其置于蒸馏水中水合1 h，将样本置于I型胶原酶溶液中，37 ℃恒温摇床酶解24 h。蒸馏水冲洗残留酶解液，干燥后重新对牙本质条称重，测量3次取平均值，记为m_1。按以下公式计算每块脱矿牙本质条的干燥质量损失率：

$$干燥质量损失率(\%)=(m_0-m_1)/m_0\times100\%$$

1.7　膨胀率测试

将剩余30块脱矿牙本质条随机分为5组(n=6)：分组及处理同1.6，5组样本分别与相应组别溶液交联60 s后冲洗3遍，在室温下将其置于蒸馏水中膨胀，磷酸盐缓冲液中平衡过夜。用吸水纸使样本干燥后立即称重，记为湿重。将脱矿牙本质条浸泡于蒸馏水中20 min以去除残余磷酸盐，随后将其置于含有二氧化硅颗粒的干燥皿中干燥24 h后再次称重，记为干重。膨胀率为脱矿牙本质条湿重与干重的比率。

1.8　傅里叶变换红外光谱检测

将24块脱矿牙本质片中的15块随机分为5组(n=3)：分组及处理同1.6，5组样本分别与相应组别溶液交联60 s后冲洗3遍后自然风干，分别对PIC粉末以及牙本质片进行FTIR测试。采集的光谱范围设定为600~4 000 cm^{-1}，分辨率为4 cm^{-1}。最后用Thermo Scientific™ OMNIC软件对所得光谱进行分析和处理。

1.9　扫描电镜观察

将剩余9块脱矿牙本质片随机分为3组(n=3)进行相应处理：蒸馏水A组(酶解前电镜观察)和蒸馏水B组(酶解后电镜观察)的样本均在室温下置于蒸馏水中处理60 s，10 mg/mL PIC组(酶解后电镜观察)的样本则在室温下置于10 mg/mL PIC溶液中处理60 s，结束后3组均用蒸馏水冲洗3遍；随后，蒸馏水A组样本直接置于碳酸氢铵缓冲液中，蒸馏水B组和10 mg/mL PIC组的样本则均置于0.1 mg/mL的I型胶原酶溶液中，37 ℃恒温摇床酶解24 h后大量蒸馏水冲洗。依次经30%、50%、70%、80%、90%、100%的乙醇溶液中浓度梯度脱水，每个浓度处理15 min，六甲基二硅氮烷干燥固定30 min，室温下干燥1 h后抽真空并喷金40 s，在SEM下仔细观察牙本质胶原酶解前后以及使用PIC交联后酶解的微观形态。

1.10　剪切粘接强度实验

将48颗乳磨牙用高速涡轮手机去除牙根，用自凝树脂将样本包埋成9 mm×9 mm×9 mm大小的约高于自凝树脂平面3 mm的统一模型。磨除牙釉质，用600目碳化硅砂纸打磨暴露出的淡黄色牙本质面，酸蚀20 s，将酸蚀剂冲洗干净后用无油无水的压缩空气吹干牙本质表面，获得统一的牙本质粘接面。将酸蚀后的48个样本随机分成4组(n=12)，Control组、2.5 mg/mL PIC组、5 mg/mL PIC组、10 mg/mL PIC组，4组样本粘接面分别用蒸馏水、2.5 mg/mL、5 mg/mL、10 mg/mL的PIC溶液预处理60 s，涂擦粘接剂20 s，轻吹5 s，光固化10 s。在直径和高均为3 mm的圆柱形塑料模具辅助下分3次分层充填复合树脂，每次充填1 mm、光固化20 s后拆除模具，得到与粘接界面垂直统一的树脂小柱。将每组12个试件随机均分，6个用来测试即刻剪切粘接强度(shear bond strength，SBS)，另外6个试件在10%次氯酸钠溶液中浸泡1 h后测试老化SBS。将电子万能试验机加速度设置为0.5 mm/min，加载剪切力的方向与粘接界面相平行，加载力量直至树脂与牙本质面断裂。SBS为树脂小柱断裂时的最大载荷除以树脂牙本质粘接表面积所得结果。

1.11　断裂模式分析

在40倍体视显微镜下观察SBS实验中样本的断裂模式，断裂面的类型分为以下4种：①粘接界面断裂；②牙本质内聚断裂；③复合树脂内聚断裂；④混合断裂。

1.12　统计学分析

实验结果采用SPSS 26.0软件进行统计学分析，采用Shapiro-Wilk检验和Levene检验方差的正态分布和齐性。所得数据均符合正态分布。干燥质量损失和膨胀率测试实验数据采用单因素方差分析(One-way ANOVA)和Tukey's检验，SBS实验数据采用双因素方差分析(Two-way ANOVA)，P<0.05为差异具有统计学意义。

2　结　果

2.1　白皮杉醇与I型胶原的分子对接结果

表1所示为PIC与I型胶原分子对接的最低结合能排列模式，其中最佳模型结合能为-6.4 kcal/mol。图1为PIC与I型胶原对接复合物的三维、二维结构示意图。在此结构下，PIC可以与分子口袋中的I型胶原蛋白进行非键接触，形成以静电势能、疏水

作用力为代表的力。此外，PIC还与I型胶原蛋白中的氨基酸残基ARG-1252、LYS-1288形成了3个氢键，表现出较好的分子亲和力。

2.2　白皮杉醇与MMPs的分子对接结果

PIC与MMP-2、MMP-9的分子对接结合能分别为-8.755、-9.529 kcal/mol，两者结合亲和力均在-8 kcal/mol以下。

PIC与MMP-2对接复合物的三维、二维结构示意图见图2a、2b。PIC与MMP-2蛋白上的氨基酸残基ALA-217、ALA-165、THR-229形成了3个氢键，与残基HIS-201发生了pi-pi共轭作用，与HIS-201、TYR-223、VAL-198、LEU-197、LEU-218形成了疏水作用。

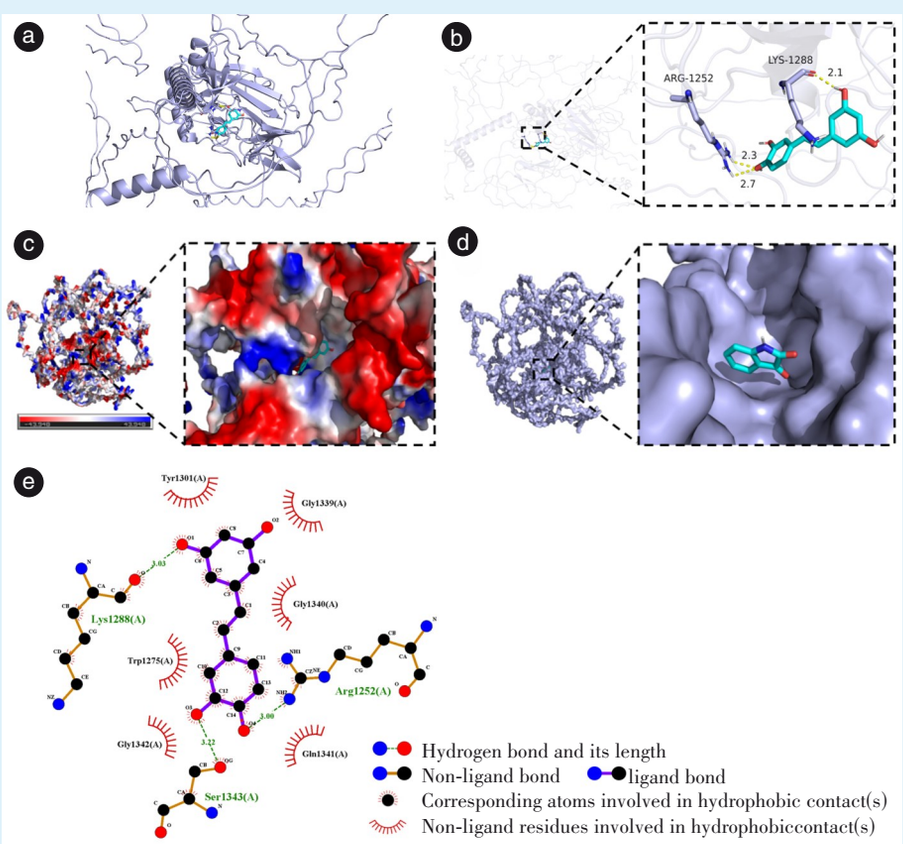
PIC与MMP-9对接复合物的三维、二维结构示

表1　PIC与I型胶原分子对接的最低结合能排列

Table 1　Alignment of the lowest binding energy for PIC-type-I collagen molecular docking

Mode	Affinity/(kcal/mol)	RMSD 1.b.	RMSD u.b.
1	-6.4	0.000	0.000
2	-6.2	18.398	19.638
3	-6.2	28.167	29.633
4	-6.2	19.349	20.404
5	-6.2	0.965	7.445
6	-6.2	18.364	20.520
7	-6.0	18.684	20.888
8	-6.0	5.310	7.584
9	-5.8	19.203	21.339

RMSD l.b.: lower limit root mean square deviation; RMSD u.b.: upper limit root mean square deviation. PIC: piceatannol

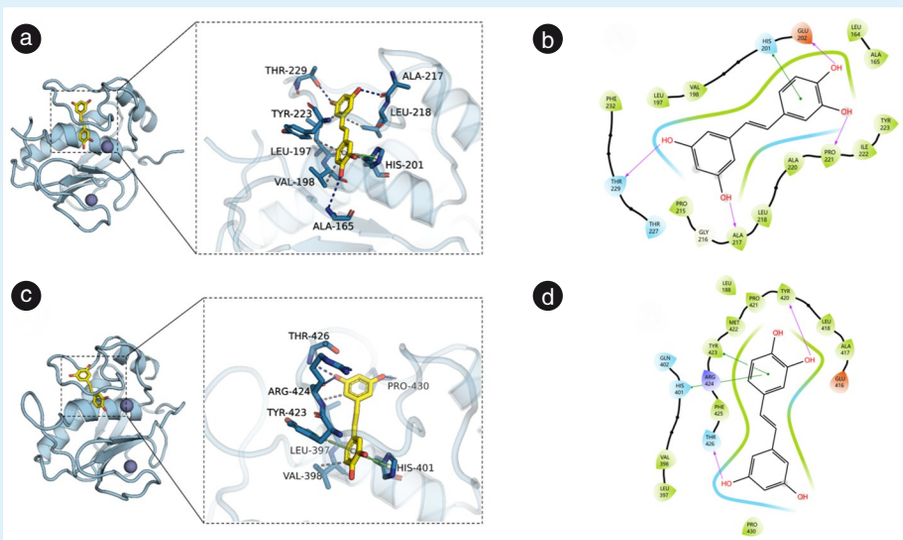


a: overall structure of the PIC-Type-I collagen docking complex. Type I collagen is a purple cartoon structure, and PIC is a blue rod structure. b: interaction diagram of the binding interface in the PIC-Type-I collagen docking complex. Type I collagen is a purple rod structure, and PIC is a blue rod structure. The hydrogen bond is represented by a yellow dotted line, with the unit in A°. c: electrostatic surface potential at the interface of the PIC-Type-I collagen docking complex. The color indicates the charge distribution: red area represents negatively charged; blue area represents positively charged; white area represents neutral or hydrophobic area. d: surface representation of the PIC-Type-I collagen docking complex. e: two-dimensional structure diagram. Arg: arginine. Gly: glycine. Gln: glutamine. Lys: lysine. Ser: serine. Trp: tryptophan. Tyr: tyrosine. PIC: piceatannol

Figure 1　The three-dimensional and two-dimensional structure diagram of PIC-type-I collagen docking complex

图1　PIC-I型胶原对接复合物的三维、二维结构示意图

意图见图2c、2d。PIC与MMP-9蛋白上的氨基酸残基ARG-424、THR-426、PRO-430形成了3个氢键，与氨基酸残基TYR-423、HIS-401发生了与MMP-2结合时类似的pi-pi共轭作用，与LEU-397、VAL-398、HIS-401形成了疏水作用，为分子提供了强大的范德华力。



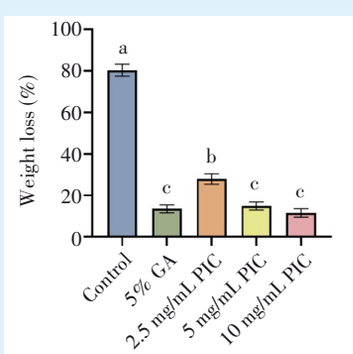
a&b: three-dimensional structure (a) and two-dimensional structure (b) of PIC-MMP-2 molecular docking. c&d: three-dimensional structure (c) and two-dimensional structure (d) of PIC-MMP-9 molecular docking. In figure a, c, the yellow rod structure is PIC, the cyan cartoon structure is MMP-2, 9. The blue dotted line represents the hydrogen bond, the gray dotted line represents the hydrophobic effect, and the green dotted line represents the pi-pi conjugate effect. In figure b & d, the purple line represents the hydrogen bond, and hydrophobic residues are indicated in green. PIC: piceatannol. MMP: matrix metalloproteinases

Figure 2　The three-dimensional and two-dimensional structure diagram of PIC-MMP-2, PIC-MMP-9 docking complex

图2　PIC-MMP-2、PIC-MMP-9对接复合物的三维、二维结构示意图

2.3　干燥质量损失测试结果

图3所示为各组处理后脱矿牙本质条的干燥质量损失测试结果。Control组、5% GA组、2.5 mg/mL PIC组、5 mg/mL PIC组、10 mg/mL PIC组质量损失率分别为80.40%±2.88%、13.55%±1.89%、27.92%±2.53%、14.91%±1.93%、11.61%±2.03%。2.5 mg/mL、5 mg/mL、10 mg/mL PIC组和5%GA组的质量损失率均显著降低，与Control组相比差异均具有统计学意义（P<0.05）。5 mg/mL、10 mg/mL PIC组与5%GA组相比差异无统计学意义（P>0.05），但随着PIC浓度的升高，质量损失率随之降低。其中，10 mg/mL PIC组的质量损失率变化最为显著，由Control组的80.40%±2.88%降低至11.61%±2.03%（P<0.05）。



Variations in lowercase letters signify notable disparities among the groups (P<0.05). Control group: not crosslinked; PIC group: 2.5, 5 and 10 mg/mL PIC were used for crosslinking for 60 s. GA group: crosslinked with 5% (w/w) GA for 60 s. PIC: piceatannol. GA: glutaraldehydeafter 24 h enzymatic digestion by group

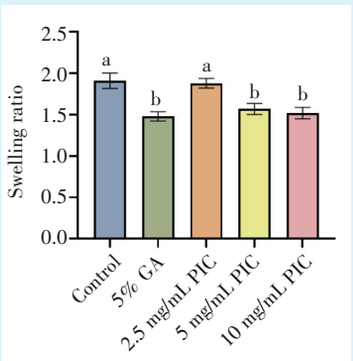
Figure 3　The percentage loss of dry mass of demineralized dentin beams after 24 h of enzymatic hydrolysis in groups

图3　各组脱矿牙本质条酶解24 h后的干燥质量损失百分比

2.4　膨胀率测试结果

各组膨胀率测试结果如图4所示。Control组、5% GA组、2.5 mg/mL PIC组、5 mg/mLPIC组、10 mg/mL PIC组的膨胀率分别为1.91±0.09、1.48±0.06、1.88±0.06、1.57±0.07、1.52±0.07。相较于Control组和2.5 mg/mL PIC组，5 mg/mLPIC组、10 mg/mL PIC组和5% GA组膨胀率降低，其中，5% GA组膨胀率降低最为明显，差异具有统计学意义（P<0.05）。2.5 mg/mL PIC组与Control组相比可使膨胀率由1.91±0.09降低至1.88±0.06，但差异无统计学意义（P>0.05）。与5% GA组相比，PIC浓度的升高并未引起5 mg/mL PIC组、10 mg/mL PIC组膨胀率的降

低，组间差异无统计学意义（P>0.05）。



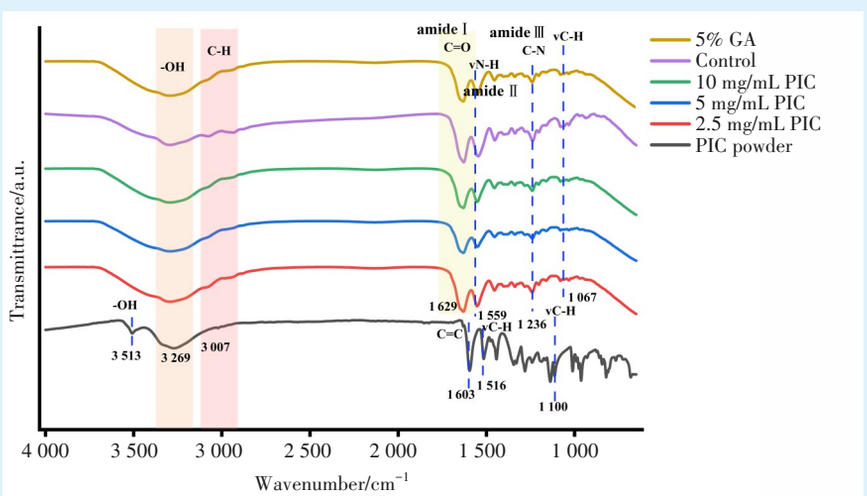
Variations in lowercase letters signify notable disparities among the groups (P<0.05). Control group: not crosslinked; PIC group: 2.5, 5 and 10 mg/mL PIC were used for crosslinking for 60 s. 5% GA group: crosslinked with 5% GA for 60 s. PIC: piceatannol. GA: glutaraldehyde

Figure 4　Swelling ratio of demineralized dentin beams in groups

图4　各组脱矿牙本质条的膨胀率

2.5　傅里叶变换红外光谱检测结果

图5所示为各组脱矿牙本质片以及PIC粉末的FTIR检测结果。PIC粉末光谱在3 513 cm^{-1}和3 269 cm^{-1}的伸缩振动对应PIC的酚羟基结构，3 007 cm^{-1}代表乙烯基C-H键的伸缩振动，1 629 cm^{-1}和1 516 cm^{-1}分别对应乙烯基C=C双键和苯环骨架的伸缩振动，进一步印证PIC的芳香环结构。Control组光谱显示出牙本质胶原三螺旋的特征峰，他们分别为位于1 632 cm^{-1}处C=O基团伸缩振动所代表的酰胺Ⅰ带、1 559 cm^{-1}处C-N和N-H键振动所代表的酰胺Ⅱ带以及1 236 cm^{-1}处C-N基团振动所代表的酰胺Ⅲ带。PIC处理组光谱在1 636 cm^{-1}处的酰胺峰形相较于Control组加宽，峰位置发生移动。且随着PIC浓度的提高，C=O峰位置也发生改变。5% GA组在1 632 cm^{-1}处峰位置的移动对应其醛基-CHO的C=O伸缩振动。相较于Control组，PIC处理组（2.5 mg/mL PIC组、5 mg/mL PIC组、10 mg/mL PIC组）的酰胺Ⅱ带呈现峰形加宽，而酰胺Ⅲ带峰强度降低。



Compared with the control group, the PIC group retained the characteristic amide Ⅰ, Ⅱ and Ⅲ peaks, indicating that PIC did not destroy the natural triple helix structure of dentin collagen. PIC powder showed characteristic peaks at 3 513 cm^{-1}, 3 269 cm^{-1}, 1 629 cm^{-1}, and 1 516 cm^{-1}, which further confirmed the aromatic ring structure of PIC. Control group: not crosslinked; PIC group: 2.5, 5 and 10 mg/mL PIC were used for crosslinking for 60 s. GA group: crosslinked with 5% GA for 60 s; The infrared spectrum of PIC powder was also collected as a reference. PIC: piceatannol. GA: glutaraldehyde

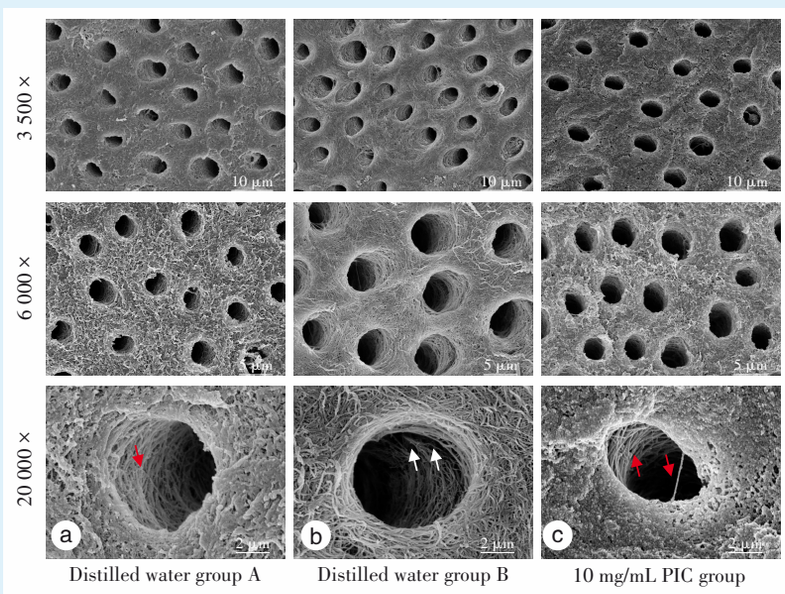
Figure 5　Representative spectra of demineralized dentin slices and PIC powder in groups

图5　各组脱矿牙本质片及PIC粉末的典型光谱

2.6　扫描电镜观察结果

各组脱矿牙本质片酶解前后的代表性微观形态如图6所示。蒸馏水A组在没有胶原酶降解的情况下，未经交联处理的脱矿牙本质胶原结构完整，可见图中红色箭头所示的纤维绳状结构连续紧密地排列在牙本质小管周围，随着无机物的流失，可观察到胶原纤维间的缝隙相应增宽（图6a）。蒸馏水B组酶解后，未经交联处理的脱矿牙本质胶

原结构严重塌陷，胶原纤维因脱水与管壁分离，断裂的胶原纤维末端松散排列（图6b）。然而，与蒸馏水A组和B组相比，10 mg/mL PIC组酶解后的脱矿牙本质很好地保留了胶原蛋白的三维网络，管间和管周结构清晰可见，纤维支架连接紧密，基质间孔隙度降低，并表现出明显的胶原交联，与未经酶解处理的蒸馏水A组胶原形态相似（图6c）。



a: the collagen fiber structure is intact, with the fibers arranged in tight, cord-like structures around the dentinal tubules. b: after enzymatic hydrolysis, the fibers are severely collapsed, fragmented, and loosely arranged, and have separated from the tubule walls. c: following enzymatic hydrolysis after PIC crosslinking, the collagen fiber network remains intact, resembling the morphology observed in figure a, demonstrating the protective effect of PIC crosslinking against enzymatic degradation. Distilled water group A: uncrosslinked demineralized dentin matrix. Distilled water group B: uncrosslinked demineralized dentin matrix was digested by collagenase for 24 h. 10 mg/mL PIC group: demineralized dentin matrix was crosslinked with 10 mg/mL PIC for 60 s, and then digested with collagenase for 24 h. Red arrow: cord-like structures of collagen fibers. white arrow: broken collagen fibrous network. SEM: scanning electron microscopy. PIC: piceatannol

Figure 6　Representative scanning electron microscopy images of demineralized dentin slices before and after enzymatic hydrolysis digestion in groups

图6　各组脱矿牙本质片酶解前后的代表性SEM图像

2.7　剪切粘接强度测试结果

各组SBS测试结果如表2所示，无论即刻SBS还是老化SBS，10 mg/mL PIC组与Control组相比，差异均有统计学意义（$P<0.05$）；10 mg/mL PIC组与2.5 mg/mL PIC组即刻SBS相比差异也具有统计学意义（$P<0.05$）；其余组间相比差异无统计学意义（$P>0.05$）。表明PIC预处理不会影响乳牙牙本质的即刻SBS和老化SBS，并且经10 mg/mL PIC预处理后SBS有所提高。4组PIC处理组的即刻SBS、老化SBS间差异均无统计学意义（$P>0.05$）。

表2　各组即刻SBS与老化SBS

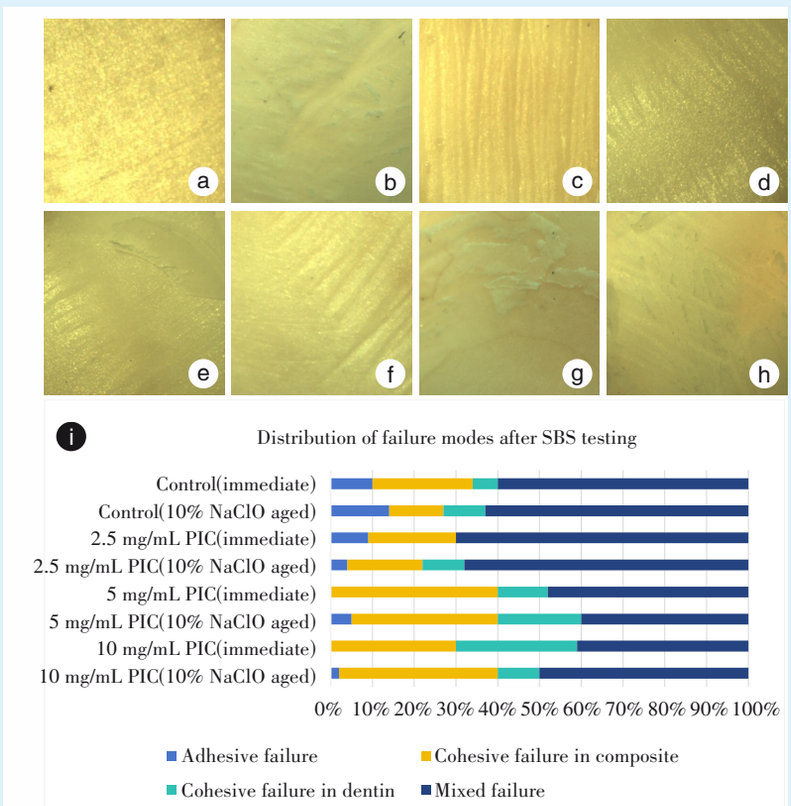
Table 2　The immediate SBS and aged SBS in groups

Group	Immediate SBS	Aged SBS	*F*	*P*
0 mg/mL PIC	26.12±5.06^{a}	14.42±3.63^{a}	0.315	0.587
2.5 mg/mL PIC	28.56±2.35^{a}	20.02±1.97^{a}	0.059	0.813
5 mg/mL PIC	31.58±7.50^{ab}	29.24±7.14^{a}	0	0.998
10 mg/mL PIC	54.92±18.62^{b}	46.96±15.39^{b}	0.039	0.848
F	9.74	15.971	—	—
P	<0.001	<0.001	—	—

Wihin the same row, statistically significant ($P<0.05$) between-group diferences are represented by different lowercase letters. SBS: shear bond strength. PIC: piceatannol

2.8　断裂模式分析

各类断裂界面代表图像及断裂模式分布见图7，各组主要断裂模式均为混合断裂，与Control组相比，随着PIC溶液浓度增加，粘接界面断裂模式比例减少，复合树脂内聚断裂模式比例增加。



a: adhesive failure; b: cohesive failure in composite; c: cohesive failure in dentin; d: adhesive failure and cohesive failure in dentin; e: adhesive failure, cohesive failure in composite and dentin; f: adhesive failure and cohesive failure in dentin; g: adhesive failure and cohesive failure in composite; h: cohesive failure in dentin and cohesive failure in composite; SBS ×40. SBS: shear bond strength. PIC: piceatannol

Figure 7　Representative graphs of failure modes after SBS testing and the distribution of failure modes in groups

图7　SBS测试后各组断裂模式代表图像及断裂模式分布

3　讨　论

3.1　选择白皮杉醇作为交联剂以期提高乳牙牙本质粘接耐久性的依据

I型胶原是牙本质有机基质的主要组成部分，其可通过支持矿化组织的支架来维持牙本质胶原的耐酶解性能和机械强度[12]。然而，酸性环境会激活以酶原形式存在的MMPs，导致牙本质胶原的快速降解，破坏混合层的稳定性[13]。乳牙牙本质较恒牙更薄，矿化程度及耐酸性更差，胶原网络的完整性更易受到侵害[7]。因此，通过保护乳牙牙本质胶原来提高树脂-牙本质粘接耐久性，延长修复体的使用寿命是长期追求的目标。

近年来，学者们研究并提出了众多稳定牙本质胶原的方法，包括使用交联剂、再矿化液和胶原酶抑制剂等[14-16]。PIC是一种具有四个单位酚羟基的天然多酚类化合物，其可能与原花青素[17]、单宁酸[18]以及茶黄素[19]等结构类似的多酚类交联剂一样，通过酚羟基和没食子酸基与胶原纤维相互作用，形成共价键、氢键以及疏水作用，改善胶原蛋白的机械性能。戊二醛作为化学合成交联剂的代表，是交联剂的黄金标准。然而，由解聚残留物及未固化分子引发的细胞毒性是限制其临床应用的主要原因[20]，因此戊二醛主要作为阳性对照溶液来比较各类交联剂的生物改性作用。本实验中使用无水乙醇溶解PIC粉末来配制不同浓度的溶液，结合乙醇湿粘接技术[21]可更高效地交联牙本质胶原，减少胶原纤维的酶解现象。本课题组前期采用CCK-8法和死/活染色法筛选出了PIC的适宜浓度：2.5 mg/mL、5 mg/mL、10 mg/mL，其细胞毒性远低于戊二醛，表明在此浓度范围内PIC具有良好的局部应用安全性，且作为预处理剂用量微少，操作后即被冲洗，其引发全身毒性的风险较低。

3.2　白皮杉醇与牙本质胶原及MMPs相互作用的机理分析

首先应用分子对接技术预测了PIC与I型胶原的相互作用。一般认为，对接能量值小于-5.0 kcal/mol表示有较好的结合活性[22]，小于-7.0 kcal/mol表示有强烈的结合活性[23]。胶原与PIC受体结合口袋表现出较高的契合度，结合能为-6.4 kcal/mol，表明PIC与I型胶原具有较好的结合活性，能在自然条件下自发结合。除此之外，PIC与I型胶原可通过多种作用力结合，包括氢键、静电势能和疏水作用力，表明PIC这一类多羟基化合物可与胶原形成更大的结合面积和更好的空间互补性[24]。

乳牙牙冠牙本质中含量最多的MMPs为MMP-2和MMP-9[25]，为探究PIC是否也能进一步交联MMPs，抑制其活性并阻止胶原降解，使用分子对接模拟了PIC与MMP-2、MMP-9的作用关系。对接结果显示，PIC-MMP-2、PIC-MMP-9复合体可通过氢键、pi-pi共轭作用以及疏水作用紧密结合，使MMPs活性位点的分子运动性降低。同时，PIC可能改变牙本质胶原的天然构象，阻碍酶的特异性识别位点，使MMPs无法最佳地识别Ⅰ型胶原纤维并与其形成复合体[26]。二者的协同作用抑制了胶原酶的活性，从而提高胶原网络的稳定。这与Lee等[27]使用岩藻甾醇和MMPs进行对接的结果一致。此外，牙本质基质中的非胶原蛋白对MMPs具有重要的调节功能，PIC可通过与这些蛋白交联，使其功能域失活，间接阻断MMPs的活性。但应该注意的是，由于胶原残基的化学特性差异和超分子组装方式的复杂性，分子对接模拟可能无法完全复现真实环境中复杂的相互作用[28]，其具体作用机制仍有待深入研究。

通过FTIR检测证实了分子对接所模拟的相互作用。PIC处理组和Control组相比均保留了特征性酰胺Ⅰ、Ⅱ和Ⅲ峰，表明PIC没有破坏牙本质胶原的天然三螺旋结构。多酚类化合物含有的亲水性酚羟基是其与蛋白质结合的基础，酚羟基的含量越高，其形成的氢键作用力越强[29]。PIC处理组在1 636 cm^{-1}处的酰胺Ⅰ峰吸收带加宽，且峰位置发生移动，说明PIC的酚羟基与胶原的酰胺基团形成了氢键，改变了其分子间作用力的均匀性。这种作用机制同样可能存在于PIC与MMPs活性位点的氨基酸残基之间。随着PIC浓度的升高，更多酚羟基与胶原C=O键形成更强的氢键。氢键会降低C=O键的力常数，使其伸缩振动频率降低[30]。此外，酚羟基可与胶原分子中的N-H基团形成多位点氢键相互作用，使N-H弯曲振动所对应的微观环境趋于异质化，进而导致PIC处理组酰胺Ⅱ带峰型出现明显增宽。5% GA组1 632 cm^{-1}处峰位置改变，可能归结于其含有的醛基与胶原氨基反应形成的仲胺或叔胺基团中C-N的延伸振动[31]。而PIC组的酰胺Ⅲ带峰强度降低，这可能是由于胶原的相互作用改变了胶原天然三螺旋结构中C-N/N-H键的协同振动[32]，削弱了该组合峰的吸收信号。此外，酰胺Ⅲ带峰强度的降低提示PIC诱导了胶原构象的改变，而胶原构象的稳定性会进一步影响MMPs对其识别与降解。已有研究表明，多酚类化合物可与MMPs的催化结构域结合，诱导其构象改变或遮盖酶切位点，从而抑制其活性[27]。结合本课题组前期研究，PIC可能通过类似的机制，在交联胶原的同时抑制内源性MMPs的活性，协同增强脱矿牙本质基质的稳定性。

3.3　白皮杉醇对乳牙脱矿牙本质基质机械性能和抗酶解能力的影响

基于可溶性胶原的降解速率与其从不溶性脱矿牙本质基质中的释放速率处于动态平衡[33]，干燥质量损失测试可以间接评价脱矿牙本质胶原蛋白对外源性Ⅰ型细菌胶原酶的耐受能力。本实验结果显示，PIC组较Control组质量损失率明显减少，并随着其浓度的增加而减少，呈浓度依赖性。10 mg/mL的PIC对胶原的交联效果甚至优于5% GA，证实了PIC具有抑制胶原酶降解的作用。溶组织梭状芽胞杆菌胶原酶是一种细菌型胶原酶，属于MMP-9家族[34]。小分子化合物长期抑制酶的主要机制是药物与蛋白质形成共价键[35]。已有研究表明，二氢青蒿素可通过羟基与MMP-2和MMP-9的锌离子螯合而形成共价键，减弱酶的活性[16]。由此推断，PIC的四个酚羟基也可能与胶原酶的锌离子和钙离子螯合，改变蛋白质的空间构象，从而干扰胶原的生物降解过程。

交联剂能够调节胶原蛋白的亲水性质，而膨胀率与亲水性呈正相关[36]。本实验中5 mg/mL PIC组、10 mg/mL PIC组及5% GA组脱矿牙本质条膨胀率均显著低于Control组，说明戊二醛对胶原具有显著的交联效果，而PIC也可促进胶原交联并提升牙本质的稳定性。2.5 mg/mL PIC并未显著改变膨胀率的原因可能是本实验对胶原的处理时间较短以及PIC浓度较低所致。

为了更直接地评价PIC对脱矿牙本质胶原结构的影响，使用扫描电镜对胶原表面进行观察。图6a显示了完全脱矿后牙本质胶原的特征性纤维网络，相邻纤维间间隙增宽，胶原表面光滑致密。经10 mg/mL PIC处理后酶解的牙本质胶原表面虽略有粗糙，但胶原结构保存完好，仍可见胶原特征性的绳状纤维结构（图6c）。而图6b所示的未经任何交联剂处理并酶解后的胶原网络严重塌陷，部分绳状纤维结构断裂，胶原排列松散。表明PIC交联后的牙本质胶原具有良好的酶解耐受力和优越的机械性能。

3.4　白皮杉醇对乳牙牙本质粘接耐久性的影响

SBS是评估树脂-牙本质粘接性能最常用的指

标。本实验中SBS测试结果表明PIC预处理不影响牙本质的粘接强度，10 mg/mL PIC组与Control组相比，无论即刻SBS还是老化SBS均表明PIC作为牙本质预处理剂具有显著提高SBS的能力，与本研究中干燥质量损失测试和膨胀率测试结果相符。通过对断裂面的观察，可以看到各组断裂模式大多为混合断裂。

与Control组相比，经过PIC预处理后，粘接界面断裂相对减少，表明PIC在一定程度上可以保护粘接界面的完整性，以期提高乳牙牙本质的粘接耐久性。

3.5　白皮杉醇应用于乳牙牙本质生物改性的未来展望

综上所述，本研究证实PIC能有效地交联脱矿牙本质胶原，显著增强其对外源性胶原酶降解的抵抗力，改善其生物力学性能，并提高树脂-牙本质的粘接强度。除此之外，本实验对可以应用于牙本质生物改性的天然多酚类化合物进行了补充，并对PIC未来应用于口腔其他方面提供了理论依据。未来本课题组可以继续优化和研究PIC在更长的交联时间内对胶原热稳定性、亲疏水性以及胶原表面硬度的影响，或将其引入到粘接剂中，对粘接剂进行化学改性，以及与其他化合物联合应用于牙本质粘接等方面继续研究。

【Author contributions】 Wang MZ performed the experiments, analyzed the data and wrote the article. Li RH, Lv J, Lv XC analyzed the data and revised the article. Jin XA designed the study and revised the article. All authors read and approved the final manuscript as submitted.

【Ethics Statement】 The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University (Approval No. 2025589), and written informed consent was obtained from all participants.

【Funding】 This study was supported by the grant from National Natural Science Foundation of Heilongjiang Province (No. LH2022H042). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

【Competing interests】 All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare: support from National Natural Science Foundation of Heilongjiang Province (No. LH2022H042) for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

【Data availability statement】 Part of the data used in this study were obtained from the Protein Data Bank (PDB ID: 1QSU; https://www.rcsb.org/structure/1QSU), (PDB ID: 1QIB; https://www.rcsb.org/structure/1QIB), (PDB ID: 2OW0; https://www.rcsb.org/structure/2OW0). The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author upon reasonable request.

【Generative AI statement】 The authors declared that generative AI was not used in the creation of this manuscript.

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（编辑　张琳）

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