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· 基础研究 ·

不同预处理剂对乳牙牙本质粘接耐久性的影响

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【摘要】 目的 探讨不同预处理剂对乳牙牙本质粘接耐久性的影响。方法 选取42颗因滞留而拔除的乳磨牙, 其中24颗沿近远中向切开为颊舌侧两部分得到48个样本, 用于剪切粘接强度实验; 另外18颗用于纳米渗漏实验。两项实验均按照不同预处理方式将样本随机分为3组, A组: 蒸馏水预处理组, B组: 2%氯己定预处理组, C组: 10 mg/mL白藜芦醇预处理组。制备实验试件, 分别在即刻和10%次氯酸钠溶液老化1 h后进行剪切粘接强度检测、界面纳米渗漏评估和扫描电子显微镜观察, 评价不同预处理剂对粘接界面的影响。**结果** 即刻测试各组的剪切粘接强度无显著差异。老化后C组的剪切粘接强度显著高于A组和B组($P < 0.05$)。A组老化后的剪切粘接强度显著低于即刻($P < 0.05$), B组和C组在老化前后的剪切粘接强度无显著差异($P > 0.05$)。C组的界面纳米渗漏程度在老化前后比较无显著差异, 老化后C组的纳米渗漏程度最低($P < 0.05$)。**结论** 氯己定和白藜芦醇预处理剂均可以改善乳牙牙本质的粘接耐久性, 但白藜芦醇的效果优于氯己定。

【关键词】 乳牙牙本质; 预处理剂; 氯己定; 白藜芦醇; 剪切粘接强度; 纳米渗漏; 树脂-牙本质粘接界面; 粘接耐久性

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【Abstract】 Objective To explore the effects of different pretreatment agents on primary tooth dentin bonding durability. **Methods** Forty-two retained primary molars were selected, 24 of which were cut along the mesial and distal directions; thus, 48 samples were obtained for shear bond strength tests, and the other 18 teeth were used for nanoleakage tests. According to different pretreatments, both experimental samples were divided randomly into three groups (Group A: distilled water pretreatment group; Group B: 2% chlorhexidine pretreatment group; Group C: 10 mg/mL resveratrol pretreatment group). The test specimens were prepared, the shear bond strength was tested, and interfacial nanoleakage evaluation and scanning electron microscope observation were performed to evaluate the effects of different pretreatment agents on the bonding interface immediately and after aging for one hour with 10% sodium hypochlorite aqueous solution. **Results** The immediate shear bond strength results showed that there was no significant difference among the three test groups. After aging, the shear bond strength of Group C was significantly higher than that of Group A and Group B ($P < 0.05$). After aging, the shear bond strength of Group A was significantly lower than the immediate shear bond strength ($P < 0.05$), whereas there was no significant difference in shear bond strength before and after aging in Group B and Group C ($P > 0.05$). For Group C, there was no significant difference in interfacial nanoleakage before



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and after aging. In addition, among the three groups, Group C had the lowest interfacial nanoleakage ($P < 0.05$). **Conclusion** Both chlorhexidine and resveratrol pretreatment can improve the adhesion durability of deciduous dentin, but the effects of resveratrol are better than those of chlorhexidine.

【Keywords】 primary dentin; pretreatment agents; chlorhexidine; resveratrol; shear bond strength; nanoleakage; resin-dentin interface; bonding durability

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树脂粘接修复术是儿童口腔科治疗乳牙龋病的常用方法之一,牙本质粘接力主要来源于粘接剂向脱矿的牙本质胶原纤维网中渗透固化后形成的混合层所提供的微机械嵌合力。乳牙牙本质中存在的基质金属蛋白酶(matrix metalloproteinases, MMPs)在酸性环境下容易被激活降解胶原纤维^[1]。现有的粘接技术和粘接剂都不能完全包裹暴露的胶原纤维,暴露的胶原纤维容易发生酶解和水解,加速混合层退变,降低修复体的使用寿命^[2]。氯己定作为抗菌剂在牙科领域应用广泛,具有抗菌和抑制内源性蛋白酶活性的作用,是众多蛋白酶抑制剂中研究最多的一种。2%氯己定预处理可以改善乳恒牙牙本质粘接界面的稳定性^[3-4],但其细胞毒性不容忽视^[5]。

近年来,天然多酚成为牙本质粘接领域的研究热点,白藜芦醇(resveratrol)是一种含有芪类结构的非黄酮类多酚化合物,具有抗癌、保护神经和心脏的作用^[6]。研究发现白藜芦醇能抑制MMP-2、MMP-9的活性^[7-8],抑制变异链球菌的致龋性^[9],具有良好的生物相容性^[10]。研究表明使用白藜芦醇/乙醇溶液预处理恒牙牙本质可以提高树脂-牙本质粘接耐久性,降低粘接界面的纳米渗漏^[11]。与恒牙牙本质相比,乳牙牙本质的矿化程度低,有机物含量高,二者的组织结构和微观形态均存在差别,因此乳恒牙牙本质粘接效果可能会有一定差异。探寻能够有效提高树脂-乳牙牙本质粘接耐久性的预处理剂具有重要意义。目前关于白藜芦醇对乳牙牙本质粘接效果的研究报道较少,因此本实验选择人乳牙作为实验对象,观察不同预处理剂对乳牙牙本质剪切粘接强度和界面纳米渗漏的影响,以期为临床选择合适的乳牙牙本质表面预处理剂提供参考。

1 材料和方法

1.1 实验材料和主要设备

白藜芦醇粉末(Sigma Aldrich,美国);2%氯己定(朗力生物,中国);人工唾液(LANSO,中国);37%磷酸酸蚀剂(SPIDENT,韩国);Single bond Universal Adhesive(3M ESPE,美国);复合树脂 Z250(3M ESPE,美国);无水乙醇;10%次氯酸钠溶液;氯化硝酸银溶液;电子万能试验机(Instron,美国);低速金刚石切割机(沈阳科晶自动化设备有限公司,中国);扫描电子显微镜(JEOL,日本);体视显微镜(OLYMPUS SZ61,日本);能量色散X射线光谱仪(Hitachi,日本)。

1.2 实验牙收集

本研究通过了哈尔滨医科大学附属第一医院伦理委员会的批准(编号:2021XS27-02),收集了42颗因滞留拔除的无龋或早期龋乳磨牙,要求牙冠完整,无裂纹和充填物,去除色素、牙石、牙周膜等软组织保存于4℃的生理盐水中。

1.3 预处理剂配制

将白藜芦醇粉末溶于无水乙醇,在37℃恒温水浴箱水浴15 min,配制成10 mg/mL的白藜芦醇预处理剂,4℃避光保存备用。

1.4 实验分组

本研究共分为3组,A组为对照组(蒸馏水预处理),B组为氯己定预处理组,C组为白藜芦醇预处理组,实验分组与预处理剂应用顺序见表1。

1.5 剪切粘接强度实验

选取24颗乳磨牙,用高速涡轮手机冷却条件下去除牙根。用硬组织切割机沿牙冠近远中方向切割成颊、舌侧两部分,自凝树脂将样本包埋成9 mm × 9 mm × 9 mm的规则模型,样本略高于自凝塑料平面3 mm,在流水降温条件下用高速涡轮手机磨除釉质暴露牙本质面,600目碳化硅水砂纸打

磨 1 min, 超声荡洗 8 min, 获得统一标准的牙本质粘接面。各实验组按照表 1 的方法制备 48 个剪切实验试件, 形成与粘接界面垂直的树脂小柱(直径和高均为 3 mm)。将制备好的试件在 37 ℃人工唾液中储存 24 h; 每组 16 个试件中取 8 个试件进行即刻剪切粘接强度测试, 其余 8 个保存在 10%次氯酸

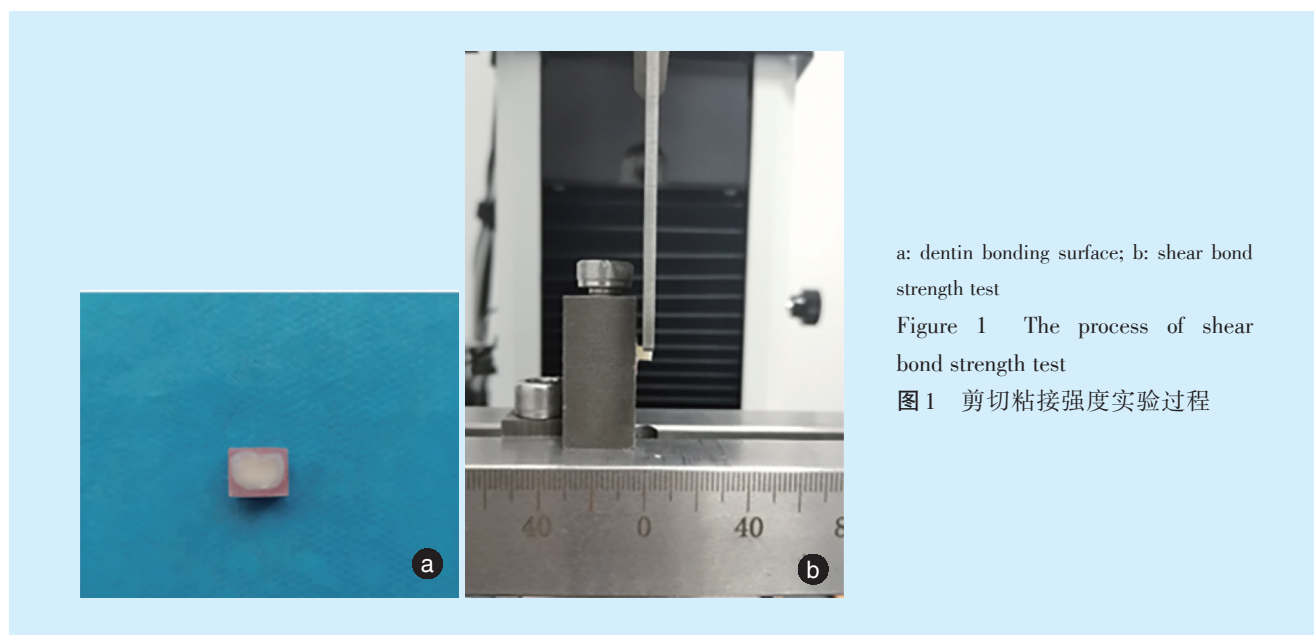
钠溶液中浸泡 1 h 后测试。用电子万能试验机测试剪切粘接强度, 剪切力的方向平行于粘接界面, 加载速度为 1 mm/min, 直至树脂与牙面断裂, 记录实验数据, 计算剪切粘接强度值=树脂小柱断裂时的最大载荷(N) / 粘接面积(mm²)。剪切粘接强度实验过程见图 1。

表 1 实验分组和预处理剂的应用顺序

Table 1 Experimental groups and application sequence of pretreatment agent

Group	Application mode	Group characteristic
Group A: control	a, b, c, d1, e, f, g, h, i	Rewetting with distilled water
Group B: 2% chlorhexidine	a, b, c, d2, e, f, g, h, i	Rewetting with 2% chlorhexidine
Group C: 10 mg/mL resveratrol	a, b, c, d3, e, f, g, h, i	Rewetting with 10 mg/mL resveratrol

a: 37% conventional acid etching (15 s); b: washing with air and water spray (15 s); c: drying with air (30 s); d1: dentin rewet with distilled water for 60 s; d2: dentin rewet with 2% chlorhexidine for 60 s; d3: dentin rewet with 10 mg/mL resveratrol for 60 s; e: the adhesive system single bond universal applied for 10 s; f: application of air for 5 s at 5 cm; g: light activation (10 s, 600 mW/cm²); h: insertion of 3 increments of resin composite 3M Z250; i: light activation of each increment (20 s, 600 mW/cm²)



a: dentin bonding surface; b: shear bond strength test

Figure 1 The process of shear bond strength test

图 1 剪切粘接强度实验过程

1.6 断裂模式分析

体视显微镜下(40倍)评估剪切实验测试后样本的断裂模式, 断裂面的类型分为 4 种: ①粘接剂界面断裂; ②牙本质内聚断裂; ③复合树脂内聚断裂; ④前三种断裂面形态的任意组合(混合断裂)。

1.7 界面纳米渗漏实验

选取 18 颗离体乳磨牙, 用高速涡轮手机冷却条件下去除牙根, 流体树脂封闭髓腔。用自凝塑料将样本包埋成 1.2 cm × 1.2 cm × 1.2 cm 的规则模型, 牙齿殆面高于自凝塑料平面 3 mm, 磨除殆面釉质暴露牙本质粘接面(方法同前), 按表 1 方法处理

粘接面, 分次放置复合树脂, 形成 3 mm 高的树脂修复体。将制备好的试件在 37 ℃人工唾液中储存 24 h; 每组随机取 3 个样本用于即刻检测, 其余 3 个在 10%次氯酸钠溶液中老化 1 h。用硬组织切割机将样本沿牙齿长轴方向切割成 2 mm 厚的片状试件。每组试件在距离树脂-牙本质粘接界面 1 mm 以外均匀涂布两层指甲油, 自然干燥后避光保存在质量分数为 50% 氨化硝酸银溶液中(pH=9.5) 24 h, 蒸馏水冲洗, 浸泡于显影液中荧光灯照射 8 h, 定影液固定 8 h。所有试件依次使用 600、800、1 500、2 000 目碳化硅砂纸在流水条件下打磨抛

光,超声荡洗 15 min,干燥,喷金,场发射扫描电子显微镜背散射模式下观察粘接界面纳米渗漏情况,每个样本随机选取 5 个视野(每组 15 张图像),使用 Image J 计算每个图像中银颗粒占整个图像面积的百分比,其分级表示如下:0,无纳米渗漏;1,渗漏 ≤ 25%;2,25% < 渗漏 ≤ 50%;3,50% < 渗漏 ≤ 75%;4,渗漏 > 75%。使用能量色散 X 射线光谱仪面扫分析粘接界面 Si 元素、Ca 元素、Ag 元素的分布情况。

1.8 粘接界面观察

从 1.7 实验余留样本中每组随机选取 2 个试件,待观察面依次用 600、800、1 500、2 000 目碳化硅砂纸流水条件下抛光,37% 磷酸酸蚀观察面 10 s,冲洗,浸入 5% 次氯酸钠溶液中 2 min,乙醇溶液梯度脱水,喷金,扫描电子显微镜下观察粘接界面情况。

1.9 统计学分析

各组剪切粘接强度值数据采用平均值 ± 标准差表示,采用 SPSS26.0 软件进行数据分析,剪切粘接强度实验数据采用方差分析,三组组间比较采用 LSD 检验,纳米渗漏实验等级资料采用 Kruskal-Wallis 检验进行分析, $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 剪切粘接强度测试结果

各组剪切粘接强度测试结果如表 2 所示,A 组的即刻粘接强度为 (33.62 ± 13.67) MPa,老化后的粘接强度为 (17.71 ± 3.82) MPa,二者比较具有显著差异 ($P < 0.05$)。B 组、C 组的即刻粘接强度与老化后的粘接强度无显著差异 ($P > 0.05$)。A、B、C 三组即刻粘接强度无显著差异 ($P > 0.05$),老化后的粘

接强度组间两两比较具有显著差异 ($P < 0.05$),且 C 组的粘接强度值最高,B 组次之,A 组最低。

表 2 各组剪切粘接强度值比较

Table 2 Comparison of shear bond strength between groups

	$\bar{x} \pm s$, MPa		
	Group A: control	Group B: 2% chlorhexidine	Group C: 10 mg/mL resveratrol
Immediate	33.62 ± 13.67 ^{ab}	29.36 ± 16.65 ^{ab}	40.59 ± 16.72 ^{ab}
Aged	17.71 ± 3.82 ^{cb}	30.46 ± 5.77 ^{ba}	39.64 ± 10.84 ^{ba}
<i>P</i>	0.013	0.858	0.878

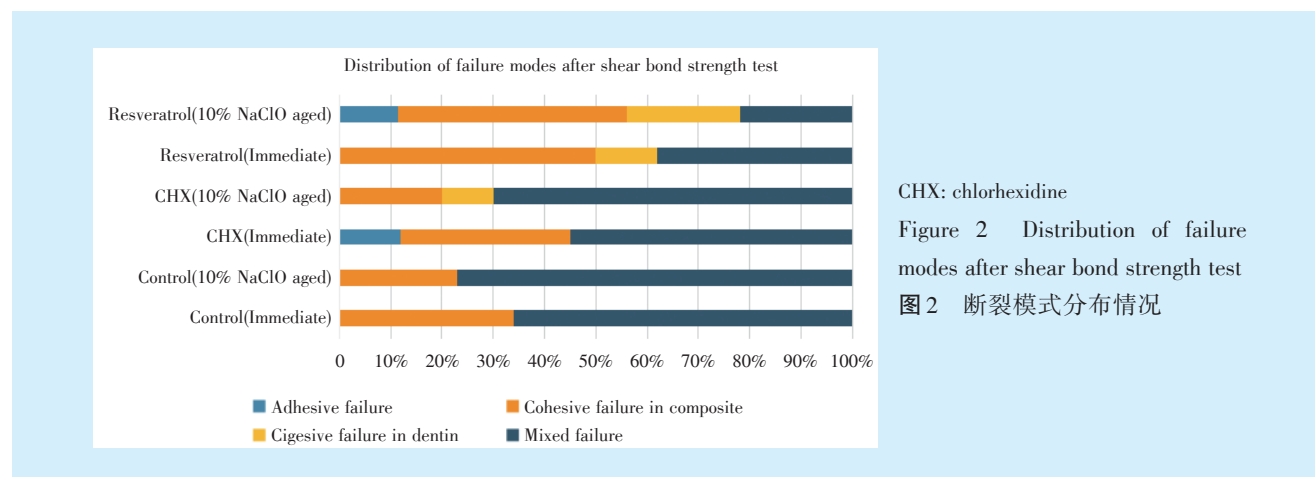
Immediate groups: $P_{(A vs B)}=0.386$; $P_{(B vs C)}=0.168$; $P_{(A vs C)}=0.594$; Aged groups: $P_{(A vs B)}=0.002$; $P_{(B vs C)}=0.022$; $P_{(A vs C)}=0.000$; Aa、Cb、Ba: different capital letters show differences regarding rows, and different small letters shows differences regarding columns, $P < 0.05$

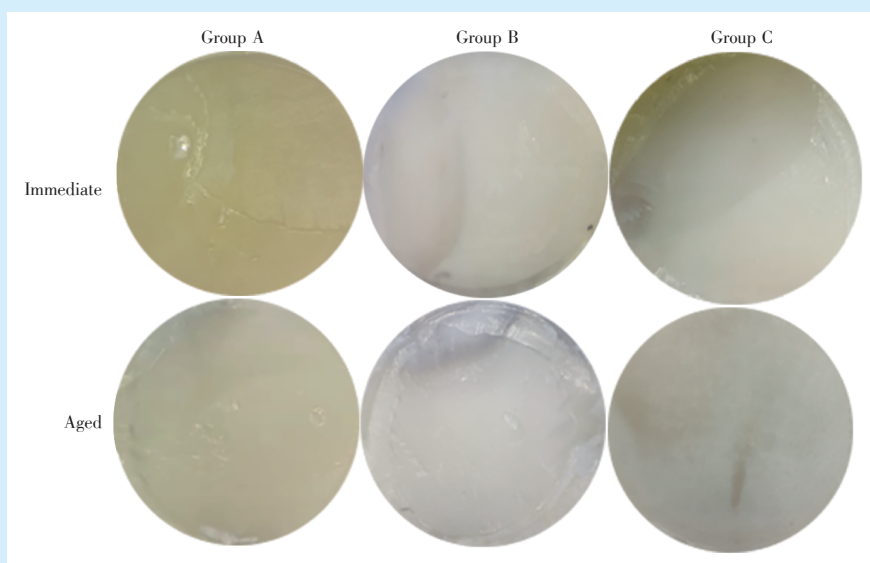
2.2 断裂模式分析

各组的断裂模式分析见图 2,A 组、B 组主要为混合断裂,C 组主要为复合树脂内聚断裂。各组体视显微镜图像如图 3 所示:A 组即刻断裂模式为粘接剂界面断裂和牙本质内聚断裂;B 组即刻断裂模式为牙本质内聚断裂和复合树脂内聚断裂;C 组即刻断裂模式为粘接剂界面断裂、复合树脂内聚断裂以及牙本质内聚断裂;A、B 组老化断裂模式为粘接剂界面断裂和复合树脂内聚断裂,C 组老化断裂模式为牙本质内聚断裂。

2.3 界面纳米渗漏结果

界面纳米渗漏结果见表 3,A 组、B 组的纳米渗漏结果显示在即刻和老化时有显著差异;C 组在即刻和老化时无显著差异。三组纳米渗漏程度即刻检测结果为 A 组 > C 组 > B 组,但三组组间比较无显著差异。经 10% 次氯酸钠溶液老化 1 h 后,三组纳米渗漏程度 A 组 > B 组 > C 组,A 组与 B 组、C 组比较差异均具有统计学意义,B 组与 C 组比较无显





The immediate fracture modes in group A were adhesive failure and cohesive failure in dentin; the immediate fracture modes in group B were cohesive failure in dentin and cohesive failure in composite; the immediate fracture modes in group C were adhesive failure, cohesive failure in composite and dentin. The aged fracture modes in group A and group B were adhesive failure and cohesive failure in composite; the aged fracture modes in group C were cohesive failure in dentin. Group A: control; group B: 2% chlorhexidine; group C: 10 mg/mL resveratrol

Figure 3 Representative SM images of failure modes from groups (x40)
图3 各组断裂模式的体视显微镜图(x40)

著差异。图4a为扫描电镜观察Ag离子渗漏情况,图4b~图4c为EDX分析粘接界面的纳米渗漏情况。红色区域代表Si元素,主要为复合树脂分布区域(图4b)。青色区域代表Ag元素,代表纳米渗漏情况,主要集中分布在粘接界面混合层底部(图4c)。黄色区域代表Ca元素,主要为牙本质分布区域(图4d)。

2.4 粘接界面形态观察

扫描电子显微镜下观察各组的粘接界面形态如图5,各组牙本质小管内均有不同长度的树脂突,C组的树脂突最长,与A组和B组比较更加致

密。A组和B组老化后均在混合层顶部出现裂隙。

3 讨论

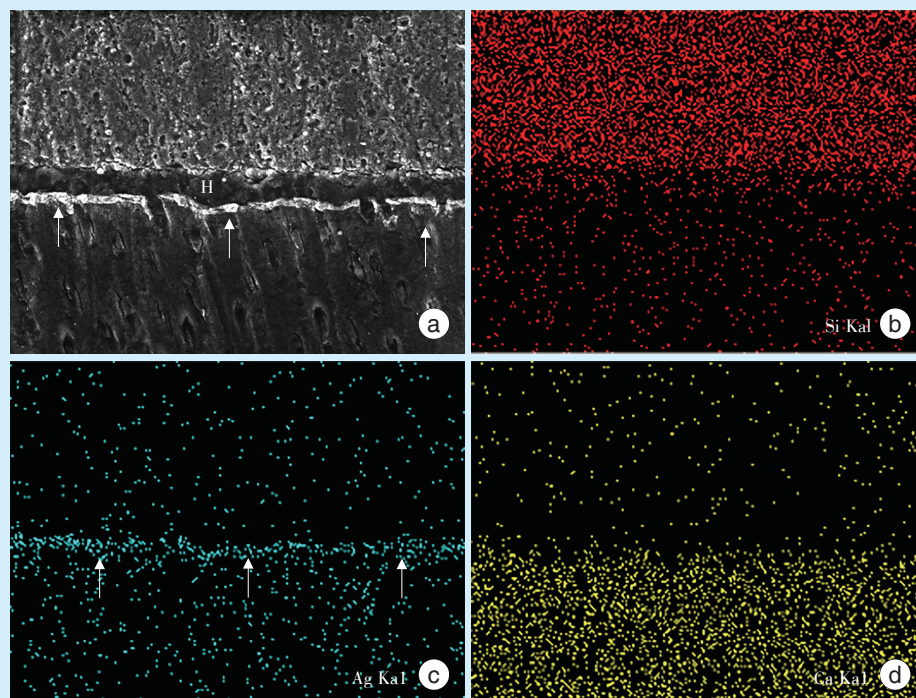
牙本质粘接界面退变是影响粘接耐久性的根本原因,而口腔环境会加速其退变的进程。粘接界面的退变包括粘接树脂的浸出、老化和胶原纤维的降解^[12]。牙本质预处理是改善粘接耐久性的方法之一,理想的牙本质预处理剂对牙髓细胞、成牙本质细胞的毒性作用应该很低或无毒性。氯己定是非特异性基质金属蛋白酶抑制剂和抗菌剂,用于预处理牙本质可以改善牙本质的粘接耐久

表3 各组纳米渗漏结果比较

Table 3 Comparison of nanoleakage results in groups

Groups	Aging mode	Score percentage (%)					Statistical difference	P
		0	1	2	3	4		
Group A: Control	Immediate	0	73.3	20	6.7	0	B	0.002
	Aged	0	13.3	66.7	20	0	Aa	
Group B: 2% chlorhexidine	Immediate	0	100	0	0	0	B	0.016
	Aged	0	66.7	33.3	0	0	Ab	
Group C: 10 mg/mL resveratrol	Immediate	0	93.3	6.7	0	0		1.000
	Aged	0	93.3	6.7	0	0	b	

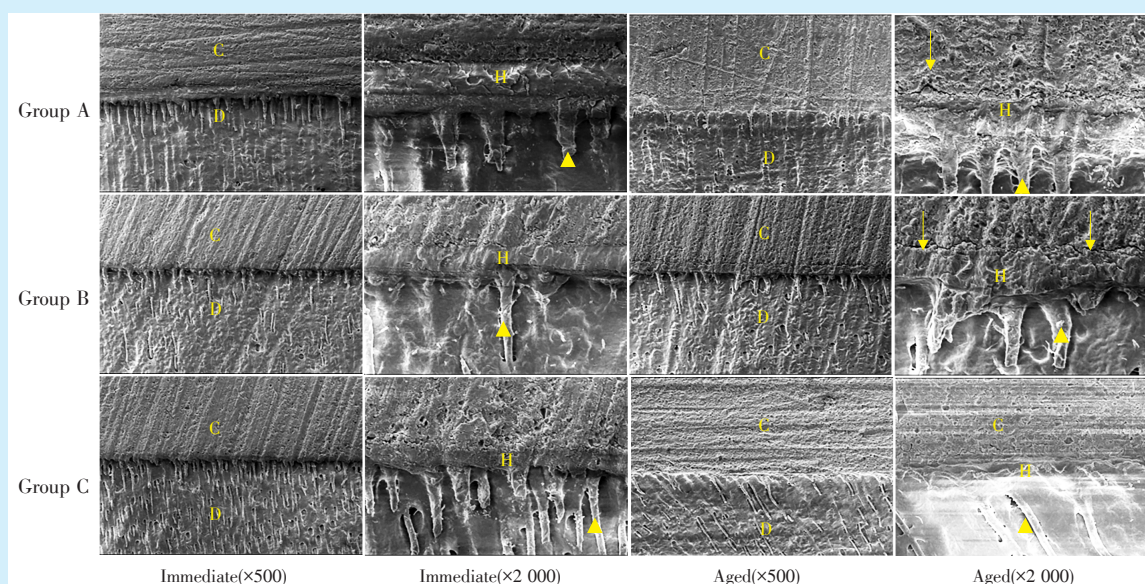
Different capital letters in statistical difference cloum show differences regarding different aging modes, and different small letters shows differences regarding different groups, $P < 0.05$, $n=15$. Aged group (A vs. B): $P=0.005$; Aged group (A vs. C): $P=0.000$; Aged group (B vs. C): $P=0.543$



a: scanning electron microscope images, white arrows: distribution area of Ag, the area indicated by the white arrows was the silver ion deposition at the bottom of the hybrid layer; b: distribution area of Si, the red area was mainly the area of composite resin distribution; c: distribution area of Ag, the area indicated by the white arrows was the silver ion deposition at the bottom of the hybrid layer; d: distribution area of Ca, the yellow area was mainly the dentin distribution area; H: hybrid layer; white arrows: distribution area of Ag, the area indicated by the white arrows was the silver ion deposition at the bottom of the hybrid layer; group A: control; group B: 2% chlorhexidine; group C: 10 mg/mL resveratrol

Figure 4 Representative scanning electron microscope images (×1 000) and energy dispersive X-ray spectroscopy analysis of dentin-bonding interface (×1 000)

图4 牙本质粘接界面的扫描电子显微镜(×1 000)和能量色散X射线光谱仪能谱分析(×1 000)



C: composite resin; D: dentin; H: hybrid layer; triangle: resin tags; arrows: crack; resin tags were observed in the dentin tubules of all groups; the resin tags of group C were longer and denser than group A and B; irregular cracks were observed at the top of the hybrid in aged group A and group B (as arrows showed); group A: control; group B: 2% chlorhexidine; group C: 10 mg/mL resveratrol

Figure 5 Representative scanning electron microscopy images resin-dentin interfaces from groups

图5 各组粘接界面的代表性扫描电镜图像

性,但其气味大、味微苦,儿童难以接受,且对成牙本质样细胞有较强的细胞毒性作用^[5,13]。有研究表明低于10 mg/mL白藜芦醇和10 mg/mL白藜芦醇改性粘接剂的细胞毒性和生物相容性均在可接受范围^[11,14],因此本实验选择白藜芦醇的浓度为10 mg/mL。白藜芦醇可以抑制MMPs的活性^[7-8],低浓度的白藜芦醇具有基因保护活性,能减低牙本质粘接剂成分的细胞毒性和DNA损伤^[15],抑制变异链球菌的活性^[9],提高恒牙牙本质粘接耐久性^[16],乳恒牙的牙本质微观形态和组织结构存在较大差异,乳牙牙本质粘接混合层更容易降解^[17],因此,评估白藜芦醇预处理对乳牙牙本质粘接性能的影响具有重要意义。

粘接强度是评估树脂-牙本质粘接性能最常用的指标。评估粘接强度常用方法为微拉伸强度和微剪切强度测试,微拉伸强度测试对试件制备要求高,技术敏感性强,而微剪切粘接强度测试相较于微拉伸强度测试更为简单方便^[18],试件制备后无需进一步切割成一定形状。本实验选择乳磨牙为研究对象,乳牙的体积比恒牙小,牙本质的厚度远不如恒牙牙本质,微拉伸强度实验的试件制备存在一定难度,因而本实验选择剪切强度测试评估粘接强度。

患者口腔是测试和评估粘接修复体的最终环境。模拟口内老化的常用方法有水储存、冷热循环、次氯酸钠溶液浸泡、pH循环等。研究表明10%次氯酸钠溶液浸泡1 h相当于冷热循环10 000次、水储存半年、pH循环15次的老化程度,10%次氯酸钠溶液浸泡快速高效^[19]。因此本实验采用10%次氯酸钠浸泡1 h模拟修复体在口内的老化环境。

实验结果显示各组即刻粘接强度无显著差异,说明氯己定或白藜芦醇预处理不会影响乳牙牙本质的即刻粘接性能,这与Giacomini等^[20]和Porto等^[21]以恒牙为研究对象的结果一致。经过10%次氯酸钠溶液老化后,对照组的粘接强度显著降低,2%氯己定组和白藜芦醇组的即刻粘接强度和老化后的粘接强度比较无显著差异,说明2%氯己定和10 mg/mL白藜芦醇均可以改善老化后乳牙牙本质粘接强度。本实验中2%氯己定组和白藜芦醇组经10%次氯酸钠溶液老化后粘接强度无明显降低,这与先前的研究结果有差别,可能是由于老化方式不同,次氯酸钠主要降解有机相,而脱矿牙本质表面经氯己定或白藜芦醇预处理后更多的胶原纤维被粘接树脂包裹。老化后,对照组、2%氯

己定组和白藜芦醇组的粘接强度均有显著差异,白藜芦醇组的粘接强度最高。白藜芦醇含有3个酚羟基,可通过氢键与胶原纤维发生交联作用,无水乙醇作为白藜芦醇的溶剂,可以通过降低介电常数来促进多酚与胶原的交联作用,从而改变胶原纤维的机械性能。乙醇或以乙醇为溶剂的预处理剂处理酸蚀脱矿的牙本质后,脱矿胶原基质悬浮于乙醇溶液中使得胶原纤维直径缩小,增大纤维网间的间隙,增加牙本质表面的疏水性并引起水分含量的变化,促进疏水树脂单体向更深层渗透和浸润,提高粘接界面的稳定性^[22]。

牙本质酸蚀脱矿的深度大于粘接树脂的渗透深度,混合层底部未被粘接树脂包裹而暴露的胶原纤维区域即纳米渗漏区域。该区域机械性能较弱,且存在游离水分子能促进混合层水解作用,是影响粘接耐久性的因素之一。纳米渗漏是评估粘接界面封闭性的重要指标,本实验使用氯化硝酸银进行试件银染,使用扫描电子显微镜观察和EDX能谱分析两种方法结合可以减少假阳性和假阴性成像,提高结果可信度^[23]。结果显示2%氯己定组、白藜芦醇组的即刻纳米渗漏程度低于对照组,对照组和2%氯己定组老化后纳米渗漏程度显著增加,白藜芦醇组纳米渗漏程度在老化前后无显著差异,老化后白藜芦醇组纳米渗漏程度最低,这证实了氯己定和白藜芦醇预处理可不同程度降低乳牙牙本质纳米渗漏情况,维持粘接界面稳定。

本实验中对对照组、2%氯己定组的断裂模式主要为混合断裂,白藜芦醇组主要为复合树脂内聚断裂,扫描电子显微镜观察白藜芦醇组的树脂突长且致密,进一步证明白藜芦醇预处理后粘接界面的混合层完整性更高。

综上所述,氯己定和白藜芦醇均可以保护粘接界面胶原纤维降解,维持粘接强度,在一定程度上改善乳牙牙本质粘接耐久性,且白藜芦醇对改善乳牙牙本质的粘接效果优于氯己定。

【Author contribution】 Liu ED designed the study, performed the experiments, analyzed the data and wrote the article. Lv J performed the experiments, analyzed the data and wrote the article. Liu YQ and Jin XA revised the article. All authors read approved the final manuscript as submitted.

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