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·基础研究·

两种止血剂对乳牙牙本质全酸蚀及自酸蚀粘接效果的影响

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【摘要】　目的　探讨两种止血剂对不同粘接系统下乳牙牙本质粘接效果的影响。**方法**　选取72颗滞留乳磨牙，其中48颗用于构建微渗漏模型，另外24颗沿近远中切开后得48个样本，构建剪切粘接强度实验模型。两实验模型均分为A、B 2组，A组即全酸蚀组：A1组（ViscoStat止血剂+NT全酸蚀粘接剂）、A2组（ViscoStat Clear止血剂+NT全酸蚀粘接剂）、A3组（无止血剂+NT全酸蚀粘接剂）；B组即自酸蚀组：B1组（ViscoStat止血剂+3M通用型粘接剂）、B2组（ViscoStat Clear止血剂+3M通用型粘接剂）、B3组（无止血剂+3M通用型粘接剂）。分别进行微渗漏实验和剪切粘接强度实验，扫描电镜观察断裂面形态。**结果**　A1、A2、A3 3组微渗漏差异无统计学意义（$P>0.05$）。B1、B2、B3 3组微渗漏差异无统计学意义（$P>0.05$）。A1、A2、A3组剪切粘接强度值比较差异无统计学意义（$P>0.05$），B1和B2组剪切粘接强度值显著低于B3组（$P<0.05$），B1和B2组间差异无统计学意义（$P>0.05$）。**结论**　ViscoStat止血剂和ViscoStat Clear止血剂对不同粘接系统下乳牙牙本质的边缘封闭性无影响。两种止血剂会明显降低自酸蚀粘接剂对乳牙牙本质的剪切粘接强度，对全酸蚀粘接剂的剪切粘接强度无影响。

【关键词】　止血剂；　乳牙牙本质；　全酸蚀粘接系统；　自酸蚀粘接系统；　氯化铝；　硫酸铁；　粘接强度；　微渗漏
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Effect of two hemostatic agents on the bonding strength of total-etch and self-etch adhesive systems in primary tooth dentin

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【Abstract】　Objective　To explore the effects of two hemostatic agents on the bonding strength of different bonding systems in primary tooth dentin. **Methods**　Seventy-two retained deciduous teeth were randomly selected. Forty-eight teeth were used to construct the microleakage model, the other 24 teeth were cut along the mesial and distal directions and 48 samples were obtained to construct the shear bond strength model. The two experiments were divided into 2 groups. Group A was the total-etch group: A1 (ViscoStat + Spectrum Bond NT); A2 (ViscoStat Clear + Spectrum Bond NT); and A3 (Non + Spectrum Bond NT); Group B was the self-etch group: B1 (ViscoStat + Single bond Universal Adhesive); B2 (ViscoStat Clear + Single bond Universal Adhesive); and B3 (Non + Single bond Universal Adhesive). Microleakage experiments and shear bond strength experiments were carried out respectively and the morphology of the fracture surface was observed by scanning electron microscopy. **Results**　There was no significant difference in microleakage among groups A1, A2, and A3 ($P>0.05$). There was no significant difference in microleakage among groups B1, B2,

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and B3 ($P > 0.05$). There was no significant difference in the shear bond strength among groups A1, A2 and A3 ($P > 0.05$). The shear bond strength of groups B1 and B2 was significantly lower than that of group B3 ($P < 0.05$). There was no significant difference between groups B1 and B2 ($P > 0.05$). **Conclusion**　ViscoStat and ViscoStat Clear had no effect on the marginal integrity of deciduous tooth dentin under the different bonding systems. The two hemostatic agents reduced the shear bonding strength of deciduous tooth dentin under the self-etch adhesive system, but had no effect on the shear bonding strength of deciduous tooth dentin under the total-etch adhesive system.

【Key words】 hemostatic agents;　primary teeth dentin;　total-etch adhesive system;　self-etch adhesive system;　aluminum chloride;　ferric sulfate;　bonding strength;　microleakage

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乳牙龋病修复治疗最常使用的材料为复合树脂，复合树脂具有美观、操作简单、粘接强度高等优点[1]。如何控制唾液、龈沟液和血液污染直接影响到复合树脂的临床粘接效果。对深达龈下的龋损，备洞过程中因牙龈出血，橡皮障无法使用或无法起到有效的隔湿作用时，常需要使用止血剂来控制血液污染，以提高临床操作效率。目前临床常用的止血剂有ViscoStat止血剂、ViscoStat Clear止血剂等，其主要成分为酸性氯化铝和硫酸铁。止血剂止血效果好，临床操作简单快捷，可明显缩短治疗时间。但在使用过程中很容易接触到牙本质粘接界面，对粘接强度产生不利影响。研究表明，止血剂可使自酸蚀粘接系统下的恒牙牙本质粘接强度显著降低，而对全酸蚀粘接系统影响不大[2]。与恒牙牙本质相比，乳牙牙本质矿化程度低，有机物含量高，化学反应性活跃，易被酸溶解。关于止血剂对乳牙牙本质粘接效果的研究报道较少，本研究探讨两种止血剂对不同粘接系统下乳牙牙本质粘接效果的影响，以期为止血剂应用于乳牙龋病修复治疗提供参考。

1　材料和方法

1.1　实验牙收集

收集哈尔滨医科大学附属第一医院儿童口腔科因乳牙滞留拔除的乳磨牙72颗，要求牙冠完整、无龋坏、无裂纹、无充填物，去除牙颈部软组织、牙石、软垢后置于4 ℃生理盐水中保存。

1.2　实验材料及设备

ViscoStat止血剂（Ultradent，美国）；ViscoStat Clear止血剂（Ultradent，美国）；Spectrum Bond NT多功能纳米粘接剂（Dentsply，美国）；Single bond Universal Adhesive粘接剂（3M ESPE，美国）；FiltekTM Z250纳米光固化复合树脂（3M ESPE，美国）；37%磷酸酸蚀剂（Heraeus，德国）；2%亚甲基蓝溶液；砂纸；指甲油等。

电热恒温水温箱（HF90，中国）；冷热循环仪（Hanon，中国）；硬组织切片机（Buehler，德国）；电子万能试验机（Instron，美国）；体视显微镜（Olympus，日本）；扫描电子显微镜（Hitachi，日本）。

1.3　实验方法

1.3.1　实验分组　本研究共分2组，A组为全酸蚀组（A1、A2、A3），B组为自酸蚀组（B1、B2、B3），见表1。

1.3.2　微渗漏实验　48颗离体乳磨牙，高速涡轮手机于𬌗面制备3 mm × 3 mm盒状洞型，洞深达牙本质层1 mm。各实验组按照表1处理方法处理窝洞后用3M250树脂充填，置于37 ℃蒸馏水中保存24 h。将所有样本置于0～5 ℃、55～60 ℃水中冷热交替循环60次，每次2 min。用流体树脂封闭根尖孔，于洞缘1 mm外均匀涂布2层指甲油，干燥后置于2%亚甲基蓝溶液中浸泡24 h，彻底冲洗吹干。用硬组织切割机沿颊舌向切开，40倍体视显微镜下观察并记录染料渗入情况。微渗漏分级标准：0级，没有染料渗入；1级，有少量染料渗入，但没超过釉牙本质界；2级，有染料渗入，且超过釉牙本质界，但没超过充填体牙本质1/2的深度；3级，有染料渗入牙本质，超过充填体牙本质1/2的深度；4级，染料全部渗入，并与对侧染料融合。

1.3.3　剪切粘接强度实验　选取24颗离体乳磨牙，用高速涡轮机磨除颊舌面牙釉质，使牙本质完全暴露。截根后用硬组织切割机沿近远中方向将牙冠分成两部分，共得48个样本。用自凝塑料包埋成9 mm × 9 mm × 9 mm的规则模型，确保牙本质完全暴露并高于自凝塑料表面0.2 mm。用600目

表1　实验分组

Table 1　Experimental groups　$n = 8$

Groups	Hemostatic agents	Adhesives	Application procedures
A1	ViscoStat (20% ferric sulfate)	Spectrum Bond NT	Apply ViscoStat for 2 min, rinse the etching agent for 1 min, bond with Spectrum Bond NT after the surface is dried
A2	ViscoStat Clear (25% aluminum chloride)	Spectrum Bond NT	Apply ViscoStat Clear for 2 min, rinse the etching agent for 1 min, bond with Spectrum Bond NT after the surface is dried
A3	–	Spectrum Bond NT	Bond with Spectrum Bond NT directly
B1	ViscoStat (20% ferric sulfate)	Single bond Universal Adhesive	Apply ViscoStat for 2 min, bond with Single bond Universal Adhesive after the surface is dried
B2	ViscoStat Clear (25% aluminum chloride)	Single bond Universal Adhesive	Apply ViscoStat Clear for 2 min, bond with Single bond Universal Adhesive after the surface is dried
B3	–	Single bond Universal Adhesive	Bond with Single bond Universal Adhesive directly

Application procedure for Spectrum Bond NT: acid etch for 15 s; pressure wash for 15 s, dry gently with a syringe; apply adhesives for 20 s repeatedly; blow gently for 5 s; light cure for 10 s. Application procedure for Single bond Universal Adhesive: apply adhesives for 20 s repeatedly; blow gently for 5 s; light cure for 10 s

碳化硅砂纸于流水下打磨牙本质面，形成粘接面。各实验组分别按照表1处理方法处理粘接面，将直径3 mm、高3 mm的圆柱形塑料模具置于粘接面上，模具与粘接面垂直，将3M250树脂充填于塑料模具内，光固化后取下模具，形成直径3 mm的树脂小柱，树脂小柱的长轴与粘接面垂直。将制备好的实验样本置于37 ℃蒸馏水中保存24 h。

用电子万能试验机测定剪切粘接强度，剪切方向平行于粘接面，加载点位置距离粘接面1 mm，加速度为1 mm/min，记录树脂小柱断裂时的最大载荷力。剪切粘接强度（shear bond strength，SBS）=树脂小柱断裂时的最大载荷力（N）/粘接面积（mm^2）。

1.3.4　扫描电子显微镜观察　将剪切粘接强度测试结束后24 h的样本干燥、喷金，扫描电子显微镜下观察断裂面形态：①牙本质内聚断裂；②树脂内聚断裂；③树脂和牙本质界面断裂；④混合断裂，内聚断裂和界面断裂均存在。以上所有实验操作、数据记录和显微镜观察均由同一人完成。

1.4　统计学分析

采用SPSS23.0软件对数据进行统计学分析。微渗漏实验等级资料采用秩和检验，剪切粘接强度的组间比较采用方差分析，组间两两比较采用LSD检验，$P < 0.05$为差异有统计学意义。

2　结　果

2.1　微渗漏实验结果

如表2所示，各组微渗漏结果差异无统计学意义（$P > 0.05$）。

表2　各组微渗漏结果比较

Table 2　Comparison of microleakage results in groups　n（%）

Groups	n	Microleakage level					Mean rank	P
		Level 0	Level 1	Level 2	Level 3	Level 4		
A1	8	0(0.00)	1(12.50)	2(25.00)	3(37.50)	2(25.00)	29.81	> 0.05
A2	8	0(0.00)	2(25.00)	2(25.00)	2(25.00)	2(25.00)	26.88	
A3	8	1(12.50)	3(37.50)	2(25.00)	1(12.50)	1(12.50)	18.69	
B1	8	0(0.00)	3(37.50)	2(25.00)	1(12.50)	2(25.00)	23.94	> 0.05
B2	8	0(0.00)	1(12.50)	2(25.00)	4(50.00)	1(12.50)	28.44	
B3	8	2(25.00)	2(25.00)	1(12.50)	2(25.00)	1(12.50)	19.25	

A1 *vs.* A2, $P = 0.682$; A1 *vs.* A3, $P = 0.107$; A2 *vs.* A3, $P = 0.223$; B1 *vs.* B2, $P = 0.540$; B1 *vs.* B3, $P = 0.415$; B2 *vs.* B3, $P = 0.157$

2.2　剪切粘接强度实验结果

各组剪切粘接强度结果如表3所示。A1、A2、A3组间比较差异均无统计学意义（$P > 0.05$）。B1和B3、B2和B3组间比较差异有统计学意义（$P < 0.05$），B1和B2组间比较差异无统计学意义（$P > 0.05$）。

2.3　扫描电子显微镜观察结果

各组扫描电镜观察结果见图1，断裂模式多为

表3　各组剪切粘接强度值比较

Table 3　Comparison of shear bond strength in groups

$\bar{x} \pm s$, MPa

Groups	n	Shear bond strength	t	P
A1	8	19.92 ± 3.79		
A2	8	20.37 ± 6.92	17.010	> 0.05
A3	8	23.17 ± 7.26		
B1	8	17.80 ± 6.24[a]		
B2	8	21.56 ± 5.35[b]	15.460	0.014
B3	8	29.17 ± 5.29[ab]		

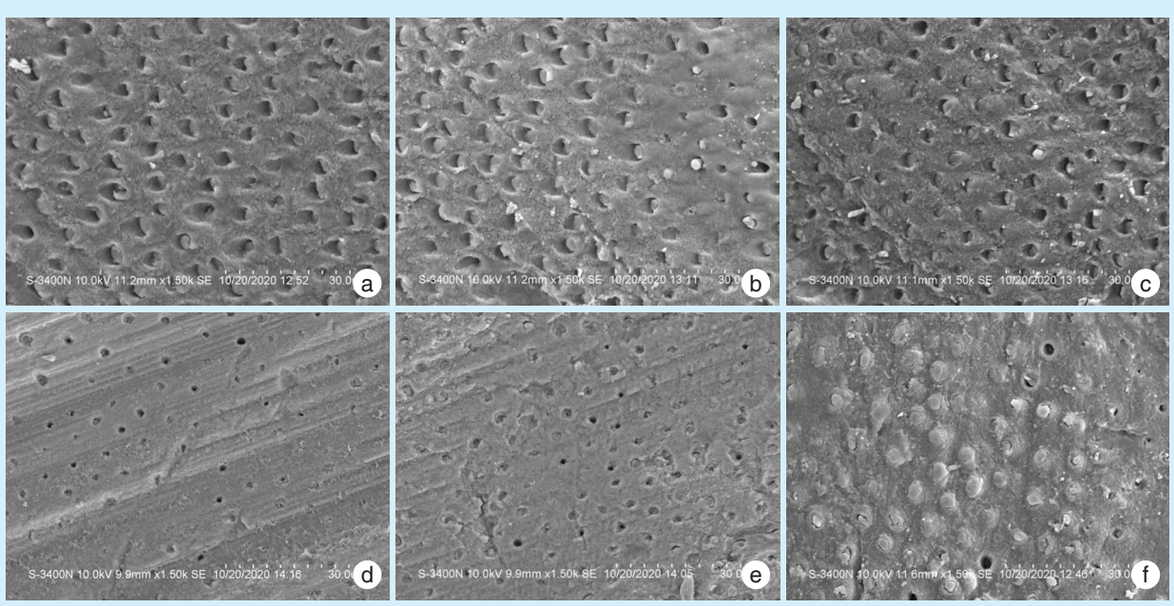
a: B1 *vs.* B3, $P < 0.001$; b: B2 *vs.* B3, $P = 0.014$

树脂和牙本质界面断裂。A1、A2、A3组树脂突断裂位置多在混合层底部，树脂突低于或平行于牙本质小管口，管间牙本质脱矿较明显，可见胶原纤维网暴露。B1、B2组多数牙本质小管空虚，树脂突较少。B3组树脂突断裂位置在混合层中上部，可见树脂突明显突出于牙本质小管口，管间牙本质脱矿明显，胶原纤维网暴露。

3　讨　论

目前口腔科常用止血剂主要为氯化铝和硫酸铁，其作用机制是与血液中蛋白质发生反应，使蛋白质沉积、血管收缩，起到止血作用。氯化铝和硫酸铁均为酸性物质，pH值为0.7～2.0，这些化合物遇水发生水解产生盐酸和硫酸，可能会酸蚀牙本质表面[3]。止血剂残留在牙本质表面的颗粒可能渗入牙本质小管中而影响混合层的结构形成。对于氯化铝类止血剂，未结合的铝在牙本质表面沉积和残留物层的形成可能是粘接强度降低的原因。与硫酸铁有关的粘接强度降低的可能解释为，硫酸铁与胶原蛋白或血浆蛋白凝结使牙本质表面结构发生改变[4]。

本研究结果显示，A1、A2、A3组剪切粘接强度值组间两两比较差异无统计学意义（$P > 0.05$），3组扫描电镜结果相近，即ViscoStat止血剂和ViscoStat Clear止血剂均不会影响全酸蚀粘接系统下的乳牙牙本质剪切粘接强度。Shadman等[5]研究表明，



The fracture mode of each group mostly occurred between the resin and dentin interface. The resin-tags of groups A1(a), A2 (b) and A3 (c) were mostly at the bottom of the mixed layer and were lower than or parallel to the dentin tubules. Most of the dentin tubules in group B1 (d) and group B2 (e) were empty, with fewer resin-tags; the fracture position of the resin-tags in group B3 (f) was in the middle and upper parts of the mixed layer; the resin-tags clearly protruded from the dentin tubules, the intertubular dentin was obviously demineralized, and the collagen fiber network was exposed. A1: ViscoStat + Spectrum Bond NT; A2: ViscoStat Clear + Spectrum Bond NT; A3: Non + Spectrum Bond NT; B1: ViscoStat + Single bond Universal Adhesive; B2: ViscoStat Clear + Single bond Universal Adhesive; B3: Non + Single bond Universal Adhesive

Figure 1　Effect of hemostatic agents on the shear bond strength of the total-etch and self -etch adhesive systems in primary tooth dentin

图1　止血剂对全酸蚀及自酸蚀粘接乳牙牙本质剪切强度的影响

ViscoStat止血剂对全酸蚀粘接系统下的恒牙牙本质粘接强度无显著影响。Khoroushi等[6]研究发现ViscoStat止血剂和ViscoStat Clear止血剂对全酸蚀粘接系统下的恒牙牙本质粘接强度均无影响。37%磷酸能够使牙本质脱矿，清除牙本质表面污染物。磷酸酸蚀足以消除止血剂对牙本质表面结构的不良影响，使粘接强度不发生改变。

B1、B2、B3组的剪切粘接强度值差异较大，B1、B2组显著低于B3组，即在自酸蚀粘接系统下，施加止血剂会显著降低乳牙牙本质的剪切粘接强度。扫描电镜结果可见B1、B2组的树脂突显著少于B3组。这与以恒牙为研究对象的研究结果相似[7-8]，学者们认为自酸蚀粘接剂的相对弱酸性不易酸蚀已被止血剂污染的更耐酸的牙本质表面，难以清除止血剂对牙本质表面的结构改变，使粘接强度降低。乳牙牙本质有机物含量较高，牙本质小管密度大，管周牙本质较厚[9]，自酸蚀粘接剂的弱酸性单体无法有效去除玷污层，止血剂对牙本质表面的污染和对粘接强度的不利影响可能更加明显。

有学者将ViscoStat止血剂和ViscoStat Clear止血剂进行比较，结果显示ViscoStat Clear止血剂对恒牙牙本质粘接强度的影响较ViscoStat止血剂小[10]。本实验中，A1和A2、B1和B2组间比较差异均无统计学意义，ViscoStat止血剂和ViscoStat Clear止血剂对乳牙牙本质的剪切粘接强度影响较一致。这种差异可能与实验所用粘接剂种类和研究对象不同有关。

本研究中止血剂对两种粘接系统的边缘封闭性无影响。Groddeck等[11]将氯化铝和硫酸铁分别用于酸蚀-冲洗粘接系统和自酸蚀粘接系统，结果表明两种止血剂对恒牙牙釉质和牙本质的边缘封闭性均无影响。有研究报道，复合树脂粘接强度下降与边缘封闭性降低之间无必然联系[12]。

本研究结果表明，ViscoStat止血剂和ViscoStat Clear止血剂对不同粘接系统下乳牙牙本质的边缘封闭性无影响。两种止血剂会明显降低自酸蚀粘接剂对乳牙牙本质的剪切粘接强度，对全酸蚀粘接剂的剪切粘接强度无影响。乳牙龋齿治疗需使用止血剂时，建议选择全酸蚀粘接系统。

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