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·综述·

粪肠球菌牙本质黏附及其影响因素的研究进展

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【摘要】 粪肠球菌是难治性根尖周炎(refractory apical periodontitis，RAP)的主要致病菌，该细菌可耐受严苛环境，引发根尖周免疫炎症反应，造成根管内外持续性感染。粪肠球菌黏附于根管牙本质壁形成生物膜，其耐药和抗冲刷能力显著增强，是介导其致病的关键因素。粪肠球菌与牙本质的黏附包括非特异性和特异性黏附，后者由黏附相关毒力因子介导，主要包括肠球菌胶原结合蛋白(adhesin of collagen from enterococci，Ace)、表面蛋白(extracellular surface protein，Esp)、明胶酶(gelatinase，GelE)和丝氨酸蛋白酶(serine prtease，SprE)、菌毛以及聚集物质，且受到多个双组份系统调控。Fsr双组份系统在群体密度增加时可以促进*gelE*及*sprE*的表达，GelE进一步减少表面肠球菌胶原结合蛋白Ace，而GrvRS双组份系统则在响应血清环境时直接下调*ace*的表达。CroRS双组份系统及WalRK双组份系统也可能分别促进和抑制包括*ace*及*gelE*在内的多种毒力因子的表达，进而影响粪肠球菌的黏附性。此外，根管机械/化学预备、根管内环境因素等均可对粪肠球菌牙本质黏附产生影响。根管治疗中避免粪肠球菌的引入和使用干扰黏附的药物可以有效预防粪肠球菌的黏附，而多种活化荡洗方法也可以有效增加根管内粪肠球菌的清除率。针对粪肠球菌牙本质黏附关键因子与调控因素为靶点设计合理药物，有望为根管感染控制提供新的思路与手段。本文就粪肠球菌与牙本质的黏附性及其影响因素进行综述。

【关键词】 难治性根尖周炎；　粪肠球菌；　生物膜；　细菌黏附；　牙本质；　影响因素；　黏附基因；　双组份系统；　Fsr双组份系统；　GrvRS双组份系统；　CroRS双组份系统；　WalRK双组份系统；　活化荡洗
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Research progress on the dentin adhesion of *Enterococcus faecalis* and its influencing factors

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【Abstract】 *Enterococcus faecalis* is the main pathogen causing refractory apical periodontitis (RAP). This bacterium can tolerate harsh environments and trigger periapical immune inflammatory responses that result in persistent infection inside and outside the root canal. Adhesion to the dentin wall of root canals and the subsequent formation of biofilms significantly enhances the drug resistance and anti-erosion ability of *Enterococcus faecalis*, which is the key factor mediating its pathogenesis. The adhesion of *Enterococcus faecalis* to dentin involves non-specific adhesion and specific adhesion, and the latter is mediated by adhesion-related virulence factors, mainly including the adhesin of collagen from en-

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terococci (Ace), extracellular surface protein (Esp), gelatinase (GelE), serine protease (SprE), endocarditis and biofilm associated pilus (Ebp) and aggregation substance (AS), which is regulated by multiple two-component systems. The two-component system Fsr can promote the expression of *gelE* and *sprE* when the cell population density increases. GelE can further reduce Ace, while the two-component system GrvRS directly downregulates ace expression in response to the serum environment. The two-component systems CroRS and WalRK may also promote and inhibit the expression of various virulence factors, including *ace* and *gelE*, thus affecting the adhesion of *Enterococcus faecalis*. In addition, the mechanochemical preparation and the internal environment of the root canal can also influence the adhesion of *Enterococcus faecalis* to dentin. Avoiding the introduction of *Enterococcus faecalis* and using adhesion-interfering medications during root canal treatment can effectively prevent the adhesion of *Enterococcus faecalis*, and a variety of activated irrigation protocols can also be effective at increasing the clearance of *Enterococcus faecalis* from the root canal. The design of rational drugs targeting key factors involved in and regulators of the adhesion of *Enterococcus faecalis* to dentin is expected to provide new ideas and strategies for root canal infection control. The present paper reviews the adhesion of *Enterococcus faecalis* to dentin and its influencing factors.

【Key words】 refractory periapical periodontitis; *Enterococcus faecalis*; biofilm; bacterial adhesion; dentin; influencing factors; adhesion-related genes; two-component system; two-component system Fsr; two-component system GrvRS; two-component system CroRS; two-component system WalRK; activated irrigation

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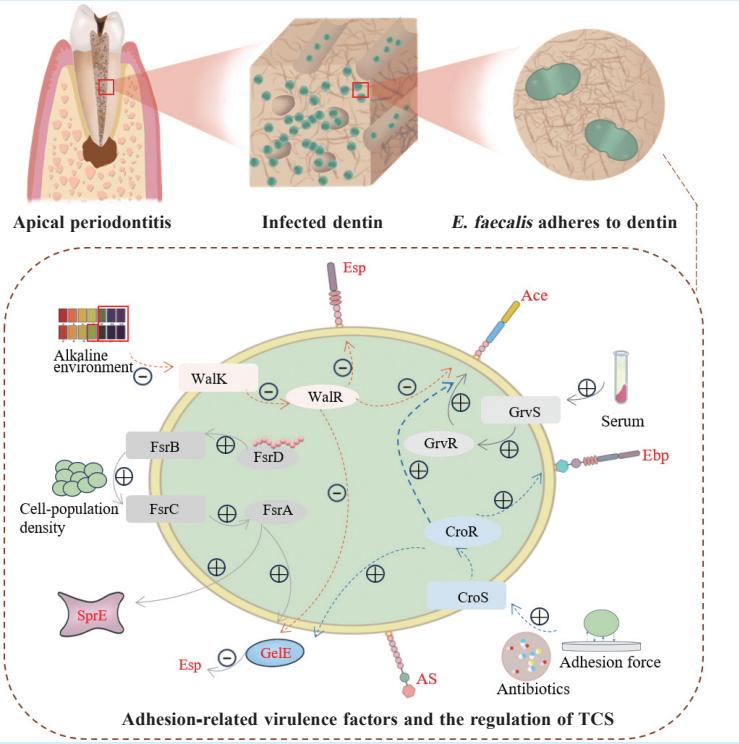
粪肠球菌为低GC含量的厚壁革兰氏阳性球菌[1]，是根管治疗失败后患牙根管中最常分离到的细菌，分离率达24%～77%，相对丰度占根管内细菌总量的1%～100%，是难治性根尖周炎的核心微生物[2-3]。粪肠球菌在外环境胁迫条件下可进入一种存活但不可培养（viable but non-culturable，VBNC）状态，后伺机复苏，进一步造成持续性感染[4]。粪肠球菌黏附于根管壁牙本质形成生物膜并侵袭牙本质小管是其导致牙齿根管系统及根尖周组织感染的关键[5-6]，而胶原是粪肠球菌与牙本质特异性结合的靶点，所以粪肠球菌更易黏附于胶原含量更多的管间牙本质。体外实验表明，粪肠球菌在根尖区的定植往往少于根中和根冠区，且更易侵袭颊舌侧的牙本质小管[7]。此外，有研究发现，体外培养21 d后粪肠球菌侵入牙本质小管的深度可达到400 μm，黏附深度可达200 μm，其中黏附于1～100 μm深度的粪肠球菌黏附性更高[3]。除牙本质外，粪肠球菌对牙胶、根管封闭剂等牙科材料也有较好的亲和力，也是再治疗患牙感染牙胶内的优势菌株[8-9]。在难治性根尖周炎患牙根管内分离的粪肠球菌中可检测到大量与细菌黏附相关的毒力因子表达，且其表达水平与临床症状密切相关，提示粪肠球菌黏附相关因子在根管感染中发挥重要作用[10]，深入研究其介导细菌黏附的分子机制及影响因素，有利于对该菌引起的难治性根尖周炎的精准防治。本文就粪肠球菌对根管牙本质的黏附机制及其影响因素进行综述。

1　粪肠球菌黏附相关毒力因子及双组份调控系统

粪肠球菌与黏附相关的毒力因子主要包括肠球菌胶原结合蛋白（adhesin of collagen from enterococci，Ace）、表面蛋白（extracellular surface protein，Esp）、心内膜炎和生物膜相关菌毛（endocarditis and biofilm associated pilus，Ebp）、聚集物质（aggregation substance，AS）、明胶酶（gelatinase，GelE）和丝氨酸蛋白酶（serine prtease，SprE）等，其介导了粪肠球菌的黏附过程，而双组份系统（two-component systems，TCS）参与了部分毒力因子的调控（图1）。

1.1　肠球菌胶原结合蛋白

肠球菌胶原结合蛋白（Ace），又称黏附素，由粪肠球菌*ace*基因编码，属于MSCRAMM（microbial surface component recognizing adhesive matrix molecules）家族，属肠球菌细胞壁锚定蛋白[4]，可黏附于细胞外基质中的各种胶原蛋白（主要是Ⅰ型和Ⅳ型胶原）[11]。*Ace*基因的缺失可显著降低粪肠球菌与牙本质的黏附性[12]，Ace以“拥抱”的方式结合牙本质胶原，通过壳聚糖处理后的牙本质可以通过位阻效应及中断疏水作用来减少粪肠球菌Ace对牙本质胶原的黏附[13]。此外，*ace*基因与粪肠球菌对根管内消毒药物（碘制剂、次氯酸钠及氢氧化钙）



"⊕" refers to promotion, "Θ" refers to inhibition, the solid arrow refers to the explicit regulation, and the dashed arrow refers to the possible regulation. Ace: adhesin of collagen from *enterococci*. Esp: extracellular surface protein. GelE: gelatinase. SprE: serine prtease. Ebp: endocarditis and biofilm associated pilus. AS: aggregation substance. TCS: two-component systems. WalK: Wal kinase. WalR: Wal regulator. Fsr A/B/C/D: *E. faecalis* sensor regulator A/B/C/D. CroS: Cro senser. CroR: Cro regulator. GrvS: Grv senser. GrvR: Grv regulator

Figure 1　Virulence factors related to dentin adhesion of *Enterococcus faecalis* and two-component regulatory systems

图1　粪肠球菌牙本质黏附相关毒力因子及双组份调控系统

的敏感性密切相关，*ace*缺失可显著提高细菌对上述消毒药物的敏感性，提示粪肠球菌可能通过表达肠球菌胶原结合蛋白使细菌表面疏水性及电荷发生变化，进而影响细菌与消毒剂间的相互作用[14]。

1.2　表面蛋白

表面蛋白（Esp）由粪肠球菌*esp*基因编码，与葡萄球菌表面蛋白Bap同源，其N端的DEv-Ig样结构域具有黏附功能，并能增强生物膜抵抗机械或降解破坏的能力，促进粪肠球菌在生物膜中的滞留，*esp*基因的存在是生物膜形成能力的重要标志，与抗菌药物耐药性相关[15-16]。Francisco等[10]研究发现，从难治性根尖周炎患牙中分离的粪肠球菌菌株*esp*基因检出率高达56%，提示其是该细菌导致根管感染的重要毒力因子。

1.3　明胶酶及丝氨酸蛋白酶

明胶酶（GelE）是一种锌金属蛋白酶，由位于染色体上的*gelE*基因编码，可以与多种毒力因子协同，在粪肠球菌生物膜形成及促进根尖周炎发展中起着重要的作用[17-18]。Rana等[19]通过体外牙本质感染模型研究发现，产GelE的粪肠球菌对牙本质的黏附性显著高于缺乏GelE的菌株，且更能耐受根管冲洗液的冲刷作用。除*gelE*基因外，位于其下游的丝氨酸蛋白酶基因*sprE*编码的丝氨酸蛋白酶（SprE）与粪肠球菌的牙本质黏附性也有着重要关系，*sprE*缺陷株对牙本质的黏附性显著低于野生菌株，且该酶耐受碱性环境的特性提高了该酶在根管内的作用效力[12]。不过目前，GelE及SprE增强粪肠球菌与牙本质黏附性的机制尚不明确。

1.4　菌毛

革兰氏阳性菌的菌毛在介导细菌定植和生物膜形成中起着重要作用。粪肠球菌心内膜炎和生物膜相关菌毛（Ebp）由*ebp*基因编码，含*ebpA*、*ebpB*和*ebpC* 3个基因，分别编码菌毛表面亚基、细胞壁锚定亚基和菌毛主体亚基，其定位独立于肽聚糖的发生，其中位点相对保守的EbpA起到重要的黏附作用，具有抗原表型，可以作为疫苗开发的潜在靶点[20-21]。*ebp*基因突变株会导致菌毛产生减少以及对阴道上皮的初始黏附降低，菌毛还与心内膜炎、尿路感染相关[22-23]。菌毛与细胞外基质的黏附性提示其在牙本质黏附中可能有一定的作用，但其在介导粪肠球菌根管内定植和感染中的具体作用未见报道。

1.5　聚集物质

粪肠球菌受体菌可分泌性信息素，诱导含有共轭质粒的供体菌表面形成聚集物质（AS），进而产生黏附和聚集，其由质粒编码，包括pAD1编码的Asa1，pPD1编码的Asp1，pAM373编码的Asm373，

以及研究较多的pCF10编码的Asc10等。pCF10上含有*prgQ*操纵子及下游的*prgA*、*prgB*（编码聚集物质）和*prgC*基因，PrgB N端含有黏附素结构域[24]，而PrgA则为细胞黏附提供了物理屏障，减少了细胞聚集[25]。AS可以结合细胞外基质蛋白，促进细菌聚集物及生物膜的形成，增加对宿主组织的附着[26-27]。不过同菌毛一样，其在介导粪肠球菌根管内定植和感染中的作用尚未见报道。

1.6　其他黏附相关毒力因子

与链球菌口腔相关黏附素高同源性的心内膜相关抗原（endocardial fibroelastosis-associated antigen A，efaA）在牙髓治疗失败患牙根管内检出率高，以*efaA*基因为靶点的肽核酸可以显著抑制粪肠球菌生物膜的形成，被认为是粪肠球菌黏附及生物膜相关毒力因子之一[10, 28]。Korir等[29]发现粪肠球菌*epaQ*基因突变株表面疏水性增加，生物膜形成过程中初始黏附降低，该基因在粪肠球菌初始黏附中可能发挥了重要的作用。Ladjouzi等[30]构建了多核苷酸磷酸化酶PNPase的突变粪肠球菌菌株，并与野生株进行转录组学分析，在生长3 h和6 h后，发现众多参与细胞壁合成、黏附、生物膜形成的基因差异性表达，提示其参与了粪肠球菌的黏附过程。

1.7　粪肠球菌双组份调控系统

粪肠球菌双组份系统（TCS）是响应外环境调控转录的重要信号通路，通常由识别特定环境信号的传感器组氨酸激酶（histidine kinase）和介导基因表达反应的反应调节因子（response regulator）组成[31]。研究发现，TCS在粪肠球菌黏附相关毒力因子的表达调控中发挥了重要作用。

粪肠球菌fsr（*E. faecalis* sensor regulator）双组份系统为群体感应系统，能通过细胞密度来调节群体行为，起到环境监测作用，其编码基因位于*gelE*（明胶酶）和*sprE*（丝氨酸蛋白酶）基因上游，编码FsrC感觉激酶、FsrA反应调节因子、FsrB及FsrD衍生的自身诱导物，可调节下游GelE和SrpE的表达，进一步调节粪肠球菌的初始黏附[32]。在转录后水平，GelE可以裂解菌体表面的Ace蛋白，fsr双组份系统通过影响细菌*gelE*的表达，减少菌体表面Ace的量，进而降低粪肠球菌的黏附[11]。Fsr双组份系统不仅是GelE和SrpE的激活剂，对其他生物膜相关因子以及生物膜基质如eDNA及胞外多糖等也起到了重要的调节作用[33]。此外，双组份系统LuxS也属于群体感应系统，可调节粪肠球菌生物膜形成和细菌的表面疏水性，该系统在fsr双组份系统缺乏的粪肠球菌中起到了重要作用[34]。

GrvRS双组份系统也参与调控了不同应激条件下*ace*的表达，*grvR*突变株*ace*表达显著下调，而该双组份系统在响应血清环境时，*ace*表达上调[35]。有研究表明，粪肠球菌黏附力的增加以及抗生素的存在可以激活CroRS双组份系统，并可能通过增加黏附与生物膜形成相关基因*gelE*、*ebpA*及*ace*的表达，进而增加其抗菌性[36]。此外，WalRK双组份系统作为粪肠球菌唯一必须的双组份系统，在碱性胁迫条件下，WalRK双组份系统下调，*ace*、*gelE*、*esp*基因表达也下调，提示该系统也可能参与碱性胁迫条件下黏附相关因子的调节[31, 37]。

上述相关毒力因子介导了粪肠球菌对牙本质的黏附，从而进一步发挥致病作用，这些毒力因子还能够进行水平基因转移，实现种内和种间的传播，增加了防治其感染的难度[25, 38]。

2　影响粪肠球菌与牙本质黏附的因素

2.1　根管机械/化学预备

粪肠球菌可黏附于牙本质内壁，而根管机械预备可以改变根管内壁微观形态，从而进一步影响粪肠球菌的黏附。Xu等[39]研究发现根管机械预备后的牙本质表面粗糙度更小，表面接触角更大，表面附着菌量反而更多，基于此研究，作者认为临床上应加大化学预备来降低残留菌的致病性。

化学预备能够改变根管内壁粗糙度、润湿性及表面成分，进一步改变粪肠球菌对牙本质的黏附。金属离子螯合剂EDTA和次氯酸钠能够分别暴露和溶解胶原纤维，从而分别增加和降低粪肠球菌对牙本质的黏附，沈晓霞等和Tartari等[40-41]的研究验证了上述结论。目前，次氯酸钠配伍EDTA作为最为经典的冲洗方案被广泛使用，但是此种配伍可能造成牙本质微观结构改变，如牙本质侵蚀及粗糙度变化，有研究发现“原花青素（proanthocyanidin，PA）+羧甲基壳聚糖/无定形磷酸钙（carboxymethyl chitosan/amorphous calcium phosphate，CMC/ACP）纳米配合物”在保证良好的根管内消毒作用外，还可以维持牙本质有机-无机结构完整性[42]。而目前研究较多的“连续螯合冲洗策略”，即次氯酸钠与较为温和的螯合物如依替膦酸配伍也能够避免根管冲洗液对牙本质的侵蚀[43]。此外，Titato等[44]研究发现EDTA与苯扎氯铵配伍可以干扰粪肠球菌对牙本质的黏附，避免二次生物

膜的形成，有利于根管治疗的远期成功率。Bayatipour等[45]研究发现，在亚抑菌浓度下的氯己定、过氧化氢溶液及其组合均能够上调AS及esp的表达，对粪肠球菌形成了选择性压力。随着根管冲洗液的不断发展，新型抗菌剂如抗菌肽因其具有抗菌浓度低、辅助生物活性等特点被广泛研究，其中，抗菌肽GH12在体外对粪肠球菌有较强的抗菌活性，并且抑制了黏附相关毒力因子*efaA*、*esp*、*gelE*及部分应激蛋白的表达，具有较强的黏附抑制及抗生物膜潜力[46]。

此外，经合适功能的半导体激光照射后，粪肠球菌参与黏附及生物膜形成的基因*gelE*、*ace*、*esp*显著下调，并抑制了细胞外多糖的合成，从而降低了粪肠球菌与牙本质的黏附性[47]。抗菌光动力疗法有较强的杀灭粪肠球菌的作用，包括根管内分离的临床株，但是却显著上调了残留浮游菌黏附相关毒力因子表达，包括群体感应系统*fsr*及其下游黏附相关毒力因子*gelE*[48]，但是当光动力疗法作用于粪肠球菌体外生物膜后，与黏附相关的毒力因子*efaA*、*esp*、*gelE*和fsr表达水平却显著降低[49]。

2.2　根管内环境

Ran等[50]发现粪肠球菌在碱性及饥饿环境下对牙本质小管的侵袭能力显著降低，与黏附相关的基因*ace*、*esp*、*gelE*、*frsB*显著下调，但在部分碱性及饥饿条件下可能首先表现出黏附性增加，然后再降低。学者研究发现，持续性根尖周炎患牙中分离的粪肠球菌菌株在葡萄糖缺乏条件下*ace*、*efaA*和*fsrB*基因转录水平均上调，而*gelE*和*esp*基因的转录水平在不同的临床分离株中存在差异[51]。而在血清和唾液环境下，牙本质块黏附了更多的粪肠球菌[52]。影响粪肠球菌牙本质黏附的外部因素及其机制总结见表1。

3　粪肠球菌黏附于牙本质的预防及处理方法

研究发现，根管治疗失败患牙内粪肠球菌与患者唾液内粪肠球菌存在遗传相关性，提示根管内感染的来源可能是唾液中粪肠球菌[53]，建议使用橡皮障隔离来避免引入粪肠球菌。此外，干扰

表1　影响粪肠球菌牙本质黏附的外部因素及其机制

Table 1　External factors influencing the adhesion of *Enterococcus faecalis* to dentin and the underlying mechanisms

Influencing factor		Adhesion	Mechanism	Reference
Mechanical preparation	Ni-Ti instrument	↑	Reduces roughness and increases the contact angle	[39]
Chemical preparation	NaClO	↓	Dissolves collagen	[40]
	EDTA	↑	Exposes collagen	[41]
	Proanthocyanidin (PA)+carboxymethyl chitosan/amorphous calcium phosphate (CMC/ACP) nanocomplexes	—	Maintains the original microstructure of dentin	[42]
	NaClO + etidronate	—	Avoids excessive collagen dissolution and erosion of dentin	[43]
	EDTA + Benzalkonium chloride	↓	Interferes in the adhesion of *E. faecalis* to dentin	[44]
	CHX/H_2O_2	—	Up-regulation of *esp* and AS	[45]
	Antimicrobial peptide GH12	↓	Down-regulation of *esp*, *gelE* and *efaA*	[46]
	Diode laser	↓	Down-regulation of *ace*, *esp* and *gelE*	[47]
	Antimicrobial photodynamic inactivation	—	Plankton: up-regulation of *gelE* and *fsr* Biofilm: down-regulation of *esp*, *gelE*, *efaA* and *fsr*	[48][49]
Root canal environment	Alkaline and energy-starvation condition	↓	Down-regulation of *ace*, *esp*, *gelE* and *frsB*	[50]
	Glucosedeprivation condition		Up-regulation of ace, *efaA* and *fsrB*	[51]
		—	Affects the expression of *esp* and *gelE* (This varies among different clinical strains)	
	Serum and saliva	↑	Provides better adhesion conditions	[52]

"↑" indicates increased adhesion, "↓" indicates decreased adhesion,"—" indicates that this research has not been conducted. Ni-Ti: nickel-titanium. NaClO: sodium hypochlorite. EDTA: ethylenediamine tetraacetic acid. CHX: chlorhexidine. esp: extracellular surface protein. AS: aggregation substance. *gelE*: gelatinase. *efaA*: endocardial fibroelastosis-associated antigen A. *ace*: adhesin of collagen from enterococci. *fsr*: *E. faecalis* sensor regulator. *fsrB*: *E. faecalis* sensor regulator B

粪肠球菌牙本质黏附的相关冲洗液也可以预防根管内粪肠球菌的二次黏附，如前文所述EDTA与苯扎氯铵配伍、抗菌肽GH12等，而使用石墨烯等材料对牙本质表面进行改性后，粪肠球菌也较难进行二次黏附[54]。

由于根管系统的复杂性，附着在根管峡部、侧支根管以及根尖分歧等结构中的粪肠球菌往往难以彻底清除，粪肠球菌还能够定植于牙本质小管内部，有研究发现，再治疗患牙在经过常规根管预备和封药后根管内仍能够检出粪肠球菌[55]，所以常规的机械化学预备很难实现根管内感染的彻底清除。而辅助根管荡洗方法可以通过活化冲洗液、增加冲洗液流速等方式进一步清除牢牢黏附于牙本质的生物膜。研究表明，超声活化荡洗可以增加冲洗液的作用深度，进而实现粪肠球菌的“深度清洁”[56]。而相比于单纯使用次氯酸钠溶液冲洗，配合使用声波激活或者光动力作用的次氯酸钠溶液也能更有效地减少根管内细菌负荷[57-58]。临床新型的激光活化荡洗技术也可以通过空泡内爆和冲击波产生高速流体运动来增加冲洗液与牙本质内壁的剪切力，进而增加根管内细菌的清除率[59]。然而，目前尚没有一种冲洗方法能够将构建的离体牙粪肠球菌感染模型内的粪肠球菌完全去除，体现出粪肠球菌在根管内黏附的持久性，所以，需要研究新的冲洗液及活化荡洗方法来进一步控制根管内粪肠球菌的黏附和感染。

4　总结及展望

综上所述，粪肠球菌与黏附相关的毒力因子在介导其与牙本质黏附和根管感染中发挥了重要作用，针对上述因子开发合理药物或者疫苗对根管感染控制有重要意义。尽管目前已发现众多与粪肠球菌黏附相关的毒力因子，但各因子在介导细菌牙本质黏附中的作用与具体机制尚不清楚，常见根管化学预备手段对粪肠球菌黏附相关毒力因子影响的分子机制也有待进一步研究。而根管的宏观形态、常见根管消毒药物及根充糊剂等对粪肠球菌黏附性的影响相关研究也可以为临床决策提供重要的参考意义，也期待开发更多有效的药物和临床新技术来预防和处理根管内粪肠球菌的黏附。此外，从根管治疗失败患牙根管及根尖周病损组织中分离、鉴定、保藏粪肠球菌临床菌株，建立该菌临床菌株生物样本库，借助泛基因组学与转录组、代谢组等多组学联合研究，深入解析粪肠球菌牙本质黏附与致病的分子机制，有望为难治性根尖周炎的临床防治提供新的思路与途径。

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