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·基础研究·

根管冲洗药物对动态氧条件下口腔混合菌群生物膜的杀菌效果及菌群结构的影响

师保江[1]，　杨子[2]，　苏征[2]
1.首都医科大学附属北京口腔医院牙体牙髓科，北京（100070）；　2.首都医科大学附属北京口腔医院国际医疗部及保健中心，北京（100070）

【摘要】　目的　探讨目前临床常用根管冲洗药物对动态氧条件下口腔混合菌群生物膜的杀菌效率及菌群结构的影响，为临床根管冲洗药物选择提供实验依据。**方法**　本研究已通过单位医学伦理委员会审查批准，并获得研究参与者的书面知情同意。选取于首都医科大学附属北京口腔医院就诊的2名口腔健康志愿者，提取其下颌的前磨牙及磨牙龈上及龈下菌斑，以构建口腔混合菌生物膜模型。在羟基磷灰石片上分别培养1周（厌氧条件培养3 d+有氧条件培养4 d）和3周（厌氧条件培养3 d+有氧条件培养18 d），以构建年轻和成熟的口腔混合菌型生物膜。采用无菌水（对照组）、2% 氯己定（CHX组）及1% 次氯酸钠（NaOCl组）处理分别生物膜3 min，通过活/死细菌染色结合激光共聚焦显微镜观察并计算死菌比例；利用16S rRNA高通量测序分析菌群结构，重点评估冲洗药物对感染根管内常见优势菌的抗菌效果。**结果**　3种根管冲洗药物对于1周和3周生物膜，死菌比例从高到低均为：1% NaOCl > 2% CHX > 对照组（$P < 0.001$）；而1% NaOCl对3周成熟生物膜的杀菌率显著低于1周年轻生物膜（$P < 0.001$）。高通量测序结果显示2% CHX及1% NaOCl对生物膜群落结构影响较小，但对其中根管内优势菌属，例如链球菌属及梭菌属杀菌作用较差，处理后其相对丰度未见降低甚至略有升高。**结论**　在前期厌氧、后期有氧的培养环境下，1% NaOCl冲洗对年轻细菌生物膜的杀菌作用较强，但对成熟生物膜及部分根管优势菌（如链球菌属、梭杆菌属）的效果有限。

【关键词】　根管；　细菌生物膜；　根管冲洗药物；　厌氧培养；　有氧培养；　杀菌率；　高通量测序；　群落结构
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Influence of root canal irrigants on the antimicrobial efficacy and bacterial communities of oral mixed biofilms under dynamic oxygen conditions

SHI Baojiang[1], YANG Zi[2], SU Zheng[2]. 1. Department of Operative Dentistry and Endodontics, Beijing Stomatological Hospital, Capital Medical University, 100070, Beijing; 2. International Medical Services and Healthcare Center, Beijing Stomatological Hospital, Capital Medical University, 100070, Beijing
Corresponding author: SU Zheng, Email: endosuzheng@163.com

【Abstract】　Objective　To investigate the bactericidal efficacy and impact on oral mixed biofilm microbial structure of commonly used clinical root canal irrigants under dynamic oxygen conditions, and to provide experimental evidence for clinical root canal irrigant selection. **Methods**　This study has been reviewed and approved by the Medical Ethics Committee, and written informed consent has been obtained from all of the participants. Two healthy oral volunteers from Beijing Stomatological Hospital, Capital Medical University were selected, and subgingival and supragingival

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【作者简介】师保江，副主任医师，硕士研究生，Email：13801201212@qq.com
【通信作者】苏征，主任医师，博士研究生，Email：endosuzheng@163.com

plaque from their mandibular premolars and molars were extracted to construct an oral mixed microbial biofilm model. The plaque was cultivated on hydroxyapatite slices for 1 week (anaerobic conditions for 3 days + aerobic conditions for 4 days) to construct a young oral mixed bacterial biofilm model, and cultivated for 3 weeks (anaerobic conditions for 3 days + aerobic conditions for 18 days) to construct a mature oral mixed bacterial biofilm model. Three root canal irrigating solutions were used: sterile water (control), 2% chlorhexidine (CHX), and 1% sodium hypochlorite (NaOCl). The bacterial biofilms were treated for 1 week and 3 weeks, respectively, and the overall bactericidal rate was assessed using live/dead bacterial staining and laser confocal microscopy. Concurrently, high-throughput 16S rRNA sequencing was performed to analyze the microbial community structure and to evaluate the antibacterial effects of irrigation solution, on common dominant pathogens. **Results** The proportion of dead bacteria in the biofilm treated with three types of root canal irrigation drugs at 1 week and 3 weeks, from high to low, were as follows: 1% NaOCl >2% CHX >control group ($P < 0.001$). The bactericidal rate of 1% NaOCl on mature biofilms at 3 weeks was significantly lower than that of light biofilms at 1 year ($P < 0.001$). High-throughput sequencing revealed minimal effects of 2% CHX and 1% NaOCl on biofilm community structure but demonstrated poor bactericidal activity against dominant root canal pathogens, such as *Streptococcus* and *Fusobacterium*. **Conclusion** Under the initial anaerobic and subsequent aerobic culture conditions, 1% NaOCl exhibits potent bactericidal effects on young bacterial biofilms, but demonstrates limited efficacy against mature biofilms and certain root canal dominant bacteria (*Streptococcus* and *Fusobacterium*).

【Key words】 root canal; microbial biofilm; root canal irrigating solution; anaerobic culture; aerobic culture; antimicrobial efficacy; high-throughput sequencing; bacterial communities

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持续性根尖周炎是指患牙经过反复根管治疗后根尖周的感染仍难以控制，病变不能愈合，是长期以来困扰临床医师的疑难问题，引起持续性根尖周炎的主要病因是根管系统内外细菌生物膜的存在[1-3]。细菌生物膜可增强细菌的致病性与耐药性[4-6]。根管不同区域氧气浓度差异显著，并随感染进程动态变化，影响菌群构成与多样性[7]。然而，现有体外研究多采用单一厌氧条件下的单菌种培养模式[8-10]，难以模拟临床微生态环境。因此，建立氧动态变化的混合菌生物膜模型对评估根管冲洗药物的抗菌效应至关重要。根管冲洗剂次氯酸钠（sodium hypochlorite，NaOCl）和氯己定（chlorhexidine，CHX）具有良好的抗菌性能[11-12]，但当前研究聚焦于其对生物膜的杀菌率[11，13-14]，对菌群结构的影响报道相对较少。为此，本研究构建前期厌氧、后期有氧的口腔混合菌生物膜模型，采用1% NaOCl和2% CHX分别处理年轻及成熟生物膜，评估杀菌效率并利用高通量测序分析菌群结构变化，以期为牙髓根尖周病的根管治疗中根管冲洗药物的选择提供实验依据。

1　材料和方法

1.1　材料和设备

羟基磷灰石片（hydroxyapatite，HAP，生物材料工程研究中心，四川大学）；无菌过滤器（22 μm，Merck，德国）；1% NaOCl冲洗液（郎力生物，中国）；2% CHX冲洗液（朗力生物，中国）；脑心浸液琼脂（brain heart infusion agar，BHI）；固体培养基（Thermo Fisher，美国）；BHI液体培养基（Thermo Fisher，美国）；密封培养罐（三菱，日本）；厌氧产气袋（三菱，日本）；DNA QIAamp DNA Mini Kit（Qiagen，GmbH，Germany）；LIVE/DEAD BacLight™ Bacterial Viability Kit（Thermo Fisher，美国）；TruSeq™ DNA Sample Prep Kit试剂盒（Illumina公司，美国）；激光共聚焦显微镜（LSM910，蔡司，德国）；多功能酶标仪（SpectraMax Paradigm，Molecules Device Company，美国）。

1.2　细菌培养

本研究实施严格遵守《赫尔辛基宣言》，已通过首都医科大学附属北京口腔医院伦理委员会审查（审批号：CMUSH-IRB-KJ-PJ-2024-79），并已获得研究参与者的书面知情同意。选取首都医科大学附属北京口腔医院就诊的2名口腔健康志愿者。纳入标准：①年龄20~40岁，性别不限；②无牙龈炎、牙周炎等牙周疾病；③龋失补指数＜2。排除标准：①严重全身系统性疾病者；②精神障碍者及智力障碍者；③处于妊娠期或哺乳期者；④3个月内有抗生素摄入史者；⑤皮肤或其他器官的急性

炎症病变者。最终纳入研究参与者男性1名，年龄25岁以及女性1名，年龄30岁。对源自2名研究参与者的牙菌斑分别进行如下相同的处理，使用无菌牙签刮取研究参与者下颌前磨牙及磨牙龈上及龈下菌斑，立即置于BHI液体培养基内制成菌悬液，进行37 ℃厌氧培养。培养后利用BiTek多功能酶标仪将菌液浓度调整为3×10^7菌落形成单位（colony-forming unit，CFU）/ mL，使用BHI液体培养基1∶10稀释菌液备用。

1.3　细菌生物膜模型建立

选取直径10 mm厚度2 mm的羟基磷灰石片分别作为2名研究参与者口腔混合菌液细菌生物膜载体，使用超纯水对羟基磷灰石片进行超声清洗，随后进行高温高压消毒。无菌羟基磷灰石片浸泡于2 mL浓度为10 mg / mL鼠尾Ⅰ型胶原（353236，BDFalcon，美国）溶液中，4 ℃冷藏过夜。随后将胶原包被羟基磷灰石片放置于无菌24孔板内，每孔内加入0.2 mL菌悬液（3×10^6 CFU / mL）及1.8 mL BHI液体培养基，保证培养基覆盖羟基磷灰石片表面。细菌生物膜于37 ℃条件下采用厌氧产气袋内培养3 d，后为大气氧浓度下有氧培养4 d或18 d，每2~3 d使用2 mL BHI液体培养基进行换液，1周及3周后可见羟基磷灰石片向上侧可形成较厚的细菌生物膜[5]。

1.4　样本处理及活死细菌染色

采用无菌PBS清洗羟基磷灰石片去除其表面浮游细菌，随后按照不同冲洗液处理将载有2名研究参与者口腔细菌生物膜的羟基磷灰石片分为3组，分别为对照组、2% CHX组及1% NaOCl组，分别浸泡于无菌水（使用无菌过滤器过滤的双蒸水）（n=5）、2% CHX冲洗液（n=5）及1% NaOCl冲洗液（n=5）中3 min。处理后采用活死细菌染色试剂盒，采用SYTO 9及碘化丙啶染色剂进行细菌活死染色。采用激光共聚焦显微镜扫描每个羟基磷灰石片上细菌生物膜的5个随机区域，计算活死细菌比例。

1.5　样本DNA提取及聚合酶链式反应（polymerase chain reaction，PCR）扩增

2名研究参与者的龈上及龈下混合菌分别培养3周（3 d厌氧+18 d有氧）后形成的细菌生物膜，各自随机分为对照组、2% CHX组及1% NaOCl组，分别浸泡于无菌水（使用无菌过滤器过滤的双蒸水）、2% CHX冲洗液及1% NaOCl冲洗液中3 min，随后刮取羟基磷灰石片上细菌生物膜，进行涡旋振荡，12 000 r / min离心5 min后弃上清，依据试剂盒说明书使用DNA QIAamp DNA Mini Kit试剂盒（Qiagen，GmbH，Germany）提取样本细菌DNA，并测定DNA浓度。以样本稀释基因组DNA为模板，扩增区域为细菌16S rRNA基因V3-V4区域，进行PCR，并依照PCR产物浓度进行等量混样。琼脂糖凝胶电泳对PCR产物进行检查，并对目的条带进行回收。

1.6　建库及测序

参照电泳初步定量结果，对PCR产物进行定量。利用TruSeq DNA Sample Prep Kit试剂盒（Illumina公司，美国）进行建库及定量，文库合格后采用Illumina Miseq PE250平台进行高通量测序。

1.7　数据分析及可视化展示

利用I-Sanger网络云平台及R软件（The R Project for Statistical Computing）对测序结果进行统计分析以及可视化展示。群落结构分析利用Usearch平台对97%相似水平下的操作分类单元（operational taxonomic units，OTU）聚类进行生物信息统计分析。对OTU代表序列进行物种注释，与人类口腔微生物组数据库（human oralmicrobiome database，HOMD）进行对比，在菌门及菌属层面统计各样本的细菌群落组成。对OTU代表序列进行物种注释在菌门及菌属层面统计各样本的细菌群落组成。

1.8　统计学分析

使用SPSS 22.0（SPSS GmbH，Munich，Germany）进行统计分析。数据以均值 ± 标准差表示。数据分布采用Shapiro-Wilk检验，方差齐性采用Levene检验。对于符合正态分布和方差齐性的数据，两组间比较采用student t检验，多组组间比较采用单因素方差分析，然后进行Tukey事后检验，$P<0.05$被认为差异具有统计学意义。

2　结　果

2.1　总体杀菌率

3种根管冲洗药物对于1周和3周生物膜，死菌比例从高到低均为：1% NaOCl＞2% CHX＞对照组（$P<0.001$）；而1% NaOCl对3周成熟生物膜的杀菌率显著低于1周年轻生物膜（$P<0.001$）。见表1。

2.2　细菌生物膜培养3周后微生物群落结构分析

2名研究参与者的龈上及龈下混合菌体外分别培养3周（3 d厌氧及18 d有氧）后，进行无菌水、2% CHX及1% NaOCl处理，随后通过高通量测序

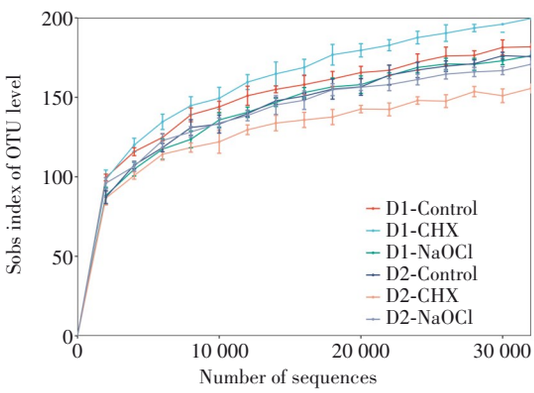
表1　不同根管冲洗药物对2名研究参与者龈上及龈下混合菌群在动态氧培养不同时间形成的生物膜的杀菌率比较

Table 1　Comparison of bactericidal rates of different root canal irrigation drugs on biofilms formed by mixed bacterial communities from supra and subgingival plaque samples of two participants under dynamic oxygen culture at different times　$\bar{x}\pm s$

Group	Proportion of dead bacteria		t	P
	1 week	3 weeks		
Control	6.2% ± 1.2%^{A}	7.9% ± 1.5%^{A}	2.215	0.051
2% CHX	37.9% ± 3.2%^{B}	28.8% ± 2.6%^{B}	5.378	< 0.001
1% NaOCl	91.3% ± 1.8%^{C}	38.4% ± 3.6%^{C}	31.79	< 0.001
F	2 162	200.1		
P	< 0.001	< 0.001		

The data of the two participants were merged and analyzed, n = 10 biologically independent samples. The biofilm was treated with sterile water (control), 2% chlorhexidine (CHX), and 1% sodium hypochlorite (NaOCl) for 3 minutes. Different capital letters within the same column indicate significant differences between the irrigation solutions (P < 0.05)

进行微生物群落结构分析。高通量测序各样本稀释度曲线可见各样本稀释度曲线趋于平坦，说明本次测序数据量可反映样品中绝大多数的微生物物种信息（图1）。所有组中共检出251个OTU，每个样本平均检出190.5个OTU。



D1: participant 1; D2: participant 2. The biofilm was treated with sterile water (control), 2% chlorhexidine (CHX), and 1% sodium hypochlorite (NaOCl) for 3 minutes

Figure 1　High-throughput sequencing sample rarefaction curve

图1　高通量测序各样本稀释度曲线

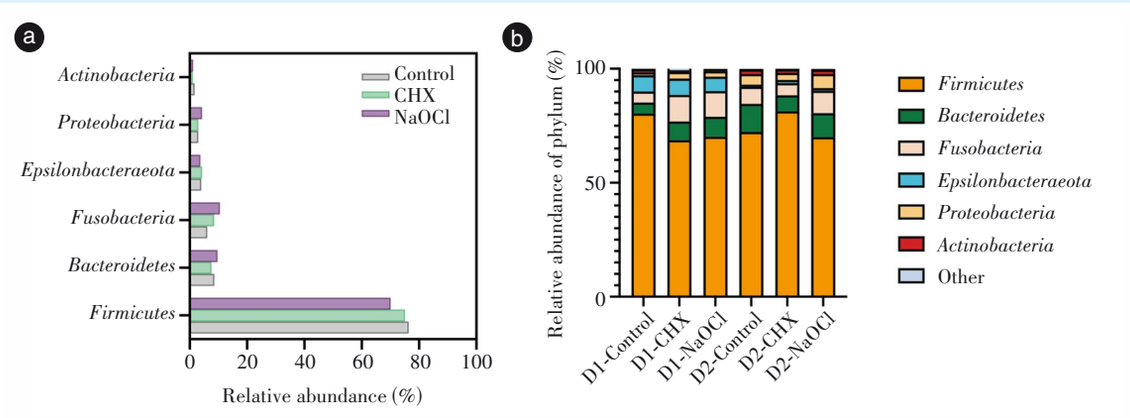
从菌门水平共检出9个菌门，按照丰度从高到低排序前6位为厚壁菌门、拟杆菌门、梭杆菌门、弯曲菌门、变形菌门及放线菌门，占总体比例的99.7%。厚壁菌门在对照组、2% CHX组及1% NaOCl组均为丰度最高的菌门，相对丰度分别为76.4%、75.1%及70.1%，不同冲洗液处理后细菌的菌门结构未见明显差异（图2）。

所有样本共检出56个菌属，按照丰度从高到低排序前10位为韦荣菌、*[Eubacterium] yurii group*、微单胞菌、链球菌、梭杆菌、小杆菌、颗粒链球菌、月形单胞菌3、弯曲杆菌、普氏菌2，总占比达83.8%（图3）。

如图4所示，针对对照组、CHX及NaOCl组进行菌属水平分析，发现3组内菌属分布未见明显差异。2名研究参与者细菌生物膜菌群结构冲洗液处理后具有相同趋势，使用CHX及NaOCl后可见*[Eubacterium] yurii group*菌属的相对丰度均减小。链球菌作为根管感染的优势菌，在2名研究参与者细菌生物膜培养3周后占比分别为5.05%及3.46%，使用CHX处理后相对丰度为7.81%及7.84%，使用NaOCl后则为10.77%及8.80%。梭杆菌属具有相似的趋势，对照组分别为3.80%及5.92%，CHX处理后为11.10%及4.17%，NaOCl处理后则为10.17%及8.08%。微单胞菌的相对丰度在3组间无明显差异。菌群结构分析提示2% CHX及1% NaOCl对生物膜群落结构影响较小，但对其中根管内优势菌属，例如链球菌及梭杆菌杀菌作用较差。

3　讨　论

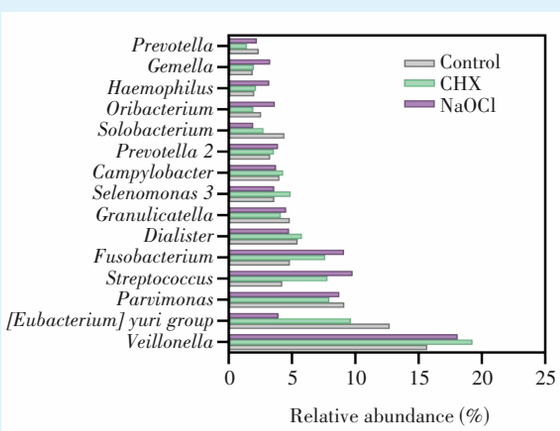
根管系统感染的核心特征在于形成结构复杂、菌种多样、耐药性强的混合菌群生物膜[3-4, 6, 9, 15]，这些细菌生物膜并非处于静态的厌氧环境，其氧气张力随感染进程和解剖位置动态变化。在感染早期或近根管口区域，残存的微量氧气有利于兼性厌氧菌的定植；随着感染进展和向根尖区深入，微环境逐渐转变为严格厌氧状态，促进卟啉单胞菌、具核梭杆菌等严格厌氧菌的定植[7]；此外，髓腔的开放亦可使局部环境由厌氧转变为有氧，这



D1: participant 1; D2: participant 2. a: the relative abundance distribution of bacterial phyla within different groups; b: the relative abundance distribution of bacterial phyla across samples. The biofilm was treated with sterile water (control), 2% chlorhexidine (CHX), and 1% sodium hypochlorite (NaOCl) for 3 minutes

Figure 2　The effect of different root canal irrigants on the phylum distribution of biofilms formed by mixed bacterial communities from supra and subgingival plaque samples of two participants under dynamic oxygen culture at different times

图2　不同根管冲洗药物对2名研究参与者龈上及龈下混合菌群在动态氧培养不同时间形成的生物膜的菌门分布的影响



The data of two participants were merged and analyzed. The biofilm was treated with sterile water (control), 2% chlorhexidine (CHX), and 1% sodium hypochlorite (NaOCl) for 3 minutes

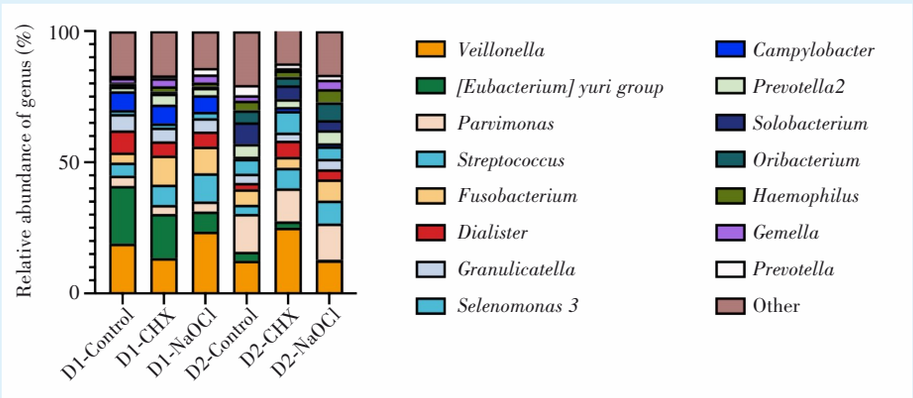
Figure 3　The effect of different root canal irrigants on the relative abundance distribution of dominant bacterial genera in biofilms formed by mixed bacterial communities from supra and subgingival plaque samples of two participants under dynamic oxygen culture at different times

图3　不同根管冲洗药物对2名研究与者龈上及龈下混合菌群在动态氧培养不同时间形成的生物膜的优势菌属相对丰度分布的影响

种动态的氧气梯度是决定根管微生物生态的关键决定因素。因此，在体外研究中构建模拟根管氧动态变化的微环境至关重要。本研究设定厌氧培养3 d到后续有氧培养的环境，初步模拟初期根管从初期封闭感染到后期可能开放状态下的根管细菌生物膜结构变化。

以往体外研究多采用粪肠球菌等单菌种生物膜模型[8-9]，但临床根管感染被证实为多种细菌参与的混合感染[7, 16-19]。相较于单菌种生物膜，多菌种生物膜在代谢活性、毒力及耐药性等方面均显著增强[5]。为模拟临床感染状态，目前常通过以下途径构建口腔多菌种生物膜模型，包括直接采集感染根管内样本[20]、获取患者龈上及龈下菌斑[13, 21-23]或在患者的口腔内置入装置以促进细菌生物膜形成[24]。此类模型所包含的细菌种类及其相互作用与天然牙感染根管更为接近，目前已逐渐成为根管微生物学研究中的主流建模策略[25-27]。因健康人群口腔龈下菌斑的总体差异较小，本研究选取2名口腔健康志愿者进行菌斑采集。本实验通过菌群结构分析发现，龈上及龈下菌斑前期厌氧+后期有氧培养后，体外混合生物膜的优势菌包括韦容菌、*[Eubacterium] yurii group*、微单胞菌、链球菌、梭杆菌、小杆菌及颗粒链球菌，与以往研究报道根管系统持续性感染优势菌属相似[7, 18-19, 28]。

细菌生物膜的培养时间是影响其成熟度的重要因素。培养数小时至数天的年轻细菌生物膜，在厚度、结构复杂性、耐药性以及清除难度等方面，均远不及培养时间更长的成熟细菌生物膜[5]。



D1: participant 1; D2: participant 2. The biofilm was treated with sterile water (control), 2% chlorhexidine (CHX), and 1% sodium hypochlorite (NaOCl) for 3 minutes

Figure 4　The effect of different root canal irrigants on the relative abundance distribution of bacterial genera in biofilms formed by mixed bacterial communities from supra and subgingival plaque samples of two participants during dynamic oxygen culture at different times

图4　不同根管冲洗药物对2名研究参与者龈上及龈下混合菌群在动态氧培养不同时间形成的生物膜的菌属相对丰度分布的影响

既往研究多选用培养1周及3周作为年轻及成熟细菌生物膜的时间节点[14, 27, 29-30]。因此,本研究选取研究参与者下颌龈上/龈下菌斑作为菌种来源,通过前期厌氧、后期转为有氧的培养条件,分别建立年轻(培养1周)与成熟(培养3周)的细菌生物膜,以模拟不同发展阶段的根管感染状态。本研究发现1% NaOCl对年轻细菌生物膜的杀菌率高达91%,而对于成熟细菌生物膜作用3 min的杀菌率为38.4%,上述结果表明,细菌生物膜的成熟度显著影响冲洗液的杀菌效率。尽管1% NaOCl在常用根管冲洗液中杀菌效果最佳,但针对成熟细菌生物膜,其清除能力仍有待进一步提升。因此在临床中针对根管长期感染,可通过增加次氯酸钠冲洗温度、时间及冲洗频率[11, 13, 31],并使用多种荡洗技术提高细菌生物膜的清除效率[11-12, 32]。

CHX是最常用的口腔抑菌药物,既往研究报道CHX与NaOCl具有相似的治疗效果[33-34],在本研究构建的前期厌氧、后期微需氧培养环境下,针对口腔混合菌生物膜,1% NaOCl表现出优于2% CHX的杀菌效果。Prasad等[35]纳入80例患者进行随机对照研究,分为两组使用2.5% NaOCl溶液及2% CHX溶液进行冲洗,发现NaOCl冲洗后平均菌落计数达到190万,细菌载量减少96%,而CHX冲洗后平均CFU计数为670万,菌量减少86%,证实NaOCl比CHX能更有效地减少根管内微生物数量,与本研究结果相似。

目前,关于根管治疗过程中根管内细菌微生态群落及代谢产物的变化研究相对较少。Zandi等[36]研究发现,根管治疗中使用NaOCl与CHX冲洗后总菌量及链球菌数量均显著减少,但其菌群结构无显著改变。本研究借助体外模型进一步探究了不同冲洗液对细菌群落结构的影响,高通量测序结果同样发现2种冲洗液处理后菌群结构并未见显著差异。在此基础上,本研究结合以往报道,筛选出根管感染中的优势菌,包括链球菌、梭杆菌及微单胞菌[4, 16-17],并深入分析2种冲洗液对特定菌种的影响,为针对性杀灭持续性感染细菌提供了实验依据。

链球菌是根管初始感染及持续性感染的重要优势菌[4],本研究发现,尽管冲洗药物处理后总菌量下降,但经CHX及NaOCl处理后,链球菌属的相对丰度均明显升高。这一结果提示,CHX及NaOCl对链球菌的杀菌效果可能相对欠佳,与既往报道中链球菌是持续性根尖周炎根管内重要优势菌的结论相符[11-12, 37-38]。Zandi等[39]对10例慢性根尖周炎再治疗患牙行1% NaOCl或2% CHX冲洗前后根管菌群进行焦磷酸测序,发现冲洗前后样本中最常见且最丰富的菌属为链球菌和梭杆菌。Vieira等[14]采用离体牙及龈下菌斑生物膜建立体外模型,发现NaOCl冲洗液可显著降低梭杆菌、放线菌、

卟啉单胞菌及嗜二氧化碳噬细胞菌相对丰度。Amaral等[40]采集患有慢性根尖周炎单根管患牙样本，根管治疗中镍钛锉进行根管预备及荡洗，选择5.25% NaOCl作为冲洗液，通过高通量测序发现根管荡洗后梭杆菌为根管内最丰富的优势菌，与以往研究相似[41-42]。本研究中同样发现，梭杆菌与链球菌呈现出相似的变化趋势，对照组梭杆菌的相对丰度分别为3.80%及5.92%，CHX处理后变为11.10%及4.17%，经NaOCl处理后则变为10.17%及8.08%，由此提示目前临床冲洗药物无法完全杀灭持续性感染相关的优势菌种，有必要选用具有针对性的临床冲洗方案。

此外，本研究存在一定局限性。首先，体外进行冲洗时间固定为3 min与临床存在差异，目前临床实践中常采用高频率、长时间或超声/激光辅助荡洗[43-44]，实际杀菌效果可能优于本模型。其次，本模型中菌斑来源于研究参与者龈上/龈下菌群，采用动态氧培养条件，与慢性根尖周炎根管内以严格厌氧菌为主的菌群构成存在差异，未能完全模拟根管内原位氧条件，可能影响冲洗药物对某些致病菌的疗效评价，可在后续依据离体牙模型进行进一步根管原位动态模拟。最后，本研究发现冲洗处理后链球菌及梭杆菌相对丰度升高，可能源于敏感菌被清除后的被动上升，而非绝对耐药，因本研究的样本量较少缺乏菌群相关统计学分析，因此在后续研究中将进一步扩大样本量，并结合绝对定量方法进行进一步验证。

综上所述，本研究在体外成功构建了前期厌氧、后期有氧培养的龈上及龈下混合菌生物膜模型，并评价了1% NaOCl及2% CHX的抗菌效果。结果表明，相比于2% CHX，1% NaOCl对年轻及成熟细菌生物膜均表现出更优的杀菌效果，但针对成熟生物膜其杀菌效率显著下降，仍需借助辅助手段进一步提升。2种冲洗药物对细菌生物膜的菌群结构的影响有限，但根管感染优势菌链球菌及梭杆菌在冲洗处理后的相对丰度上升，提示针对与持续性感染相关的特定菌种仍需强化清理策略。未来研究应进一步模拟不同根管部位的氧条件，采用感染根管原位样本、延长冲洗时间并引入活化技术，开展原位菌群结构分析以及冲洗药物的杀菌效率评估，以更精准地模拟临床根管微生态环境，提高复杂根管感染的控制效果。

【Author contributions】 Shi BJ designed the study, performed the experiments and wrote the manuscript. Yang Z performed the experiments and wrote the manuscript. Su Z designed the study, evaluated the data, and reviewed the manuscript. All authors read and approved the final manuscript as submitted.

【Ethics statement】 The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Stomatological Hospital, Capital Medical University (No. CMUSH-IRB-KJ-PJ-2024-79), and written informed consent was obtained from all participants.

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【Data availability statement】 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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