doi: 10.13241/j.cnki.pmb.2018.13.009

## Micro-CT 监测尼古丁对大鼠正畸牙移动过程中牙周改建的影响\*

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摘要目的:探讨不同摄取量的尼古丁对大鼠正畸过程牙周改建的影响。方法:选择120只雄性Wistar大鼠并将其随机分为四组: A 组 - 空白对照,B 组 - 正畸模型,C 组 - 正畸并 0.01 mg/mL 尼古丁给药,D 组 - 正畸并 1 mg/mL 尼古丁给药。分别于实验开始后 第 1、3、7、14、21 天通过 Micro-CT 和 HE 染色观察模型牙齿移动距离和牙周组织改变并通过 ELISA 实验检测 IL-17 的表达。结 果:Micro-CT 扫描显示:正畸建模组相对于空白对照组在牙移动距离、骨体积分数、骨密度等指标均有明显变化,变化最大幅度发 生在 D 组,B、C 两组之间的差异没有统计学意义(P>0.05)。21 天,D 组移动距离达到 0.80± 0.06 mm,明显高于 B、C 组(P<0.05)。相 较于空白对照组(A 组),B、C、D 三组 Micro-CT 测量的骨体积分数、骨密度、骨小梁厚度均降低,D 组骨密度值降至 1108.36± 8.86 mg/cm<sup>3</sup>。HE 染色结果显示:D 组在 21 天时破骨细胞增多并出现牙根吸收陷窝伴牙周膜纤维排列混乱;ELISA 检测显示 B、C 组 IL-17 的含量在第 7 天时达到峰值,D 组则在 14 天含量最高。结论:高浓度的尼古丁可加速正畸牙齿的移动速度及牙槽骨吸收,增 加牙周组织中的破骨细胞及 IL-17 表达水平。

关键词:尼古丁;正畸;Micro-CT;骨小梁;IL-17

中图分类号:R-33; R783.5 文献标识码:A 文章编号:1673-6273(2018)13-2448-04

# Effect of Nicotine on the Peridentium during Orthodontic Tooth Movement in Rats Monitored by Micro-CT\*

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**ABSTRACT Objective:** The study the effect of differentdosis of nicotine on the periodontium during orthodontic treatment in adult rats. **Methods:** 120 cases of Wistar male rats were segmented into four groups stochastically: A-control; B-orthodontic model group; C-orthodontic + 0.01 mg/kg nicotine group; D-orthodontic+1 mg/kg nicotine group. Micro-CT was used to measure tooth movement and the changes on microstructure of trabecular, the variety of periodontal microstructures and expression of IL-17 on day 1, 3, 7, 14 and 21 were detected and compared. **Results:** Micro-CT scanning showed that the orthodontic groups changed largely on observed indicators, such as tooth movement, BVF, and BMD, when compared to control groups. There were no significant changes between B and C groups (P>0.05), D group changed mostly. The tooth movement of D group reached  $0.80 \pm 0.06$  mm, which was obviously higher than B and C groups at day 21 (P<0.05). The BVF, BMD and Tb.Th in all groups lessened apart from A group. HE staining of A - D groups indicated that the ligament of D group was disordering and more osteoclasts. The expression level of IL-17 reached its peak at day 7 in B, C groups, but it was at day 14 for D group. **Conclusion:** High-dose nicotine can not only accelerate the speed of tooth movement and the absorption of alveolar bone; in addition, but also increase the quantity of osteoclast and IL-17 expression.

Key words: Nicotine; Orthodontic; Micro-CT; Trabecular bone; IL-17

Chinese Library Classification(CLC): R-33; R783.5 Document code: A Article ID: 1673-6273(2018)13-2448-04

#### 前言

成人正畸治疗已占牙齿矫治总数的15%~20%,临床上吸烟的成年患者并不少见<sup>11</sup>。尼古丁作为烟草的主要成分,易通过口腔粘膜被机体吸收,长期接触会损害牙周组织引起骨丧失

<sup>[2,3]</sup>。另外,吸烟可以导致口腔炎症,增加白细胞介素 17(IL-17, interleukin -17)的表达水平,IL-17 又可直接促进破骨细胞形成, 而破骨细胞是牙槽骨动态平衡的重要介质<sup>[44]</sup>。以往对牙周骨改 建情况的评价指标多为骨量或组织病理改变,但有研究证实骨 小梁的显微结构是判断骨重建的一个重要指标<sup>[7]</sup>。微型计算机

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(收稿日期:2018-03-05 接受日期:2018-03-28)

<sup>\*</sup>基金项目:黑龙江省教育厅基础研究项目(2016-KYYWF-0611)

断层成像(Micro computed tomography, Micro CT)基于 X 线成 像原理可在不破坏样本的情况下对牙槽骨进行三维扫描,同时 对结构、密度等指标进行定量分析<sup>[8-10]</sup>。本实验将不同浓度尼古 丁涂抹于大鼠口腔,并通过 Micro-CT 等监测骨小梁的显微结 构指标变化,探讨正畸过程中尼古丁对牙周组织改建的影响, 旨在为吸烟者接受更科学有效的正畸治疗提供理论依据。

#### 1 材料与方法

#### 1.1 主要试剂和仪器

尼古丁溶液(上海抚生实业有限公司);0.9%生理盐水;10% 水合氯醛溶液;多聚甲醛;EDTA;HE 染色试剂及电子显微镜; IL-17 酶联免疫吸附实验(ELISA)试剂盒(上海研生实业有限公 司),iMark 酶标仪(Bio-Rad 公司,美国);Micro-CT(Super Argus, Spanish)。

#### 1.2 方法

1.2.1 正畸模型建立和给药方式 雄性 Wistar 大鼠 120 只(3 月龄,350~400 g)随机分四组:A 空白组,B 正畸组,C 正畸并 0.01 mg/kg 尼古丁给药组,D 正畸并 1 mg/kg 尼古丁给药组。大 鼠以 10%水合氯醛(0.3 mL/100 g)腹腔注射麻醉后,仰卧位用慢 速金刚砂车针于上颌两中切牙龈缘及左上第一磨牙近中颈缘 处预备一深约 0.3 mm 的沟用于固位,两牙之间用正畸拉簧牵 拉,0.2 mm 结扎丝固定于沟处,力值约 30 g<sup>[11]</sup>。口腔涂抹给药, 每天 2 次,间隔 6 h,正畸组同频次涂抹生理盐水。

1.2.2 标本采集 各时间点分别于每组随机选取 6 只大鼠处死,随机选取 3 只剥取左侧上颌第一磨牙压力侧牙龈组织,编号后放入 -20 ℃冰箱保存待用,另外 3 只分离左上颌骨,浸入 4%多聚甲醛固定液,24 h 后进行 Micro-CT 扫描,完成后放入 10%EDTA 脱钙液,室温脱钙。

1.2.3 Micro-CT 扫描及视图分析 将上颌骨牙冠平行地面固定在扫描床上,用以下参数扫描:最高分辨率(Max Res),静态调强模式(step & shot),720°,50 KV,300 μA,39 ms,扫描用时约 20 min。图像重建采用配套 Sedecal RECON 软件,视图分析采用配套 MMWKS 软件及 ImageJ 软件。CT 图上第一磨牙远中和第二磨牙近中的距离即为牙移动距离(mm)。选择左上颌第一磨牙远中颊根根中 1/3 的近中牙槽间隔骨松质为感兴趣区(region of interest, ROI),三维重建后对骨小梁的显微结构进行定量分析,包括:骨密度(BMD,mg/cm<sup>3</sup>)、骨体积分数(BVF,指ROI 内的骨小梁体积与所选 ROI 体积比值)、骨小梁厚度(Tb. Th,mm)<sup>[12-14]</sup>。

1.2.4 HE 染色 上颌骨标本脱钙 45 天,OCT 包埋后 -80℃冷 冻 lh,于冰冻切片机内复温(OT -20℃,CT -20℃) lh 切片,厚度 5 μm。将切片依次进行苏木素染色、分化、伊红染色、透明化、封片操作,在× 400 电子显微镜下观察。

1.2.5 ELISA 实验 将冰箱内牙龈组织取出复温,加入盛有 0.5 mL 二甲基甲酰胺(DMF)的研磨杵,制作组织匀浆,高速离 心(1500 rpm,15 min)。取上清液 100 μL,按照大鼠 IL-17 ELISA 检测试剂盒标准步骤进行操作,将样品按 1:2 倍稀释,最后用 酶标仪在 450 nm 波长依序测量各孔的 OD(平均光密度)值,根 据标准品 OD 值和浓度描绘标准曲线,将样品 OD 值代入标准 曲线公式得到对应浓度,乘稀释倍数 2 则为该样本检测浓度。

#### 1.3 统计学分析

采用 GraphPad Prism 5 统计软件处理。所有数据均以 x̄±s 表示(n≥ 3),多组间比较采用方差分析,两组间比较采用 t 检验,以 P<0.05 为差异具有统计学意义。

#### 2 结果

#### 2.1 Micro-CT 扫描测量结果

2.1.1 **牙移动距离** 在实验开始后 1-21 天时间点,A 组在基 线范围内变化 (0.08± 0.01~0.12± 0.03),B、C、D 三组的移动距 离随时间而增加,并且 14 天后增速有所放缓,分别于 21 天达 到最大值(B:0.4± 0.07,C:0.45± 0.045,D:0.80± 0.06),其中 14 天、21 天 D 组移动距离高于 B、C 两组具有统计学意义 (*P*< 0.05),表明高剂量尼古丁局部作用可以促进正畸牙移动。牙移 动 CT 检测距离见图 1(a、b、c、d),具体数值变化趋势见图 3a。

# 2.1.2 大鼠牙槽骨 Micro-CT 图像及显微结构的骨参数分析 21 天矢状位 CT 图像显示:A 组牙槽骨骨质紧密;B、C

组牙齿移动距离略有增加,骨质密度有所降低;D组移动距离 最为明显,骨质密度降低显著,见图 1。其余骨小梁细微结构的 具体指标变化趋势见图 3(a、b)及图 4(a、b),A 组各指标的变化 范围不明显,B、C、D 三组由于加力等原因导致各项指标均有 变化,其中 B、C 之间的微小差距没有统计学意义(P>0.05)。D 组变化最为明显,7 天、14 天、21 天的 BMD、BVF、Tb.Th 均低 于 B、C 两组(P<0.05),差异有统计学意义。

#### 2.2 HE 染色结果

A 组牙周膜间隙等宽,胶原纤维排列规则,无破骨细胞出现;B 组和 C 组牙周膜变窄,牙根及牙槽骨边缘粗糙,周围可见少量破骨细胞,D 组牙周膜明显缩窄,周围有较多的成熟多核破骨细胞聚集,牙根可见吸收馅窝。如图 2(a、b、c、d)。





#### 2.3 牙龈组织 IL-17 的表达

ELISA 检测结果显示:各组均有 IL-17 表达,其中 A 组为 正常生理水平,且基本维持稳定,B、C、D 三组均有先升高后降 低的趋势;B、C 组表达水平基本一致,并于第 7 天达到峰值,其 后下降;D组在第14天时达到最高表达水平,且明显高于BC

两组(P<0.01),见表1。



图 2 21 天各组牙齿标本的 HE 染色图像

Fig.2 The HE staining of samples in each group at Day 21. (M-alveolar bone, F-tooth root, P-parodontium, Black arrow- osteoclast; a-A Group, b-B Group, c-C Group, d-D Group)



### 图 3 牙齿移动距离和骨体积分数的 Micro-CT 测量指标变化

Fig.3 The changes of measurement index of Micro-CT for tooth movement and BVF







正畸治疗的生物学基础是牙周组织改建,压力侧牙槽骨吸收,张力侧新骨生成,最终使牙齿达到理想位置<sup>15]</sup>。尼古丁可对

表1各	组大國	鼠不同时间	]点 IL-	17 的表	达水平	(单信	Ì:pg∕	$(mL)(\bar{x})$	±s, n=3)	
11 1 5		CTT 17	1.00		• ,	c		1	_	

Table 1 Expression of IL-17 at different time points of rats in each group( $\bar{x}\pm s$ , n=3)											
Groups	Day 1	Day 3	Day 7	Day 14	Day 21						
A Group	22.871± 1.047	22.077± 2.653	24.670± 2.655	21.794± 4.081	24.454± 0.925						
B Group	23.415± 2.471	27.552± 3.542	37.915± 5.300	36.812± 4.588	29.502± 6.160						
C Group	24.089± 4.005	30.212± 3.977	40.668± 7.025	37.070± 4.175	33.440± 5.1784 6.050± 2.935*						
D Group	20.463± 1.680	33.235± 3.824	50.969± 4.521	53.904± 2.357*	46.050± 2.935*						

Note: compared with theB and C group at the day 14,\*P<0.01; at the day 21,\*P<0.05.

骨质改建产生不利影响,吸烟人群牙周附着丧失率约为未吸烟 人群的 3~4 倍,表明尼古丁是加重牙周损伤的危险因素之一 <sup>[2]</sup>。Mizrak S、Esfahrood ZR 等学者通过尼古丁腹腔注射的方法 研究其对正畸牙移动的影响,证实尼古丁可以通过血液循环进 入到牙周组织来影响正畸过程<sup>[16,17]</sup>。本实验采用口腔涂抹的给 药方式,更能模拟真实吸烟环境。此外,本实验采用新型影像技 术 Micro-CT 对牙周改建各项指标进行检测,可精准测量牙齿 移动距离,并可对骨质显微结构进行重建、定量分析<sup>[18,19]</sup>。

本研究结果显示正畸模型组由于加力因素破骨细胞数量、 牙槽骨显微结构指标、IL-17水平均较正常对照出现明显变化; 正畸模型组和低浓度尼古丁处理正畸模型组变化基本一致,说 明低浓度尼古丁正畸过程中牙槽骨改建没有明显影响;高浓度 尼古丁处理正畸模型组破骨细胞数量、牙槽骨显微结构指标的 变化幅度、IL-17水平均较任何一组更为明显,而且压力侧的牙 根表面出现吸收陷窝。另外,根据牙槽骨显微结构指标的变化 趋势,可以发现正畸模型组和低浓度尼古丁处理正畸模型组在 14 天达到改建的最大程度,并在此后转入修复期,而高浓度尼 古丁处理正畸模型组则在 21 天时间点,牙槽骨依旧呈现出改 建趋势。以上结果表明高浓度尼古丁涂抹给药可以明显加快大 鼠正畸牙的牙周改建过程,并可延长改建过程。相对于其他类 似实验[20],本实验结果变化更为明显,其原因可能在于给药作 用方式不同,口腔涂抹的尼古丁溶液可以被粘膜组织等充分吸 收并直接作用于正畸牙及其附着组织,故造成的影响也较为明 显。分子生物学检测结果显示 IL-17 水平及破骨细胞数量的变 化趋势与 CT 检测指标所反映的牙槽骨改建过程一致,进一步 验证了高浓度尼古丁涂抹给药可以明显加快大鼠正畸牙的牙 周改建过程。

综上所述,高浓度尼古丁可以加快大鼠正畸牙的牙周改建 过程,并可在一定程度上延长改建过程,这为正畸医师引导患 者戒烟提供理论依据。低浓度尼古丁组对正畸改建影响不大, 与单纯正畸的牙周改建接近,说明少量吸烟的患者也可以进行 正畸治疗,该结论对于正畸治疗具有一定的临床指导意义。

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