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·基础研究·

低能量激光对大鼠牙移动后保持过程中破骨细胞和胶原纤维改建的影响

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【摘要】 目的 探讨低能量激光（low level laser，LLL）对大鼠牙移动后保持过程中破骨细胞和胶原纤维改建的影响，为临床应用提供实验依据。**方法** 8周龄Wistar大鼠20只，随机选取5只为基线组：不施加正畸力，作为空白对照。其余15只建立上颌第一磨牙近中移动模型，加力结束去除加力装置后随机3组，每组各5只。对照组：即刻拆除加力装置后，不采用任何保持措施；保持组：拆除口内装置后，结扎丝拧成麻花状作为固定保持，维持大鼠第一磨牙与切牙之间的距离；保持+激光组：加力结束拆除口内装置后，结扎丝拧成麻花状作为固定保持，并在去除加力装置后的第0天、第3天、第6天、第9天、第12天应用LLL照射。2周后处死所有大鼠，取第一磨牙组织块，用HE染色、TRAP染色、Masson染色法观察破骨细胞以及胶原纤维的分布，分析牙槽骨及胶原纤维改建的过程。**结果** 去除加力装置后2周，基线组牙根两侧可见胶原纤维沉积，牙根远中侧未见明显破骨样细胞；对照组牙根两侧未见明显胶原纤维沉积，远中侧破骨细胞活动活跃；保持组牙根两侧可见胶原纤维沉积，远中侧亦可见破骨细胞活动，但不如对照组活跃；保持+激光组牙根两侧胶原纤维合成明显，未见明显破骨细胞分布，且保持+激光组与其余各组差异具有统计学意义（$P<0.05$）。**结论** 固定保持同时进行低能量激光照射能有效促进大鼠牙移动后保持阶段内胶原纤维的合成，抑制破骨细胞活动，从而减少磨牙复发的可能性。

【关键词】 大鼠；正畸；牙移动；低能量激光；破骨细胞；胶原纤维；Masson染色；TRAP染色
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Effect of low level laser on osteoclasts and collagen fiber remodeling during the process of tooth retention after tooth movement in rats

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【Abstract】 Objective To investigate the effect of low level laser on osteoclast and collagen fiber remodeling during the process of tooth retention after tooth movement in rats and to provide the experimental basis for clinical application. **Methods** In total, 20 eight-week-old Wistar rats were selected to establish a mesial movement model of the maxillary first molar and then randomly divided into four groups after the appliance was removed. In total, 5 rats were included in each group, including baseline group (without force as blank control), control group (without any intervention after re-

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moving the force appliance), retention group (teeth were wrapped with orthodontic ligature wires that were screwed into hemp flower as fixed retention to maintain the space between the first molar and incisor after appliances were removed) and retention and low energy laser irradiation group (teeth were wrapped with the orthodontic ligature wires that were screwed into hemp flower as fixed retention and low energy laser irradiation was applied on days 0, 3, 6, 9 and 12 after appliance removal). Two weeks later, all the rats were sacrificed and the first molar tissue blocks of each group were collected. The distribution of osteoclasts and collagen fiber were studied by HE staining, TRAP staining and Masson staining to illustrate the process of alveolar bone and collagen fiber remodeling. **Results** Two weeks after appliances were removed, collagen fibers were deposited on both sides of the root in the baseline group, but no osteoclasts were observed in the distal side of the root. In the control group, collagen fibers on the two sides of the root were not obvious and osteoclasts were active on the distal side. In the retention group, collagen fibers were obvious on the two sides of the root and the osteoclasts on the distal side were less active than the control group. Regarding the retention and low energy laser irradiation group, collagen fibers were significantly obvious and osteoclasts were not seen. The difference was statistically significant between the retention and low energy laser irradiation group and the other three groups ($P < 0.05$). **Conclusion** These results suggest that fixed retention with simultaneous low level laser can effectively promote the synthesis of collagen fibers and inhibit the activity of osteoclasts during the process of tooth retention after movement, thus reducing the possibility of molar recurrence.

【Key words】 rat; orthodontics; tooth movement; low level laser; osteoclasts; collagen fiber; Masson staining; TRAP staining

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正畸治疗结束后，被移动的牙齿具有即刻回到最初位置的趋势，即复发的趋势。这一现象被认为与牙周组织的改建密切相关。如何在正畸治疗结束后促进牙周组织改建，缩短保持期，减少错𬌗畸形的复发是研究热点。低能量激光（low level laser，LLL）具有良好的生物学作用，包括光生物促进作用、光生物学调节功能等。既往研究显示，LLL能够增强细胞代谢，促进成骨细胞增殖以及成骨分化[1-2]。LLL促进正畸牙移动的报道较多，而对正畸牙保持的研究甚少，基于LLL的生物促进作用，本项研究将探讨LLL对大鼠牙移动后保持过程中胶原纤维和破骨细胞的影响，为后续临床研究提供实验依据。

1　材料和方法

1.1　材料

本实验选取8周龄雄性健康Wistar大鼠20只，动物合格证号：SCXK（京）2016-0006，体重210～300 g，均由中国医学科学院放射医学研究所（天津）提供，常规饮食，普通喂养。本实验经天津市医学伦理委员会审核通过。

直径0.2 mm、0.25 mm结扎丝（天天齿科，中国）、镍钛拉簧（有研亿金，中国）、测力计、京津化学固化粘结剂（天津市合成材料工业研究所）、注射器、棉线、鼠板、胶布、一次性器械盘、天平（上海越平科学仪器有限公司）。808 nm波长激光器[（国医华科（天津）医疗科技集团有限公司）]、病理切片机（RM2235，LEICA，德国）、光学显微镜（Nikon，日本）。水合氯醛（中国医药集团化学试剂有限公司）、EDTA脱钙液、TRAP试剂盒（北京索莱宝科技有限公司）、Masson试剂等。

1.2　方法

1.2.1　建立大鼠第一磨牙近中移动模型　20只8周龄雄性Wistar大鼠，随机分为4组：基线组5只，对照组5只，保持组5只，保持+激光组5只。基线组不施加正畸力，作为空白对照，另外3组共15只大鼠建立大鼠第一磨牙近中移动模型：10%水合氯醛麻醉下仰卧于鼠板上。用直径0.2 mm结扎丝结扎上颌第一磨牙后，与镍钛螺旋拉簧一端结扎，直径0.25 mm结扎丝穿过镍钛螺旋拉簧另一端并结扎至切牙牙颈部，粘接剂覆盖其表面增强固位。测力计下50 g力牵拉上颌第一磨牙近中移动（图1）。主动加力时间为3周，每周主动加力1次，期间检查所有样本的力值是否维持稳定，若发现口内装置脱落，重新安装加力装置。

1.2.2　加力结束后保持和激光照射　加力结束后拆除口内装置，进行以下操作。①对照组：加力结束即刻拆除口内装置后，不采用任何保持措施。②保持组：加力结束拆除口内装置后，结扎丝拧成麻花状作为固定保持器，维持大鼠第一磨牙与切

Figure 1　Establishment of mesial movement model of rat first molar

图1　建立大鼠第一磨牙近中移动模型

牙之间的距离，定期检查保持装置是否脱落，如发现脱落，应及时安装。③保持+激光组（retention and LLL，RL group）：加力结束拆除口内装置后，结扎丝拧成麻花状作为固定保持器，并在去除加力装置后的第0天、第3天、第6天、第9天、第12天应用中国医学科学院生物医学研究所提供的二极管激光器进行照射，照射波长808 nm，输出功率100 mW。兼顾生物安全性和穿透能力两个方面，确定照射光斑直径2 mm，照射光斑面积合约0.03 cm^2。照射部位为第一磨牙颊侧、腭侧牙龈约平牙根中部高度两个位点。照射时间为每个位点7 s，剂量23 J/cm^2，符合以往文献[3]中20～25 J/cm^2的剂量范围。

以上所有样本在去除加力装置后的第14天全部处死，并立即对第一磨牙牙周组织进行取材。

1.2.3　样本切片与染色　处死大鼠后，采集第一磨牙所在的牙槽骨骨块样本，4%多聚甲醛固定24～48 h，10%EDTA脱钙3个月，脱水，透明，常规浸蜡包埋。以第一磨牙近远中方向为横切面，5 μm厚度进行切片，切片内需同时包含牙根及牙根周围牙槽骨。常规脱蜡、水合，HE染色、TRAP染色、Masson染色。HE染色切片观察破骨样细胞的组织学形态。TRAP染色切片观察破骨细胞活跃度。Masson染色切片观察胶原纤维的沉积。

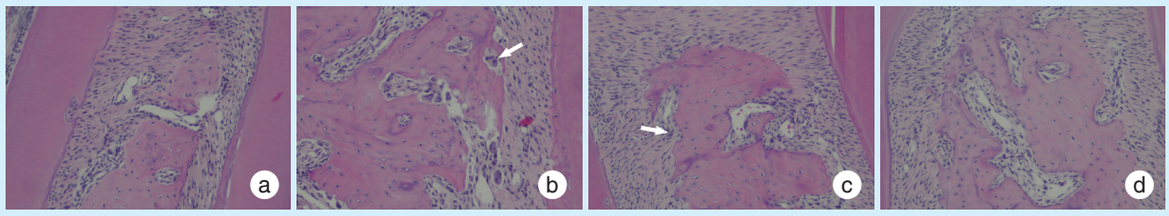
1.3　统计学分析

应用Image Pro Plus6.0软件半定量分析胶原纤维面积及破骨细胞数，将分析结果导入Graph Pad Prism5软件，生成柱状图。胶原纤维面积及破骨细胞数在各组间的差异采用方差分析，$P<0.05$为差异有统计学意义。

2　结　果

2.1　HE染色结果

基线组牙根远中侧未见明显破骨样细胞，牙周膜内未见明显异常；对照组局部区域牙周膜内炎症，偶见玻璃样变，牙根远中侧可见明显破骨样细胞；保持组未见明显牙周膜炎症及玻璃样变，牙根远中侧偶见破骨样细胞；保持+激光组几乎无破骨样细胞，牙周膜内无局部炎症反应及玻璃样变（图2）。



a：distal side of the root in the baseline group，normal tissue；b：distal side of the root in the control group，osteoclasts（white arrow）and local inflammation；c：distal side of the root in the retention group，occasional osteoclasts（white arrow）and local inflammation；d：distal side of the root in the retention and LLL group，no osteoclasts and local inflammation

Figure 2　HE staining images of 4 groups　×100

图2　4组HE染色观察照片　×100

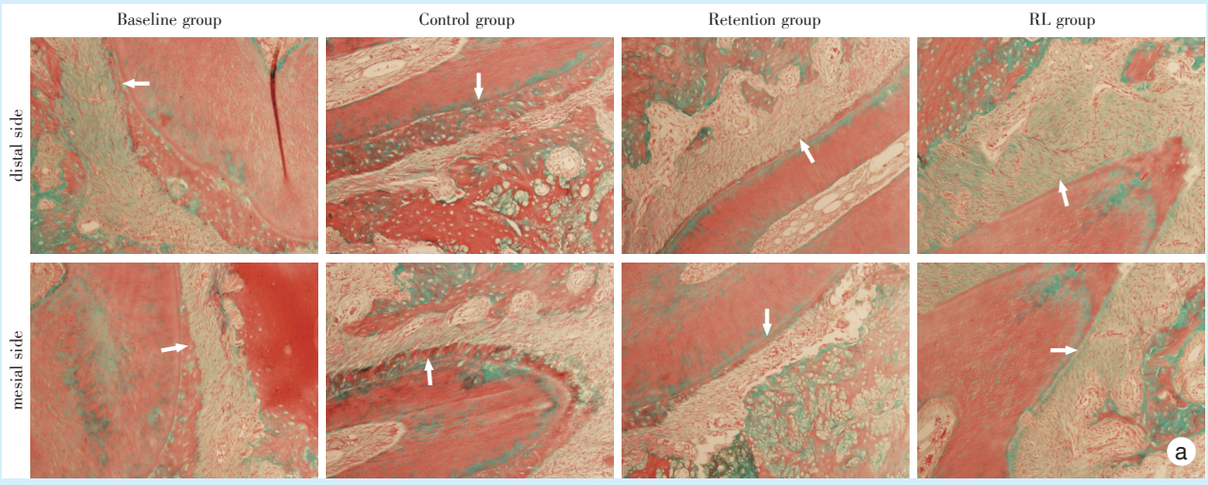
2.2　Masson染色结果

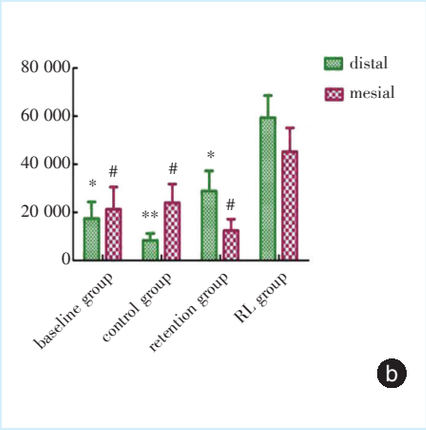
基线组牙根两侧可见胶原纤维沉积，对照组牙根两侧未见明显胶原纤维沉积，保持组牙根两侧胶原沉积较明显；与以上三组相比，保持+激光组胶原沉积更为明显（图3）。

2.3　TRAP染色结果

基线组牙根远中侧未见明显破骨细胞活动，对照组远中侧破骨细胞活动活跃；保持组牙根远

中侧偶见破骨细胞活动，但不如对照组活跃；保持+激光组牙根远中侧未见破骨细胞活动（图4）。

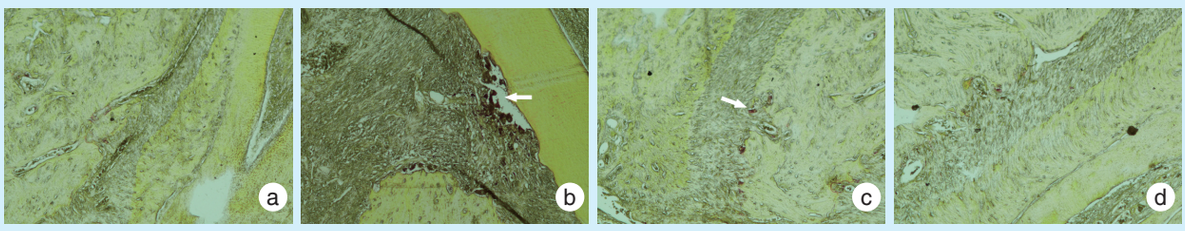


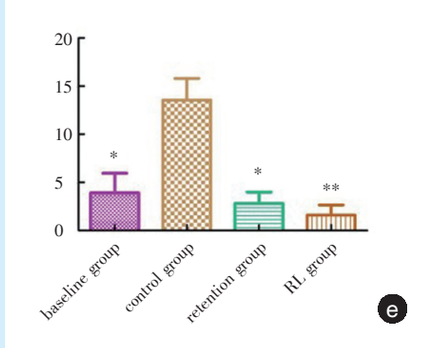


a：collagen fiber（green staining as the white arrow showed）remodeling was observed in both sides（distal side and mesial side）of the root in the baseline group；limited collagen fiber（white arrow）remodeling was observed in both sides of the root in the control group；obvious collagen fiber（white arrow）remodeling was observed in both sides of the root in the retention group；more obvious collagen fiber（white arrow）remodeling in both sides of the root in the RL group（the retention and LLL group）；b：comparison of collagen fiber areas in 4 groups；*：compared with the RL group（the retention and LLL group）（$P < 0.05$）；#：compared with the RL group（$P < 0.05$）

Figure 3　Observation of collagen fiber remodeling during the process of tooth retention after tooth movement of maxillary first molars of rats in each group by Masson staining　× 100

图3　Masson染色观察各组大鼠上颌第一磨牙牙移动后保持过程中胶原纤维改建情况　× 100





a：distal side of the root in the baseline group，no obvious osteoclasts；b：distal side of the root in the control group，obvious osteoclasts（white arrow）；c：distal side of the root in the retention group，occasional osteoclasts（white arrow）；d：distal side of the root in the RL group（the retention and LLL group），no osteoclasts；e：comparison of osteoclasts counts in 4 groups；* compared with the control group（$P < 0.05$）

Figure 4　Observation of osteoclasts during the process of tooth retention after tooth movement of maxillary first molars of rats in each group by TRAP staining　× 100

图4　TRAP染色观察各组大鼠上颌第一磨牙牙移动后保持过程中破骨细胞情况　× 100

3　讨　论

工程、科技、医学界把低功率密度或低能量辐射波长500～1 100 nm、输出功率10～90 mW、能量密度为1～20 J/cm²的激光称之为低能量激光[2]。低能量激光的最大特点是良好的穿透性和生物安全性，直接照射生物组织时，不仅不会使生物组织产生不可逆损伤[4-6]，而且能减轻炎症反应、促进伤口愈合、加速正畸牙移动、促进血液微循环等。

国内有学者发现低能量激光具有促进成骨分化的作用[7]。Franzen等[3]的动物实验证实，照射低能量激光可以加快牙齿移动的速度，促进骨折区新骨形成，从而加速骨折愈合。另外还有研究结果表明，激光治疗可通过加速新骨沉积以及活化成骨因子如RUNX-2和骨形成蛋白-9（bone morphogenetic protein-9，BMP-9）等方式来促进骨愈合[8]。

Trelles等[9]报道，低能量激光可能通过刺激张力侧成骨细胞和压力侧破骨细胞的增殖分化来加速牙槽骨改建的过程，从而缩短了正畸治疗的时间。史瑞新等[10]发现，在正畸牙齿的压力侧，照射低能量激光可降低由压力引起的成骨细胞活性增加，减少成骨活动、而破骨活动相对增加，从而促进了牙齿的快速移动。

Lee等[11]的动物实验发现激光照射组牙周膜中BMP-2、MMP-9基因表达水平显著增加，提示激光照射的确加速了牙周纤维的沉积。牙齿移动时，在基质金属蛋白酶抑制剂-1的反向调控下，牙周膜成纤维细胞分泌MMPs，明胶酶亚组MMP-2、MMP-9能够清除分解后的胶原片段和正畸牙齿移动过程中出现的透明样变性区域。MMPs转录和翻译的活性均可作为牙周纤维改建的生物学标志。与此同时，在张力侧有明显的Ⅰ型胶原生成，形成新生牙周纤维的主体，从而加速了牙周组织的改建。

本研究中，保持+激光组表现为牙根两侧有更多的胶原纤维沉积，远中侧未见破骨细胞，表明低能量激光在保持阶段促进了牙周纤维的改建，一定程度上抑制了破骨活动，即加力结束后使用固定保持器同时进行低能量激光照射，可以促进大鼠第一磨牙胶原纤维沉积、抑制牙齿复发，更好地维持牙齿位置稳定。

本研究结果显示，正畸牙齿保持过程中，固定保持同时进行激光照射可促进胶原纤维的沉积、抑制破骨活动，有助于更高效地使牙齿达到稳定状态，减少正畸复发，缩短保持期。

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