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改良丝线结扎法建立大鼠牙周病模型的实验研究*

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摘要 目的:探讨改良丝线结扎法建立大鼠牙周病模型的可行性。**方法:**本实验创新一种新的手段,利用玻璃离子水门汀结合传统丝线结扎法建立大鼠牙周病模型,即在丝线结扎大鼠第一磨牙之后,在大鼠磨牙牙颈部丝线附近堆砌有黏着性的玻璃离子水门汀制造利于丝线固定的外形高点并同时稍微垫高咬合面,制造咬合创伤,加快牙周病的发生发展。**结果:**改良丝线结扎法建立牙周病模型的成功率为 95%,相比传统丝线结扎法的 65%,具有更高的成功率,模型建立更稳定,相比现有各种改良方法操作更简便易行。**结论:**改良丝线结扎法相比传统丝线结扎法建立大鼠牙周病模型具有更高的稳定性和成功率。

关键词:牙周病;Wistar 大鼠;丝线结扎;玻璃离子;牙槽骨

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Modified Thread Ligation Establishes Rat Periodontal Disease Model*

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ABSTRACT Objective: To explore the feasibility of establishing a rat model of periodontal disease by the method of modified silk suture. **Method:** A new method of the innovation, using of glass ionomer cement to establish a rat model of periodontal disease with traditional silk ligation, after the ligation of rat first molars in the vicinity of rat molar tooth neck threads are piled glass ionomer cement adhesive manufacturing for fixed shape and slightly high silk pad the occlusal surface, making occlusal trauma, accelerate the occurrence and development of periodontal disease. **Results:** The success of modified silk ligation model of periodontal disease rate was 95%, compared with the traditional silk ligature of the 65%, has a higher success rate, model is more stable, compared with the existing various improved methods of operation more easy. **Conclusion:** Compared with the traditional method, the method of improving the periodontal disease in the rat model of periodontal disease is more stable and successful.

Key word: Periodontal disease; Wista Rat; Silk ligature; Glass ion; Alveolar bone

Chinese Library Classification(CLC): R-33; R781.4 **Document code:** A

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前言

牙周病是一种由局部细菌刺激而引起的感染性的牙周组织慢性炎症,其发病机制复杂,一直以来在口腔医学领域备受关注^[1]。而牙周病的研究中,相关稳定动物模型的建立,在整个实验里起着至关重要的作用^[2]。传统大鼠牙周病模型的建立即使用丝线结扎的方法结扎大鼠上颌第一磨牙,同时辅以高糖饮食,在局部创造一个利于细菌滞留的环境同时结扎丝线本身对牙龈组织也是一种直接的刺激,引起大鼠牙周组织的持续炎症反应,从而破坏牙周组织^[3]。但是传统方法成功率较低,操作难度大,即使用丝线结扎的方法建立大鼠牙周病模型,过程精细,费时,且由于建模时间较长,极易造成丝线脱落,导致建模失败^[4]。多年来多种改良方法的出现并没有彻底的简化造模过程,大幅提高成功率^[5]。因此,本实验在反复的经验教训中,结合使用玻璃离子成功的探索了一种新的方法有效的简化了操作,提高了

成功率,即称为改良丝线结扎法。

1 材料与方法

1.1 实验材料

Wistar 大鼠 40 只(来自哈尔滨医科大学附属第四医院分子影像中心),体重(200±50)g;3-0 手术缝合线;玻璃离子水门汀(粉剂+溶剂);舒泰 50(肌肉麻醉剂)自制鼠架;自制大鼠开口器;环形灯;止血钳子;持针器;1ML 注射器等。

1.2 方法

1.2.1 麻醉 选择 6-8 周龄健康大鼠,实验前一天禁食不禁水。按体重比例肌肉注射舒泰 50 麻醉剂约 0.2 mL,3-5 min 大鼠麻醉。取麻醉大鼠固定于自制鼠架,开口器暴露大鼠上颌第一磨牙。

1.2.2 丝线结扎 用持针器夹持 3-0 缝合线,将丝线压入大鼠第一磨牙与第二磨牙邻间隙内,尽量将丝线压入牙龈方向并紧

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密结扎,于大鼠第一磨牙近中出打结,打一外科滑结,便于调整进一步使结扎线稳固。丝线紧密贴合牙颈部,轻探牙颈部丝线,丝线不脱落即为结扎成功。

1.2.3 玻璃离子水门汀的使用 于大鼠第一磨牙近中丝线以上牙面部位黏着玻璃离子水门汀。首先用棉球擦干大鼠第一磨牙附近唾液,棉签撑开大鼠颊侧黏膜,保持上颌第一磨牙表面干燥,调适玻璃离子水门汀用探针取少量玻璃离子水门汀黏着于大鼠上颌第一磨牙近中丝线处的咬合方向,人造丝线咬合面方向的外形高点一保证丝线不脱落。

1.2.4 分组设计 按上述改良丝线结扎法,建立 20 只大鼠。传统丝线结扎法建立 20 只的大鼠模型,一个月后检查大鼠口腔内丝线情况,及牙周组织炎症情况。

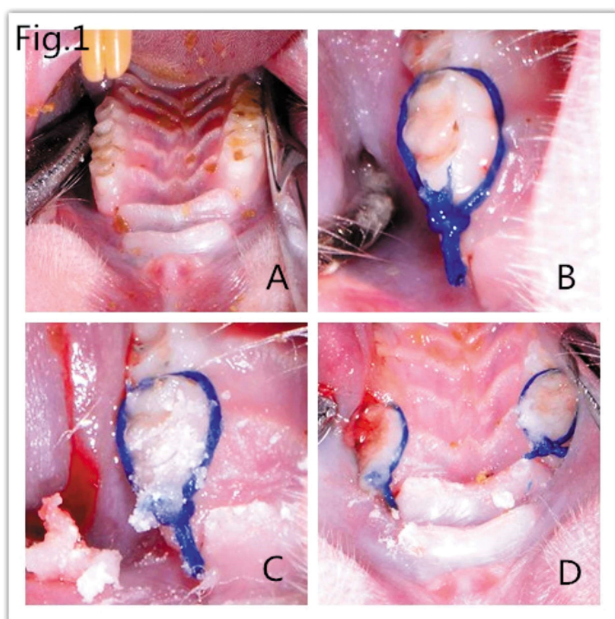


图 1 大鼠口内丝线结扎

A 为结扎前大鼠口内状态;图 B 为传统丝线结扎法;图 C, D 为辅助使用玻璃离子的改良丝线结扎法

Fig.1 Rat mouth thread ligation

A is the state of the mouth of the rat before ligation; B is for the traditional thread ligation; C, D is modified silk ligation method for the use of glass ions

2 结果

改良组 20 只大鼠丝线稳固,除 2 只单侧丝线脱落外,其他大鼠造模成功,牙龈红肿并附着大量软垢,探诊出血严重,组织病理学可见牙龈组织炎症及牙槽骨吸收。建模成功率为 95%。对照组有 12 只大鼠单侧或双侧丝线脱落,造模失败,8 只大鼠牙周病模型建立成功。建模成功率为 65%。

3 讨论

大鼠牙周病模型的建立,不仅在牙周病学中是一项重要的实验技术,在口腔正畸学以及口腔颌面外科学中也有着同样重要的地位^[6,7]。牙周病的病理特点即牙周附着丧失和牙槽骨吸收,而口腔正畸学和口腔颌面外科学中对于牙槽骨吸收模型的

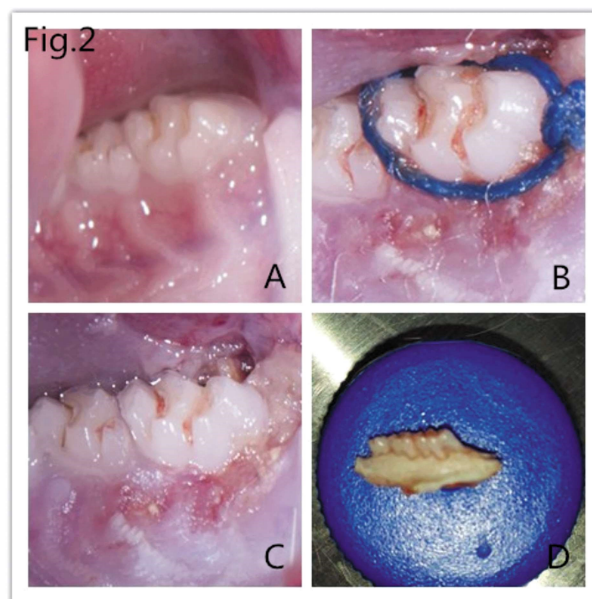


图 2 丝线结扎引起牙周组织病变

A 为大鼠健康牙周组织(可见牙龈致密,有光泽,状态良好);B,C 为通过结扎造成的牙周炎组织(牙龈暗红,水肿,并有溃疡,轻探极易出血);D 为牙槽骨吸收的骨组织标本(可见上颌第一磨牙牙槽嵴顶吸收严重)

Fig.2 Periodontal tissue lesions caused by silk ligation

A is healthy periodontal tissue in rats (visible gum, shiny, good condition); B, C is ligation caused by periodontitis (gum red, edema, and ulcer, light probe bleeding); D is the alveolar bone resorption of the bone tissue samples (visible maxillary first molar alveolar crest absorption is serious)

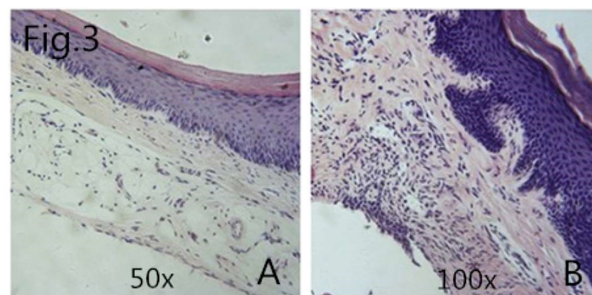


图 3 牙龈组织 HE 染色片

A 为正常大鼠牙龈上皮的 HE 染色;图 B 是牙周炎大鼠模型牙龈上皮的 HE 染色;可见上皮丁突不规则排列,上皮结缔组织中大量炎症细胞浸润

Fig.3 HE staining of gingival tissue

A is HE staining of gingival epithelium in normal rats; A is the HE stain of the gingival epithelium of the rat model of periodontitis. The irregular arrangement of epithelial, a large number of inflammatory cell infiltration in the epithelial connective tissue

建立,多在牙周炎的基础上建立糖尿病大鼠模型从而造成大鼠的牙槽骨吸收的病理表现。由此可以看出大鼠牙周病模型的建立在整个口腔医学动物实验领域极其重要^[8]。只有建立一种稳定,可靠的牙周病动物模型,才能为接下来探究牙周组织各种病理变化提供支持^[9]。

传统大鼠牙周病模型的建立即使用丝线结扎的方法结扎

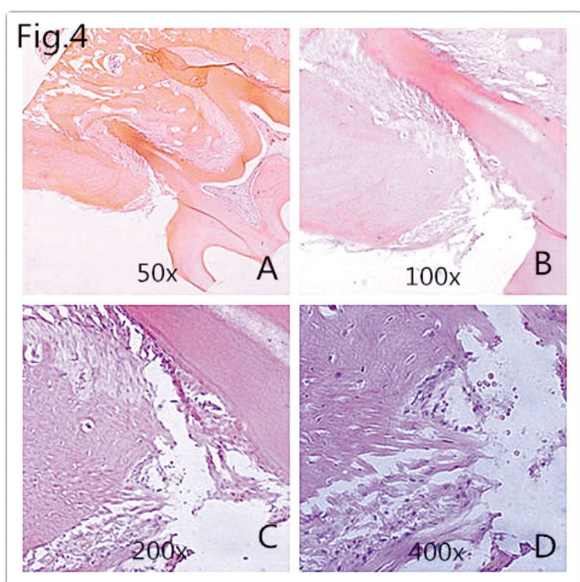


图4 骨组织 HE 染色病理片

A.B.C.D 为从低倍镜到高倍镜下观察牙槽骨组织的 HE 染色,可见近中牙槽骨嵴顶炎症性吸收,骨组织破坏,组织中炎症细胞浸润

Fig.4 HE staining of alveolar bone

A.B.C.D HE staining of alveolar bone was observed from low power microscope to high magnification. Inflammatory resorption, destruction of bone tissue and inflammatory cell infiltration in the alveolar bone near the middle of the visible

大鼠上颌第一磨牙^[10]。大鼠上颌磨牙与人磨牙的牙周组织结构基本相同,所以多采用磨牙进行模型建立^[11]。大量文献选用正畸结扎丝或外科缝合线进行磨牙结扎,同时辅以高糖饮食,目的是使用结扎线既在局部创造一个利于细菌滞留的环境同时结扎丝本身对牙龈组织也是一种直接的刺激,经过一段时间,就可以引起大鼠牙周组织的持续炎症反应,从而破坏牙周组织^[12]。但牙周组织破坏的前提是需要长时间保持这种对牙周组织缓慢的刺激,而大鼠磨牙龈上距离短,磨牙牙体形态呈锥形无明显外形高点,磨牙邻面接触点紧密等一系列客观条件的存在,导致结扎在大鼠牙齿上的丝线极易脱落,从而影响牙周病模型建立的进程^[13]。

玻璃离子水门汀是含氟化物的硅酸铝玻璃粉,与浓缩的聚羧酸水溶液经酸碱反应形成的水门汀。呈半透明状态^[14]。可用于牙体修复、窝沟封闭、冠桥粘固等方面。固化反应本质上为酸碱反应,玻璃粉溶解,离子(Ca^{2+} , Al^{3+})析出,与羧基生成交链状的聚羧酸盐。与牙齿粘接机理是,有机酸作用于牙釉质和牙本质释放出磷和钙离子,这些游离离子进入水门汀产生一层富含离子的结构,形成化学键牢固地附着于牙齿结构^[15]。玻璃离子水门汀可以持续释放氟离子,生物相容性,边缘封闭性好,同时,其操作过程不需要制备洞缘斜面 and 酸蚀釉质,可直接充填粘结修复,简便快捷省时省力^[16,17]。利用玻璃离子水门汀结合传统丝线结扎法建立大鼠牙周病模型,即在丝线结扎大鼠第一磨牙之后,在大鼠磨牙牙颈部丝线附近堆砌有黏着性的玻璃离子水门汀制造利于丝线固定的外形高点并同时稍微垫高咬合面,制造咬合创伤,加快牙周病的发生发展。

通过实验结果可以看出,改良丝线结扎法相比传统丝线结扎法建立大鼠牙周病模型具有更高的稳定性和成功率。传统大鼠牙周病的模型的建立,即利用丝线结扎大鼠上颌第一磨牙,建立有利于菌斑滞留的局部环境,持续刺激大鼠牙周组织,使其产生炎症。但由于大鼠磨牙牙体结构形态,口腔内环境小,以及操作误差等方面的影响,常常导致牙周病模型建立期间丝线脱落,严重影响造模进度从而影响相关实验的顺利进行。

现有众多相关文献改进大鼠牙周病造模方法^[18]。如在大鼠磨牙近中位置用细车针磨一处倒凹以稳定丝线,丝线位置种植牙周致病菌,植入牙石,增加饲料黏着性,体内注射相关免疫因子等方法^[19,20]。但纵观这些方法,尚未发现可以即简化造模流程且能有效提高成功率的方法。故本实验通过验证一种新的造模方法来简化牙周病造模方法,提高模型成功率。为接下来进一步对于牙周病学的研究奠定基础。

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