

声带组织切片方法的比较 *

韩 鹏¹ 赵 昱¹ 王延辉² 梁媛媛¹ 刘 阳¹ 杨润琴¹ 邓志宏^{1△}

(1 第四军医大学西京医院耳鼻咽喉头颈外科中心 陕西 西安 710032; 2 西安市中心医院耳鼻咽喉头颈外科 陕西 西安 710003)

摘要 目的 探讨犬声带冠状位切片与水平位切片各自的特点,为声带实验提供合适的切片方法。方法 家犬 4 只,2 只取材后行冠状位石蜡切片,2 只取材后行水平位石蜡切片。通过 HE 染色观察声带固有层的一般组织结构,Masson 三色染色观察固有层中胶原的排列情况。结果 HE 染色示冠状位、水平位切片均可见声带表面被覆复层鳞状上皮,固有层内有大量排列紧密的纤维组织,纤维组织夹杂少量腺体,固有层下方为肌层。冠状位切片可观察声带某一点冠状面固有层的情况,若观察整个声带的情况需声带连续切片。水平位切片可在一张切片中观察到前联合、声带膜部及声带突部位的固有层情况,解剖标志明显,利于定位。Masson 三色染色示冠状位、水平位切片均可见固有层浅层有较细的胶原纤维束,中层有较粗的纤维束与较细的纤维束交织排列,深层纤维束排列更紧密。结论 冠状位切片可观察声带某一点冠状面固有层的整体情况,水平位切片可在一张切片中观察到前联合、膜部及声带突部位的固有层情况。

关键词 声带 水平位切片 冠状位切片 Masson 三色染色

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Comparison of Vocal Folds Section Methods in a Canine Model*

HAN Peng¹, ZHAO Yu¹, WANG Yan-hui², LIANG Yuan-yuan¹, LIU Yang¹, YANG Run-qin¹, DENG Zhi-hong^{1△}

(1 Department of Otorhinolaryngology, Xijing Hospital of Forth Military Medical University, Xi'an, 710032, China;

2 Department of Otorhinolaryngology, Xi'an Central Hospital, Xi'an, 710003, China)

ABSTRACT Objective: To investigate the methods of vocal folds sections in a Canine model, and provide reliable section methods for vocal fold experiment. **Methods:** 4 dogs were chosen, 2 underwent coronal paraffin sections, while 2 underwent horizontal paraffin sections. The general structure and the collagen of lamina propria (LP) were observed by HE staining and Masson trichrome staining. **Results:** In both coronal sections and horizontal sections the surface of vocal folds were covered with stratified squamous epithelium. There was a large number of tightly packed fibrous tissue mixed with a small amount of glands in the lamina propria, and beneath the LP was muscle layer. The coronal situation of LP can be observed in coronal sections, and serial sections will be needed to detect the whole situation of vocal folds. The anterior parts, membranous parts and vocal process of vocal folds can be observed in horizontal sections with obvious anatomical landmarks. Masson trichrome staining shows thinner collagen fiber bundles can be observed in the superficial layer of the LP, in the Middle layer thicker and thinner fiber bundles are arranged together, and in the deep layer the thick fiber bundles are arranged closely. **Conclusion:** The coronal situation of LP can be observed in coronal sections, and serial sections will be needed to observe the whole situation of vocal folds. The anterior parts, membranous parts and vocal process of vocal folds can be observed in horizontal sections with obvious anatomical landmarks.

Key words: Vocal folds; Coronal sections; Horizontal sections; Masson trichrome staining

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前言

反复的声带损伤、炎症均可导致声带瘢痕,引起声嘶、声音沙哑等症状,了解声带瘢痕的组织学变化对声带瘢痕的修复有着极其重要的意义。声带固有层的组织结构是正常发声的基础,胶原纤维是声带固有层最主要的细胞外基质成分(ECM),起到 ECM 支架的作用,当声带固有层受到损伤时,胶原纤维增生并排列紊乱,影响声带的粘弹性,从而产生发声障碍。因此,胶原纤维的改变可以大体反映声带固有层瘢痕的形成情况^[1-4]。

为了更好的观察声带损伤后瘢痕形成的情况,选择合适的声带切片方式十分重要。目前常用的声带切片方式有冠状位切片和水平位切片,但很少有文献报道这两种切片方式的不同^[5-7]。犬因其声带结构与人相似,是理想的实验动物,因此本研究选用犬声带为实验对象,冠状位及水平位切片后 HE 染色观察固有层的一般组织结构,Masson 三色染色观察固有层中胶原纤维的排列,为声带瘢痕修复实验提供合适的切片方式。

1 材料与方法

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作者简介 韩鹏,男,硕士研究生,医师,研究方向 喉部组织工程,电话:15829925420, E-mail: mriver2010@qq.com

△通讯作者:邓志宏,教授,博士生导师, E-mail: dengzh@fmmu.edu.cn

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1.1 实验动物

健康成年家犬 4 只,体重 10-15 kg,随机分为 2 组。实验动物由第四军医大学实验动物中心提供。

1.2 主要材料及器材

石蜡切片机 (Leica RM2125)、速眠新 II (沈阳畜牧公司)、Masson 三色染色套装 (珠海贝索生物)、图像采集电脑及显微镜 (Olympus DP2BSW)。

1.3 实验方法：

动物麻醉前禁食 12 h,经后肢肌肉注射速眠新 0.1 mg/kg,麻醉后声带取材,4%多聚甲醛固定,2 对声带行冠状位石蜡切片,2 对声带行水平位石蜡切片,切片厚 3 μm,切片常规脱蜡至水,分别行 HE 染色及 Masson 三色染色。通过 HE 染色观察声带固有层的一般组织结构,Masson 三色染色观察固有层中胶原的排列情况。

2 结果

2.1 组织学切片

2.2.1 HE 染色结果 光镜下 HE 染色示冠状位、水平位切片均可见声带表面被覆复层鳞状上皮,固有层内有大量排列紧密的纤维组织,纤维组织中夹杂少量腺体,固有层下方为肌层。

冠状位切片可观察到声带某一点冠状面固有层的情况,声带冠状面上端 (近室带部) 及下端 (近环状软骨部) 的固有层较薄,分层不明显,冠状面中部 (声带闭合时的接触面) 固有层较厚,分层明显 水平位切片可在一张切片中观察到前联合、声带膜部及声带突部位的固有层情况,声带突在此切片中可见 (图 1)。

2.2.2 特殊染色结果 Masson 三色染色示胶原纤维呈蓝色,肌肉组织呈红色。冠状位、水平位切片均可见固有层浅层有较细的胶原纤维束,中层较细的纤维束与较粗的纤维束交织排列,深层纤维束排列更紧密。在胶原纤维的分布及排列上,冠状位与水平位未见明显的区别 (图 2)。

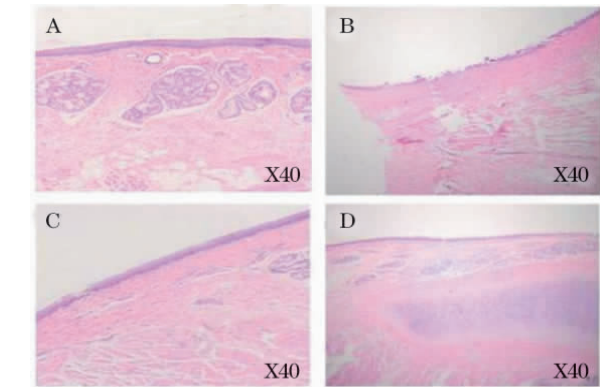


图 1 声带石蜡切片 HE 染色

A: HE 染色示声带冠状位切片,固有层分层清楚,利于固有层结构的观察 B: HE 染色示声带水平位切片 (前联合处) C: HE 染色示声带水平位切片 (膜部中点) ;D: HE 染色示声带水平位切片 (声带突部,声带突为蓝色软骨组织)

Fig.1 Paraffin sections of the vocal folds by HE staining

A: The coronal section of vocal fold (HE staining) B: The horizontal section of vocal fold (Anterior part, HE staining) C: The horizontal section of vocal fold (Membranous part, HE staining) ;D: The horizontal section of vocal fold (Vocal process, HE staining)

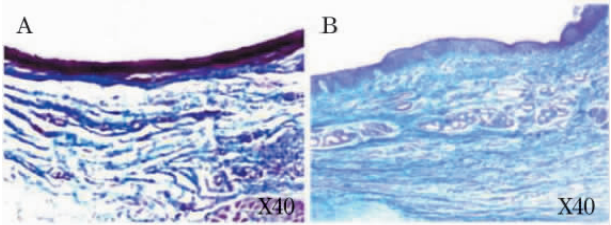


图 2 声带石蜡切片 Masson 三色染色

A: Masson 三色染色示声带冠状位切片胶原排列情况 (胶原纤维呈蓝色) B: Masson 三色染色示声带水平位切片胶原排列情况

Fig.2 Paraffin sections of the vocal folds by Masson trichrome staining

A: The collagen arrangement of vocal fold in coronal sections(Collagen is blue bundle, Masson trichrome staining) B: The collagen arrangement of vocal fold in horizontal sections(Collagen is blue bundle ,Masson trichrome staining)

3 讨论

声带位于喉腔室带下方,左右各一,从浅至深由上皮层、固有层和肌层组成,前起甲状软骨板交角内面,后端止于杓状软骨的声带突。为观察声带损伤修复的情况,研究人员不仅要大体形态上观察声带损伤愈合的情况,还要从组织水平观察声带愈合情况^[8-15]。而声带组织的解剖结构是研究声带损伤的关键,尤以声带前 2/3,即膜部最重要,经研究证实,声音是由膜部震动产生,声带后 1/3 的软骨部只对发声起支持作用。固有层位于声带上皮及肌层之间,是产生正常黏膜液的重要组织结构,因此固有层的情况是保证声带正常震动的基础^[16-20]。

然而,声带的形状为长条形,组织切片的方式有冠状位、水平位及矢状位。由于解剖的因素,矢状位切片无法满足对声带固有层结构的观察,因此应用很少。冠状位、水平位为最常用的切片方式,但至今无文献报道两种方式的不同。本实验从组织学水平观察两种切片方式的特点,通过 HE 染色观察固有层的一般组织结构,Masson 三色染色观察固有层中胶原的排列情况。HE 染色示冠状位、水平位切片均可见声带表面被覆复层鳞状上皮,固有层内有大量排列紧密的纤维组织,纤维组织中夹杂少量腺体,固有层下方为肌层。冠状位切片可观察声带某一点冠状面固有层的情况,若观察整个声带的情况需声带连续切片,声带冠状面顶端 (近室带部) 及底端 (近环状软骨部) 的固有层较薄,分层不明显,冠状面中部 (声带闭合时的接触面) 固有层较厚,且分层清楚,利于固有层结构的观察 水平位切片可在一张切片中观察到前联合、声带膜部及声带突部位的固有层情况,解剖标志明显,声带突清晰可见,利于定位,但水平位切片的不同层面固有层厚度不一,若双侧声带切片进行比较时,两侧切片固有层厚度可能不一致,需连续水平位切片选择厚度相近切片进行比较。Masson 三色染色示冠状位、水平位切片均可见固有层浅层有较细的胶原纤维束,中层有较粗的纤维束与较细的纤维束交织排列,深层纤维束排列更紧密,两种切片方式在胶原纤维的观察上无明显区别。

4 结论

冠状位切片可观察声带某一点冠状面固有层的整体情况,

水平位切片可在一张切片中观察到前联合、膜部及声带突部位的固有层情况。本实验探讨了冠状位与水平位切片观察声带固有层结构的特点,为声带实验提供了可借鉴的切片方式。

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