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异种脱钙骨基质、异种骨基质胶加释放系统移植的组织学观察*

林贵德 柯新华 王声文** 周 萍** (第三军医大学大坪医院骨科) 630042

提 要 Wistar大鼠24只分4组,分别将4种复合异种骨植入大鼠股四头肌内,定期取材制片,光镜及TEM-10SX透射电镜观察。结果:2 d巨噬细胞等密集成团,细胞器发达;4 d间充质细胞伪足伸入异种脱钙骨基质(Demineralized xenogeneic bone matrix,XDBM)、异种骨基质胶(Xenogeneic bone matrix gelatin,XBMG)隙内;8 d间充质细胞增多、细胞器发达,常与成软骨细胞、软骨细胞并存在一个网眼内;14 d软骨细胞团增多并出现软骨内化骨,有的软骨细胞固缩;22 d成骨细胞增多且细胞器丰富,骨细胞之细胞突起明显;34 d新生骨组织增多,骨细胞突起相互联结成网,骨髓细胞出现,始终未见破骨细胞。结果表明:诱导成骨的细胞来源——间充质细胞;成骨方式——软骨内化骨。

关键词 骨基质;释放系统*;骨发生;移植,异体
中图法分类号 R322.71

异种脱钙骨基质(Demineralized xenogeneic bone matrix,XDBM),异种骨基质胶(Xenogeneic bone matrix gelatin,XBMG)加释放系统(Deliverly system,DS)移植成功后[1,2];为观察早期的组织学和超微结构变化,深入认识诱导成骨的细胞来源和成骨方式而进行本实验。

1 材料与方法

1.1 XDBM、XBMG制备

取小公牛四肢长管状骨按实验方法制成[1~4];聚乙烯薄膜封装,环氧乙烷消毒,培养无菌贮4℃冰箱。

1.2 DS配制

配成25 mmol/L $CaSO_4$、25 mmol/L $CaCl_2$、5 mmol/L Na_2HPO_4、5 mmol/L KH_2PO_4,高压消毒。

1.3 分组

Wistar大鼠24只,体重110~145.5 g(平均130.8 g),分4组每组6只:(1)XDBM 40 mg加DSA($CaSO_4$、Na_2HPO_4、KH_2PO_4);(2)XDBM 40 mg加DSB($CaCl_2$、Na_2HPO_4、KH_2PO_4);(3)XBMG 40 mg加DSA;(4)XBMG 40 mg加DSB,不设对照。

1.4 移植

1%戊巴比妥钠0.35 ml/100 mg腹腔内麻醉,分别植入4种移植物于股四头肌内。

1.5 观察

术后第2、4、8、14、22、34天取材,每个标本分成2份,一份作光镜观察,另一份用TEM-10SX透射电镜观察。

2 结果

综观4组的组织学与超微结构基本相似,描述以(1)组为主,各组相异处作补充。

第2天,巨噬细胞、淋巴细胞、浆细胞等密集成团,XDBM周围较多,部分呈索条状伸入XDBM、XBMG隙内;少量间质细胞贴附在XBMG、XDBM表面。电镜下巨噬细胞体积大,细胞表面有较多微绒毛状突起,有的伸入XDBM、XBMG孔隙内,细胞核大小、形态均不甚规则,异染色质呈块状,胞质内大量线粒体、溶酶体及粗面内质网,有的扩大成池,高尔基氏体较发达。

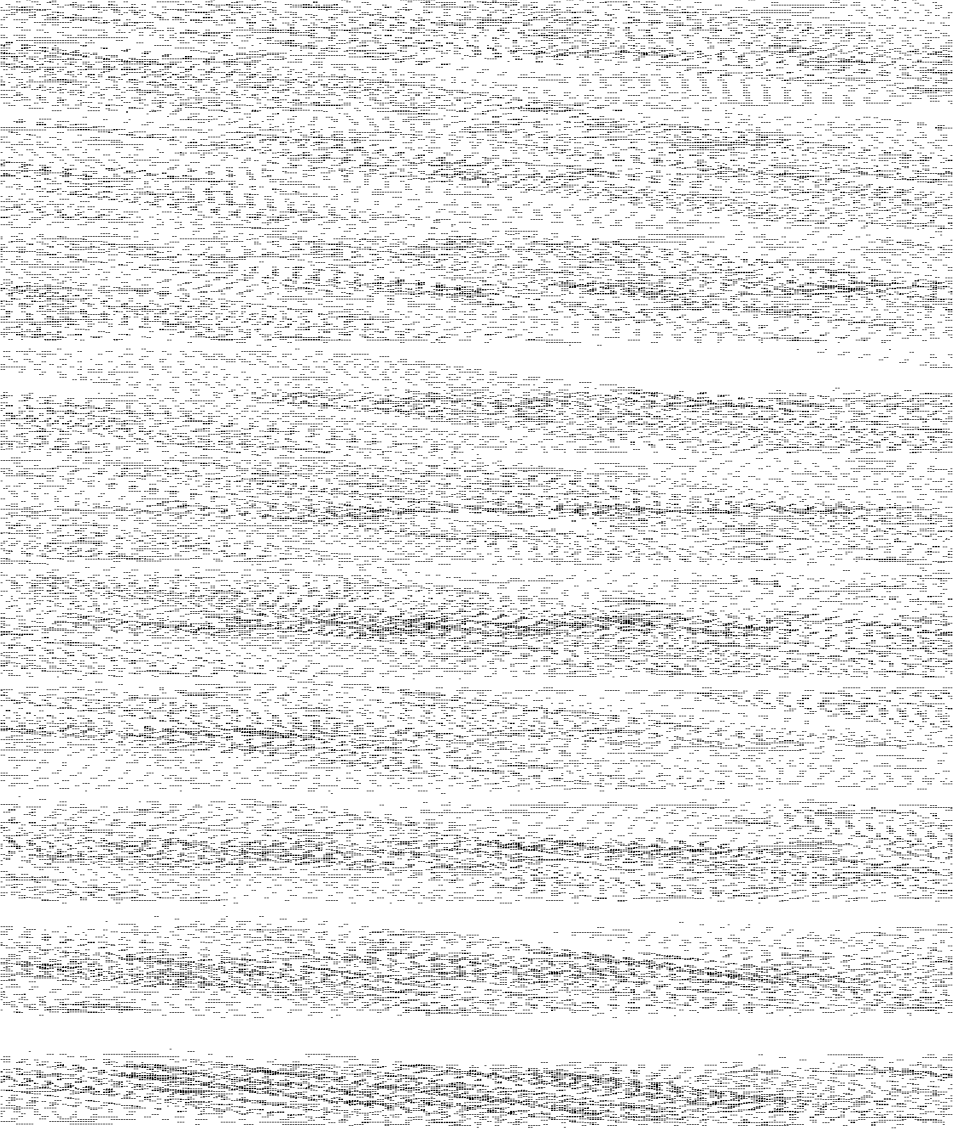
第4天,间充质细胞增殖呈条索状排列并伸入XDBM、XBMG隙内。电镜下巨噬细胞内见吞噬的XDBM、XBMG残片;血管内皮细胞未见肿胀,形态正常;间充质细胞(图1)呈梭形或长圆形,核偏心形状不规则,异染色质围在核膜周围,线粒体,短管状粗面内质网不多;XBMG组间充质细胞的细胞器较为发达。

第8天,少量成软骨细胞、软骨细胞出现。电镜下间充质细胞与XDBM、XBMG间出现吸收间隙,线粒体、溶酶体增多,粗面内质网扩大成池,池内出现低电子密度物质,高尔基氏体发达;有的间充质细胞与成软骨细胞、软骨细胞并存一个网眼内。成软骨细胞(图2)体积大,圆形或卵圆形,表面微绒毛状突起较多,常染色质丰富,线粒体、溶酶体较多,粗面内质网扩大成囊成池内有低电子密度物质,高尔基氏体发达。软骨细胞圆形,少量微绒毛状突起,有的如扇蛤状。(3)、(4)组的细胞器较(1)、(2)组发达,周围胶原纤维较多,羟基磷灰石沉积。

第14天,软骨细胞团增多,边缘出现成骨细胞群且向初期骨小梁过渡呈软骨内化骨演变。电镜下少数软骨细胞肥大、变性、溶解,核固缩,成骨细胞呈多边形,核圆,常染色质多;线粒体、溶酶体、粗面内质网丰富,有的扩大成囊,高尔基氏体发达;细胞周围胶原纤维排列规则,羟基磷灰石沉积;骨细胞少而幼

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monphogenetic protein，BMP）加速释放的作用；而 DS 中的另一组成份 Na_2HPO_4、KH_2PO_4 具有促进羟基磷灰石沉积的能力；而整个 DS 可能还备有削弱 XDBM、XBMG 移植的免疫原性的功用；这从巨噬细胞较长时间的吞噬 XDBM、XBMG 残片，且一直未转化为破骨细胞也是一个旁证。

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Histologic observation on the manifestations after the grafting of demineralized xenogeneic bone matrix and xengogeneic bone matrix gelatin with delivery system※

Lin Guide, Ke Xinhua, Wang Shengwen, Zhou Ping (Department of Orthopedics, Daping Hospital, Third Military Medical College, Chongqing, 630042)

Abstract Twenty-four Wistar rats were randomized into 4 groups and 4 types of grafts were transplanted into the quadriceps of the rats respectively. Tissue samples were taken from quadriceps regularly for histological examination under electron microscopy. It was found that many phagocytes accumulated in groups with well developed organelles on the 2nd day; the pseudopods of the mesenchymal cells extended into the interstices of the demineralized xenogeneic bone matrix (XDBM) and xenogeneic bone matrix gelatin (XBMG) on the 4th day; mesenchymal cells increased in number with well developed organelles and they usually existed with chondroblasts and chondrocytes in a same field on the 8th day; the number of chondrocyte masses was increased and endochondral ossification was seen, and some chondrocytes underwent pyknosis on the 14th day; osteoblasts increased in number with well developed organelles and osteocytes were shown to have prominent projections on the 22nd day; the amount of newly formed bone increased, the projections of osteocytes were connected with each other, bone marrow cells began to appear and no osteoclasts were seen on the 34th day. The findings indicate that the source of cells in induced bone formation is the mesenchymal cells and the way of bone formation is endochondral ossification.

Key words bone matrix; delivery system*; osteogenesis; transplantation, heterologous

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