中南大学学报(医学版) 2012, 37(3) 260-266 DOI: 10.3969/j.issn.1672-7347.2012.03.009 ISSN: 1672-7347 CN: 43-1427/R

论著

肝素铁复合物纳米修饰提高异种移植血管的血液相容性

陶运明[1,2], **胡铁辉**[1], **吴忠仕**[1], **唐浩**[1], **胡野荣**[1], **谭琦**[1]

1. 中南大学湘雅二医院胸心外科, 长沙 410011;
2. 江西省吉安市中心人民医院, 江西 吉安 343000

摘要:

目的:采用肝素铁复合物纳米修饰去细胞异种血管以提高其血液相容性。方法:采用层层自组装技术将二羟基铁和肝素交替固定在去细胞牛颈静脉(DC-BJV) 表面,构建一种新型的多层肝素铁复合物(HICMs) 抗凝表面,并通过扫描电镜(SEM) 检测其表面微结构、拉力器检测其生物力学稳定性、抗凝血活性实验和血小板黏附实验检测其血液相容性。结果:每组装一次,约有(808±86) μg/cm^2 肝素固定在BJV 表面;SEM 图片显示HICMs 是均匀地包裹在胶原纤维表面并形成纳米膜;组织切片甲苯胺蓝染色提示肝素主要结合在DC-BJV 的表层;拉力试验提示实验组的生物力学稳定性有显著提高;经过1 d 及1,2,3,4,6,8 周的洗脱,肝素的释放量分别为(281±43),(422±60),(729±81),(1053±116),(1317±157),(1618±187),(1945±228) μg/cm^2;抗凝血活性检测显示实验组凝血酶原时间(PT)和活化部分凝血酶原时间(APTT) 高于正常值范围;血小板黏附试验显示每10000 μm^2 HICMs 层层自组装修饰的牛颈静脉(LBL-BJV) 和DC-BJV 的血小板计数分别为8±4 和48±16。结论:HICMs 牢固地结合在DC-BJV 表层,形成纳米厚度的抗凝表面,并长时间地缓慢释放肝素。HICMs 纳米修饰能够提高去细胞异种血管的血液相容性。

关键词: 肝素 自组装 纳米修饰 血液相容性 牛颈静脉

Heparin-iron complex multilayer nanomodification improves hemocompatibility of decellular xenograft

TAO Yunming[1,2], HU Tiehui[1], WU Zhongshi[1], TANG Hao[1], HU Yerong[1], TAN Qi[1]

1. Department of Cardiothoracic Surgery, Second Xiangya Hospital, Central South University, Changsha 410011;
2. Department of Cardiothoracic Surgery, Ji'an Central People's Hospital, Ji'an Jiangxi 343000, China

Abstract:

Objective: To improve the hemocompatibility of decellular vascular matrix via heparin-iron complex multilayers (HICMs) nanomodification.
Methods: A novel thrombo-resistant surface for decellular xenograft was developed by alternating linkage of dihydroxy-iron and heparin to decellular bovine jugular vein (DC-BJV), and its surface characterization, biomechanical stability and hemocompatibility were detected by scanning electron microscopy, tensile test and hemocompatibility evaluation, respectively.
Results: A toluidine blue colorimetric method indicated the amount of linked heparin was about (808±86) μg/cm^2 per assembly-cycle. Scanning electron microscopic (SEM) images proved that HICMs were uniformly linked to and formed nanoscale films around the fibrils of DC-BJV. Toluidine blue staining histologic images showed that HICMs were linked mainly to DC-BJV surfaces. Washing test showed that the release of heparin was (281±43), (422 ± 60), (729±81), (1053±116), (1317±157), (1618±187) and (1945 ± 268) μg/cm^2 at 1 day, 1, 2, 3, 4, 6 and 8 week washing, respectively. Tensile tests showed an increased biomechanical stability. Hemocompatibility evaluations showed that PT and APTT of all the trial groups were above the normal reference ranges and that mean platelet count per 10000 μm^2 area was 8±4 for HICMs layer-by-layer modified BJV (LBL-BJV) vs 48±16 for DC-BJV.
Conclusion: HICMs are firmly linked to DC-BJV, and can form nanoscale thrombo-resistant films, which yield a sustained release of heparin. HICMs nanomodification improves the hemocompatibility of decellular xenograft.

Keywords: heparin self-assembly nanomodification hemocompatibility bovine jugular vein

收稿日期 2011-05-05 **修回日期** **网络版发布日期**

DOI: 10.3969/j.issn.1672-7347.2012.03.009

基金项目:

国家高新技术研究发展计划项目(2007AA071900);国家自然科学基金(81071275)。

通讯作者: 吴忠仕,Email: owenzswu@sina.com

作者简介: 陶运明,博士,副主任医师,主要从事生物材料和纳米材料方面研究。

作者Email: owenzswu@sina.com

参考文献:

1. Lu WD,Zhang M,Wu ZS,et al.Decellularized and photooxidatively crosslinked bovine jugular veins as potential tissue engineering scaffolds
[J].Interact Cardiovasc Thorac Surg,2009,8(3):301-305.
2. Lu WD,Zhang M,Wu ZS,et al.The performance of photooxidatively crosslinked acel lular bov ine jugular vein conduits in the reconstruction of connections between pulmonary arteries and right ventricles
[J].Biomaterials,2010,31(10):2934-2943.
3. Schmidt CE,Baier JM.Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering
[J].Biomaterials,2000,21(22):2215-2231.
4. Tschoeke B,Flanagan TC,Cornelissen A,et al.Development of a composite degradable/ nondegradable tissue-engineered vascular graft
[J].Artif Organs,2008,32(10):800-809.
5. Crikis S,Cowan PJ,d'Apice AJ.Intravascular thrombosis in discordant xenotransplantation
[J].Transplantation,2006,82(9):1119-1123.
6. Liao D,Wang X,Lin PH,et al.Covalent linkage of heparin provides a stable anti-coagulation surface of decellularized porcine arteries
[J].J Cell Mol Med,2009,13(8B):2736-2743.
7. Wang XN,Chen CZ,Yang M,et al.Implantation of decellularized small-caliber vascular xenografts with and without surface heparin treatment
[J].Artif Organs,2007,31(2):99-104.
8. Basmadjian D,Sefton MV.Relationship between release rate and surface concentration for heparinized materials
[J].J Biomed Mater Res,1983,17(3):509-518.
9. Balamurugan K,Kalaichelvan AD.Isolation and identification of heparin like substances from the body tissue of conus musicus
[J]. Internat J PharmTech Res,2009,1(4):1650-1653.
10. Smith PK,Mallia AK,Hermanson GT.Colorimetric method for the assay of heparin content in immobilized heparin preparations
[J]. Anal Biochem,1980,109(2):466-473.
11. Liu M,Yue X,Dai Z,et al.Novel thrombo-resistant coating based on iron-polysaccharide complex multilayers
[J].ACS Appl Mater Interfaces,2009,1(1):113-123.
12. Rabenstein DL.Heparin and heparan sulfate: structure and function
[J].Nat Prod Rep,2002,19(3):312-331.
13. Ozkan S,Akay TH,Gultekin B,et al.Xenograft transplantation in congenital cardiac surgery at baskent university: midterm results
[J]. Transplant Proc,2007,39(4):1250-1254.
14. Fiore AC,Ruzmetov M,Huynh D,et al.Comparison of bovine jugular vein with pulmonary homograft conduits in children less than 2 years of age
[J].Eur J Cardiothoracic Surg,2010,38(3):318-325.
15. Lee WK,Park KD,Han DK,et al.Heparinized bovine pericardium as a novel cardiovascular bioprosthesis
[J].Biomaterials,2000,21(22):2323-2330.
16. Wissink MJ,Beernink R,Pieper JS,et al.Immobilization of heparin to EDC/NHS- crosslinked collagen.Characterization and in vitro evaluation
[J].Biomaterials,2001,22(2):151-163.
17. Nugent MA.Hepar in s equenc ing br ings st r uc ture to the function of complex oligosaccharides
[J].Proc Natl Acad Sci USA,2000,97(19):10301-10303.
18. Wagenseil JE,Mecham RP.Vascular extracellular matrix and arterial mechanics
[J].Physiol Rev,2009,89(3):957-989.
19. Mark K,Park J,Bauer S,et al.Nanoscale engineering of biomimetic surfaces: cues from the extracellular matrix
[J].Cell Tissue Res,2010,339(1):131-153.

本刊中的类似文章

1．张浩； 季龙振； 戴中伟； 伍锟； 季迎； .低分子量肝素对增生性肾炎尿白细胞介素-6的影响[J]. 中南大学学报(医学版), 2001,26(5): 443-
2．李伟1，2， 杨丽娟3， 吴忠仕1， 张庆广2， 刘哲亮1， 胡建国1， 刘文亮1.去细胞光氧化处理牛颈静脉带瓣管道的形态学研究[J]. 中南大学学报(医学版), 2008,33(06): 500-506
3．蒋玲,喻杰锋.感染性心内膜炎患者体外循环手术中ACT的监测[J]. 中南大学学报(医学版), 2007,32(01): 167-169
4．吴忠仕*，胡建国，杨一峰，王晖，邓又华，刘文亮，胡野荣，杨晓涵.带瓣牛颈静脉在治疗复杂先天性心脏病中的应用[J]. 中南大学学报(医学版), 2005,30(4): 471-473
5．冯耀光，吴忠仕*，胡建国，胡铁辉，张竟超，徐朝军，王晖，马忠夏.光氧化反应处理牛颈静脉带瓣管道的形态学与理化性能研究[J]. 中南大学学报(医学版), 2004,29(4): 429-431
6．黄增辉， 曾珊， 欧阳淼， 等.肝素联合肝干细胞经脾移植治疗SD大鼠急性肝损伤[J]. 中南大学学报(医学版), 2011,36(5): 411-
7．张浩； 季龙振； 伍锟； 季迎； 杨君； .低分子量肝素延缓进行性肾小球硬化的实验研究[J]. 中南大学学报(医学版), 2001,26(1): 7-

---