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血管内皮生长因子(VEGF)乳液法电纺纤维膜的体外研究 *

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摘要 目的:研究担载血管内皮生长因子(VEGF)的乳液法电纺纤维膜的亲水性能、外观形态和机械性能,纤维膜中 VEGF 的包封率和体外释放动力学,为评价其能否应用于血管再生领域的研究奠定基础。**方法:**将 VEGF 水溶液通过 W/O 乳液法制备成缓释 VEGF 的生物可降解的丙交酯 - 乙交酯共聚物(PLGA)静电纺丝纤维膜,对该纤维膜的接触角、外观形态、机械性能进行表征,Elisa 法测定该纤维膜的体外 14 天的释放行为,分别观察纤维膜释放 0 天、7 天、14 天后的电镜图。**结果:**加入 VEGF 后,纤维膜的接触角由 140.0° 减小到 136.1°,亲水性增强,具有类似细胞外基质(ECMs)网状结构和良好的力学性能,纤维膜第 1 天的突释不超过载药量的 50%,电镜图下显示纤维膜释放 1 周时纤维发生断裂。**结论:**通过乳液法制备的担载 VEGF 的电纺纤维膜具有良好的物理性能,能够持续缓释 VEGF,可作为血管再生的组织工程支架进行深入研究。

关键词:血管内皮生长因子;乳液法;电纺纤维膜;PLGA;血管再生

中图分类号:R318;R945 **文献标识码:**A **文章编号:**1673-6273(2014)28-5443-03

Study on the Vascular Endothelial Growth Factor (VEGF) emulsion Electrospun fiber Membrane in Vitro*

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ABSTRACT Objective: To investigate the hydrophilic performance, morphology and mechanical properties of emulsion electrospun fiber membrane loading vascular endothelial growth factor (VEGF), the coating rate and release kinetics of VEGF in vitro in the fiber membrane, and evaluate whether it could be applied in the research of angiogenesis. **Methods:** VEGF aqueous solution was prepared into a VEGF sustained-release PLGA electrospun fiber membrane by W/O emulsion method. The contact Angle, exterior morphology, and mechanical properties of the fiber membrane were characterized. Elisa method was to detect the release behavior of the fiber membrane in vitro. The fiber membrane SEM 0 day, 7 days, and 14 days after release were observed respectively. **Results:** After adding VEGF, the contact Angle of fiber membrane reduced from 140.0° to 136.1°, its hydrophilicity enhanced, the fiber membrane had similar extracellular matrix (ECMs) mesh structure and good mechanical properties, initial burst release fiber membrane was no more than 50% drug loading capacity on the first day, fiber membrane had a break under SEM in a week. **Conclusion:** VEGF emulsion electrospun fiber membrane had good physical properties, continuous sustained release of VEGF, which could be used as angiogenesis tissue engineering scaffolds for further research.

Key words: VEGF; Emulsion method; Electrospun fiber membrane; PLGA; Revascularization

Chinese Library Classification(CLC): R318; R945 Document code: A

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前言

20 世纪 80 年代,随着生物学技术的进步,血管组织工程(tissue-engineered vascular grafts,TEVG)也发展起来,其主要任务是构建生物血管,包括血管种子细胞、血管生物材料支架和细胞因子[1-3]。在 TEVG 中,血管支架材料的构建尤其重要,其可为组织工程血管提供一定的机械强度和力学特性,支撑活细胞的生长,为细胞的生长提供适宜的三维空间,以便细胞的黏附、生长和代谢[4-8]。静电纺丝技术能够制备出连续的纳米级或亚微米级超细纤维,具有较大的比表面积和孔隙率,能够模仿天然细胞外基质(ECMs)的纳米网状结构和生物功能,为细胞获取营养、生长、代谢和分泌细胞外基质,最终形成相应的组织或器官提供良好的环境,这些特点使静电纺丝技术在血管组织工程支架制备方面具有广泛的应用前景[9-13]。生物可降解的丙交酯 - 乙交酯共聚物(PLGA)因其优异的生物相容性和可控的降解性能在组织工程材料、药物释放领域及静电纺丝纳米技术领域得到了广泛的应用[14-17]。VEGF 能特异性地作用于血管内皮细胞,具有维持血管功能、提高血管的通透性、促进血管生成的作用,在血管再生、组织修复等方面的作用引起了极大关注[18]。如何将 VEGF 加入到血管支架材料中并使其保持有效浓度的持续释

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放是目前血管组织工程领域急需解决的问题[19]。本研究将VEGF水溶液通过W/O乳液法制备成缓释VEGF的PLGA静电纺丝纤维膜，并对该纤维膜的接触角、外观形态和机械性能以及体外释放动力学进行研究，以期为评价其能否应用于血管再生领域奠定基础。

1 材料与方法

1.1 仪器与试剂

静电纺丝机(ss-2534H，北京永康乐业科技有限公司)；冷冻干燥机(CHRIST Alpha 1-4，德国CHRIST公司)；场发射扫描电子显微镜(S-2700，日本Hitachi公司)；恒温空气浴摇床(HZ-8812S，太仓市科教器材厂)；酶标仪(芬兰Thermo Labsystem公司)；Theta Lite光学接触角仪(Optical Tensionmeter)(瑞典百欧林科技有限公司)；万能电子拉力机(美国Instron公司)；聚乳酸-羟基乙酸共聚物(PLGA)(PLA/PGA=75:25，美国Lakeshore Biomaterials公司)；重组人血管内皮细胞生长因子蛋白(VEGF165)(R&D system公司)；VEGF165 ELISA试剂盒(R&D system公司)；葡聚糖(MW 64,000-70,000)，聚乙二醇6000(PEG 6000)均购自SIGMA公司；四氢呋喃，N,N-二甲基甲酰胺，二氯甲烷等试剂均为分析纯，购自国药集团化学试剂有限公司。

1.2 实验方法

1.2.1 纤维膜的制备 VEGF的乳液法电纺纤维膜的制备：配置200 μL一定浓度的蛋白水溶液作为内水相，常温下用1.5 mLN,N-二甲基甲酰胺(DMF)：四氢呋喃(THF)=1：3(体积比)溶解300 mgPLGA，配制浓度为0.20 g/mL的PLGA溶液作为油相。将内水相逐滴加至外油相中，采用磁力搅拌1000 rpm，30 min，形成W/O乳液。将上述制备好的浓度为0.20 g/mL的PLGA溶液转入静电纺丝设备的注射泵中，喷丝头与接受板的间距为15 cm，电场强度为10 KV，在1 mL/h的流速下静电纺丝，收集、在室温下晾干，获得空白电纺纤维膜；将上述W/O乳液转入静电纺丝设备的注射泵中，同样的纺丝条件下制备得到乳液法电纺纤维膜。

1.2.2 静电纺丝纤维膜的表征 将制备好的纤维膜样品平铺固定于载样台的导电胶上，表面喷金后于扫描电镜(SEM)下观察纤维膜的表面形态和直径大小。将制备好的乳液法和空白纤维膜平铺置于载样台，液滴滴于纤维膜表面，通过光学接触角仪测定纤维膜的接触角，以表征纤维的亲水性能。将制备好的两种种纤维膜样品制成哑铃状，使用拉力机测定两种种样品的力学性能参数，以表征纤维是否具备成为组织工程支架所需的机械性能。

1.2.3 VEGF纤维膜体外释放研究 精密称量乳液法纤维膜50 mg放入释放瓶中，加入1 mL PBS释放液(pH 7.4)中，加盖密封，置于37℃恒温振荡摇床中(100 r/min)。分别在第1、2、3、4……14天取出1 mLPBS释放液，并补充1 mL新鲜PBS释放液，放回摇床。VEGF165 ELISA试剂盒检测释放液中VEGF165的含量，计算各时间点的累积释放量，并绘制出纤维膜的累积释放曲线。

2 结果

2.1 纤维的表征

如图1所示，纤维膜呈现互相相通的三维网状结构，能够

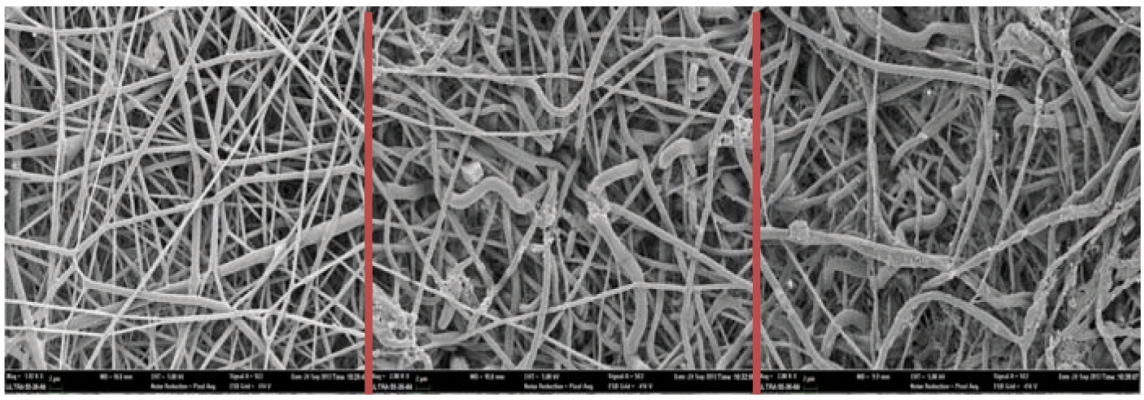

图1 乳液法纤维膜体外释放0、7、14天后的扫描电镜图

Fig.1 SEM image of emulsion method fiber membrane 0,7,14 days after release

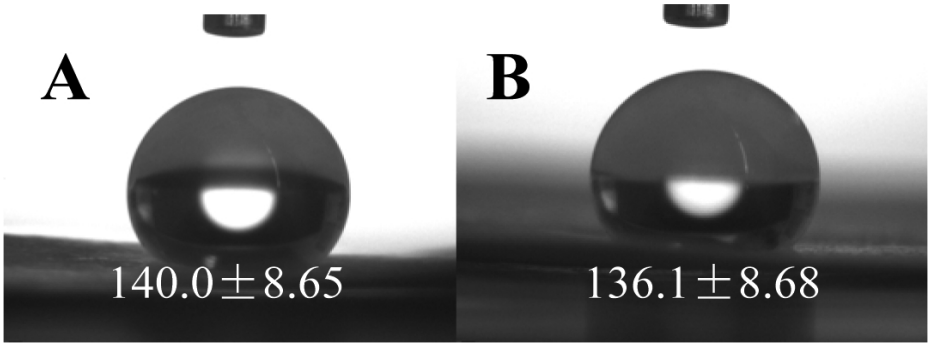

图2 纤维膜的接触角

Fig.2 Contact angle of the fiber membrane

较好地模拟天然细胞外基质(ECM)的结构,纤维的直径分布在200 nm-1000 nm之间,电镜图下显示纤维膜释放一周时纤维就发生断裂(图1C)。图2是乳液法纤维膜的接触角测试结果图,A图为空白PLGA纤维膜的接触角,B图为VEGF乳液法纤维膜的接触角,PLGA本身是疏水性材料,加入了内水相VEGF,纤维膜的接触角由140.0°减小到136.1°,接触角有所减小,亲水性增强。表1为纤维膜的力学性能,A图为空白PLGA纤维膜,B图为VEGF乳液法纤维膜,包括膜的厚度、弹性模量、断裂时的拉伸应变、拉伸应力和位移等参数。由表中数据可见,纤维膜具有良好的弹性和抗拉伸性能。

表1 纤维膜的力学性能

Table 1 Mechanical properties of the fiber membrane

Sample	Thinkness(mm)	Modulus of elasticity (MPa)	Tensile strain(mm/mm)	Maximum tensible strength(MPa)	Displacement(mm)
A	0.11	81.07± 10.05	5.25± 0.66	3.65± 0.23	54.56± 6.2
B	0.11	62.1± 7.5	3.21± 0.42	3.36± 0.63	53.78± 9.6

2.2 VEGF纤维膜的体外释放动力学

乳液法纤维VEGF体外累积释放曲线如图3所示,VEGF的累积释放时间为14天,纤维膜的第一天释放量到达总释放量的45%,存在着一定的突释,但是14天中的释放量能维持血管再生所需VEGF的有效浓度。

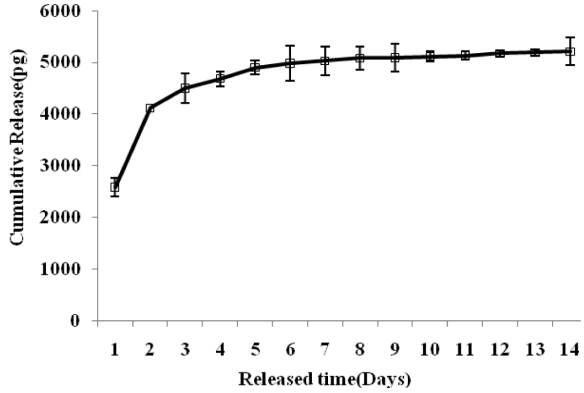

图3 VEGF纤维膜的体外累积释放曲线

Fig.3 Cumulative release of the VEGF fiber membrane in vitro

3 讨论

理想的血管组织工程支架材料需要具备良好的生物相容性、生物可降解性、低免疫原性、可塑性,为组织工程血管提供一定的机械强度和力学特性,以支撑活细胞的生长,为细胞的生长提供适宜的三维空间,便于细胞的黏附、生长和代谢,并能够持续缓慢释放血管内皮细胞生长因子促进血管再生[20]。本研究通过乳液法制备的静电纺丝纤维膜具有良好的外貌形态,能够模仿天然细胞外基质(ECMs)的三维网状结构,有利于细胞的黏附和增殖,加入VEGF后的纤维膜的接触角由140.0°减小到136.1°,亲水性增强,具有良好的力学性能。将血管内皮生长因子加入血管组织工程支架材料的关键在于如何维持血管内皮生长因子持续释放有效的浓度以促进血管再生,生物体内,血管内皮生长因子仅需极少的浓度就能够达到促进血管再生的功能,本研究制备的纤维膜第一天的突释低于50%,能够维持14天的有效浓度释放。通过后期实验方法改进,可能实现更长时间VEGF缓慢释放,可进一步通过血管内皮细胞和动物实验验证。总之,本研究制备的乳液法VEGF静电纺丝纤维膜的具有良好的组织相容性和血管再生的效果,有望在血管再生领域发挥一定的应用价值。

参 考 文 献(References)

[1] Huina Zhang, Francesco Migneco, Chia Ying Lin. Chemically-Conjugated Bone Morphogenetic Protein-2 on Three-Dimensional Polycaprolactone Scaffolds Stimulates Osteogenic Activity in Bone Marrow Stromal Cells [J]. Tissue Engineering Part A, 2010, 16(11): 3441-3448

[2] Lin D. Wu H. Zhang R. Pan W. Enhanced Pholocatalysis of Electrospun Ag-ZnO Heleroslructured Nanofibers [J]. Chemistry of Materials. 2009; 21:3479-3484

[3] Jongman Lee, Won Il Choi, Giyoong Tae. Enhanced regeneration of the ligament-bone interface using a poly(L-lactide – co-e-caprolactone) scaffold with local delivery of cells/BMP-2 using a heparin-based hydroge l [J], Acta Biomaterialia, 2011, 7(1): 244-257

[4] Thomas H, Alexander, August B Sage. Insulin-like Growth Factor-I and Growth Differentiation Factor-5 Promote the Formation of Tissue-Engineered Human Nasal Septal Cartilage [J]. Tissue Engineering:Part C, 2010, 16(5): 1213-1221

[5] Wang Y, Liu XC, Zhao J, et al. Degradable PLGA scaffolds with basic fibroblast growth factor experimental studies in myocardial revascularization [J]. Tex Heart Inst, 2009, 36: 89-97

[6] L L Luo, G X Wang, Y L Li, et al. Layer-by-layer assembly of chitosan and platelet monoclonal anti body to improve biocom patibility and release character of PLLA coated stent [J]. Journal of Biomedical Materials Research Part A, 2011, 97(4): 423-432

[7] Stoop R. Smart biomaterials for tissue engineering of cartilage [J]. Injury, 2008, 39(S1): S77-S87

[8] Hojjat Naderi, Maryam M Matin, Ahmad Reza Bahrami. Critical issues in tissue engineering: biomaterials, cell sources, angiogenesis, and drug delivery systems [J]. Journal of Biomaterials Applications, 2011, 26(4): 383-417

[9] Yamamoto M, Ikada Y, Tabata Y. Controlled release of growth factors based on biodegradation of gelatin hydrogel [J]. J Biomater Sci Polym Ed, 2001, 12(1): 77-88

[10] L. Buttafoco, N.G. Kolkman, P. Engbers-Buijtenhuijs, A.A. Poot, P.J. Dijkstra, I. Vermes, J. Feijen, Electrospinning of collagen and elastin for tissue engineering applications[J]. Biomaterials, 2006, 27: 724-734

[11] Soker S, Fidder H, Neufeld G, et al. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165via its exon 7-encoded domain [J]. J Biol Chem, 1996,271 (10):5761-5767

(下转第5506页)

[3] Jun Kimura. Electro diagnosis in diseases of nerve and muscle: principles and practice [M]. 2nd Ed, FA Davis. Philadelphia, 1989

[4] Ferry S, Hannaford P, Warskyi M, et al. Carpal tunnel syndrome: a nested case-control study of risk factors in women[J]. Am J Epidemio, 2000,151:566

[5] 韩贞和, 金春玉, 柳振宇. 健康人的末梢神经传导参数与生活习惯的关系[J].中国民族民间医药, 2010, 19(3):77-79
Han Zheng-he, Jin Chun-yu, Liu Zhen-yu. Relations between peripheral nerve conduction parameter and living habits of healthysubjects [J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2010, 19(3): 77-79

[6] Aroori S, Spence RA. Carpal tunnel syndrome [J]. Ulster Med J,2008, 77(1): 6-17

[7] Cartwright MS, Hobson Webb LD, Boon AJ, et al. Evidence-based guideline: neuromuscular ultrasound for the diagnosis of carpal tunnel syndrome[J]. Muscle Nerve, 2012, 46(2): 287-293

[8] Kelly CP, Pulisteti D, Jamieson AM. Early experience with endoscopic carpal tunnel release[J]. J Hand Surg(Br), 1994, 19(1):18-21

[9] Ginameechi F. Short tem electrophysiologieal conduction change in median nerve fibree aftercarpal tunnel rdease [J]. Clin Nemd Neurosung, 2008, 29(110):1025-1030

[10] Dellon A L. Neurosurgical prevention of ulceration and amputation by decompression of lower extremity peripheral nerves in diabetic neuropathy:update 2006 [J]. Acta Neurochir Suppl, 2007, 100(1): 149-151

[11] Practice parameter for electro diagnostic studies in carpal tunnel syndrome (summary statement). American Academy of Neurology, American Association of Electro Diagnostic Medicine, and AmericanAcademy of Physical Medicine and Rehabilitation [J]. Neurology, 1993, 43(11): 2404-2405

[12] Chow JCY. Endoscopic release of the carpal ligament for carpal tunnel syndrome:22-month clinical result [J]. Arthroscopy, 1990, 6 (4): 288-296

[13] Stamboulis E, Voumvourakis K, Andrikopoulou A, et al. Association between asymptomatic median mononeuropathy and diabetic polyneuropathy severity in patients with diabetes mellitus [J]. J Neurol Sci, 2009, 278(1-2):41-43

[14] 罗雁威, 李昌琪. 脑源性神经营养因子前体蛋白生物学效应研究进展[J]. 现代生物医学进展, 2012, 12(1):151-154
Luo Yan-wei, Li Chang-qi. Advancement in Research on Biological Effects of ProBDNF[J].Progress in Modern Biomedicine,2012,12(1): 151-154

[15] Bartels R H. Verbeek A L. Risk factors for ulnar nerve compression at the elbow: a case control study [J]. Acta Neurochir, 2007, 149(7): 669-674

[16] Mitsionis GI, Manoudis GN, Paschos NK, et al. Comparative study of surgical treatment of ulnar nerve compression at the elbow [J]. J Shoulder Elbow Surg, 2010, 19(04): 513-519

[17] 何晓云,陈世文,张树芳,等. 肌苷促进脑损伤后神经功能恢复的实验研究[J].现代生物医学进展, 2011,11(22):4240-4243
He Xiao-yun, Chen Shi-wen, Zang Shu-fang, et al. Inosine promotes recovery of neural function after traumatic brain injury[J]. Progress in Modern Biomedicine, 2011,11(22):4240-4243

[18] Balci K, Utku U. Carpal tunnel syndrome and metabolicsyndrome[J]. Acta Neurol Scand, 2007, 116(2):113-117

[19] Kivmura, J. Electrodiagnosis in diseases of nerve and muscle:Principles and practice[M]. 2nd ed.Philadelphia: Davis FA,1989: 288-304

[20] 党静霞. 尺神经肘部病变患者的神经电生理定位诊断特征 [J]. 西安交通大学学报(医学版), 2007, 28(4):427-429
Dang Jing-xia. The electrophysiological features for localizing ulnar neuropathy at the elbow [J]. Journal of Xi'an Jiaotong University (Medical Sciences), 2007, 28(4): 427-429

(上接第 5445 页)

[12] D.I. Zeugolis, S.T. Khew, E.S.Y. Yew, A.K. Ekaputra, Y.W. Tong, L.-Y.L. Yung, D.W. Hutmacher, C. Sheppard, M. Raghunath, Electrospinning of pure collagen nano-fibres-just an expensiveway tomake gelatin [J]. Biomaterials, 2008, 29:2293

[13] S.J. Lee, J. Liu, S.H. Oh, S. Soker, A. Atala, J.J. Yoo, Development of a composite vascular scaffolding system that withstands physiological vascular conditions [J]. Biomaterials, 2008, 29:2891-2898

[14] B.W. Tillman, S.K. Yazdani, S.J. Lee, R.L. Geary, A. Atala, J.J. Yoo, The in vivo stability of electrospun polycaprolactone-collagen scaffolds in vascular reconstruction [J]. Biomaterials, 2009, 30:583-588

[15] M.S. El-Kurdi, Y. Hong, J.J. Stankus, L. Soletti, W.R. Wagner, D.A. Vorp, Transient elastic support for vein grafts using a constricting microfibrillar polymer wrap [J]. Biomaterials, 2008, 29:213-3220

[16] N. L'Heureux, S. Paquet, R. Labbe, L. Germain, F.A. Auger, A completely biological tissue-engineered human blood vessel [J], Faseb J, 1998, 12 :7-56

[17] Chai, C. and Leong, K.W. Biomaterials approach to expand and direct differentiation of stem cells [J]. Mol. Ther., 2007, 15:467-480

[18] Bondar, B. et al. Functionality of endothelial cells on silk fibroin nets: comparative study of micro-and nanometric fibre size [J]. Biomaterials, 2008, 29:561-572

[19] Engler, A.J. et al. Matrix elasticity directs stem cell lineage specification [J]. Cell, 2006,126:677-689

[20] Mironov, V. et al. Fabrication of tubular tissue constructs by centrifugal casting of cells suspended in an in situ crosslinkable hyaluronan-gelatin hydrogel [J]. Biomaterials, 2005,26:7628-7635