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基于壳聚糖导管的周围神经再生研究进展

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摘 要

如何促进周围神经损伤修复是一个棘手的临床难题，特别是长距离周围神经缺损。近年来随着材料科学的发展，已有多种不同材料制造的神经导管被用于治疗周围神经缺损，其中，壳聚糖由于其优良的生物性能而被广泛应用，研究表明联合使用各种可促神经再生的物质，可进一步提高治疗效果。本文就基于壳聚糖导管的混合策略、细胞接种、生物因子负载三个方面展开阐述。

关键词

周围神经再生，壳聚糖，导管，组织工程

Research Progress of Peripheral Nerve Regeneration Based on Chitosan Catheter

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Abstract

How to promote the repair of peripheral nerve injury is a difficult clinical problem, especially for long-distance peripheral nerve defect. In recent years, with the development of materials science, nerve catheters made of various materials have been used to treat peripheral nerve injury, among which chitosan has been widely used due to its excellent biological properties. Studies have shown that the combined use of various substances that can promote nerve regeneration can further improve the therapeutic effect. In this paper, the mixing strategy, cell inoculation and biological factor loading based on chitosan catheter are described.

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Keywords

Peripheral Nerve Regeneration, Chitosan, Catheter, Tissue Engineering

1. 引言

周围神经再生和功能恢复是当前临床上面临的一大挑战，特别是长距离周围神经缺损(大鼠：>10 mm，人类：>30 mm)，由于周围神经的牵引弹性有限，通过手术方法无法实现断端缝合[1]。目前在临床上，自体神经移植仍然是治疗长距离周围神经缺损的金标准。但自体神经移植会导致供体神经支配区域的功能丧失和二次手术创伤[2]。

近年来，壳聚糖由于具有优异的可加工性、稳定性、良好的生物相容性和广谱抗菌活性等而引起了广泛关注[3] [4]。此外，壳聚糖在生物体内的降解产物是中性葡糖胺，不影响体内酸碱平衡，从而不会导致体内的无菌性炎症。因此，壳聚糖已被广泛应用于组织工程研究，特别是用于神经组织损伤的修复[5]。但由单一壳聚糖材料制备的神经导管的功能有限，为了改善导管性能，近年来，关于壳聚糖导管的研究逐渐从早期使用单一材料的神经导管向使用复合神经导管发展[6]，各项研究中通常通过组合生物材料[7]、负载细胞[8]或生物活性物质[9]制备神经导管，发现这些复合导管比中空导管具有更好地促进神经再生的能力。本文接下来将从基于壳聚糖导管的混合策略、细胞接种、生物因子负载三个方面展开阐述。

2. 基于壳聚糖导管的混合策略促进周围神经再生

2.1. 胶原(Collagen, COL)/壳聚糖

由于生物降解过程的时间可控性以及无毒性，胶原蛋白成为支持周围神经再生的宝贵材料并已被充分研究。Li 等[10]通过溶液共混、原位冻干和表面生物化等方法构建了一种具有双重生物功能的壳聚糖/胶原复合支架材料，在 10 mm 坐骨神经缺损的大鼠体内评估其功能修复能力。发现双重生物功能支架支持施万细胞的增殖，与神经再生和血管化相关的多个基因和蛋白的表达水平上调，表现出较于单一支架更强的血管化、髓鞘化和功能恢复作用。

2.2. 聚乙醇酸(PolyGlycolic Acid, PGA)/壳聚糖

研究发现填充了经层粘连蛋白浸泡过的胶原支架的 PGA 导管，或填充了 PGA 胶原纤维的 PGA 导管，可以促进长达 80 mm 的神经缺损的轴突再生[11]。因此，将 PGA 的治疗潜力与壳聚糖的优良性能相结合或可进一步改善神经导管的性能[12]。Wang 等[13]开发了一种双组分的人工神经移植物，该导管具有壳聚糖的外部微孔和 PGA 的细丝内层。桥接 30 mm 比格犬坐骨神经缺损模型并恢复 6 个月后，与自体神经移植结果相比，壳聚糖/PGA 组显示出更多的有髓神经纤维。

2.3. 聚乳酸(PolyLactic Acid, PLA)/壳聚糖

与单独的壳聚糖相比，壳聚糖和聚乳酸组成的复合纤维(CH-PLA)具有更高的拉伸强度和更低的溶胀率。基于壳聚糖导管的外层与 CH-PLA 纤维内层而制备的神经导管，能够引导轴突再生。Shen 等[14]利用 CH-PLA 纤维制备壳聚糖导管，在 25 mm 犬胫神经缺损治疗中取得了显著的效果。

2.4. 聚乙二醇(PolyEthylene Glycol, PEG)/壳聚糖

另一种有应用前景的混合策略是在壳聚糖导管的基础上填充聚乙二醇溶液，用于桥接大鼠 10 mm 坐骨神经缺损，具有显著的治疗效果。然而，与自体神经移植相比，功能恢复方面，实验组结果明显较差[15]。

2.5. 氨基酸、多肽、蛋白/壳聚糖

神经导管对神经细胞的亲和力在引导神经再生的过程中起关键作用。与壳聚糖导管相比，负载了层粘连蛋白或聚赖氨酸的壳聚糖导管能够诱导神经突向外生长[16]。Cheng 等[17]将 PC12 细胞与不同混合量的聚-L-赖氨酸壳聚糖神经导管共培养来测试导管的细胞亲和力。有趣的是，聚-L-赖氨酸的疏水性与表面电荷，可能促进了细胞的黏附、生长和分化。此外，聚-D-赖氨酸浓度的增加虽然不会影响细胞存活，却会抑制神经突向外生长[18]。

3. 基于壳聚糖导管的细胞接种促进周围神经再生

3.1. 施万细胞(Schwann Cell, SC)接种

Huang 等[19]已经证明了壳聚糖对 SC 增殖的促进作用和对成纤维细胞生长的抑制作用。因此，壳聚糖有可能通过增加 SC 的数量来支持轴突再生，并且可以防止周围神经损伤后瘢痕组织的形成。Yuan 等[2]对 SC-壳聚糖支架或纤维的生物相容性进行了进一步研究，结果显示壳聚糖具有优异的神经胶质细胞亲和力，可作为优良的细胞载体。MTT 测试结果也表明，壳聚糖膜或纤维无明显细胞毒性。

3.2. 骨髓基质细胞(Bone Arrow Stromal Cell, BMSC)接种

骨髓基质细胞接种已被证明是增强周围神经再生和促进轴突再生的有效策略[20] [21]。Moattari 等[22]将壳聚糖/PLGA(聚乳酸－羟基乙酸共聚物(poly(lactic-co-glycolic acid))神经支架与 BMSC 结合，用于桥接 50 mm 甚至 60 mm 的狗坐骨长距离周围神经缺损。结果发现接种 BMSC 的壳聚糖/PLGA 神经导管组的功能再生程度与自体神经移植组几乎相当[23] [24]。然而，由于潜在的供体部位发病率，植入前的长时间培养时间和细胞分化的不稳定性，目前周围神经手术中关于细胞接种的大多数策略仍然有待进一步研究。因此，基于壳聚糖导管的细胞接种策略目前受到严格限制。

4. 基于壳聚糖导管的生长因子负载以促进周围神经再生

4.1. 神经生长因子(Nerve Growth Factor, NGF)

Ruil 等[25]的最新研究通过选择性提取大鼠血浆中的神经生长因子，制备了修饰有 NGF 的壳聚糖电纺纤维网(eFM-NGF)。在不更换培养基的情况下，将 PC12 细胞与支架共培养 7 天，观察 eFM-NGF 诱导神经突起生长的情况。结果表明，这种神经生长因子传递系统能够刺激神经细胞分化，更能促进神经突生长。

4.2. 胶质细胞源性生长因子(Glial-Derived Neurotrophic Factor, GDNF)

文献中已知 GDNF 有利于周围神经再生进程，如促进运动神经元再生，加速轴突再生和预防肌肉萎缩[26]。随着组织工程的进一步发展，人们制备了富含层粘连蛋白和 GDNF 的壳聚糖导管。将未负载层粘连蛋白的壳聚糖管与层粘连蛋白负载的壳聚糖管进行比较，掺入 GDNF 用于桥接 10 mm 的周围神经缺损。与未掺入 GDNF 的壳聚糖导管相比，GDNF 的掺入促进了神经功能恢复[27]。

4.3. 碱性成纤维细胞生长因子(Basic Fibroblast Growth Factor, BFGF)

碱性成纤维细胞生长因子(BFGF)是成纤维细胞生长因子家族的重要成员，对神经组织具有营养和保护作用[28]。Li 等[29]开发了一种 BFGF 缓释系统，用于诱导骨髓间充质干细胞分化为神经谱系细胞，并促进神经干细胞(Neural stem cell, NSC)分化为功能性神经元[30]。该系统将 BFGF 掺入壳聚糖载体中，可以实现至少 5 周的缓慢释放，这可以进一步促进周围神经再生。

4.4. 成纤维细胞生长因子-2 (Fibroblast Growth Factor-2, FGF-2)

成纤维细胞生长因子-2 是成纤维细胞生长因子家族的 22 个成员之一。在体外条件下，与未掺入 FGF-2 的壳聚糖支架相比，掺入了 FGF-2 的壳聚糖支架更能促进细胞存活和神经干细胞生长[31]。

5. 讨论

壳聚糖在周围神经再生领域的应用具有巨大的前景，通过给予机械支撑(如壳聚糖管，内填充物或神经外膜缝合保护剂)或用作药物输送系统来引导轴突再生。壳聚糖具有良好的生物相容性，抗菌性能，促细胞黏附等优良特性。在组织工程领域，已经探索了各种不同的方法改良壳聚糖导管的性能。其中，基于壳聚糖导管的混合模型可以适应周围神经再生的需求，并且在周围神经再生的所有水平(如组织学、感觉恢复、知觉恢复等)均显示良好的效果。然而，临床上研究有限，未来的研究应侧重于将壳聚糖作为药物递送系统或机械引导/保护材料用于周围神经损伤后的临床治疗效果。

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