

人工神经导管原材料选择与功能设计的研究进展

刘经纬¹, 王健^{1,2*}, 王琳^{1,2*}

1 华中科技大学同济医学院附属协和医院 再生医学交叉与转化湖北省重点实验室, 湖北 武汉 430022

2 华中科技大学同济医学院附属协和医院检验科, 湖北 武汉 430022

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摘 要: 人工神经导管(nerve guidance conduits, NGCs)作为一种合成的神经移植物, 为神经再生提供结构与营养支持。理想的神经导管对生物相容性、机械强度、拓扑结构和导电性等均有较高要求, 因此需对神经导管的设计不断改进并建立更完善的周围神经再生策略, 以期满足临床需求。虽然 NGCs 在周围神经损伤的治疗中已经取得一定进展, 但其对长距离神经离断伤的结构与功能修复仍不理想。本文分别从原材料选择、结构设计、治疗因子搭载及自供电元件集成 4 个方面对神经导管的设计进行综述, 归纳总结 NGCs 在周围神经损伤治疗中的研究进展, 以期推动 NGCs 的迭代更新与临床转化。

关键词: 人工神经导管; 周围神经损伤; 生物材料; 拓扑结构; 神经再生; 组织工程

Advances in the raw material selection and functional design of artificial nerve guidance conduits

LIU Jingwei¹, WANG Jian^{1,2*}, WANG Lin^{1,2*}

1 Hubei Key Laboratory of Regenerative Medicine and Multi-disciplinary Translational Research, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China

2 Department of Clinical Laboratory, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China

Abstract: Artificial nerve guidance conduits (NGCs) are synthetic nerve grafts that are capable of

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*Corresponding authors. E-mail: WANG Jian, jianwang_wh@hust.edu.cn; WANG Lin, lin_wang@hust.edu.cn

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providing the structural and nutritional support for nerve regeneration. The ideal NGCs have plenty of requirements on biocompatibility, mechanical strength, topological structure, and conductivity. Therefore, it is necessary to continuously improve the design of NGCs and establish a better therapeutic strategy for peripheral nerve injury in order to meet clinical needs. Although current NGCs have made certain process in the treatment of peripheral nerve injury, their nerve regeneration and functional outcomes on repairing long-distance nerve injury remain unsatisfactory. Herein, we review the nerve conduit design from four aspects, namely raw material selection, structural design, therapeutic factor loading and self-powered component integration. Moreover, we summarize the research progress of NGCs in the treatment of peripheral nerve injury, in order to facilitate the iterative updating and clinical transformation of NGCs.

Keywords: artificial nerve guidance conduits; peripheral nerve injury; biomaterials; topological structure; nerve regeneration; tissue engineering

周围神经损伤(peripheral nerve injury, PNI)是一种临床常见的疾病,多由交通事故、坠落伤、地震灾害、火器伤以及医源性损伤等引发。据统计约 2.8%的外伤患者会累及周围神经,其中近 4 成患者出现神经器质性损伤^[1],主要表现为运动与感觉功能部分或全部丧失、慢性疼痛和靶肌肉萎缩等^[2]。目前 PNI,尤其是神经离断伤会导致残疾,严重危害公共健康。

临床上短距离 PNI 的治疗多采用无张力神经吻合术,而长距离 PNI 治疗的金标准为自体神经移植。腓肠神经是最为常用的供体神经,手术方式为从腓肠神经中获取多束感觉神经移植到周围神经缺损处^[3]。但是该疗法仍存在着诸多限制,如获取供体神经需要二次手术且会损害患者浅表感觉功能;自体神经的供给有限,无法有效治疗长段的周围神经离断伤^[3-4]。因此 PNI 的治疗仍面临严峻挑战。

近年来随着组织工程学的发展,学者们提出开发人工神经导管(nerve guidance conduits, NGCs)用于替代自体神经移植。NGCs 主要由天然或合成聚合物制备,旨在为神经再生提供引导与支持,并克服自体神经移植的限制^[5]。20 世纪 70 年代,第一代硅胶神经导管应运而生,随

后各种材质、不同设计的 NGCs 迭代更新。第一代 NGCs 的设计宗旨是提供一种支撑结构,引导横断神经的再生并阻止结缔组织的浸入^[6]。其中,典型的这类导管包括硅胶神经导管、聚四氟乙烯神经导管等^[7-8],但它们在体内移植后均无法降解,会引发局部神经压迫以及纤维包膜的形成^[9]。在此基础上,第二代神经导管提出使用可生物吸收、生物相容性好的原材料构建 NGCs^[10],这类导管包括聚乙交酯(polyglycolic acid, PGA)神经导管^[11-12]、I 型胶原神经导管^[13]、己内酯神经导管^[14-15]和聚乙烯醇(polyvinyl alcohol, PVA)神经导管^[16]等。它们虽然能高效修复短距离的周围神经缺损,但是在治疗中、长距离 PNI 时神经再生与功能修复效果均不理想。针对上述问题,研究者提出在导管内搭载支持细胞、提供可控的治疗因子递送、给予物理电刺激、填充微-纳纤维导向材料或提供拓扑微结构等以研发第三代神经导管,目前该种导管的设计与制备仍为神经再生领域的研究热点。本文重点介绍第三代 NGCs 的研发以及其在 PNI 中的治疗现状,并将从原材料选择、结构设计、治疗因子搭载以及自供电元件集成等多个方面进行综述。

1 制备 NGCs 原材料

1.1 天然生物材料

天然生物聚合物作为支架材料具有生物相容性好、支持细胞黏附与(部分)促细胞增殖等多种生物活性^[17]。目前用于制备 NGCs 的天然生物材料包括多糖类生物材料如透明质酸、海藻酸盐和壳聚糖等与蛋白多肽类生物材料如胶原蛋白、丝素蛋白和纤维蛋白等(表 1)。对近年制备 NGCs 天然生物材料的优缺点进行归纳总结(表 2)。基于不同生物来源,天然生物材料有着各自独特的优势,如透明质酸的抗瘢痕组织再生、壳聚糖的抗菌活性与丝胶蛋白的促神经再生作用等。可惜天然生物材料都有相似的缺陷,即机械强度难以满足神经再生的需求。同时天然蛋白与多糖成分复杂,纯化工艺难以标准化,无法完全去除其免疫原性,因此天然生物材料的应用受到限制^[44]。交联多种天然生物材料以互补彼此缺陷,如壳聚糖/海藻酸盐、壳聚糖/丝胶蛋白等,是目前克服上述困难的主要解决方案之一。

1.1.1 透明质酸

透明质酸是一种天然线性多糖,由葡萄糖醛酸与 N-乙酰氨基葡萄糖组成,属于细胞外基质中的糖胺聚糖成分^[17-18]。在促进细胞信息交流中发挥重要作用。同时,透明质酸与血管形成、肿瘤发生和炎症反应等生理病理过程息息相关^[18]。作为早期天然导管材料代表,透明质酸的优势在于可以有效减少神经溶解后的疤痕与神经再生后的粘连^[34]。同时来源于细胞外基质,使其生物相容性与降解性优异,但无法满足神经导管对机械强度的需求。因此,透明质酸常与人工合成材料联合使用以达到更好的机械性能。Entekhabi 等^[19]利用了人工聚合物聚己内酯作为透明质酸神经导管的外壳,克服了纯

透明质酸导管易形变的缺点,通过评价支架上的施万细胞增殖与轴突的生长情况,证明了其具有促神经再生的潜力。Li 等^[18]采用透明质酸/壳聚糖神经导管治疗周围神经卡压伤,发现透明质酸的引入有效抑制了瘢痕组织的形成与损伤处的组织黏附,有效促进神经再生。

1.1.2 壳聚糖

壳聚糖是由甲壳素经过脱乙酰作用得到的碱性多糖。壳聚糖与糖胺聚糖的结构相似,故壳聚糖可以与细胞外基质中的分子相互作用。另外,壳聚糖具有多种生物学特性,且相较于透明质酸更易加工^[35]。壳聚糖的物理性能比透明质酸更优秀,在为轴突再生提供物理支撑的同时,其降解产物壳寡糖可促进神经的再生^[36]。但是壳聚糖在干燥状态下易碎,因此多与其他材料联合使用。Lu 等^[20]开发聚乳酸-羟基乙酸共聚物[poly(lactic-co-glycolic acid), PLGA]与壳聚糖复合的神经导管,其降解产物改变局部的酸碱度从而调控神经再生中的炎症水平与再生微环境。Rao 等^[21]利用壳聚糖与细胞外基质类似的特点,将细胞因子模拟肽结合在壳聚糖导管上以模拟神经再生微环境,修复了大鼠 15 mm 的坐骨神经缺损。

1.1.3 海藻酸盐

海藻酸盐主要来自于褐藻,与其他的天然生物材料相比,古洛糖酸醛与甘露糖酸醛的含量较高,赋予海藻酸盐生物材料更好的韧性。海藻酸盐可以通过钙离子进行物理交联,避免了戊二醛等共价交联剂引起的化学毒性。海藻酸盐作为支架材料的优势在于生物相容性好、可生物降解和螯合能力强,促细胞黏附。不同于壳聚糖的脆性,海藻酸盐凝胶有着更好的韧性与黏附性。因此海藻酸盐是一种极具潜力的组织修复与再生的支持基质或递送系统^[37]。Salehi 等^[22]构建了一种海藻酸盐/壳聚糖支架,

表 1 人工神经导管原材料选择

Table 1 Raw material selection for NGCs

Materials	Type	Animal model	Effects	Functional outcome	References
Chitosan/ Hyaluronic acid	Natural- composite	Rats with sciatic nerve crush	Decrease of the formation of nerve scars and adhesions, improved neural regeneration and repair	N.A.	[18]
Collagen/ Hyaluronic acid/PCL	Natural- synthetic composite	N.A.	Improved attachment and proliferation of Schwann cells, and enhanced axon outgrowth	N.A.	[19]
Chitosan/ PLGA	Natural- synthetic composite	Rats with sciatic nerve transection	Improved migration and maturation of Schwann cells, and reduced inflammatory response	N.A.	[20]
Chitosan	Natural	Rats with sciatic nerve transection	Angiogenesis and improved regeneration of nerve and myelin	Sciatic nerve function index was improved	[21]
Alginate/ Chitosan	Natural composite	Rats with sciatic nerve crush	Improved cell viability of the transplanted extraolfactory mesenchymal stem cells	Sciatic function nerve index was improved and heat pain latency was shortened	[22]
Alginate/ Chitosan	Natural composite	Rats with sciatic nerve crush	Decreased levels in gastrocnemius atrophy and of nerve fiber demyelination	Sciatic function nerve index was improved and heat pain latency was shortened	[23]
Silk fibroin	Natural	N.A.	Improved migration and adhesion of Schwann cells	N.A.	[24]
Silk fibroin/ Magnesium filaments	Natural- synthetic composite	Rats with sciatic nerve transection	Improved adhesion and proliferation of Schwann cells, regeneration of nerve and myelin sheath	Sciatic nerve function index was improved	[25]
Sericin/ Chitosan	Natural composite	Rats with sciatic nerve crush	Improved regeneration of nerve and myelin sheath, reduced levels of inflammation and gastrocnemius atrophy	Better performances in mechanical withdrawal threshold test and thermal withdrawal latency test	[26]
Sericin/ CNT	Natural- synthetic composite	Rats with sciatic nerve transection	Myelin sheath regeneration, electrophysiological reconstruction and muscle reinnervation	Sciatic nerve function index was improved and thermal withdrawal latency was shortened	[27]
PLA/CNT	Synthetic composite	Rats with sciatic nerve transection	Myelin sheath and axon regeneration	Sciatic function nerve index was improved and heat pain latency was shortened	[28]
PLA	Synthetic	Rats with sciatic nerve transection	Myelin sheath and axon regeneration	Sciatic nerve function index was improved	[29]
PCL/PPy	Synthetic composite	N.A.	Improved cell proliferation and differentiation of human embryonic stem cells-derived neural crest stem cells	N.A.	[30]
PCL/ Gelatin	Synthetic composite	Rats with sciatic nerve transection	Neurotrophic factor expression and axon regeneration	Sciatic nerve function index was improved and hot plate latency time was shortened	[31]
PLGA/ Collagen/ Hyaluronic acid	Natural- synthetic composite	Rats with sciatic nerve transection	Controlled release of neurotrophic factors	Better performances on electrical stimulation test	[32]
PLGA	Synthetic	Rats with sciatic nerve crush	The reduced release of proinflammatory factors	Sciatic nerve function index and BBB scores were improved, and withdrawal reflex latency was shortened	[33]

N.A.: Not applicable.

表 2 人工神经导管天然生物材料比较

Table 2 Comparison of natural biomaterials for artificial nerve conduits

Materials	Source	Advantage	Disadvantage	References
Hyaluronic acid	ECM	Hypo-immunogenicity Overcoming extra-neural scarring	Very low mechanical strength Biodegradation	[18-19,34]
Chitosan	Carapace	Antibacterial activity Reducing neuroma formation and fibrosis	Low mechanical strength High brittleness	[20-21,35-36]
Alginate	Brown algae	Remarkable chemical flexibility Physical crosslinking	Low mechanical strength High degradation rate	[22-23,37]
Silk fibroin	Silk	Suitable mechanical strength Economically advantageous High resistance to fracture and compression	Silk fibroin derived from solutions are generally weak and fragile.	[24-25,38-41]
Sericin	Silk	Hypo-immunogenicity Suitable degradation rate Degradation products promoting nerve regeneration	Low mechanical strength	[26-27,42-43]

搭载嗅间充质干细胞用于治疗坐骨神经缺损。基于海藻酸盐的良好生物相容性与促细胞黏附的能力,该复合水凝胶显著延长了间充质干细胞在损伤部位的存活时间。此外,Rahmati等^[23]利用海藻酸盐/壳聚糖支架搭载小檗碱治疗坐骨神经损伤,海藻酸盐提供了支架足够的机械强度与韧性,通过水凝胶的溶胀与降解实现小檗碱的缓释。

1.1.4 丝素蛋白

蚕丝作为手术缝合线的原材料在医学领域被广泛应用。近10年来,蚕丝的核心蛋白——丝素蛋白作为第三代导管材料在生物医学工程领域受到关注。丝素蛋白具有良好生物相容性、低免疫原性、适宜的机械强度与韧性等优点^[38-40]。丝素蛋白拥有促进神经再生的特性,其已被证明可支持神经轴突延伸与施万细胞增殖^[41]。基于此,丝素蛋白也常作为组织工程神经导管的材料。Zhang等^[24]开发了一种负载甲钴胺的丝素蛋白神经导管,丝素蛋白有效促进了施万细胞的黏附与迁移。Zhang等^[25]构建了一种丝素蛋白/镁金属丝复合神经导管,丝素蛋白的包裹有效提高镁金属丝的生物相容性并且增强导管的机械强度,实现 Mg^{2+} 缓释以促进神经再生。

1.1.5 丝胶蛋白

丝胶蛋白作为一种水溶性黏蛋白,在天然蚕丝中维持其核壳结构的完整性。武汉协和医院王琳团队^[42]发现温和的溴化锂提取法可以获取结构完整的丝胶蛋白,并证实丝胶水凝胶具有良好的生物相容性、可降解性与低免疫原性。丝胶水凝胶可以支持神经细胞的黏附和生长,并通过降解产物抑制细胞凋亡通路发挥神经保护作用^[43];开发了一种壳聚糖/丝胶蛋白复合神经导管,通过丝胶蛋白的降解产物诱导神经营养因子与髓鞘生成相关蛋白表达升高,有效地促进神经的再生^[26];构建了碳纳米管/丝胶蛋白神经导管,其中的丝胶蛋白提供了良好的神经保护与营养作用,促进神经轴突的延伸与髓鞘再生^[27]。丝胶蛋白作为一种新兴的再生修复材料,在神经导管制备与研发方面极具潜力。

1.2 人工合成材料

不同于天然生物材料的组分复杂,人工合成材料因为其可控的理化性能,在组织工程领域中拥有独特的优势^[35]。但是,相较于天然生物材料,其生物相容性较差,需要进行表面修饰以改进生物亲和力。早在1970年,有学者尝试使用有机硅导管在猴子身上进行正中神经修

复^[45]。有机硅导管在短距离的周围神经修复中取得了效果^[46]，此后包括聚丙烯酸、聚乙烯等不可降解材料也被用于 NGCs 的制备^[47]。但是，这些材料会引发慢性异物反应，同时阻碍导管内外物质的交换，这导致其临床应用受到限制。

为克服不可降解人工合成材料的上述缺点，研究者将目光转向了可降解人工合成材料的研发和应用上。可降解合成材料具有低免疫原性、降解产物可吸收等优点，并且可制备成疏松多孔结构，以支持轴突再生中代谢产物的交换^[37]。常见的可降解合成材料包括聚乳酸 (polylactic acid, PLA)、聚己内酯(polycaprolactone, PLC)、PLGA、聚丙交酯己内酯[poly(L-lactide-co-caprolactone), PLCL]及其它们的衍生物等(表 1)。不同共聚物单体组成与晶体结构不同，决定其物理性质的差异(表 3)。PLA 的杨氏模量通常在 2.7–4.1 GPa，拉伸强度在 15–150 MPa，这使 PLA 导管拥有着更强的机械强度与抗拉伸性能，在体内不易形变^[48–49]。PCL 的杨氏模量与拉伸强度偏低，但是其高熔点温度与玻璃化转变温度分别为 224 °C 与 36 °C，因此 PCL 易于加工并适配于 3D 打印技术^[50,54]。而 PLGA 作为 PLA 与 PGA 的共聚物，其物理性能受到两者配比调控，在保证杨氏模量与拉伸强度可控的同时，可以实现快速降解^[54]。

1.2.1 聚乳酸

PLA 是一种生物降解材料，可使用淀粉为原料进行合成。PLA 的杨氏模量与拉伸强度显

著高于多数合成导管材料，这意味着 PLA 神经导管在复杂体内环境中可以有效维持形貌并支持神经再生。其降解产物为乳酸，可迅速被机体代谢，但是其较差的细胞黏附性与极高的疏水性限制了 PLA 的应用^[28,55]。Zheng 等^[29]构建了一种有序 PLA 纤维神经导管，利用去细胞化的基质水凝胶改善导管的疏水性，这种神经导管为神经髓鞘的再生与功能恢复提供了拓扑信号。Jahromi 等^[28]将表面改性碳纳米管掺入 PLA 神经导管，以改善导管的机械性能，同时在导管中填充含有施万细胞与姜黄素的纤维蛋白水凝胶。这种复合支架可获得与自体神经移植相媲美的修复效果。

1.2.2 聚己内酯

PCL 是一种脂肪族聚酯，降解时间要远长于 PLA 等人工合成材料，完全降解时间长达 24 个月。所以对于 PCL 是否属于可降解材料仍存在争议^[49,56]。但是，PCL 具有低熔点与易于加工定型等特点。伴随着 3D 打印技术的发展，PCL 重新成为神经导管研发的热门材料^[50]。Vijayavenkataraman 等^[30]利用 3D 打印技术制备 PCL/聚吡咯复合导电神经导管，PCL 的使用使得三维多孔结构得以实现。Qian 等^[31]利用 PCL 在 3D 打印技术上的优势构建了一种多孔复层的神经导管，将多巴胺、氨基酸、石墨烯与 PCL 按照设计逐层铸造喷涂，利用复杂的导管结构实现了细胞黏附、导电性与机械强度等优势有机统一。

表 3 人工神经导管人工合成材料比较

Table 3 Comparison of synthetic materials for artificial nerve conduits

Materials	Tensile modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)	Degradation time (months)	Degradation products	References
PLA	2.70–4.10	15–150	3–10	>24	Lactic acid	[48–49]
PCL	0.20–0.35	20–34	300–1 000	>24	Acetic acid	[50–51]
PLGA (80% PDLA)	3.30–3.50	40–55	2–10	>1.5	Lactic acid and glycolic acid	[52–53]

1.2.3 聚乳酸-羟基乙酸共聚物

PLGA 是由 PLA 与聚乙醇酸以不同比例共聚而成,这使得其物理性能可控。虽然 PLGA 的降解速率随着其中 PLA 含量升高而放缓,但其降解速率远高于 PLA 与 PCL 材料^[53]。由于神经营养因子的半衰期短,而且在时间维度上不同神经营养因子有各自的分泌峰,所以 PLGA 这种可控降解对于实现神经营养因子多相释放有重要价值。Lackington 等^[32]开发一种 PLGA 微粒,使其中包裹的 NGF 与 GDNF 在 28 d 内依次缓释到神经再生微环境中,契合神经再生的时空过程。同样,PLGA 也可作为一种优良的药物缓释平台,Haidar 等^[33]将 α -硫辛酸与阿托伐他汀负载纳米颗粒加载在 PLGA 神经导

管上以实现药物的局部长期缓释。

2 NGCs 的物理结构与设计

随着电纺与生物 3D 打印技术的成熟与发展,拓扑结构的概念被引入神经导管的设计之中(表 4)。神经元拥有独特的极化特性,这使神经轴突再生方向有重要意义。在体内外实验中,拓扑形貌信号对神经突的引导作用均被证实,并有效促进了神经再生^[65]。图 1 展现了 4 种主流的 NGCs 拓扑形貌设计。

2.1 多通道结构

单通道作为神经导管最基础的设计之一,凭借其制备方式简单、技术门槛低的优势,仍是神经导管设计中的主流。王琳团队^[42]研发的

表 4 人工神经导管的结构设计

Table 4 Structure design of NGCs

Materials	Type	Animal model	Effects	Functional outcome	References
Collagen/ Hyaluronic acid/PCL	Natural- synthetic composite	N.A.	Improved cell attachment and proliferation of Schwann cells, and enhanced axon outgrowth	N.A.	[57]
Chitosan/ Hyaluronic acid	Natural composite	Rats with sciatic nerve crush	Reduction of extraneural scarring and adhesion, and improved nerve regeneration	N.A.	[58]
Chitosan/PLGA	Natural- synthetic composite	Rats with sciatic nerve transection	Neurotrophic factor release and nerve regeneration	N.A.	[59]
Silk fibroin/ Collagen	Natural- synthetic composite	Rats with sciatic nerve transection	Axonal regeneration that was induced by the NGF gradient and longitudinal oriented microstructure	Static sciatic function index was improved	[60]
PLGA	Synthetic	N.A.	This system delivering FK506 that also guides enhanced axon growth induced by released FK506 and the microgroove microstructures	N.A.	[61]
PLCL	Synthetic	Rats with sciatic nerve severe traction injury	The groove and ridge morphologic surfaces of the NGCs	N.A.	[62]
Collagen or Laminin	Natural	Rats with facial nerve transection	The ordered growth of axons	Whisker movements index was improved	[63]
Spider fibroin/Silk fibroin/Hyaluronic acid	Natural composite	Rats with sciatic nerve transection	Regeneration of nerve and myelination	Better performances on footprint analysis and von frey test	[64]

N.A.: Not applicable.

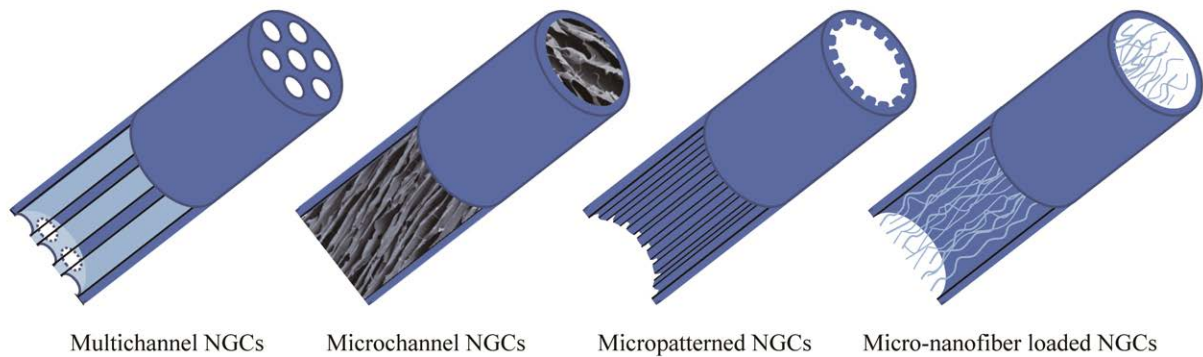


图1 人工神经导管的结构设计示意图
Figure 1 Structure design of NGCs.

单通道丝胶神经导管成功修复 5 mm 的周围神经缺损。但是周围神经在生理结构上是由多条神经束共同组成,单通道结构未考虑仿生理念,因此模拟神经束膜将神经导管分隔成多通道结构的 NGCs 受到越来越多的关注^[66]。

多通道导管的制造工艺复杂,目前多以注塑、电纺以及 3D 打印等技术来实现^[57]。Yao 等^[58]研究不同通道数的多通道胶原导管与商业化单通道导管的治疗效果差异,发现多通道 NGCs 可以促进神经再支配,达到更好的术后运动功能恢复。Wang 等^[57]将一种形状记忆性聚合物聚丙交酯与共三甲基碳酸酯制备成一种四通道的神经导管,形状记忆性使其在生物体内自形成四通道的结构,同时多通道的设计促进了神经轴突的再生。

2.2 微通道结构

相较于宏观尺度上的多通道,微观尺度上的微通道结构与神经轴突的真实尺寸更加匹配,可给予新生的轴突更加仿生的指引信号^[67]。微通道结构导管多采用冻干技术,利用冰晶生长方向与尺寸来控制微通道的方向与内径大小,形成单方向的多孔结构^[68]。Manoukian 等^[59]通过液氮速冻制备具有微通道结构的壳聚糖神经导管,搭载 4-氨基吡啶实现神经电生理的恢

复,而有序微通道结构提供了轴突延伸的物理导向信号。Huang 等^[60]制备了一种具有神经营养因子梯度分布的有序微通道结构神经导管,实现化学与物理线索协同促进神经再生的效果。

2.3 微槽结构

微槽结构是在材料平面上印刻出一系列平行规整的纵向沟槽,通过导管表面的形貌设计来影响细胞的生理活动,进而促进神经轴突的再生。微槽结构的构建主要采用刻蚀与冲压技术^[61-62]。Davis 等^[61]利用光刻蚀技术在搭载了有他克莫司的 PLGA 薄膜上刻蚀了不同宽带比的微槽,发现微槽的形貌不仅会影响神经再生的效率还会影响导管搭载药物的释放曲线。Yu 等^[62]利用冲压技术在搭载了人工多肽与 NGF 的多孔 PCL 膜上印刻纵向分布的微槽,这些微槽在神经再生过程中可有效引导施万细胞迁移,促进大鼠坐骨神经的再生。

2.4 微-纳纤维结构

微-纳纤维的设计理念是为神经再生提供支持并引导信号,微丝与纳米纤维在直径上的差异与排布密度会直接影响神经修复效果^[69]。早在 1946 年 Weiss 等^[70]就展开了对于微丝的探究。Li 等^[71]通过在 PLGA 导管中填充基于自组装肽的有序纳米纤维,证实这种有序纤维结构

有利于施万细胞的增殖与迁移。Cao 等^[63]将神经营养因子交联在层粘连蛋白与胶原蛋白上,并构建微丝填充的 NGCs,该导管有效促进了大鼠面神经再生与功能恢复。Huang 等^[64]利用透明质酸包裹的蛛丝作为微丝填充到丝素神经导管中,发现这种天然的蛛丝纤维具有促神经轴突与施万细胞附着的作用,同时也证实过多或过少地填充纤维都会影响到微-纳纤维导管的治疗效果。这表明为神经再生提供物理支持与再生空间之间存在着平衡,需要进一步研究与探索。

3 支持细胞搭载

3.1 施万细胞

施万细胞是周围神经修复的主要支持细胞,可形成神经髓鞘并分泌多种神经营养因子。将自体施万细胞搭载于 NGCs 中,通过在神经损伤处补充施万细胞来提高神经再生效果。但是,自体施万细胞存在提取与纯化困难、细胞扩增时间长以及难以标准化等缺陷,而同种异体施万细胞存在免疫原性及疫源性问题,这限制了它们的临床应用^[72]。Wang 等^[73]利用 *Büngner* 带中成纤维细胞与施万细胞的协同作用,将二者共同搭载于 NGCs 中,成纤维细胞增强施万细胞的迁移和分泌行为,促进神经再生与功能修复。

施万细胞的基因改造也是近年研究热点。PNI 发生后,施万细胞上调多种神经再生相关细胞因子的表达。通过病毒转染等方式进行施万细胞基因改造,使其过表达神经再生细胞因子以促进神经的再生。Wu 等^[74]通过慢病毒转染使施万细胞过表达血管内皮生长因子,随后负载于 NGCs 中,促进周围血管再生与神经修复。

3.2 干细胞

干细胞发挥促神经再生的机制与施万细胞

相似,它们递送到损伤部位后可分化为施万细胞样细胞并分泌神经营养因子。胚胎干细胞、诱导多能干细胞、间充质干细胞(mesenchymal stem cells, MSCs)和神经干细胞等多种干细胞已被应用到 NGCs 的设计中^[4]。其中 MSCs 因易于获取而成为被广泛搭载的干细胞^[75]。而在不同来源的间充质干细胞中,又以骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)与脂肪来源间充质干细胞(adipose-derived stem cells, ADSCs)研究得最为广泛。

虽然未分化 BMSCs 的疗效存在着争议,但是施万细胞样分化 BMSCs (d-BMSCs)的治疗效果已被证实^[76]。d-BMSCs 被认为与轴突再生蛋白以及神经营养因子高表达密切相关^[77-78]。Wang 等^[79]通过体外实验发现了缺氧处理有利于 BMSCs 向 d-BMSCs 分化并释放神经营养因子,负载缺氧处理 BMSCs 的 NGCs 取得更好的促神经再生效果。同时 Zhou 等^[80]对 BMSCs 与 ADSCs 的应用进行比较,发现二者在疗效上没有显著的差异,且 ADSCs 有着更加安全易行的获取方式。

4 治疗因子递送

神经营养因子作为一种高生物活性的蛋白多肽,调控多种神经再生相关的生理活动,但是其存在价格高昂以及生物半衰期短等局限性^[81]。这注定神经营养因子的递送需要精准且缓释的递送系统,因此神经导管便成为了一种理想的载体。王琳团队^[26]在壳聚糖/丝胶导管上搭载了神经生长因子,通过导管的缓慢降解实现了神经生长因子的缓释,调控了施万细胞的基因表达并促进了神经再生。

在神经再生过程中,神经营养因子之间存在着时间与空间上的动态变化。设计搭载神经营养因子的导管需要兼顾时间和空间维度的药

物控释。Hong 等^[82]开发一种由 PCL 与 PLGA 不同构成比的 3 层复合神经导管。他们将神经营养因子-3、脑源性神经营养因子以及血小板源生长因子搭载在不同的层面上,利用不同层面的降解速率差异实现了这 3 种神经营养因子的时空控释,并证实了这 3 种神经营养因子在神经再生的不同阶段发挥不同作用。

除去神经营养因子,也有少量的临床药物被证明有益于神经的再生,如阿托伐他汀、他克莫司与氯倍他索等^[33,59,83]。但是这些药物常是其他疾病的临床用药,对于 PNI 治疗的效果尚不明确。氯倍他索先前被证明可以促进少突胶质细胞的增生,进而影响中枢神经的髓鞘再生。王琳团队^[83]首次将其搭载于丝胶导管上,证明了氯倍他索在促外周神经再生中的作用。

5 外泌体递送

迄今为止,已有研究证实外泌体在细胞信息交流中发挥关键作用。在神经再生相关细胞中,MSCs、施万细胞与巨噬细胞都可以通过分泌外泌体影响相关细胞增殖与迁移行为、损伤处血管再生以及免疫炎症反应等,达到调控神经再生的目的^[84]。

搭载外泌体的 NGCs 实现疗效的关键是实现外泌体与支持细胞的长时间交互,这要求导管在拥有孔隙的同时也具有缓释能力,因此天然生物材料是制备这类导管的主要原材料。Hsu 等^[85]开发一种海绵状的海藻酸盐导管以缓释 MSCs 的外泌体,该外泌体作用于再生微环境的多种成分,抑制炎症因子和疼痛相关分子的表达,并显著促进髓鞘再生。在坐骨神经损伤模型的治疗中,Rao 等^[86]开发的甲壳素 NGCs 缓释 MSCs 外泌体显著促进施万细胞增殖与背根神经节的生长。

6 电刺激在 NGCs 中的运用

近年几项随机临床试验已证实围手术期的低频电刺激可促进轴突生长与神经功能恢复,目前也有临床 I 期的积极试验结果^[87]。但是目前临床电刺激均需术中外接电极并且难以在术后维持刺激,这限制其治疗效果。为了实现体内长程电刺激,多种自供电元件集成的 NGCs 被陆续研发(表 5)。

6.1 导电 NGCs

电信号传递是神经纤维的基本功能之一,因此导电神经导管具有仿生概念。在过往 10 年间,学界已经证明了包括聚吡咯、聚噻吩、碳纳米管与石墨烯在内的诸多导电材料可以有效促进横断神经纤维之间的神经冲动传递并促进神经再生^[27,88]。Wang 等^[88]选用氧化石墨烯掺入 PLCL 导管以赋予其导电性,利用大鼠体内生物电加速了神经修复过程。王琳团队^[27]选择了性质类似的碳纳米管来增强神经导管的导电性,通过术后结合单次电刺激显著改善了髓鞘再生与神经再支配。

6.2 体外无线供电 NGCs

Macewan 等^[89]将硅胶导管与无线电接收线圈相连接,构建了一个基于电磁耦合原理的体外无线供电 NGCs。将无线电接收器植入小鼠的皮下可以有效地提高能量转化效率,而基础导管结构则提供电极平台支持并引导神经生长的作用。同样的策略 Koo 等^[90]也有采用,进一步使用 PLGA、氧化硅与镁等可降解材料制备了柔性无线电接收器,克服了植入无线线圈易形变损耗、诱导排异反应与不可降解等缺陷,使得整个体系生物相容性更加优异。

6.3 化学原电池供电 NGCs

除去无线供电的方式,Wang 等^[91]将原电池与 NGCs 相结合以实现自供电刺激。镁电极与

表 5 自供电神经导管的研究进展

Table 5 Research progress of self-powered NGCs

Power supply	Animal model	Electrical stimulation parameter	Design of self-powered components	References
Electric conduction	Rats with sciatic nerve transection	N.A.	CNTs were dispersed into sericin solution to construct hollow tubular catheter with genipin as a cross-linker	[27]
Electric conduction	Rats with sciatic nerve transection	N.A.	The reduced graphene oxide (RGO) was coated onto ApF/PLCL nanofibrous scaffolds	[88]
Electromagnetic coupling	Rats with sciatic nerve transection or sciatic nerve crush	2.5 V voltage, 20 Hz frequency, immediate stimulation for 1 h after operation	The wireless receiving coil was connected with the silicone nerve catheter through Pt/Ir microwire	[89]
Electromagnetic coupling	Rats with sciatic nerve transection	Over minimum threshold voltage, 20 Hz frequency, daily stimulation after operation	The wireless receiving coil and wire was constructed by biodegradable metals such as magnesium	[90]
Primary battery	Rats with sciatic nerve transection	0.984 V on day 1, 0.450 V on day 2, and 0.068 V on day 3	The electroactive NGCs consisted of thin-film magnesium and iron-manganese alloy electrodes on a polymer-based biodegradable scaffold	[91]
Primary battery	Rats with sciatic nerve transection	350 mV maximum voltage, maintaining the electrical stimulation of 100 mV voltage for 6 h	The multiblock NGCs realized self-powered electrical stimulation by the consumption of glucose and oxygen	[92]
Mechanical energy	Sciatic nerve of rat	Extracorporeal ultrasound stimulation, 50 μ A to 400 μ A of current amplitude	As cuff-type nerve attachment, the system for wireless neural stimulation elicited highly repeatable compound action potentials in the sciatic nerve	[93]
Mechanical energy	Rats with sciatic nerve transection	Thoracic friction stimulation, 2.7 V, 0.12 μ A at the active state and 2.2 V, 0.1 μ A at the calm state	A self-powered system developed by integrating a novel tribo/piezoelectric hybrid nanogenerator and a multifunctional nanoporous NGCs	[94]

N.A.: Not applicable.

铁锰合金电极位于两端并用多孔膜相夹，最终形成一种双层导管结构。这样的复合结构可以在植入导管后，以原电池供电原理持续给予神经微弱的电刺激促进神经的再生。Sun 等^[92]采用了燃料电池的原理设计了原电池供电 NGCs，由 Pt 纳米粒子与碳纳米管构成的电极对可以有效氧化体液中的葡萄糖。葡萄糖转化为葡萄糖酸内酯并在导管内形成电流，同样实现导管的自供电与对神经的电刺激。

6.4 机械能供电 NGCs

机械能供电 NGCs 的构建主要基于压电材料，这类材料在发生机械形变时出现电荷分离而形成局部电场，给予其超声、振动刺激或者生理压力变化即可实现对组织的电刺激^[95]。常见压电材料包括压电聚合物聚偏二氟乙烯 (polyvinylidene difluoride, PVDF)、压电陶瓷钛酸钡 (BaTiO₃) 和氮化硼 (BN) 等，其中压电陶瓷纳米材料的应用最为广泛^[96]。

Piech 等^[93]利用压电陶瓷、集成电路与电容等构建了神经袖带,压电陶瓷将体外超声转换成神经电刺激。压电陶瓷实现了高达 7 V 的电压,但不得不使用惰性材料封装以提高生物相容性。而压电聚合物的生物相容性优于压电陶瓷,更适于 NGCs 的开发。Jin 等^[94]制备了一种基于 PVDF 的摩擦发电机,通过患者手腕脉搏跳动实现 90 mV 的电压,达到电刺激神经再生阈值。将发电机置于大鼠胸部皮下并连接 NGCs,成功促进大鼠坐骨神经再生的同时未引发显著的排斥反应。

7 讨论与展望

近几十年,随着导管设计理念的革新以及工程技术的进步,各式各样的 NGCs 被研发与报道。在原材料的选择方面,人们最初选用惰性生物材料(如硅胶)来制备第一代 NGCs,但是在动物实验中发现其无法降解,需要 2 次手术取出。随后,人工合成可降解聚合物和天然生物材料被用于新型 NGCs 的制备中。人工合成聚合物具有组分单一和理化性能可控等优势,而天然生物材料对支持细胞的亲和性更好,因此合成聚合物/天然复合生物材料可以继承二者优势,并越来越多地用于第三代 NGCs 的制备中。在 NGCs 的结构设计上,各种方案层出不穷,如导管内表面构建有序拓扑结构、导管内填充微-纳纤维、设计多通道及微通道的导管类型等。现有的结果显示,相较于传统的单通道神经导管,结构优化的 NGCs 具有更好的神经再生效果。但是,大部分 NGCs 的结构设计方案具有排他性,无法兼容其他设计方案。因此,在选择 NGCs 的结构设计方案时,需要统筹兼顾,不可盲目地做加法。在设计治疗因子搭载的 NGCs 时,要特别关注治疗因子的活性保持和可控释放,这对神经再生效果至关重要。而在导管内搭载施万细胞或者干细胞时,NGCs

的研发者需要考虑如下 2 个问题:(1) 如何提高植入细胞的活率并长期保持其生物活性;(2) 如何降低细胞增殖失控而引发的肿瘤发生风险。在传统 NGCs 的研发中,原材料选择、结构设计以及治疗因子搭载是需要重点考虑的问题,研究者需要在统筹兼顾这些设计的基础上,提出自己的设计理念。

自供电元件集成 NGCs 为当前神经导管的一个研究热点。研究表明,物理电刺激可诱导施万细胞分泌神经营养因子,并通过促进 Ca 离子内流上调轴突表面神经营养因子受体的表达,二者共同作用提升再生轴突延伸速率并促进神经功能修复^[97-98]。基于以上论点,多种类型的自供电元件被集成到 NGCs 中,包括磁耦合自供电,压电材料自发电和原电池自发电等。动物实验数据表明,自供电元件集成的 NGCs 可提供物理电刺激,促进神经功能恢复。但是,已报道的自供电元件均需要连接必要的配件,如金属导线、电容器何刺激电极等,这会引发人们对其降解性和生物安全性的担忧。此外,过强的电刺激会造成机体组织的损伤而过低的电刺激生物学效果不明显,如何保证自供电元件输出电刺激参数稳定并能维持在合适范围是一个需要重点关注的问题。尽管如此,自供电元件集成 NGCs 提供了一种可行的物理刺激方法促进神经再生,此外其提供的电信号可为传感微器件供能,为神经再生检测元件的集成打下基础。

未来,随着材料科学的发展和工程技术的革新,NGCs 的研发必定会迎来暴发式增长,越来越多的 NGCs 会从动物实验走向临床转化。在这个过程中,周围神经损伤与再生的基础研究至关重要,这需要多背景、多领域专家通力合作,希望早日研发出促进长距离离断神经高效再生修复的 NGCs,造福广大患者。

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