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脊髓损伤治疗策略研究进展

姚淳[a]，汤欣[a,*]，曹宇琪[a]，王绪化[c]，于彬[a,b,*]

[a] *Key Laboratory of Neuroregeneration of Jiangsu and Ministry of Education & NMPA Key Laboratory for the Research and Evaluation of Tissue Engineering Technology Products, Co-Innovation Center of Neuroregeneration, Nantong University, Nantong 226001, China*
[b] *NHC and CAMS Key Laboratory of Medical Neurobiology, MOE Frontier Science Center for Brain Science and Brain-Machine Integration, School of Brain Science and Brain Medicine, Zhejiang University, Hangzhou 310003, China*
[c] *Jiangsu Clinical Medicine Center of Tissue Engineering and Nerve Injury Repair, Affiliated Hospital of Nantong University, Nantong University, Nantong 226001, China*

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摘要

脊髓损伤（SCI）对患者的生活来说是一种灾难性的疾病。受损的脊髓会破坏脑-脊髓神经元环路，导致相关功能缺失。SCI的发病过程是一个渐进、复杂的过程。很多临床试验尝试促进SCI后神经再生和功能恢复，但效果并不理想。近年来，随着转录组测序和生物材料的发展，研究者一直在努力探索新型高效的SCI治疗方法。本文从损伤微环境、神经环路和生物材料支架等方面综述了近年来SCI修复的最新进展，并展望SCI治疗的未来发展方向，包括靶向microRNA治疗、血管干预以及多种方法联合治疗策略。总之，本文旨在为该领域的研究提供新见解，并为SCI的治疗铺平道路。

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1. 引言

全球约有300万人患有创伤性脊髓损伤（SCI），每年有25~50万新发病例[1–3]。SCI是一种严重的致残性疾病，往往导致运动和感觉神经元缺陷，不仅会降低患者的生活质量，还会带来相当大的经济和社会负担[4–5]。人们在探索SCI的有效治疗方法方面做出了巨大的努力。然而，由于SCI后的病理生理机制非常复杂，目前的治疗预后较差，功能恢复程度有限[6–7]。因此，阐明SCI的细胞和分子机制将为制定提高神经再生能力及其可塑性的新策略奠定基础[8]。

简单来说，SCI的病理生理过程包含三个连续的阶段，包括原发性损伤、继发性损伤以及最终的慢性损伤阶段[9]。物理创伤引发脊髓的机械破坏导致局部神经元和少突胶质细胞损伤，病变区域的血管和血脊髓屏障（BSCB）被破坏。这些事件触发多因素继发性损伤级联反应，并持续数周。在这个阶段，免疫细胞浸润损伤部位，释放炎性细胞因子。炎症反应导致进一步的神经元和胶质细胞死亡[10]，最后在慢性期，反应性星形胶质细胞、小胶质细胞/巨噬细胞和细胞外基质分子形成密集的胶质瘢痕，阻碍轴突再生[9]。

损伤诱导的损伤区域抑制性微环境和内在再生能力的缺乏阻碍了SCI后轴突的成功再生[11]。研究者进行了大量的研究，旨在改善SCI的神经再生和神经修复。这些治

* Corresponding authors.
E-mail addresses: Tangxin@ntu.edu.cn (X. Tang), yubin@ntu.edu.cn (B. Yu).

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疗包括非药物治疗、药物治疗、基因治疗、细胞治疗和生物材料治疗[12–15]。神经营养因子，如神经营养因子-3（NT-3）、脑源性神经营养因子（BDNF）和神经生长因子（NGF），可以调节神经元存活、轴突生长、突触可塑性和神经传递[16–17]。神经营养因子对SCI的修复作用依赖于神经营养因子类型、给药方式、位置和时间[18–19]。例如，NT-3的表达支持皮质脊髓束（CST）的生长和轴突可塑性[20–21]、改变运动神经元（MN）的突触传递[22]、改善腰椎局部神经环路[23–24]，并促进SCI后神经干细胞（NSC）的增殖和分化[25–26]。研究发现，脊髓持续退化可能会限制SCI后12周NT-3的促进再生能力[27]。各种细胞类型的细胞治疗现已应用于SCI，包括胚胎干细胞（ESC）、NSC、间充质干细胞（MSC）、诱导多能干细胞（iPSC）、嗅鞘细胞（OEC）、雪旺细胞（Schwann cell）等。细胞治疗结合生物因子或生物材料可以替代丢失的细胞，提供神经营养因子、调节病变微环境、促进SCI后轴突再生[7,28–32]。此外，影响神经环路活性的神经调节技术（如无创磁刺激或电刺激）已被用于促进SCI后的神经再生和损伤修复[33]。

一般来说，这些治疗遵循三个主要方向：①提供一个有利的微环境，减少阻止轴突出芽的排斥屏障；②重建受损的神经环路，促进功能恢复；③提供脊髓样组织移植物，支持和引导轴突再生（图1）。本文就这三个方面简要介绍脊髓损伤治疗的最新成果。同时，针对今后SCI的有效治疗提出了几个重要方向。

2. 损伤微环境

与周围神经系统（PNS）不同，中枢神经系统（CNS）损伤后再生能力有限，可能与损伤后局部微环境有关[11]。神经微环境由神经元、胶质细胞、轴突、髓鞘、血管、细胞基质、神经递质等组成，受到各种营养因子和细胞因子的调控[33]。SCI后，大量炎症反应、支持底物缺乏、抑制性生长因子和胶质瘢痕形成等均阻碍轴突再生[34]。既往研究表明，SCI后轴突与周围神经节段在桥接时具有短暂的急性再生能力[35]，这一结果为SCI修复和功能改善提供了有力证据。研究人员已经做了许多尝试来改善SCI治疗的再生抑制微环境，如靶向神经胶质细胞和炎症反应[36–37]以及促进髓鞘再生[38–39]。

SCI的病理生理过程十分复杂，涉及多种细胞类型（如神经元、神经胶质细胞、免疫细胞等）、细胞反应和生物学过程[8,11,40–41]，这给阐明SCI后的损伤微环境带来了严峻的挑战。高通量测序技术的迅速发展，如RNA测序（RNA-seq）和单细胞RNA-seq（scRNA-seq），为SCI研究提供了强有力的工具[42–43]。专家已经进行了大量的转录组分析，揭示了SCI的复杂环境[44–46]。例如，本

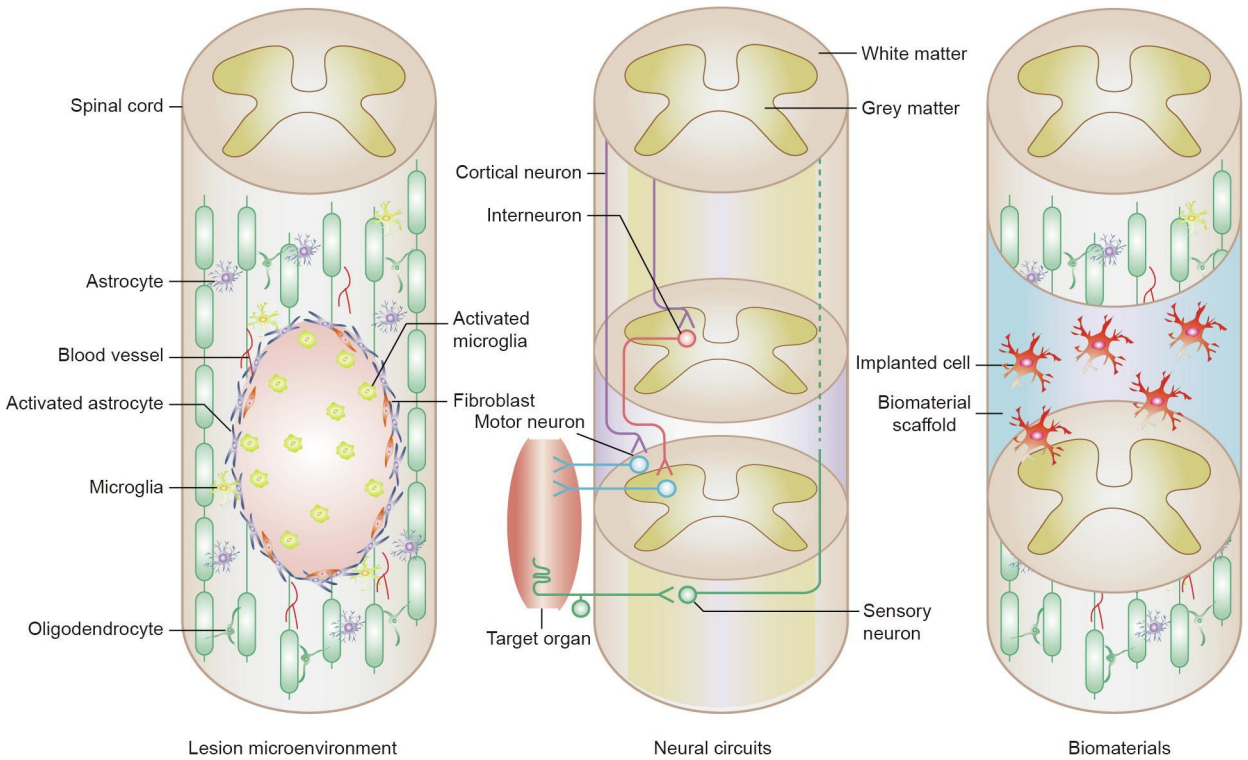

图1. 目前SCI治疗方法示意图。

文研究团队[47]通过RNA-seq鉴定了大鼠脊髓半横断损伤后28天的脊髓远近端中的显著基因和重要生物学过程。同时，该研究还在多细胞水平上检测了损伤微环境，验证了多系统之间的相互作用，对SCI后复杂生物学过程进行了综合分析[48]。由于脊髓在胚胎发育过程中具有极大的生长潜能[49]，Yang等[50]利用RNA-seq技术对从胚胎期到成年期的大鼠的脊髓进行分析，发现了在脊髓发育过程中的信使RNA（mRNA）、microRNA（miRNA）、长链非编码RNA（lncRNA）、小RNA和可变剪接模式，为脊髓研究提供了有价值的遗传学基础，并促进了脊髓相关组织工程的技术发展。

近年来，研究人员通过scRNA-seq在复杂组织中表征了细胞异质性，并确定了稀有细胞群体的分子标记[51]。目前，已有多项scRNA-seq研究揭示了脊髓发育过程中或损伤后的复杂微环境[52–56]。这些研究证实了啮齿类动物脊髓的细胞异质性，并初步描述了损伤部位细胞间的相互作用。例如，Li等[57]通过scRNA-seq技术在小鼠挤压SCI模型中发现了一种在新生阶段短暂激活的小胶质细胞子亚群。新生小胶质细胞通过分泌纤维连接蛋白形成细胞外基质，表达相关分子来抑制炎症反应，促进无瘢痕愈合[57]。该研究针对新生和成年小鼠SCI后损伤区小胶质细胞的异质性，提出通过调节损伤区域微环境来提高愈合和轴突再生的可能性，这也是scRNA-seq技术与神经功能验证相结合的一次成功尝试，为进一步的研究提供了新思路。

3. 神经环路

在脊椎动物中，位于脊髓的神经元可以接受来自大脑的激活输入，并向运动神经元和肌肉传递运动节律，从而产生精确的运动[58]。SCI通过病变下方的树突收缩或萎缩破坏神经环路，导致运动和感觉反馈受损[59]。功能恢复是SCI治疗的标准和挑战之一[60]。促进轴突再生，重新连接神经环路，增强神经元的可塑性对脊髓修复具有重要意义。

大脑皮层皮质脊髓神经元（CSN）向脊髓进行直接输出，对控制脊髓运动活动至关重要[61]。Wang等[62]通过逆行示踪标记技术鉴定了具有不同脊髓投射和不同运动模块编码的区域特异的CSN。该研究阐明了脊髓CSN与轴突之间的组织结构，有助于理解目标定向运动能力中的神经元环路。CST起源于大脑皮层的CSN，向脊髓传递大脑皮层命令。创伤性脑损伤或SCI破坏CST轴突，导致运动功能障碍[63–64]。骨桥蛋白（OPN）/胰岛素样生长因子1（IGF1）治疗可促进脊髓半切术后CST轴突的再生，提高术后的精确性运动[65]。该研究证明激活CSN的内在生长能力可实现SCI后功能恢复。与神经营养因子和生长因子相比，提高能量代谢也是促进损伤后轴突再生的有效方法[66]。Han等[66]研究发现损伤诱导的线粒体功能障碍可能是导致CNS轴突再生失败的原因之一。敲除线粒体蛋白锚合蛋白syntaphilin（Snph）可恢复损伤诱导的线粒体去极化。此外，$Snph^{-/-}$小鼠CST在T8脊髓全横断后再生能力增强，促进了功能突触的形成和运动功能的恢复。

SCI后残留的固有神经束重塑或运动神经元的可塑性调节可重塑运动回路，促进SCI后的运动恢复[67–69]。研究发现，在小鼠T10挫伤SCI模型中，将NT-3逆行运输到腰椎运动神经元可显著重塑腰部神经环路和突触连接，促进行为和电生理恢复[70]。进一步的研究表明，残留的下行脊髓固有神经束通路导致了NT-3介导的恢复。此外，NT-3通过促进树突状细胞的再生长来重塑脊髓固有神经束-运动神经元神经环路[59]。

脊髓中间神经元对于神经可塑性是必不可少的[71–72]。Chen等[73]发现了一种可用于SCI修复的小分子化合物——CLP290。CLP290是神经元特异性K^+-Cl^-共转运体（KCC2）激动剂。脊髓抑制性中间神经元阻碍损伤后下行输入整合到神经回路中，而KCC2可以通过中间神经元激活残存的神经环路。CLP290可以通过激活脊髓半切后小鼠的神经环路来恢复其行走能力。该研究通过调节抑制性神经元兴奋性，为SCI后运动功能的恢复治疗提供了新的思路。

NSC是一种自我更新的多潜能细胞，可以分化为神经元、星形胶质细胞和少突胶质细胞[74]。移植的NSC也可能重新连接神经环路。Lu等[75]发现在大鼠T3完全横断后，植入含有生长因子混合物的纤维蛋白基质的NSC可分化为成熟神经元；这些NSC来源的脊髓神经元将轴突延伸到宿主脊髓，并通过突触形成从损伤部位向宿主脊髓的投射。此外，研究发现在大鼠T3全横断SCI后，大量皮质脊髓轴突再生进入神经前体细胞（NPC）移植物，再生的皮质脊髓轴突与移植的神经元形成功能性突触，改善大鼠前肢运动[76]。

4. 生物材料支架

在过去10年中，越来越多的天然或人工合成的生物材料被开发用于SCI治疗。这些生物材料可以作为物理支架，为轴突再生提供结构支持，引导新生轴突进入损伤区

域。此外，生物材料可作为药物或细胞的转运载体，调节损伤微环境，促进SCI修复[77–79]。

如前所述，神经营养因子如NT-3在SCI治疗中非常重要。由于缺乏有效的给药途径，限制了神经营养因子的临床应用。Li等[25]和Yang等[26]设计了一种可持续14周缓释的NT-3的壳聚糖可降解载体，并将其桥接至大鼠脊髓完全横断损伤区的5 mm间隙。他们发现损伤脊髓内源性NSC被激活分化为神经元，形成功能神经网络，促进感觉和运动行为恢复[80]。转录组分析表明，这种NT-3/壳聚糖载体可以促进神经再生和血管生成，减轻炎症反应，提供了良好的再生微环境[45]。此外，这种生物材料载体在成年恒河猴T8脊髓半横断损伤中也得到了应用，并表现出良好的轴突再生能力。该结果为这种NT-3/壳聚糖载体的潜在治疗应用提供了坚实的基础[81]。

Lin等[82]研制出一种线性有序的胶原支架（NeuroRegen），具有良好的细胞相容性并能引导适当的神经生长。然后，他们在不同的SCI模型中使用与多种功能分子结合的NeuroRegen支架，如BDNF，可以显著促进运动和功能感觉恢复[83–85]。除神经营养因子，NSC也被移植到这种神经再生支架用于SCI修复。为了在微环境中促进NSC的神经元分化，Li等[86]将紫杉醇（PTX）包裹的脂质体负载到胶原微通道支架中持续释放PTX。结果表明，在大鼠T8脊髓全横断损伤模型中，这种负载NSC的胶原支架可以为NSC的神经元分化、轴突再生和运动恢复提供有利的微环境。临床上，将NeuroRegen支架与人脐MSC联合移植到慢性完全SCI患者后随访一年，结果显示出初步疗效，未见不良事件[87]。

如前所述，SCI破坏了损伤局部的上行、下行神经纤维束，导致部分或全部感觉或运动神经环路中断。因此，通过促进损伤部位轴突再生、重建突触连接和桥接神经纤维来重塑神经环路具有重要意义[88]。三维（3D）培养可以通过结合干细胞、生物材料和神经营养因子为SCI修复构建功能组织或类器官[14]。Lai等[89]提出了一种干细胞修复SCI的组织工程新策略——神经元中继器组织工程。通过在脊髓病变处植入NT-3修饰的雪旺细胞和神经营养受体酪氨酸激酶3（TrkC）修饰的NSC的明胶海绵支架，促进组织工程神经网络的构建，重塑大鼠T10脊髓横断后的神经环路。在机制上，NT-3/TrkC相互作用，激活磷脂酰肌醇3-激酶（PI3K）/Akt丝氨酸/苏氨酸激酶1（AKT）/哺乳动物雷帕霉素靶蛋白（mTOR）通路，刺激NSC源性神经元突触形成。此外，为了模拟脊髓的结构，Lai等[90]通过将从NSC中培养出的白质样组织（WMLT）和灰质样组织（GMLT）进行模块化组装，在体外建立了一种新型的脊髓样组织（SCLT）。这种SCLT可以在大鼠T10 SCI后协同作用重塑神经通路，促进功能恢复。此外，SCLT未来也可以用作研究脊髓药理和发育的体外平台。

5. 展望

5.1. 基于miRNA的疗法

大量研究表明，SCI后miRNA失调[91–93]。miRNA参与多个过程，如星形胶质细胞反应、炎症和脱髓鞘[94–95]。在大多数研究中，miRNA通常直接注射[96–98]或转染到移植细胞中[99–100]。一些研究为了实现此目的也使用了基因修饰动物模型[101–102]。然而，基于miRNA的治疗存在许多障碍：首先，miRNA对SCI修复影响的机制尚不清楚；其次，目前基于miRNA的治疗方法通常在没有细胞特异性的情况下表达或沉默miRNA。由于脊髓是一个由多种细胞类型组成的复杂组织，如果不关注特定细胞类型，很难确定miRNA的确切作用。生物材料支架系统为基于miRNA的治疗提供了希望。尝试设计新的结合识别细胞多肽的生物纳米粒子，将实现将miRNA递送到SCI后损伤区域的特定细胞类型。这种方法也将有助于阐明miRNA在SCI中的潜在机制。再次，SCI的发病机制是一个持续数月的时空过程。因此，生物材料有利于缓释miRNA，从而长期维持损伤部位中适当的局部有效浓度，促进SCI后的再生和功能恢复。

5.2. 血管介入治疗SCI

血管系统能够运输氧气和营养物质，代谢废物，维持神经系统微环境稳定[40]。SCI后损伤区域局部血管遭到破坏，血管通透性增加，进一步加速炎症反应，造成组织损伤。此外，损伤引起的内源性血管新生通常不充分且功能异常[103–104]。因此，进一步了解损伤区域血管系统的变化，制定新的策略减少血管损失，减轻BSCB破坏，促进血管再生，将是一种很有前景的SCI治疗方法[105]。近年来，SCI后血管新生的作用受到越来越多的关注。据报道，UTX/KDM6A缺失可体外增加血管内皮细胞成管，促进小鼠T10挫伤SCI后的血管再生，进而促进功能恢复[106]。

5.3. 联合多种治疗策略

虽然有许多治疗方法在动物模型中可以保护脊髓免受损伤，促进轴突再生，但在临床上并无疗效[7]，其原因之一可能是SCI是一个多方面、伴随性、连续的病理过

程，需要联合治疗。与目前的一种或两种病理生理机制的治疗策略不同，由多种治疗手段组成的综合治疗更有利于SCI的修复[9]。例如，Anderson等[107]研发了一种能够激活神经元生长能力、诱导生长支持底物和趋化脊髓轴突的治疗方法。该方法也可通过生物材料在时间和空间上控制释放因子。因此，小鼠和大鼠在完全性脊髓挤压损伤后轴突再生能力增强，重塑神经回路获得功能恢复[107]。目前的治疗方法可以促进轴突再生[79]、提高中间神经元兴奋性[108]、减轻炎症反应[109]、促进血管重建[78,110]。今后，可以将这些疗法与细胞移植[7,15]、细胞因子和生物材料支架结合起来。本文研究团队也将试图找出关键调控因子[如OPN、IFG1、BDNF、睫状神经营养因子（CNTF）、NT-3或miRNA]对不同脊髓神经束的作用机制，重新连接脑-脊髓神经环路；将改良的生物材料与干细胞、细胞因子、药物或miRNA移植至损伤区来改善损伤周围微环境，并通过进一步的康复训练和物理刺激促进SCI修复。

6. 结论

在探索能够显著促进SCI后功能恢复的治疗策略上，我们还有很长的路要走。虽然未来仍需开展更多的相关工作，但目前良好的研究基础和临床神经科学研究为SCI后的轴突再生和功能恢复提供了巨大的希望[111]。因此，本研究期望通过对当前的SCI治疗方法进行综述，以便开发出更有效的治疗方法，实现SCI后功能性神经再生（图2）。

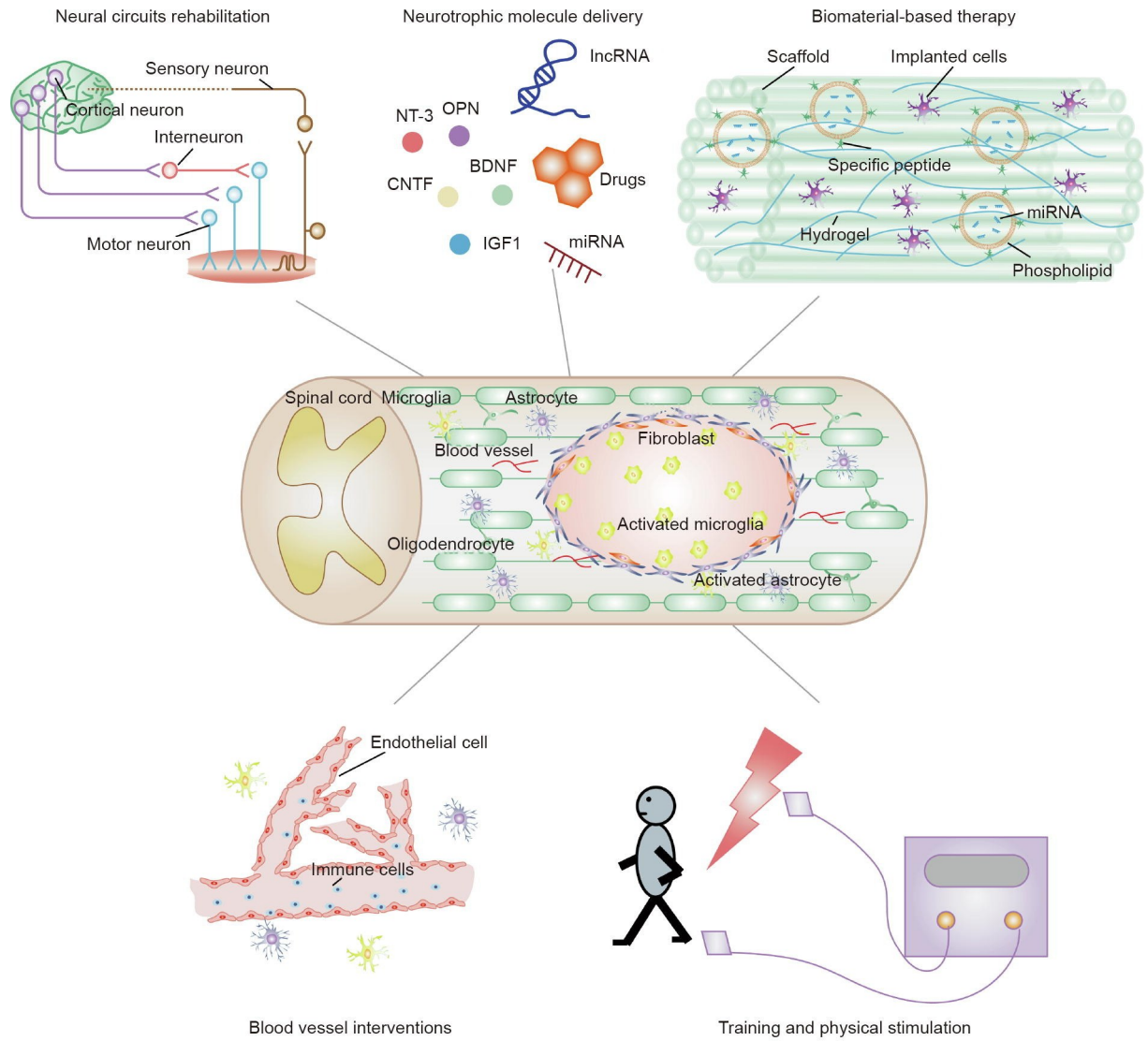

图2. 未来SCI综合治疗方法示意图。LncRNA：长链非编码RNA。

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Compliance with ethics guidelines

Chun Yao, Xin Tang, Yuqi Cao, Xuhua Wang, and Bin Yu declare that they have no conflict of interest or financial conflicts to disclose.

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