脊髓损伤的病理改变及修复策略

李建平², 何留民³, 吴武田^{1,2,3*}

1. 江苏瑞思坦生物科技有限公司, 苏州 215168;

2. 暨南大学粤港澳中枢再生研究院, 广州 510632;

3. 中山大学第三附属医院脊柱外科, 广州 510630

* 联系人, E-mail: wtwu@hku.hk

收稿日期: 2021-06-15; 接受日期: 2021-08-27; 网络版发表日期: 2021-12-03

国家自然科学基金(批准号: 81971165, 32071354, 31870964)、国家重点基础研究发展计划(批准号: 2014CB542205)和广东省重点研发计划“精准医学与干细胞”重大科技项目(批准号: 3242001)资助

摘要 脊髓损伤造成神经组织坏死, 传导通路中断, 损伤平面以下运动和感觉功能丧失, 导致瘫痪甚至死亡。脊髓损伤的病理变化极其复杂, 早期主要为分子基因水平的改变, 亚急性期主要为细胞组织水平的变化。这些变化引发继发性损伤, 致使组织坏死、神经元死亡、轴突断裂并形成由瘢痕组织包裹的囊性空洞, 抑制轴突再生。目前临床上仅能通过手术减压或者使用药物对症干预, 无法从根本上改善受损神经的功能。脊髓损伤后功能难以恢复有多方面的原因: 炎症反应贯穿脊髓损伤全过程, 炎症介质导致损伤区域的神经元及胶质细胞变性坏死, 轴突因瓦勒变性而萎缩; 神经元再生能力弱, 轴突再生乏力, 并且瘢痕组织导致轴突无法穿越损伤区域与远端的轴突形成联系。本文就脊髓损伤后的病理改变进行综述并探讨修复策略。

关键词 脊髓损伤, 修复, 干细胞, 材料

脊髓损伤(spinal cord injury, SCI)是指脊髓在外界直接或者间接因素的作用下造成脊髓受损, 患者常出现损伤平面以下感觉、运动、排尿和排便功能障碍^[1]。患者因损伤平面以下的运动功能丧失而瘫痪, 而长时间瘫痪使患者出现肌肉萎缩、血栓形成、关节变形、肺栓塞、泌尿系统反复感染、吞咽困难和疼痛等并发症, 严重影响患者的生活质量和身心健康并给患者带来了沉重的心理负担^[2-4]。因此, 寻找有效治疗脊髓损伤方法、促进神经功能恢复对家庭和社会都具有重要的意义。

1 脊髓损伤的病理改变

脊髓损伤可分为原发性和继发性。原发性脊髓损伤是由于脊柱受到外界直接或者间接的机械性撞击使脊髓受到压缩、撕裂、扭曲或剪切, 往往造成轴突断裂、神经组织破坏和神经元死亡^[5]。而原发性损伤引发一系列继发性病理生理学事件。血脊髓屏障和微血管被破坏, 触发炎症细胞向损伤区域侵入, 包括巨噬细胞、T细胞和中性粒细胞, 同时激活脊髓常驻小胶质细胞, 这些炎症细胞释放大量的炎症因子, 如肿瘤

引用格式: 李建平, 何留民, 吴武田. 脊髓损伤的病理改变及修复策略. 中国科学: 生命科学, 2022, 52: 1472–1483

Li J P, He L M, Wu W T. Pathological changes and repair strategies for spinal cord injury (in Chinese). Sci Sin Vitae, 2022, 52: 1472–1483, doi: 10.1360/SSV-2021-0065

坏死因子- α (tumor necrosis factor- α , TNF- α)、白细胞介素(interleukin, IL)-1 α 、IL-1 β 和IL-6, 在损伤后6~12小时达到峰值, 在损伤后4天仍在升高^[6]. 此外, 吞噬炎症细胞释放活性氧, 导致DNA氧化损伤、蛋白质氧化和脂质过氧化, 进一步诱导延迟性坏死和细胞凋亡^[7].

另外, 损伤激活星形胶质细胞, 激活的星形胶质细胞发生反应性增生, 在损伤早期以突起的形式长入损伤区域形成胶质瘢痕来保护受损组织以防止其进一步受损. 然而, 这种胶质瘢痕致密的三维结构在损伤后期形成了阻碍轴突再生的物理性屏障. 除了胶质瘢痕外, 脊髓损伤也会形成纤维瘢痕. 成纤维细胞在损伤后第3天从损坏的硬脊膜向损伤部位浸润, 7天后反应性增殖并分泌大量的细胞外基质, 如IV型胶原、纤维连接蛋白和层黏连蛋白形成致密的瘢痕结构^[8]. 脊髓内的病变是进行性加重, 从中心出血至全脊髓出血水肿, 从中心坏死到全脊髓坏死, 形成囊性空洞. 随着实质体积的丧失, 囊性腔合并, 最终形成由胶质-纤维瘢痕包裹的囊性空腔^[9]. 而且瘢痕中的细胞分泌硫酸软骨素蛋白多糖(chondroitin sulfate proteoglycan, CSPG)、NG2蛋白多糖、磷酸黏蛋白、脑信号蛋白3A等抑制轴突生长的化学物质^[7]. 继发性脊髓损伤导致脊髓受到第二次打击, 形成神经再生抑制性微环境, 扩大原发性损伤范围以外的脊髓组织, 持续时间长, 防止组织再生和功能恢复^[10]. 脊髓损伤的最终程度是由初级和继发机制导致的, 它们从损伤的一刻开始, 持续几天甚至几周.

1.1 脊髓损伤后轴突的反应

成像技术的发展和运用提高了人们对脊髓损伤后轴突的变化情况的认识, 轴突遭横切后其近端和远端在一小时内会发生突然碎裂样的急性轴突变性. 轴突在损伤发生6小时即观察到发芽, 重新形成生长锥, 但是表现出营养不良的形态. 在之后的48小时轴突不再延伸, 并且出现回缩, 最终近端稳定在距离病变部位约300 μm 处, 而远端则出现沃勒变性^[11]. 病变周围的瘢痕组织对营养不良的生长状态起着非常重要的作用, 有证据表明这些神经纤维仍具有活性, 但无法跨越病变中心^[12]. 体外实验显示, 胶质瘢痕能抑制大鼠海马神经元的生长, 体内实验研究表明, 成体背根神经节神经元能在中枢神经系统的白质内延伸, 但是当它们接触到瘢痕组织时, 生长会突然停止^[13].

1.2 脊髓损伤后星形胶质细胞反应

星形胶质细胞在中枢神经系统中含量非常丰富, 与神经元的代谢密切相关, 参与调节神经电生理活动、突触可塑性、神经元内外离子平衡和神经递质释放以及维持血脑屏障等^[14]. 星形胶质细胞在脊髓损伤后数小时内就已经活化并开始增殖, 损伤后3~5天增殖达到高峰, 7~10天分布于损伤区域周围, 参与胶质瘢痕的形成, 数周后神经胶质瘢痕成熟^[15,16]. 星形胶质细胞在脊髓损伤的病理演变过程中扮演着双重角色, 一方面通过形成胶质瘢痕限制炎症的扩展, 另一方面星形胶质细胞又能产生硫酸软骨素等参与构成细胞外基质的抑制分子, 抑制轴突再生. 星形胶质细胞活化为反应性胶质细胞, 在炎症因子的作用下可转化为神经毒性表型^[17,18]. 研究发现, 反应性星形胶质细胞合成并释放多种物质, 包括神经营养因子和炎症因子, 并能增加脊髓损伤小鼠的神经营养因子前体(proNGF)的表达, proNGF的转运或释放引起神经元凋亡并加重脊髓损伤的进程^[19,20].

星形胶质细胞在脊髓损伤治疗中受到广泛关注, 研究者希望通过干预星形胶质细胞反应调控其功能. 研究发现, 人参皂苷Rg1能促进体外培养星形胶质细胞分泌bFGF, GDNF和NGF, 通过激活PI3K/Akt信号通路增强星形胶质细胞的生物学活性, 改善损伤后大鼠的运动功能^[21]. 应用基因技术将星形胶质细胞向有益方向转化为治疗脊髓损伤提供一种可能. 例如, 将ADAMTS4经AAV导入星形胶质细胞后, 体外培养转导的星形胶质细胞中硫酸软骨素和糖胺聚糖含量均显著降低, 移植到脊髓损伤部位后能刺激受损脊髓中皮质脊髓束轴突的侧支发芽, 增加病灶尾部的五羟色胺能神经纤维的密度^[22].

1.3 脊髓损伤的炎症反应

在直接或者间接机械力作用下, 脊髓血管结构被破坏, 使外周炎症细胞浸润并激活中枢炎症细胞, 启动免疫反应并释放大量的促炎因子(如IFN- γ , TNF- α , IL-1, IL-6, IL-8和IL-12)、趋化因子(CXCL1和CXCL12)、一氧化氮(NO)、氧化剂、谷氨酰胺离子、蛋白酶(基质金属蛋白酶、钙蛋白酶和胰天蛋白酶)和补体等^[23]. 血管损伤后红细胞进入脊髓实质, 被巨噬细胞降解后释放有毒的铁又将巨噬细胞激活为M1促炎表型^[24]. 同时, 脊髓损伤后线粒体受损导致能

量耗竭继而形成大量的氧自由基, 缺血及再灌注后线粒体结构及功能极易受损, 某些酶类的变化亦可增加氧自由基, 如缺血时还原型辅酶 II (NADPH II) 增加, 在其作用下 O_2 变为氧自由基。而内源性抗氧化酶(例如过氧化氢酶和过氧化物酶)和低分子量抗氧化剂(如谷胱甘肽、抗坏血酸、尿酸、硫辛酸和胆红素)致使氧化还原失衡, 超过机体的清除能力, 在铁催化下产生氢氧自由基($\cdot OH$), $\cdot OH$ 除可使蛋白质及其他有机分子变性外, 还可以与细胞脂质膜的疏水部分(特别在双键部)发生反应生成脂质自由基^[25,26]。同时, 激活的胶质细胞还分泌谷氨酸导致神经元发生兴奋性中毒而死亡^[27,28]。

创伤性脊髓损伤发生缺血再灌注的同时, 外周的中性粒细胞、巨噬细胞和淋巴细胞等浸润到损伤部位, 而脊髓固有的小胶质细胞也被激活, 这些炎症细胞在脊髓损伤中存在有益和有害两方面的作用。

(1) 中性粒细胞。中性粒细胞是急性免疫炎症反应的主要炎症细胞, 脊髓损伤后首先浸润到损伤区域, 最早在24小时达到高峰, 随后一周开始迅速减少^[29,30]。它们吞噬外来细胞并清除碎片, 同时也在组织中释放各种有毒的介质从而引起继发性损伤, 被认为是有害的。但是, 并非中性粒细胞释放的所有产物都是有害的, 越来越多的证据表明, 中性粒细胞还可通过发挥与炎症有关的组织修复作用, 发挥有益作用^[31,32]。例如, 中性粒细胞通过释放分泌性白细胞蛋白酶抑制剂(secretory leukocyte protease inhibitor, SLPI)减少炎症从而促进轴突再生^[33,34], 还能释放调控肿瘤蛋白的生长因子促进中枢神经系统的轴突再生^[35]。最新研究发现, 酵母聚糖调节的中性粒细胞亚群Ly6G^{lo}能刺激体内横断视神经和脊髓损伤轴突的再生, 进一步研究显示, Ly6G^{lo}能分泌NGF和IGF-1以及其他生长因子^[36]。总之, 中性粒细胞在脊髓损伤的研究中, 如何避免其有害方面促进其有益的功能仍需要进一步探索。

(2) 巨噬细胞/小胶质细胞。损伤脊髓内的巨噬细胞/小胶质细胞有两种来源, 一种来自常驻小胶质细胞群, 另一种是外周来源的髓样巨噬细胞, 活化的小胶质细胞无论在形态还是表面抗原表达上都和血源性巨噬细胞难以分开。巨噬细胞/小胶质细胞在脊髓损伤的过程中起着清除受损组织, 抵抗感染并恢复组织稳态的作用。巨噬细胞/小胶质细胞是高度可塑性的炎症细胞, 在炎症因子的刺激下极化为M1和M2两种表型^[37]。

M1型巨噬细胞/小胶质细胞可促进先天免疫, 清除损伤部位的异物和组织碎片; M2型巨噬细胞/小胶质细胞分泌免疫抑制性细胞因子(如IL-10)和趋化因子配体(CCL17, CCL18和CCL22)吸引抗炎性白细胞, 这些介质可使M2型巨噬细胞/小胶质细胞调节炎症反应, 清除碎片并促进组织重塑和修复^[37,38]。巨噬细胞/小胶质细胞表型通过转录事件来调节, 如通过转录因子STAT1和NF- κ B等进行信号转导驱动促炎性和趋化因子(如TNF α , IL-1 β , IL-6, CCL2和ROS), 促使巨噬细胞/小胶质细胞活化为M1型^[39,40]。而STAT6, IRF4和过氧化物酶体增殖物激活受体(peroxisome proliferator-activated receptor, PPAR)等则促使巨噬细胞/小胶质细胞活化为M2型^[41,42]。脊髓损伤后M2型巨噬细胞/小胶质细胞迁移到损伤部位并迅速丢失这些表型^[43], 研究者为了恢复脊髓损伤中M2型巨噬细胞/小胶质细胞的表型, 促进其在脊髓损伤中的修复功能做出许多努力。脊髓损伤后48小时使用IL-4能增加巨噬细胞/小胶质细胞向M2型极化的作用, 减少CD45^{high}, CD11b⁺, F4/80⁺, Ly6G⁺中性粒细胞的数量, 促进脊髓损伤大鼠运动功能的恢复^[44]。阻断IL-6信号通路可促进损伤部位微环境的变化, 减少IFN- γ 水平, 增强IL-4和IL-13表达, 通过IL-4R α /JAK/STAT信号通路将巨噬细胞/小胶质细胞激活为M2型, 从而抑制炎症的发展, 清除损伤部位的髓鞘碎片瘢痕组织, 促进轴突再生和运动功能的恢复^[45,46]。IL-7阻滞剂阻断IL-7信号介导的促炎因子的释放, 促进M2巨噬细胞/小胶质细胞活化并抑制损伤部位的炎症浸润, 改善脊髓损伤后的运动功能^[47]。Ecto-5'-核苷酸酶(CD73)能抑制巨噬细胞/小胶质细胞向M1型极化, 促进其向M2型极化^[48]。研究发现, 阿奇霉素、褪黑激素在SCI后可降低M1基因表达并增强M2基因表达^[49,50]。利用类脂质纳米颗粒将转录因子干扰素调节因子5(interferon regulatory factor 5, IRF5)递送到脊髓损伤小鼠脊髓, 抑制了M1巨噬细胞/小胶质细胞标志物的表达, 促进M2型标志物的表达, 并促进了脊髓损伤后运动功能的恢复^[51]。

2 脊髓损伤的治疗策略

脊髓损伤的修复策略主要是重建中断的传导通路, 恢复损伤平面上下的传导功能。因此, 如何使损伤两端的轴突重新建立突触联系成为首要解决的问题。

但是, 脊髓损伤后轴突再生能力较弱, 并强烈依赖微环境. 针对于此, 国内外学者在提高神经元的内在再生能力和改善不良再生微环境方面做了大量的工作, 并取得了令人鼓舞的进展.

2.1 药物治疗

脊髓损伤急性期的治疗主要为抑制炎症反应, 早期临床试验表明, 使用大剂量激素能减轻次生损伤. 类固醇激素抗炎药甲基强的松龙琥珀酸钠(methylprednisolone sodium succinate, MPSS)被广泛用于脊髓损伤的治疗, 为脊髓颈段不完全损伤患者间歇性注射MPSS后能改善其运动功能, 提高其生活质量^[52]. MPSS与脂肪间充质干细胞(adipose-derived stem cells, ASCs)联合应用能降低脊髓损伤后的炎症因子表达(COX-2, IL-6和TNF α)和反应性星形胶质细胞激活, 增加少突胶质细胞的水平, 改善损伤小猎犬的运动能力, 同时也降低胃出血等不良反应的发生概率^[53]. 利鲁唑是经FDA批准的钠通道阻滞剂, 它能抑制谷氨酸释放减轻对神经元的兴奋性毒性, 用于治疗肌萎缩性侧索硬化症^[54]. 在脊髓损伤的治疗上, 利鲁唑能降低脊髓损伤后轴突变性和髓鞘蛋白坏死, 减轻脊髓损伤的炎症, 减少损伤区域细胞死亡, 保留轴突的细胞骨架完整性. 研究显示, 利鲁唑可能是通过影响P2x7受体的表达来调节神经性疼痛大鼠模型中的巨噬细胞/小胶质细胞的极化, 增加M2型巨噬细胞标记的mRNA水平, 并降低M1标记的mRNA水平^[55,56]. 大环内酯类抗生素雷帕霉素能调节细胞周期、自噬、蛋白转录和翻译^[57,58], 脊髓损伤后使用雷帕霉素能降低炎症, 减轻损伤部位星形胶质细胞反应, 增加神经元的存活率^[59]. 治疗糖尿病的药物西他列汀对脊髓损伤后的神经元存活和功能恢复均具有神经保护作用, 可以抑制神经元凋亡^[60]. GABA B受体激动剂巴氯芬在完全性脊髓损伤后抑制cAMP酶的活化并促进脑干下行神经元的轴突再生, 降低脑中HESB的表达, 并且促进完全性脊髓损伤后神经元的再生^[61]. 但是, 使用大剂量激素会带来副作用, 长期疗效欠佳也影响了临床应用. 例如甲基强的松龙是推荐治疗, 而非标准性治疗方法.

2.2 细胞移植

1975年, Bunge和Bunge^[62]开创性地将体外培养的

施万细胞(Schwann cells, SCs)用于脊髓修复临床试验, 使损伤轴突修复、再生和脊髓部分神经功能恢复成为可能. 至此, 细胞移植被广泛应用于脊髓损伤修复的基础研究和临床转化研究中. 目前应用脊髓损伤修复的移植细胞有神经干细胞/祖细胞(neural stem/progenitor cells, NSC/NPCs)、间充质干细胞(mesenchymal stem cells, MSCs)、SCs、诱导多能干细胞(induced pluripotent stem cell, iPS)、少突胶质细胞前体细胞(oligodendrocyte progenitor cells, OPCs)和嗅鞘细胞(olfactory ensheathing cells, OECs)等^[63]. 细胞移植后可通过多种机制介导脊髓损伤后的功能改善, 包括代替受损的神经元和髓鞘再生. 同时, 移植细胞能分泌多种营养因子和活性物质来提高宿主细胞的存活率, 调节炎症促进血管的再生^[64].

NSPCs是存在于神经系统中具有分裂潜能和自我更新能力的母细胞, 因与脊髓损伤区的细胞同源而具有独特的治疗优势. 大量的动物实验证实, NSPCs移植后可分化成神经元替代因损伤而丢失的神经元, 与上下游神经纤维建立新的突触联系, 改善运动及感觉功能^[65,66]. Tuszynski团队^[67]将NSPCs与负载生长因子“鸡尾酒”的纤维蛋白基质联合移植至大鼠脊髓全横断损伤处, 发现NSC/NPC能分化成大量的神经元, 轴突长距离生长并与宿主神经纤维建立突触联系, 展现出NSC治疗严重脊髓损伤的潜力. 该团队最近将人胚胎脊髓来源的NSPCs移植至恒河猴脊髓损伤部位, 发现新生神经元能与宿主神经建立联系, 帮助四肢瘫痪的恒河猴恢复前肢的部分功能. 并且NSPCs能分泌神经营养因子(BDNF, IGF-1, GDNF和VEGF-A)和抑炎因子(TGF- β 1和IL-10), 改善损伤部位微环境并促进轴突和髓鞘再生^[68].

骨髓间充质干细胞(bone marrow stem cells, BMSCs)是人们在哺乳动物的骨髓基质中发现的一种具有分化形成骨、软骨、脂肪、神经及成肌细胞的多种分化潜能的细胞亚群, 因其具有低免疫原性、取材方便、扩增迅速、遗传背景稳定等特点, 在组织工程和细胞及基因治疗等方面具有广阔的应用前景. BMSCs向神经细胞表型方向诱导后, 在体内可以分化为神经元和星形胶质细胞, 并能下调caspase-3的激活从而抑制神经细胞的凋亡^[69,70]. 近年来, BMSCs在脊髓损伤中的修复作用更多是通过分泌的营养物质和免疫调控分子. BMSCs可以分泌多种营养因子, 如睫状

神经营养因子(ciliary neurotrophic factor, CNTF)、转化生长因子 $\beta 1$ (transforming growth factor- $\beta 1$, TGF- $\beta 1$)、脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)、信号转导因子和转录激活子3(signal transducer and activator of transcription 3, STAT3)等促进轴突和神经细胞的再生, 保护残存组织细胞. BMSCs分泌的外泌体(BMSCs-exosomes, BMSCs-Exo)可以通过与小胶质细胞结合而抑制补体mRNA的合成和释放, 并抑制SCI中NF- κ B信号的激活, 在脊髓损伤中起保护作用^[71]. 腹膜内或静脉内注射BMSCs后, 可以通过局部释放BDNF, NGF, NT-3和NT-4等神经营养因子改善脊髓损伤后轴突髓鞘再生, 保存白质和改善运动功能^[72].

ASCs是来源于脂肪组织的另一种间充质干细胞, 易于获取, 与其他干细胞来源相比具有明显的优势. ASCs通过分泌细胞因子和生长因子促进多种组织再生和修复^[73]. ASCs分泌的细胞因子和生长因子参与免疫调节(HGF, PGE₂, TGF- β 和IL-6)、血管再生(FGF-2, HGF, VEGF, TGF- $\beta 2$ 和bFGF)、神经再生(GDNF, NGF, IGF-1和BDNF)、造血(HGF, GM-CSF, IL-6和IL-11等), 另外还能分泌其他因子(脂联素、血管紧张素和CXCL12)^[74-78]. 足底肌内注射ASCs可改善大鼠因烧伤诱导的脊髓腹角运动神经元和坐骨神经施万细胞的细胞凋亡, 并抑制腓肠肌的萎缩^[79]. 有关ASCs能否分化为神经元存在争议. 最近的一些研究报道ASCs在体外成功分化为神经谱系细胞和施万细胞, ASCs可表达用于识别增殖的成年神经祖细胞的nestin^[80]. 预分化的人源性ASCs(human adipose-derived mesenchymal stem cells, hADSCs)在体外转化为具有电生理功能的运动神经元样细胞, 移植后能与宿主的脊髓组织进行功能整合, 并与内源性神经元建立突触联系直接参与重建受损部位的神经回路^[81]. Ngn2基因敲入ADSCs(Ngn2-ADSCs)植入损伤脊髓后可分化为NeuN⁺和Tuj1⁺神经元, 抑制胶质瘢痕形成, 上调VEGF和BDNF的表达^[82].

SCs是周围神经的胶质细胞, 包绕周围神经纤维形成髓鞘. 近年研究发现, SCs可以通过髓鞘的形成以及对轴突的支持保护作用来促进轴突再生, 用于修复脊髓损伤^[83]. 同时, SCs能分泌多种神经营养因子、细胞外基质、细胞黏附分子. 因此, 研究认为修复脊髓损伤的机制主要是通过分泌多种神经营养因子及产生细胞外基质改善损伤部位微环境^[84]. 活化的SCs表达模

式识别受体(pattern recognition receptor, PRR), 促进吞噬细胞增殖, 吞噬髓鞘碎片并支持轴突再生^[85,86]. 但也有研究者指出, SCs能产生促炎细胞因子, 如IL-1 β , TNF- α , IL-6, 趋化因子以及一氧化氮等, 驱使免疫细胞渗透到损伤部位并放大免疫应答, 从而导致神经炎症加重^[87]. OPCs移植后主要分化为成熟的少突胶质细胞, 释放细胞因子, 包括IGF-1, GDNF和BDNF, 它们可促进神经元的存活, 维持轴突结构, 并在存活的轴突中支持突触可塑性^[88]. 营养因子的表达和释放提供了少突胶质细胞与神经元之间的相互作用, 在受损脊髓中形成功能性神经回路^[89]. OECs是在功能上介于施万细胞和少突胶质细胞之间的一种特殊的胶质细胞, 分泌BDNF, NRG和NGF, 具有神经营养, 抑制胶质增生, 减少瘢痕形成, 促进成鞘作用等功能^[90-92]. SCI后OECs移植促进残端中的神经元存活并保护病灶核心中存活的神经元, 减少病变中心轴突死亡和抑制性CSPG的表达以及髓磷脂碎片的产生^[93].

iPSC的出现为细胞移植治疗脊髓损伤带来了新的希望. iPSC可以通过诱导转录因子(Oct3/4, Sox2, c-Myc和Klf4)从体细胞中产生, 并诱导分化为具有中枢神经系统细胞特征的NPCs^[94]. 人iPSC衍生的NPCs能促进宿主脊髓损伤轴突的再生, 并与宿主神经细胞和树突形成突触结构建立神经环路^[95]. 将iPSC诱导成多种类型的神经细胞治疗脊髓损伤取得了可喜的成就, 如将iPSC派生为A2B5⁺细胞在体外和体内均显示出神经谱系的特异性分化潜能, 其生理功能类似NPC^[96].

在动物实验取得突破性进展的基础上, 干细胞移植治疗脊髓损伤的临床试验也积极开展. Ciacci团队^[97]将人脊髓来源的NSPCs系移植到慢性脊髓损伤病人T2~T12胸椎, 术后18~27个月表现出良好的安全性, 甚至获得一定的神经功能改善. MSCs可以直接从患者骨髓内提取培养进行自体移植, 是非常有吸引力的临床移植候选细胞, 已在脊髓损伤修复中展示了非常大的潜能. 来自耶鲁大学和日本的研究显示, 静脉输注患者的MSCs修复脊髓损伤, 在II期临床试验中, 所有13位患者6个月后神经功能均得到改善^[98]. 我国中山大学附属第三医院戎利民团队^[99,100]完成了41例慢性脊髓损伤受试者的完整随访, 证实蛛网膜下腔移植人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUC-MSCs)安全、有效, 能显著改善受损神经功能, 提升日常活动能力.

综上, 干细胞移植治疗脊髓损伤取得了令人瞩目的成果, 但是也存在以下几个问题需要解决: (i) 脊髓损伤急性期因损伤部位的炎症反应强烈, 此时移植细胞存活率低; 而在慢性期移植胶质瘢痕已经成熟, 细胞移植后效果不明显. 因此, 研究者将亚急性期作为细胞移植细胞的最佳时机, 但是具体时间和细胞存活问题仍无统一的标准, 需要进一步研究. (ii) 免疫排斥问题. 即使是自体细胞移植也存在免疫排斥, 自体细胞离体培养传代或定向诱导后, 会导致细胞部分抗原改变或丢失, 在进行细胞移植后可能会遭到宿主免疫细胞的攻击. (iii) 干细胞分化增殖问题. 虽然在动物体内多种干细胞并未向肿瘤方向分化, 但是这些研究在动物体内观察的时间大多有限, 而如果应用到人体就需要考虑观察更长时间以获得更为可靠的数据. 另外, 干细胞注射后因细胞自身具有移动能力, 可能会移动到移植以外的部位. 为此, 将干细胞固定在材料内再进行移植被广泛应用于脊髓损伤的治疗中.

2.3 生物材料在脊髓损伤中的应用

生物材料已经广泛应用于再生医学, 具有良好生物相容性的材料不仅能降低免疫排斥的风险, 保存宿主组织器官的解剖形态, 还能促进宿主组织器官的修复以及搭载药物、细胞因子或细胞. 目前有多种生物材料应用于脊髓损伤修复的研究, 包括天然生物材料如脱细胞基质、壳聚糖(chitosan)、透明质酸(hyaluronic acid)、胶原蛋白(collagen)、纤维蛋白(fibrin)、琼脂糖(agarose), 以及合成生物材料如聚乳酸(polylactic acid, PLA)、聚乙醇酸(polyglycolic acid, PGA)、聚己内酯(polycaprolactone, PCL)及其共聚物等^[101,102]. 这些生物材料被加工成三维支架、单/多通道导管和水凝胶, 作为“桥梁”为再生神经提供支撑.

三维多孔支架可为再生轴突提供人工细胞外基质, 为轴突延伸提供通道. 同时, 材料上能装载一些营养因子或者生长因子, 为轴突生长提供生长通道和促进生长的环境^[103]. 在支架内的孔腔内还可以注入各种细胞, 帮助轴突加速生长穿过病变区域^[104]. 多孔胶原基支架(porous collagen scaffolds, PCS)负载NSCs后治疗脊髓损伤, 结果显示, PCS移植植物可保护NSCs, 并在体内实现NSCs的神经分化和功能整合, 使NSC迁移到周围组织中, 并减少星形胶质变性; 体外实验显示, 小鼠背根神经节(dorsal root ganglion, DRG)细胞植入胶

原蛋白支架后5天, 细胞广泛附着在支架上, 神经元沿支架表面延伸了具有突触活性的长突起, 在支架内部形成DRG神经元的3D网络, 并且对神经丝重链和突触前标记突触素染色呈阳性^[105].

水凝胶是一类极为亲水的三维网络结构, 它在水中迅速溶胀并保持大量体积的水, 具有极高的柔韧性. 脊髓组织在结构上是水凝胶样的“材料”, 具有与脊髓弹性模量相匹配的水凝胶, 特别是可注射水凝胶具有无创或微创植入的优点, 可避免大型手术带来的风险, 并且原位形成凝胶非常适合临床上脊髓不规则形状的损伤部位, 可获得优异的植入物-宿主脊髓界面^[106,107]. 水凝胶还可以装载促进生长的分子或可以刺激轴突生长和组织修复的细胞, 发挥增强轴突生长潜力的作用. 由细胞外基质的胶原蛋白、纤连蛋白和透明质酸制备的水凝胶具有良好的生物相容性^[108], 可以作为细胞或者细胞因子递送的载体, 以促进神经再生及轴突生长, 同时也为再生组织提供结构支撑^[109]. 纤维蛋白基质联合MSCs移植能改善损伤区域微环境, 促进MSCs分化为神经元和损伤脊髓的修复^[110,111]. 将坐骨神经脱细胞后制成的基质支架与施万细胞移植到脊髓损伤部位后, 可支持移植的施万细胞存活和轴突生长, 从而改善运动功能^[112]. 生物材料基质可通过接触诱导机制促进轴突生长, 将三维纳米纤维与胶原蛋白水凝胶混合并装载NT3用于大鼠C5脊髓损伤的治疗, 显著提高了轴突的生长长度, 而且再生的轴突能沿纳米纤维的方向生长^[113]. 因此, 开发黏弹性能与宿主脊髓相匹配的可注射水凝胶是脊髓损伤修复材料的发展方向.

本团队^[114]前期对RADA 16-I短肽进行了深入的改性研究, 发明了一类在中性pH条件下形成纳米水凝胶的功能性自组装短肽, 解决了因溶液呈酸性而无法直接注射使用的难题, 显著促进了脊髓神经再生. 短肽纳米水凝胶具有可批量生产、重复性高、安全稳定、可降解以及黏弹性能可调控等诸多优点, 是一种极具临床转化前景的脊髓损伤修复材料. 将含血管化组装肽支架植入损伤脊髓部位后, 降低了损伤部位炎症反应和神经胶质疤痕的形成, 增加了进入损伤/移植部位神经轴突的密度. 将毛细管内皮细胞接种藻酸盐水凝胶(alginate hydrogels, AHs)可以恢复脊髓的连续性并支持轴突再生, 促进大量宿主细胞迁移到支架通道中^[115]. AH移植植物减少了病变周围的纤维胶质细胞瘢痕, 并且5-羟色胺能轴突生长到整个支架并延伸到

远端宿主实质中^[116]。此外, 植入人体的水凝胶常常会受到来自外在的机械作用而极易产生应力裂纹, 裂纹进一步发展会破坏材料内部结构的规整性和功能的完整性, 而破损的凝胶基质进入体液后还很容易引起排斥反应, 具有引发炎症的风险。水凝胶若具有自修复性能, 在被外力损坏后能自发地重新结合为一个整体, 将会有更大的应用价值^[117]。

在大量的动物实验基础上, 临床试验也逐步开展。我国学者戴建武团队^[118]开展了首例神经再生胶原支架治疗急性完全性脊髓损伤临床研究, 经过一年的康复, 受试者下肢肌力明显增强, 髋关节的活动功能大幅度改善, 可在支具的辅助下通过髋关节的活动行走。这无疑为基于生物材料的脊髓损伤治疗策略带来了希望。

3 总结与展望

恢复传导通路是功能恢复的基础和关键, 目前并无有效的方法。因此, 促进传导通路的轴突再生, 跨过损伤区域与靶区重建功能联系仍然是脊髓损伤研究的重点。损伤部位的微环境极其复杂, 为修复带来极大的挑战。单一修复方法效果不佳, 应该研究综合治疗方法。在脊髓损伤急性期应该调控炎症反应, 减少继发损伤的严重程度; 在慢性期通过康复及其他治疗激发残留的神经功能。生物材料的可塑性能有效填补损伤造成的不规则空洞, 起到消炎、搭桥、促再生、重塑损伤内环境等作用, 生物材料对损伤部位的修复最有可能尽快转化到临床应用, 与其他修复方法的协同应用具有更大的潜力。

参考文献

- McDonald J W, Sadowsky C. Spinal-cord injury. *Lancet*, 2002, 359: 417–425
- Siddall P J, Middleton J W. Spinal cord injury-induced pain: mechanisms and treatments. *Pain Manage*, 2015, 5: 493–507
- Alabed S, de Heredia L L, Naidoo A, et al. Incidence of pulmonary embolism after the first 3 months of spinal cord injury. *Spinal Cord*, 2015, 53: 835–837
- Gwak Y S, Hulsebosch C E, Leem J W. Neuronal-glial interactions maintain chronic neuropathic pain after spinal cord injury. *Neural Plast*, 2017, 2017: 1–14
- Stahel P F, VanderHeiden T, Finn M A. Management strategies for acute spinal cord injury. *Curr Opin Crit Care*, 2012, 18: 651–660
- Uldreaj A, Chio J C T, Ahuja C S, et al. Modulating the immune response in spinal cord injury. *Expert Rev Neurother*, 2016, 16: 1127–1129
- Ahuja C S, Nori S, Tetreault L, et al. Traumatic spinal cord injury—repair and regeneration. *Neurosurgery*, 2017, 80: S9–S22
- Kawano H, Kimura-Kuroda J, Komuta Y, et al. Role of the lesion scar in the response to damage and repair of the central nervous system. *Cell Tissue Res*, 2012, 349: 169–180
- Wilson J R, Forgione N, Fehlings M G. Emerging therapies for acute traumatic spinal cord injury. *CMAJ*, 2013, 185: 485–492
- Anwar M A, Al Shehabi T S, Eid A H. Inflammogenesis of secondary spinal cord injury. *Front Cell Neurosci*, 2016, 10: 98
- Kerschensteiner M, Schwab M E, Lichtman J W, et al. *In vivo* imaging of axonal degeneration and regeneration in the injured spinal cord. *Nat Med*, 2005, 11: 572–577
- Davies S J A, Fitch M T, Memberg S P, et al. Regeneration of adult axons in white matter tracts of the central nervous system. *Nature*, 1997, 390: 680–683
- Evans T A, Barkauskas D S, Myers J T, et al. High-resolution intravital imaging reveals that blood-derived macrophages but not resident microglia facilitate secondary axonal dieback in traumatic spinal cord injury. *Exp Neurol*, 2014, 254: 109–120
- Syková E, Nicholson C. Diffusion in brain extracellular space. *Physiol Rev*, 2008, 88: 1277–1340
- Ren Y, Ao Y, O'Shea T M, et al. Ependymal cell contribution to scar formation after spinal cord injury is minimal, local and dependent on direct ependymal injury. *Sci Rep*, 2017, 7: 41122
- Wanner I B, Anderson M A, Song B, et al. Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. *J Neurosci*, 2013, 33: 12870–12886
- Qian D, Li L, Rong Y, et al. Blocking Notch signal pathway suppresses the activation of neurotoxic A1 astrocytes after spinal cord injury. *Cell Cycle*, 2019, 18: 3010–3029
- Yang T, Dai Y J, Chen G, et al. Dissecting the dual role of the glial scar and scar-forming astrocytes in spinal cord injury. *Front Cell Neurosci*,

- 2020, 14: 78
- 19 Cheng Y Y, Zhao H K, Chen L W, et al. Reactive astrocytes increase expression of proNGF in the mouse model of contused spinal cord injury. *Neurosci Res*, 2020, 157: 34–43
 - 20 Lukovic D, Stojkovic M, Moreno-Manzano V, et al. Concise review: reactive astrocytes and stem cells in spinal cord injury: good guys or bad guys? *Stem Cells*, 2015, 33: 1036–1041
 - 21 Xu L, Tang Y Y, Ben X L, et al. Ginsenoside Rg1-induced activation of astrocytes promotes functional recovery via the PI3K/Akt signaling pathway following spinal cord injury. *Life Sci*, 2020, 252: 117642
 - 22 Griffin J M, Fackelmeier B, Clemett C A, et al. Astrocyte-selective AAV-ADAMTS4 gene therapy combined with hindlimb rehabilitation promotes functional recovery after spinal cord injury. *Exp Neurol*, 2020, 327: 113232
 - 23 Visavadiya N P, Patel S P, VanRooyen J L, et al. Cellular and subcellular oxidative stress parameters following severe spinal cord injury. *Redox Biol*, 2016, 8: 59–67
 - 24 Dumont R J, Okonkwo D O, Verma S, et al. Acute spinal cord injury, part I: pathophysiologic mechanisms. *Clin Neuropharmacol*, 2001, 24: 254–264
 - 25 Donnelly D J, Popovich P G. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. *Exp Neurol*, 2008, 209: 378–388
 - 26 Orr M B, Gensel J C. Spinal cord injury scarring and inflammation: therapies targeting glial and inflammatory responses. *Neurotherapeutics*, 2018, 15: 541–553
 - 27 Hall E D, Springer J E. Neuroprotection and acute spinal cord injury: a reappraisal. *NeuroRX*, 2004, 1: 80–100
 - 28 Anderson D K, Hall E D. Pathophysiology of spinal cord trauma. *Ann Emerg Med*, 1993, 22: 987–992
 - 29 Francos-Quijorna I, Santos-Nogueira E, Gronert K, et al. Maresin 1 promotes inflammatory resolution, neuroprotection, and functional neurological recovery after spinal cord injury. *J Neurosci*, 2017, 37: 11731–11743
 - 30 Kigerl K A, McGaughy V M, Popovich P G. Comparative analysis of lesion development and intraspinal inflammation in four strains of mice following spinal contusion injury. *J Comp Neurol*, 2006, 494: 578–594
 - 31 Liu C Y, Wang Y M, Wang C L, et al. Population alterations of l-arginase- and inducible nitric oxide synthase-expressed CD11b⁺/CD14⁺/CD15⁺/CD33⁺ myeloid-derived suppressor cells and CD8⁺ T lymphocytes in patients with advanced-stage non-small cell lung cancer. *J Cancer Res Clin Oncol*, 2010, 136: 35–45
 - 32 Neirinx V, Coste C, Franzen R, et al. Neutrophil contribution to spinal cord injury and repair. *J Neuroinflammation*, 2014, 11: 1–9
 - 33 Ghasemlou N, Bouhy D, Yang J, et al. Beneficial effects of secretory leukocyte protease inhibitor after spinal cord injury. *Brain*, 2010, 133: 126–138
 - 34 Hannila S S, Siddiq M M, Carmel J B, et al. Secretory leukocyte protease inhibitor reverses inhibition by CNS myelin, promotes regeneration in the optic nerve, and suppresses expression of the transforming growth factor- β signaling protein Smad2. *J Neurosci*, 2013, 33: 5138–5151
 - 35 Kurimoto T, Yin Y, Habboub G, et al. Neutrophils express oncomodulin and promote optic nerve regeneration. *J Neurosci*, 2013, 33: 14816–14824
 - 36 Sas A R, Carbajal K S, Jerome A D, et al. A new neutrophil subset promotes CNS neuron survival and axon regeneration. *Nat Immunol*, 2020, 21: 1496–1505
 - 37 Murray P J, Allen J E, Biswas S K, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*, 2014, 41: 14–20
 - 38 Van Assche T, Deschacht M, da Luz R A I, et al. Leishmania-macrophage interactions: Insights into the redox biology. *Free Radic Biol Med*, 2011, 51: 337–351
 - 39 Galli S J, Borregaard N, Wynn T A. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nat Immunol*, 2011, 12: 1035–1044
 - 40 Gensel J C, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res*, 2015, 1619: 1–11
 - 41 Mosser D M, Edwards J P. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*, 2008, 8: 958–969
 - 42 Bouhrel M A, Derudas B, Rigamonti E, et al. PPAR γ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab*, 2007, 6: 137–143
 - 43 Gallardo-Soler A, Gómez-Nieto C, Campo M L, et al. Arginase I induction by modified lipoproteins in macrophages: a peroxisome proliferator-

- activated receptor- γ/δ -mediated effect that links lipid metabolism and immunity. *Mol Endocrinol*, 2008, 22: 1394–1402
- 44 Kigerl K A, Gensel J C, Ankeny D P, et al. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci*, 2009, 29: 13435–13444
- 45 Francos-Quijorna I, Amo-Aparicio J, Martinez-Muriana A, et al. IL-4 drives microglia and macrophages toward a phenotype conducive for tissue repair and functional recovery after spinal cord injury. *Glia*, 2016, 64: 2079–2092
- 46 Guerrero A R, Uchida K, Nakajima H, et al. Blockade of interleukin-6 signaling inhibits the classic pathway and promotes an alternative pathway of macrophage activation after spinal cord injury in mice. *J Neuroinflammation*, 2012, 9: 40
- 47 Busch S A, Hamilton J A, Horn K P, et al. Multipotent adult progenitor cells prevent macrophage-mediated axonal dieback and promote regrowth after spinal cord injury. *J Neurosci*, 2011, 31: 944–953
- 48 Bao C, Wang B, Yang F, et al. Blockade of interleukin-7 receptor shapes macrophage alternative activation and promotes functional recovery after spinal cord injury. *Neuroscience*, 2018, 371: 518–527
- 49 Xu S, Zhu W, Shao M, et al. Ecto-5'-nucleotidase (CD73) attenuates inflammation after spinal cord injury by promoting macrophages/microglia M2 polarization in mice. *J Neuroinflammation*, 2018, 15: 155
- 50 Gensel J C, Kopper T J, Zhang B, et al. Predictive screening of M1 and M2 macrophages reveals the immunomodulatory effectiveness of post spinal cord injury azithromycin treatment. *Sci Rep*, 2017, 7: 1
- 51 Zhang Y, Liu Z, Zhang W, et al. Melatonin improves functional recovery in female rats after acute spinal cord injury by modulating polarization of spinal microglial/macrophages. *J Neurosci Res*, 2019, 97: 733–743
- 52 Fehlings M G, Wilson J R, Harrop J S, et al. Efficacy and safety of methylprednisolone sodium succinate in acute spinal cord injury: a systematic review. *Glob Spine J*, 2017, 7: 116S–137S
- 53 Wang W, Zuo B, Liu H, et al. Intermittent injection of Methylprednisolone Sodium Succinate in the treatment of Cervical Spinal Cord injury complicated with incomplete paraplegia. *Pak J Med Sci*, 2018, 35: 141
- 54 Miller R G, Mitchell J D, Moore D H. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev*, 2012, 2012: CD001447
- 55 Wu Y, Satkunendrarajah K, Teng Y, et al. Delayed post-injury administration of riluzole is neuroprotective in a preclinical rodent model of cervical spinal cord injury. *J Neurotrauma*, 2013, 30: 441–452
- 56 Wu Q, Zhang Y, Zhang Y, et al. Riluzole improves functional recovery after acute spinal cord injury in rats and may be associated with changes in spinal microglia/macrophages polarization. *Neurosci Lett*, 2020, 723: 134829
- 57 Tateda S, Kanno H, Ozawa H, et al. Rapamycin suppresses microglial activation and reduces the development of neuropathic pain after spinal cord injury. *J Orthop Res*, 2017, 35: 93–103
- 58 Gao K, Wang Y S, Yuan Y J, et al. Neuroprotective effect of rapamycin on spinal cord injury via activation of the Wnt/ β -catenin signaling pathway. *Neural Regen Res*, 2015, 10: 951
- 59 Goldshmit Y, Kanner S, Zacs M, et al. Rapamycin increases neuronal survival, reduces inflammation and astrocyte proliferation after spinal cord injury. *Mol Cell Neurosci*, 2015, 68: 82–91
- 60 Han W, Li Y, Cheng J, et al. Sitagliptin improves functional recovery via GLP-1R-induced anti-apoptosis and facilitation of axonal regeneration after spinal cord injury. *J Cell Mol Med*, 2020, 24: 8687–8702
- 61 Sobrido-Cameán D, Robledo D, Romaus-Sanjurjo D, et al. Inhibition of gamma-secretase promotes axon regeneration after a complete spinal cord injury. *Front Cell Dev Biol*, 2020, 8: 173
- 62 Bunge R P, Bunge M B. Interrelationship between Schwann cell function and extracellular matrix production. *Trends Neurosci*, 1983, 6: 499–505
- 63 Kim Y, Jo S H, Kim W H, et al. Antioxidant and anti-inflammatory effects of intravenously injected adipose derived mesenchymal stem cells in dogs with acute spinal cord injury. *Stem Cell Res Ther*, 2015, 6: 229
- 64 Tetzlaff W, Okon E B, Karimi-Abdolrezaee S, et al. A systematic review of cellular transplantation therapies for spinal cord injury. *J Neurotrauma*, 2011, 28: 1611–1682
- 65 Pereira I M, Marote A, Salgado A J, et al. Filling the gap: neural stem cells as a promising therapy for spinal cord injury. *Pharmaceuticals*, 2019, 12: 65
- 66 Lu P, Wang Y, Graham L, et al. Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. *Cell*, 2012, 150:

1264–1273

- 67 Rosenzweig E S, Brock J H, Lu P, et al. Restorative effects of human neural stem cell grafts on the primate spinal cord. *Nat Med*, 2018, 24: 484–490
- 68 Gao S, Guo X, Zhao S, et al. Differentiation of human adipose-derived stem cells into neuron/motoneuron-like cells for cell replacement therapy of spinal cord injury. *Cell Death Dis*, 2019, 10: 597
- 69 Muniswami D M, Kanthakumar P, Kanakasabapathy I, et al. Motor recovery after transplantation of bone marrow mesenchymal stem cells in rat models of spinal cord injury. *Ann Neurosci*, 2018, 25: 126–140
- 70 Lin L, Lin H, Bai S, et al. Bone marrow mesenchymal stem cells (BMSCs) improved functional recovery of spinal cord injury partly by promoting axonal regeneration. *Neurochem Int*, 2018, 115: 80–84
- 71 Zhao C, Zhou X, Qiu J, et al. Exosomes derived from bone marrow mesenchymal stem cells inhibit complement activation in rats with spinal cord injury. *Drug Des Devel Ther*, 2019, Volume 13: 3693–3704
- 72 Ramalho B D S, Almeida F M, Sales C M, et al. Injection of bone marrow mesenchymal stem cells by intravenous or intraperitoneal routes is a viable alternative to spinal cord injury treatment in mice. *Neural Regen Res*, 2018, 13: 1046
- 73 Salgado J A, L Reis R, Sousa N, et al. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. *Curr Stem Cell Res Ther*, 2010, 5: 103–110
- 74 Gir P, Oni G, Brown S A, et al. Human adipose stem cells: current clinical applications. *Plast Reconstr Surg*, 2012, 129: 1277–1290
- 75 Sadat S, Gehmert S, Song Y H, et al. The cardioprotective effect of mesenchymal stem cells is mediated by IGF-I and VEGF. *Biochem Biophys Res Commun*, 2007, 363: 674–679
- 76 Hong S J, Jia S X, Xie P, et al. Topically delivered adipose derived stem cells show an activated-fibroblast phenotype and enhance granulation tissue formation in skin wounds. *PLoS ONE*, 2013, 8: e55640
- 77 Kilroy G E, Foster S J, Wu X, et al. Cytokine profile of human adipose-derived stem cells: Expression of angiogenic, hematopoietic, and pro-inflammatory factors. *J Cell Physiol*, 2007, 212: 702–709
- 78 Seo M J, Suh S Y, Bae Y C, et al. Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. *Biochem Biophys Res Commun*, 2005, 328: 258–264
- 79 Wu S H, Huang S H, Lo Y C, et al. Autologous adipose-derived stem cells attenuate muscular atrophy and protect spinal cord ventral horn motor neurons in an animal model of burn injury. *Cytotherapy*, 2015, 17: 1066–1075
- 80 Carelli S, Giallongo T, Rey F, et al. Neuroprotection, recovery of function and endogenous neurogenesis in traumatic spinal cord injury following transplantation of activated adipose tissue. *Cells*, 2019, 8: 329
- 81 Arboleda D, Forostyak S, Jendelova P, et al. Transplantation of predifferentiated adipose-derived stromal cells for the treatment of spinal cord injury. *Cell Mol Neurobiol*, 2011, 31: 1113–1122
- 82 Tang L, Lu X, Zhu R, et al. Adipose-derived stem cells expressing the neurogenin-2 promote functional recovery after spinal cord injury in rat. *Cell Mol Neurobiol*, 2016, 36: 657–667
- 83 Bunge M B. Efficacy of Schwann cell transplantation for spinal cord repair is improved with combinatorial strategies. *J Physiol*, 2016, 594: 3533–3538
- 84 Kanno H, Pearse D D, Ozawa H, et al. Schwann cell transplantation for spinal cord injury repair: its significant therapeutic potential and prospectus. *Rev Neurosci*, 2015, 26: 121–128
- 85 Campana W M. Schwann cells: activated peripheral glia and their role in neuropathic pain. *Brain Behav Immun*, 2007, 21: 522–527
- 86 Tofaris G K, Patterson P H, Jessen K R, et al. Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *J Neurosci*, 2002, 22: 6696–6703
- 87 Dubový P. Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction. *Ann Anat*, 2011, 193: 267–275
- 88 Wilkins A, Majed H, Layfield R, et al. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. *J Neurosci*, 2003, 23: 4967–4974
- 89 Sun Y, Xu C C, Li J, et al. Transplantation of oligodendrocyte precursor cells improves locomotion deficits in rats with spinal cord irradiation injury. *PLoS ONE*, 2013, 8: e57534
- 90 Kim B Y, Park J Y, Kim E J, et al. Olfactory ensheathing cells mediate neuroplastic mechanisms after olfactory training in mouse model. *Am J*

- Rhinol Allergy, 2020, 34: 217–229
- 91 Gómez R M, Sánchez M Y, Portela-Lomba M, et al. Cell therapy for spinal cord injury with olfactory ensheathing glia cells (OECs). *Glia*, 2018, 66: 1267–1301
- 92 Wright A A, Todorovic M, Tello-Velasquez J, et al. Enhancing the therapeutic potential of olfactory ensheathing cells in spinal cord repair using neurotrophins. *Cell Transplant*, 2018, 27: 867–878
- 93 Muniswami D M, Tharion G. Functional recovery following the transplantation of olfactory ensheathing cells in rat spinal cord injury model. *Asian Spine J*, 2018, 12: 998–1009
- 94 Wang B, Wu L, Li D, et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Jdp2-Jhdm1b-Mkk6-Glis1-Nanog-Essrb-Sall4. *Cell Rep*, 2019, 27: 3473–3485.e5
- 95 Liu Y, Zheng Y, Li S, et al. Human neural progenitors derived from integration-free iPSCs for SCI therapy. *Stem Cell Res*, 2017, 19: 55–64
- 96 Hodgetts S I, Edel M, Harvey A R. The state of play with iPSCs and spinal cord injury models. *J Clin Med*, 2015, 4: 193–203
- 97 Curtis E, Martin J R, Gabel B, et al. A first-in-human, phase I study of neural stem cell transplantation for chronic spinal cord injury. *Cell Stem Cell*, 2018, 22: 941–950.e6
- 98 Honmou O, Yamashita T, Morita T, et al. Intravenous infusion of auto serum-expanded autologous mesenchymal stem cells in spinal cord injury patients: 13 case series. *Clin Neurol Neurosurg*, 2021, 203: 106565
- 99 Yang Y, Pang M, Chen Y Y, et al. Human umbilical cord mesenchymal stem cells to treat spinal cord injury in the early chronic phase: study protocol for a prospective, multicenter, randomized, placebo-controlled, single-blinded clinical trial. *Neural Regen Res*, 2020, 15: 1532–1538
- 100 Yang Y, Pang M, Du C, et al. Repeated subarachnoid administrations of allogeneic human umbilical cord mesenchymal stem cells for spinal cord injury: a phase 1/2 pilot study. *Cytotherapy*, 2021, 23: 57–64
- 101 Collins M N, Birkinshaw C. Hyaluronic acid based scaffolds for tissue engineering—A review. *Carbohydr Polym*, 2013, 92: 1262–1279
- 102 Peng Z, Gao W, Yue B, et al. Promotion of neurological recovery in rat spinal cord injury by mesenchymal stem cells loaded on nerve-guided collagen scaffold through increasing alternatively activated macrophage polarization. *J Tissue Eng Regen Med*, 2018, 12: e1725–e1736
- 103 Pabari A, Lloyd-Hughes H, Seifalian A M, et al. Nerve conduits for peripheral nerve surgery. *Plast Reconstr Surg*, 2014, 133: 1420–1430
- 104 Houweling D A, Lankhorst A J, Gispén W H, et al. Collagen containing neurotrophin-3 (NT-3) attracts regrowing injured corticospinal axons in the adult rat spinal cord and promotes partial functional recovery. *Exp Neurol*, 1998, 153: 49–59
- 105 Assunção-Silva R C, Gomes E D, Sousa N, et al. Hydrogels and cell based therapies in spinal cord injury regeneration. *Stem Cells Int*, 2015, 2015: 1–24
- 106 Chockalingam K, Blenner M, Banta S. Design and application of stimulus-responsive peptide systems. *Protein Eng Des Sel*, 2007, 20: 155–161
- 107 Taylor S J, Sakiyama-Elbert S E. Effect of controlled delivery of neurotrophin-3 from fibrin on spinal cord injury in a long term model. *J Control Release*, 2006, 116: 204–210
- 108 Nisbet D R, Crompton K E, Horne M K, et al. Neural tissue engineering of the CNS using hydrogels. *J Biomed Mater Res*, 2008, 87B: 251–263
- 109 Dumont C M, Carlson M A, Munsell M K, et al. Aligned hydrogel tubes guide regeneration following spinal cord injury. *Acta Biomater*, 2019, 86: 312–322
- 110 Jain A, Kim Y T, McKeon R J, et al. *In situ* gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. *Biomaterials*, 2006, 27: 497–504
- 111 Mukhamedshina Y O, Akhmetzyanova E R, Kostennikov A A, et al. Adipose-derived mesenchymal stem cell application combined with fibrin matrix promotes structural and functional recovery following spinal cord injury in rats. *Front Pharmacol*, 2018, 9: 343
- 112 Papa S, Vismara I, Mariani A, et al. Mesenchymal stem cells encapsulated into biomimetic hydrogel scaffold gradually release CCL2 chemokine in situ preserving cytoarchitecture and promoting functional recovery in spinal cord injury. *J Control Release*, 2018, 278: 49–56
- 113 Milbreta U, Nguyen L H, Diao H, et al. Three-dimensional nanofiber hybrid scaffold directs and enhances axonal regeneration after spinal cord injury. *ACS Biomater Sci Eng*, 2016, 2: 1319–1329
- 114 Sun Y, Li W, Wu X, et al. Functional self-assembling peptide nanofiber hydrogels designed for nerve degeneration. *ACS Appl Mater Interfaces*, 2016, 8: 2348–2359
- 115 Tran K A, Partyka P P, Jin Y, et al. Vascularization of self-assembled peptide scaffolds for spinal cord injury repair. *Acta Biomater*, 2020, 104: 76–84
- 116 Huang L, Wang Y, Zhu M, et al. Anisotropic alginate hydrogels promote axonal growth across chronic spinal cord transections after scar

removal. *ACS Biomater Sci Eng*, 2020, 6: 2274–2286

- 117 Luo J, Shi X, Li L, et al. An injectable and self-healing hydrogel with controlled release of curcumin to repair spinal cord injury. *Bioact Mater*, 2021, 6: 4816–4829
- 118 Zhao Y, Tang F, Xiao Z, et al. Clinical study of neuroregen scaffold combined with human mesenchymal stem cells for the repair of chronic complete spinal cord injury. *Cell Transplant*, 2017, 26: 891–900

Pathological changes and repair strategies for spinal cord injury

LI JianPing², HE LiuMin³ & WU WuTian^{1,2,3}

¹*Re-Stem Biotechnology Co., Ltd., Suzhou 215168, China;*

²*Guangdong-Hong Kong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou 510632, China;*

³*Spine Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China*

Spinal cord injury causes necrosis of nerve tissue, disruption of conduction pathways, loss of movement and sensation below the injury level, resulting in paralysis and even death. The pathological changes of spinal cord injury are extremely complex. At the early stage, the changes were mainly at the molecular/gene level, while the changes at the cell/tissue level were mainly in the subacute stage. These changes cause secondary damage, resulting in tissue necrosis, neuron death, axon rupture, the formation of cystic cavities wrapped by scar tissue and the subsequent inhibition of axon regeneration. At present, the function of the damaged nerve cannot be fundamentally improved by surgical decompression alone, or by symptomatic intervention with drugs. There are several reasons for the difficulty in functional recovery after spinal cord injury: inflammatory reactions take place through the whole process of spinal cord injury, and the inflammatory mediators lead to degeneration and necrosis of neurons and glial cells in the injured area, and axon atrophy due to valerosis. Regeneration of neurons and axons are both weak, and scar tissues prevent axons from crossing the damaged area to form connection with the contralateral axons. This article reviews the pathological changes after spinal cord injury and discusses the repair strategies.

spinal cord injury, repair, stem cells, materials

doi: [10.1360/SSV-2021-0065](https://doi.org/10.1360/SSV-2021-0065)