PERSPECTIVE ARTICLE

Rectify the Injury-Induced Microenvironment Imbalance in Peripheral Nerve Repair

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Peripheral nerve injury poses a great threat to the involved individuals' life quality. Given the situation that the current clinical treatments have limitations, it is urgent to seek for alternative therapies. Artificial nerve guide conduits can bridge nerve gaps and the underlying mechanism is worth studying. In this perspective, we propose that a high-performance nerve guide conduit repairs peripheral nerve by rectifying the injury-induced microenvironment imbalance. And we analyse four different ways to stabilize the disturbed microenvironment: (i) nourish the distal nerve end, (ii) stabilize the immune response, (iii) rectify the energy metabolic disturbance, (iv) restore the bioelectrical signal conduction.

Introduction

Severe peripheral nerve injury (PNI) results in nerve gaps. For nerve gaps over 2cm, epineural coaptation surgery cannot be performed and the standard-of-care therapy autograft transplantation brings about side effects (e.g. morbidity in donor sites and lack of resources) [**1,2**]. Luckily, peripheral nerves have the intrinsic capacity to regenerate. With the aid of an artificial nerve guide conduit (NGC), the proximal axons can regrow across the gap to complete reinnervation. To date, the reported NGCs can repair nerve gaps up to 5cm in rhesus macaques and 2cm in Sprague–Dawley rats [**3,4**]. However, there are still some limitations. For instance, the NGC implantation cannot guarantee a satisfactory outcome when bridging extensive nerve gaps and the full recovery of motor function remains challenging [**5**]. In recent decades, multiple methods have been explored to improve the NGCs' performances. Based on our previous research, we speculate that the homeostatic balance of native nerve microenvironment is disrupted when PNI occurs. A well-designed NGC can achieve satisfactory therapeutic effect via rectifying the injury-induced microenvironment imbalance in peripheral nerve regeneration (**Fig. 1**). Herein, we propose that there are four different ways for a high-performance NGC to facilitate neurogenesis.

Nourishment of distal nerve end

When a nerve is transected or even defected, the distal end loses its connection with the proximal end and it no longer receives bio-signals from the neuron. Meanwhile, the relative micro-vessels are destroyed, leading to the consequent loss of nutritional support. Thus, providing nutritional support for the distal end is among the key reasons for a high-performance NGC to repair a transected or defected nerve.

New vessel formation is of vital significance in peripheral nerve regeneration for its ability to nourish the distal nerve end. Our graphene oxide/polycaprolactone NGC helped new micro-vessels regrow in the original channels. We also identified one of the possible underlying mechanisms-the activation of AKT-endothelial nitric oxide synthase (eNOS)-vascular endothelial growth factor (VEGF) signalling cascade. Other pro-angiogenesis molecules like ephrin, Ang1, Ang2 and basic fibroblast growth factor (bFGF) participated in the regenerating process as well [**6**]. In addition, our black phosphorus nanoparticle controlled-release system created a mildly oxidative microenvironment to induce new vessel formation, and therefore effectively repaired a 20-mm rat sciatic nerve defect [**4**].

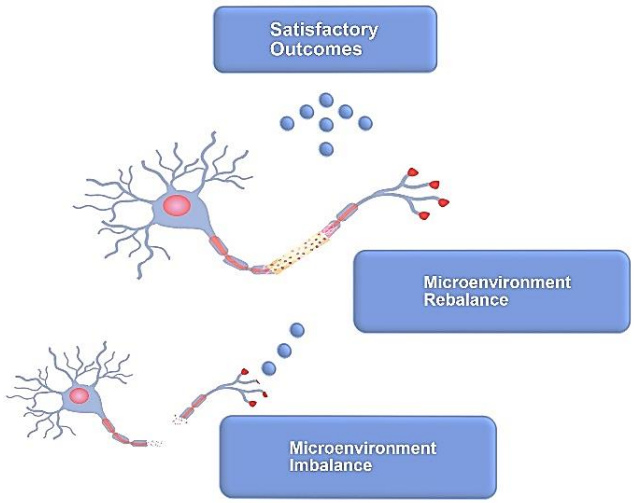

Fig. 1. The microenvironment imbalance and rebalance in peripheral nerve injury.

Stabilization of immune responses

When traumatic PNI happens, the microenvironment produces cytokines, and activates injury-induced immune responses. Immune cells like macrophages and neutrophils are recruited to the injured site. Among them, macrophages play a vital role in modulating the regenerating process. M1 macrophages, known for their pro-inflammatory effects, respond to the injury-induced microenvironment by removing myelin debris [**7**]. This process makes the microenvironment more suitable for the proximal end to reconnect with the distal end, and it is supposed to end within 48 hours post injury [**8**]. However, if we do not intervene, M1 macrophages keep recruiting chemokines and cause chronic inflammatory responses, thereby impeding the progress of regenerating axons. Therefore, researchers should pay attention to the immune responses when applying the NGC implantation therapy.

Our nano-diamonds/polycaprolactone NGC showed the ability to regulate macrophage phenotype both in vitro and in vivo. We examined the expression level of IL-6, TNF-α (classic M1 phenotype macrophage markers), CD206 and IL-10 (classic M2 phenotype macrophage markers). The results proved the effectiveness of our immune-modulating NGC. It successfully repaired 20-mm rat sciatic nerve defects by ameliorating the immune milieu [**9**].

Rectification of energy metabolic disturbance

PNI disrupts the homeostatic balance of energy metabolism near the injury site. The mitochondrial function is impaired and the regenerating microenvironment is overloaded with reactive oxygen species (ROS). Lasting ROS exposure hinders neurite outgrowth and Schwann cell remyelination. However, low-level oxidative stress helps induce angiogenesis and endothelial cell recruitment [**4**]. To conclude, a well-designed NGC could modulate the energy metabolism of the injury site.

Our group found that melatonin application could remedy the post-traumatic mitochondrial dysfunction and relieve the oxidative stress in peripheral nervous system. The expression of antioxidant markers like MnSOD (manganese superoxide dismutase), HO-1 (heme oxygenase-1) and GCLC (glutamate cysteine ligase) significantly increased in our 3D melatonin/ polycaprolactone conduit implantation group. ATP synthase and Complex I expression, indicating the function of mitochondrial was also elevated in vivo. The in vitro study supported this opinion as well [**10**].

In addition, we analysed the ability of our green tea-derived (-) -Epigallocatechin gallate-loaded NGC to scavenge free radical oxygen by examining similar biomarkers, and demonstrated the importance of rectifying the energy metabolic disturbance again [**11**].

Restoration of bioelectrical signal conduction

Nerve tissues are electroactive, but PNI blocks the bioelectrical signal conduction. According to our previous research, electrically conductive NGCs exhibited superior therapeutic effect than their nonconductive counterparts [**12**]. When the regeneration process initiates, bioelectricity is gradually restored. Electrically conductive NGCs can conduct this bioelectricity, which in turn improves axon elongation, Schwann cell remyelination and micro-vessel formation. Furthermore, external electrical stimulation is also beneficial, and higher intensity within a certain range leads to better therapeutic effects [**13**]. In vitro study showed that the external electrical stimulation could upregulate the expression of brain-derived neurotrophic factor, tyrosine kinase B, α1-tubulin and growth-associated protein 43, which are pro-regenerative proteins, in neuronal cells [**14,15**].

Although the exact mechanism remains elusive, substantial evidences support the positive role of conductive biomaterials in peripheral nerve tissue engineering [**16-19**]. We functionalized our NGCs with graphene, graphene oxide, gold nanocomposite and black phosphorus, and achieved exciting therapeutic effects in repairing extensive peripheral nerve defect and improving functional recovery [**4,6,12, 20**].

Apart from electrically conductive NGCs, we engineered self-powered NGC based on piezoelectric materials. The scaffold could apply “smart and wireless” electrical stimulation therapy in the injured microenvironment by converting ambient mechanical energy into electric signals and effectively repaired 15mm sciatic nerve defects in Sprague–Dawley rats **[21, 22]**.

Conclusion & future prospective

In conclusion, a high-performance NGC promotes peripheral nerve regeneration via rectifying the injury-induced microenvironment imbalance. In this perspective, we propose that there are four different ways to rebalance the microenvironment: (i) nourish the distal nerve end, (ii) stabilize the immune response, (iii) rectify the energy metabolic disturbance, (iv) restore the bioelectrical signal conduction. It will deepen our understanding on the PNI and shed light on the potential treatment in the future.

Fadia *et al*. demonstrated that the average functional recovery rates of NGC implantation and autograft transplantation are both <80% in nonhuman primates [**3**]. The exact explanation for this phenomenon remains controversial. It could be explained by micro structure mismatch, irreversible atrophy of dominant muscles, neural apoptosis, degeneration of neuromuscular junctions and degeneration of the distal nerve end. Although fancy technologies like immune-modulating bio-interfaces, “smart” drug delivery systems and biomimetic 3D printing seemingly hold great promises, their therapeutic effects are very likely to be limited. Our group is working on a systematic theory that thoroughly explains the question “how could NGC implantation benefit peripheral nerve regeneration”. We hope this theory will guide researchers engineer an integrated NGC that promote peripheral nerve regeneration from different aspects and push the current limits of therapeutic effects.

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Conflicts of interest

There are no conflicts to declare.

Keywords

Peripheral nerve regeneration, nerve guide conduit, regenerative medicine, microenvironment imbalance.

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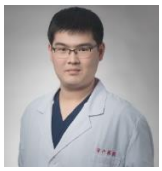
Authors biography



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Graphical abstract