Mini Review
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Recent approaches for augmenting peripheral nerve regeneration: mini-review

Abstract

Injury to superficial or deeply seated nerve is commonly reported in human and animals causing crippling morbidity. The sciatic nerve is the most frequently involved nerve in such injuries. There could be several approaches for the repair of injured peripheral nerves. However, recent therapeutic approaches include the use of molecular, cellular and electrophysical methods. Mesenchymal stem cells are the most commonly used adult stem cells for the therapeutic purpose. Several studies have shown that stem cell transplantation may promote neural regeneration by enhanced growth factor secretion, extracellular matrix production and differentiation into Schwann cell, which are primarily responsible for the axonal regeneration. Scaffolds are used to maintain cell viability, support cell proliferation and permit intercellular communication. ECM proteins, and nerve growth factors can be incorporated into nerve conduits in order to improve the nerve regenerative ability. Among the electro physical methods use of 20Hz electrical stimulation, No thermal Laser Amnion Wrap and Thermal Laser Welding have shown promising results. The present review focuses on the application and outcome of important molecular, cellular and electro physical methods used for nerve regeneration.

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Introduction

Peripheral nerve injuries are quite common in humans and animals and it has been observed that the nerves of fore and hind limbs are mostly affected.[1] Though injury can occur in any superficial or deeply seated nerve, the sciatic nerve is the most frequently involved nerve.[2–3] Diabetes and leprosy are the manin causes of generalized neuropathies but focal nerve injuries are often caused by injections, gunshot wounds, lacerations, contusions, compressions, iatrogenic causes, penetrating injury, crush, traction, ischemia, thermal and electric shock, radiation, percussion, vibration and antineoplastic drugs like Paclitaxel.[4–6] Injuries to the peripheral nerves result in partial or total loss of motor, sensory and autonomic functions due to loss of myelination, the breach in axonal continuity, degeneration of nerve fibers distal to the lesion and eventual death of axotomized neurons.[7] The capacity of the peripheral nervous system to regenerate lost axons after injuries is well recognised, but it will depend upon the type of the injury. First degree of injuries known as neurapraxia and second degree of injuries (Axonotmesis) would regenerate without surgical intervention, but sever injuries (Neurotmesis) would require surgery or even grafting, however the rate of regeneration of axons is quite slow (1–4mm/day), similar to the rate of slow axonal transport.[8,9] The chances of functional recovery are directly related to the rate of nerve regeneration. The faster the rate of nerve regeneration, the better is the chance of functional recovery. In this paper we aim to present a review of literature regarding the methods of augmentation of nerve regeneration following neurapraxia and axonotmesis.

Stem cells

Stem cells are broadly classified as Embryonic Stem cells (ESCs) and Adult Stem Cells (ASCs) depending on their sources. ESCs are totipotent, denoting their capacity to give rise to any tissue of embryo and associated structures, whereas ASCs are pluripotent/multipotent cells and may transdifferentiation into the cells and tissue of all the germ layers (pluripotent) or cells of their own germ layer. Mesenchymal stem cells, derived from the tissues of mesodermal origin, are the most commonly used adult stem cells for the therapeutic purpose. They have been have been isolated and characterised from almost every type of connective tissue, but bone marrow and adipose tissue are the main sources.[10] MSCs can self renew and characterised by their potential to differentiate into osteogenic, chondrogenic and adipogenic lineages but under appropriate conditions they have the capacity to trans-differentiate to endodermal and ectodermal lineages,[11] which make then suitable candidates for stem cell therapy in nerve injury cases. Several studies have shown that stem cell transplantation may promote neural regeneration and rescue impaired neural function after nerve injury. This could be achieved by means of secreting permissive neurotrophic molecules at the lesion site to enhance the regenerative capacity, providing a scaffold for the regeneration of axons and replacing lost neurons and other neural cells.[12] Transplanted cells may enhance growth factor secretion and extracellular matrix production.[13] The extracellular matrix proteins (such as collagen I, collagen IV, fibronectin and laminin) and neurite guidance proteins (such as netrin and ninjurin-2) have been reported to have pro-regenerative effects.[14,15] Transplanted MSCs are also capable of decreasing demyelination degeneration, reducing neural inhibitory molecules, promoting axonal regeneration, and guiding axon growth.[16] MSCs enhance nerve growth and regeneration by differentiating into Schwann cell, which are primarily responsible for the axonal regeneration and directional guidance.[17] Hence, MSC therapy has been proposed, recently, as an effectual method of regenerating injured nerves.[18] Another category of stem cell i.e. induced pluripotent stem cells (iPSC) have been the subject of several in vitro and in vivo studies investigating their suitability for nerve repair. Takahashi et al.[19] described the derivation of induced pluripotency from somatic cells following ectopic coexpression of transcription factors and now established protocols exist for the in vitro differentiation of iPSCs into neural lineages.[20] iPSCs have been shown to have a pro-regenerative effect in small animal models both in central and peripheral nervous system injury.[21]

Scaffolds and conduits

To improve the efficacy of stem cells in nerve injury treatment, a vehicle is required that could hold them, allow their growth and make them less prone to deleterious environmental effects. Scaffolds are aimed to maintain cell viability, support cell proliferation and permit intercellular communication. In addition to their supportive role, scaffolds also help prevent unwanted cell dissipation from the site of injury. Scaffolds like N-methacrylate glycol, alginate, chitosan, hyaluronic acid, fibrin, polyethylene glycol, and Matrigel have all been shown to successfully fulfil these requirements.[22–24] Scaffold selection should be made on the basis of its biocompatibility, ability to be retained at the implantation site, sufficient porosity to allow in growth of the host tissue yet maintain adequate mechanical strength and properties to deliver cells without any toxic effect upon them.[25–27]

The suitable method to directly deliver the cells to the site of nerve injury could be the microinjection of stem cells into the nerve or into the graft used.[28,29] However, this process of micro-injection could be traumatic to the intra-neural architecture and can also result in unpredictable cell distribution. Alternatively, the cells can be suspended into scaffolds matrix (like fibrin) and injected around repair sites or can be injected within the lumen of a conduit or seeded onto conduit matrix. It maintains neurotrophic factor secretion and cell migration and results in better outcomes.[30] Natural conduits including vein and artery grafts are rich in ECM proteins (such as collagen and laminin) and provide a good substrate for cell adhesion.[29–31] However, in recognition of the importance of basal lamina and other ECM framework for axonal guidance, conduits with internal structure have become more popular than hollow, single lumen tubes. Commercially available natural conduits are usually composed of collagen[32] and fibrin[33] (i.e. ECM components). Synthetic materials used for the purpose include polyglycolic acid, Poly lacticcoglycolic acid, silk fibroin, silicone tube, polytetrafluoroethylene, polyhydroxybutyrate and chitosan.[34] ECM proteins, Schwann cells, and nerve growth factors have been incorporated into nerve conduits in order to improve the nerve regenerative ability.[37] ECM molecules mediate both Schwann cell proliferation and activation to enhance neurite growth.[38] The laminin family of proteins is critical for managing a variety of cellular activities.[35] The laminin (Ln) family of ECM proteins is ubiquitously expressed but are especially abundant in the basement membrane of many epithelial and endothelial tissues, where they mediate cell attachment, migration, and tissue organization in conjunction with other ECM proteins.[36] It is the main non-collagenous component of the basal lamina that can promote neurite outgrowth and guide neurite.[39] The laminin used with stem cells applied over the nerve act as a scaffold for sustained release of the cells over the injured nerve. Laminin and fibronectin interact with cells and allow cell attachment to ECM as well as further signal transduction.[40] The rats implanted with the laminin-coated chitosan conduits carrying BMSCs showed the best results when judged by the extent of nerve regrowth, muscle mass of gastrocnemius, functional recovery, and tract tracing.[41] Besides mechanical cues, neuroactive factors and natural materials such as collagen, laminin, fibronectin and chitosan are often applied as biochemical cues to promote cell survival, attachment, proliferation and differentiation.[42] Impregnation of neurotrophic factors such as NGF into fabricated collagen/laminin fibrils represents an exciting new therapeutic paradigm.[43]

Growth factors

Neurotrophins are the molecules that are naturally upregulated in the process of nerve regeneration. They are released from the nerve endings after injury and have an impact on nerve growth, differentiation, and guidance. A number of these neurotrophic factors have been isolated and used to enhance axonal regeneration. Nerve growth factor (NGF) is one of these neurotrophins and is present at low concentrations in healthy nerves. Following nerve injury, NGF is upregulated in the distal nerve stump and plays an important role in the survival of sensory neurons and outgrowth of their neuritis.[44,45] NGF promotes proliferation and differentiation of neurons, and also modulates the repair of injured nerves.[46,47] The administration of recombinant NGF protein into injured has been found to promote nerve repair and enhanced functional restoration following nerve damages.[48] However NGF given in solution is difficult to be retained at the injury sites because it gets diffused rapidly into body fluids. Therefore, it requires periodic injection of NGF which is expensive, impractical and excessive doses may also evoke undesirable side effects.[49–51] To solve these problems, many groups are working on developing NGF delivery to the nervous system via drug delivery systems[52] or transplantation of cells with/without encapsulation.[53] These systems should be improved with regard to release control, dosing, efficacy and their safety. There are numerous other growth factors that play important roles in nerve regeneration. They include fibroblast growth factor (FGF), glial growth factor (GGF), glial cell derived neurotrophic factor (GDNF), ciliary neurotrophic factor, neurotrophin 3(NT-3), and leupeptin.[44,54] NGF, GGF, GDNF, and NT-3 have been applied in nerve conduits to models of nerve gap injury (1–4cm gap), demonstrating improved functional outcomes, electrophysiological and histological recovery, compared to conduit controls.[44]

Electrical stimulation

Animal studies have demonstrated that continuous 20Hz electrical stimulation for 30minutes to one hour could improve nerve function in injured nerves. Alrashdan et al.[55] demonstrated a significant increase in numbers of sensory neurons that regenerated their axons 10 mm into the distal nerve stump after three weeks of sciatic nerve crush injury. The regenerated axon size was increased, and myelination and significant functional improvement was recorded, as assessed with the sciatic functional index. In another study of sciatic nerve injury model, one hour of 20 Hz electrical stimulation paradigm also promoted earlier functional recovery during walking in line with accelerated reinnervation of the gastrocnemius muscles.[56]

Nonthermal laser amnion Wrap

Photochemical tissue bonding (PTB) creates a covalently bonded nerve wrap around a nerve coaptation, using an Nd/YAG laser, photoactive dye, and a nonimmunogenic amnion wrap.[57,58] The problems of unintended thermal injury to nerve tissue from traditional laser techniques are avoided. Collagen fibres in the amnion wrap are covalently bonded to collagen in the epineurium. This bond adds strength to the repair, concentrates neurotrophic and neurotropic factors inside the coaptation where they are needed, excludes inflammatory mediators from the extrinsic tissues, and contains regenerating axons, guiding them distally towards the motor/sensory target. Animal studies in rat sciatic nerve and rabbit common peroneal nerve models have demonstrated to improve axon counts at the site of

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injury and gait function after end-to-end coaptation with a PTB nerve wrap.[59] Improved gait function has also been demonstrated in a one cm rat sciatic nerve graft model.[57,58]

Thermal laser welding

Thermal laser achieves tissue bonding by denaturation of structural proteins, which anneal and join when cooled. Tse et al.[60] have reported successful nerve coaptation by laser welding; however, this was followed by reports of frequent dehiscence. To prevent dehiscence, one or two stay sutures can be placed before laser welding; however, nylon stay sutures lose their tensile strength when irradiated with a CO_2 laser.[60] Although CO2 laser-welded nerve adhesion has demonstrated favourable results in animal models, its clinical use can be cumbersome and its versatility is limited.[60] Concerns remain about the high rate of nerve dehiscence and thermal injury to axons and nerve tissue.

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Conflict of interest

The author declares no conflict of interest.

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