



## AN OVERVIEW ON NERVOUS CONDUITS AND STEM CELLS ASSOCIATION

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**Abstract.** Nowadays it is unanimously accepted in the specialty scientific medical world that an axon physical guidance is vital for the repair of injured peripheral nerves. Nerve guidance channels represent a diffusion pathway for growth factors secreted by the injured nerves and they also decrease the development of scar tissue. The researches in this domain were axed on the use of natural and synthetic materials for nervous conduits (NC) manufacture. Lately, the studies have been centered on the combination of more materials and bio-molecules in order to develop new composite materials that can stimulate nervous regeneration. Also demonstrated was the importance of neurotrophic factors (neurotrophins, neuronal growth factor, fibroblastic growth factor, etc) and cell adhesion molecules presence. To enhance the performances of the biomaterials used for the manufacture of NC, the effect of Schwann and other cell introduction in these structures was also tested. The biocompatible biomaterial domain is very dynamic, the researches leading to the achievement of new more efficient types. These will improve the prognosis of nerve sections with loss of motor, sensory, or both functions. Peripheral nerve regeneration by mean of efficient NC became a priority for plastic surgeons, neurologists and neurosurgeons. NC achievement in a shape that will ensure patients' rapid and precise healing is a challenge for the biomaterial research domain.

**Keywords:** nervous conduits, stem cells, nerve regeneration

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### Introduction

With versatile causes (penetrating injuries, avulsion and traction injuries, tissue crush and ischemia – burns, electric shock, irradiation, percussion, etc) nerve injuries are extremely frequent and represent a great number of admissions to hospitals.

Nowadays nerve lesion elective treatment is microsurgical repair, its aim being axonal growth. Nervous regeneration failure leads to neuroma formation. Surgical therapeutic options are: 1) primary

or secondary end-to-end closure, 2) nerve grafts, 3) alternate conduits (veins, silicone and biodegradable conduits, etc.) and 4) nervous transfer.

Peripheral nerve defects, varying from 15 to 30 mm cannot be usually crossed by regenerative axons due to the interposition of Schwann cells or fibroblasts that represent a physical blockade [1]. In these cases, the standard surgical treatment is represented by autologous nerve grafts. Conventional nerve grafting, regardless of graft length, tends to have better postoperative results than end to end closure done under tension, but needs a well vascularized and germ free bed [2]. It was demonstrated that excessive tension has an extremely deleterious effect on nerve regeneration leading to nerve elongation, the development of a large quantity of scar tissue and the decrease of nerve diameter [3].

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Nerve grafting has some downsides: a high tissue morbidity, graft harvest from a different site, with the consequent loss of target organ innervation, a longer surgical procedure and also the reduced quantity of the graft material provided, scarce for extensive nerve injuries [4].

These inconveniences imposed the development of alternative methods, namely allografts, degenerated muscle fibers and venous conducts, but the achieved results were not satisfactory [4]. The failure is the consequence of a combination of factors [5]: 1) the inability of neurotrophic factors secreted by the proximal stump to reach the distal stump, 2) the absence of a matrix behaving as a support structure and 3) Schwann cell absence.

### Classical and modern approaches in peripheral nerve lesion management

At present, researches are axed on tissue engineering, its specific aim being the achievement of an interactive medium between cells, matrices and bio-active molecules. To achieve this specific goal it will be necessary to transplant *ex vivo* cell-matrix blocks or alternatively non-cellular matrices at the injury site. This led researchers to design “nervous guidance channels” or nervous conduits (NC), tubular structures that lead regenerative axons to the distal stumps. Peripheral nerve tissue engineering is quite different compared to other organs because in this specific case a viable scaffold and not an already manufactured organ is implanted [4]. At present, tissue engineered artificial nerves represent the laboratory equivalent of allografts and autologous grafts [4].

To a better resemblance to natural repair, the ideal NC should have a series of features; 1) porous and bio-degradable wall, 2) the capacity to deliver bio-active growth factors, 3) support cells, 4) an oriented internal matrix that supports cell migration, 5) mimetic nervous fascicle structure internal channels and 6) electric activity [6]. One of the central problems of nervous substitute tissue engineering is also represented by optimal NC cell seeding. An ideal NC should have an equally distributed cell population to a better activation of regenerative axonal sprouts [7].

NC manufacture technologies evolved from the silicone NC (preferred in the past due to the relative flexibility of this material and to the numerous lengths and diameters) to nowadays NC manufactured from bio-degradable materials such as collagen, aliphatic polyesters or polyurethanes. Nervous synthetic tubular structures are seen as an alternative repair method to autologous grafting to avoid the immune reaction induced by the heterologous grafting [8].

Regarding the materials that constitute NC scaffold, they must present a series of features: 1) allow diffusion transport of nutrients and other molecules, not allowing the entrance of foreign cells, 2) be sufficient rapidly vascularized not to limit nutrients transport in the graft, 3) degrade in a sufficiently long time to maintain a stable support structure for the entire regeneration process but not remain in the body more than necessary to avoid the possible nervous compression syndromes, 4) the material must be immunologically compatible with the host, 5) it must be able to be surgically manipulated and 6) it must be able to permit the adherence and cell spread on its surface [9].

From the most known materials that are used for NC manufacture we shall enumerate: synthetic polymers (polylactic acid, polyglycolic acid and their copolymers, dextran and methacrylate-dextran, *polyethylene glycol*, etc.) and biologic polymers (collagen (type I and III), chitosan and chitin, alginate and hyaluronic acid) [10-12].

The use of NC adheres to the basic properties of microsurgical techniques, namely: 1) the precision of nervous stumps preparation, 2) axial orientation of nerve fascicles, 3) proper coaptation and 4) tension avoidance at the level of the microsurgical suture. Nerves are microsurgically repaired with nylon 8-0, 9-0, 10-0 (both in humans and laboratory animals). The nervous stumps are gently manipulated and placed in the tubular structure represented by the NC. Then they are sutured to the conduit with microsurgical sutures. By maintaining a gap between the conduit and the nerve stump, various neurotrophic factors, Schwann or Schwann-like cells can be introduced [13].

In the specific case of peripheral nerve tissue engineering, the central idea consists of the implantation of viable Schwann cells in adequate NC. Schwann cells have a fundamental role in nerve regeneration, nerve grafts without the presence of viable Schwann cells having a decreased axonal regeneration, due to a subliminal neurotropic influence. The controversies induced by their clinical application (short number, *in vitro* Schwann cells limited expansion) stimulated the identification of viable therapeutic alternatives [14].

An alternative strategy is offered by stem cells, due to their plasticity – meaning their ability to transdifferentiate into cells of different lineages, such as neuronal phenotypes. This capability has obvious implications for their potential application in tissue engineering and tissue regeneration [15].

Recent studies emphasize mesenchymal stem cells (MSCs) plasticity and their huge role in tissue engineering and regenerative medicine [16]. Human MSCs, due to their characteristics (easy to isolate, high proliferative potential, genetic stability,

reproducible properties from an aspirate to another, compatibility with tissue engineering principles and the capacity to stimulate tissue regeneration), represents the ideal cell model for cell therapies development [17].

In a recent report, the standard criteria for human MSCs definition were defined: (a) adherence to plastic culture plates and fibroblast-like morphology; (b) cell colonies forming, CFU-F (colony forming unit – fibroblast); (c) the expression of surface specific antigens (Ag); (d) multipotent differentiation potential [18].

MSCs can be purified by harvesting a small quantity of bone marrow [19]. They proliferate in the culture medium and afterwards they are introduced again in the body of the patient, thus avoiding immune problems. MSCs have immunomodulatory and neuroprotective features that can make them potential candidates for future therapeutic modalities in immune-mediated and neurodegenerative diseases [20]. They are multipotent and can differentiate in various cell types; adipocytes, osteoblasts, chondrocytes, cardiomyocytes, endothelial cells, neural cells, kidney cells [21]. Several possible mechanisms may be speculated for bone mesenchymal stem cell (BMSC) plasticity: one mechanism could be that bone marrow (BM) cells that (trans)differentiate into these diverse cell types represent a previously unsuspected population of highly pluripotent stem cells located in the BM that have not “committed” to becoming blood and so the gene expression pattern changes to that of a completely different cell type, including neuronal cells [22]. Thus MSCs are evaluated as an alternative source with obvious implications in peripheral nerve tissue engineering and regeneration [13].

Recent studies [23] showed that multipotent MSCs can be isolated from the endosteal surface, with similar characteristics to those isolated from bone marrow. They support haematopoietic cells and interact with blood vessels and subendosteal nervous networks [15]. *In vivo* studies have demonstrated that MSCs can differentiate in Schwann cells and can be considered the support of nervous regeneration and myelination, promoting in the same time blood vessels and connective tissue growth [24]. Hou revealed that MSCs can be induced to differentiate in cells expressing specific markers of Schwann cells, respectively S-100 and fibrillar acid glial protein, promoting peripheral nerve regeneration. They demonstrated that stromal bone marrow cells are able to differentiate into Schwann-like cells and that these Schwann-like cells are able to stimulate nerve regeneration [4].

Although Schwann cells are essential cells in peripheral nerve regeneration, by physical support and guidance, they are not ideal for tissue

engineering use due to their slow growth *in vitro*. By MSC differentiation under the action of a mixture of growth factors, including also the second glial growth factor, they showed that these express proteins for the second glial growth receptor, erb B3 and neurotrophic factors (BDNF, NGF, glial derived neurotrophic factor and leukaemia inhibitory factor), thus revealing cellular and molecular characteristics similar to Schwann cells [25].

## Conclusions

Peripheral nerve injuries represent a major health problem because they determine motor, sensory or both functions loss, leading to a severe work capacity decrease and consequently having a huge impact on patient social and professional reinsertion. Thus, peripheral nerve regeneration by efficient nervous conduits represents a priority both for plastic surgeons and for bio-material researchers. Nervous regeneration molecular mechanisms imply a variety of inner and intercellular signal pathways. Peripheral nerve classical repair methods used autologous grafts or allografts, both with their own advantages and disadvantages. Recent researches demonstrated that nervous guidance conduits can be used for the enhancement of peripheral nerve regeneration. NC are obtained from natural, synthetic or composite polymers. The researches tend to be focused on the combination of more materials and bio-molecules to create new composite materials that can stimulate nerve regeneration. The NC “ideal” structure is represented by: a support (hydrogel or fibres), extracellular matrix proteins and cells and neurotrophic factors. NC cells can be mesenchymal stem cells, glial or Schwann cells. NC biomaterials must have certain mechanical and porosity properties and need to meet the requirements for biocompatibility and biodegradability. The effects of these experimental strategies are most often evaluated by motor and electrophysiological tests and also by high specificity studies, such as histological and immunohistochemical analyses.

## References

1. Lundborg G, Dahlin LB, Danielsen N, Gelberman RH, Longo FM, Powell HC, Varon S: Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. *Exp Neurol* 1982, 76(2):361-375.
2. Millesi H: Techniques for nerve grafting. *Hand Clin* 2000, 16(1):73-91, viii.
3. Clark WL, Trumble TE, Swiontkowski ME, Tencer AF: Nerve tension and blood flow in a rat model of immediate and delayed repairs. *J Hand Surg Am* 1992, 17(4):677-687.

4. Hou SY, Zhang HY, Quan DP, Liu XL, Zhu JK: Tissue-engineered peripheral nerve grafting by differentiated bone marrow stromal cells. *Neuroscience* 2006, 140(1):101-110.
5. Anselin AD, Fink T, Davey DF: Peripheral nerve regeneration through nerve guides seeded with adult Schwann cells. *Neuropathol Appl Neurobiol* 1997, 23(5):387-398.
6. Huang YC, Huang YY: Biomaterials and strategies for nerve regeneration. *Artif Organs* 2006, 30(7):514-522.
7. Kalbermatten DE, Kingham PJ, Mahay D, Balcin H, Pierer G, Terenghi G: [Fibrin matrix enhances adherence of peripheral nerve regenerative cells]. *Handchir Mikrochir Plast Chir* 2008, 40(2):75-80.
8. Lundborg G: Brain plasticity and hand surgery: an overview. *J Hand Surg Br* 2000, 25(3):242-252.
9. Ceballos D, Navarro X, Dubey N, Wendelschafer-Crabb G, Kennedy WR, Tranquillo RT: Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. *Exp Neurol* 1999, 158(2):290-300.
10. Pfister LA, Papaloizos M, Merkle HP, Gander B: Nerve conduits and growth factor delivery in peripheral nerve repair. *J Peripher Nerv Syst* 2007, 12(2):65-82.
11. Bian YZ, Wang Y, Aibaidoula G, Chen GQ, Wu Q: Evaluation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) conduits for peripheral nerve regeneration. *Biomaterials* 2009, 30(2):217-225.
12. Xie F, Li QF, Gu B, Liu K, Shen GX: In vitro and in vivo evaluation of a biodegradable chitosan-PLA composite peripheral nerve guide conduit material. *Microsurgery* 2008, 28(6):471-479.
13. Pereira Lopes FR, Camargo de Moura Campos L, Dias Correa J, Jr., Balduino A, Lora S, Langone F, Borojevic R, Blanco Martinez AM: Bone marrow stromal cells and resorbable collagen guidance tubes enhance sciatic nerve regeneration in mice. *Exp Neurol* 2006, 198(2):457-468.
14. Ogden MA, Feng FY, Myckatyn TM, Jensen JN, Grand AG, Wood PW, Hunter DA, MacKinnon SE: Safe injection of cultured schwann cells into peripheral nerve allografts. *Microsurgery* 2000, 20(7):314-323.
15. Tohill M, Terenghi G: Stem-cell plasticity and therapy for injuries of the peripheral nervous system. *Biotechnol Appl Biochem* 2004, 40(Pt 1):17-24.
16. Hassan HT, El-Sheemy M: Adult bone-marrow stem cells and their potential in medicine. *J R Soc Med* 2004, 97(10):465-471.
17. Pittenger MF, Martin BJ: Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* 2004, 95(1):9-20.
18. Sotiropoulou PA, Perez SA, Salagianni M, Baxevanis CN, Papamichail M: Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells. *Stem Cells* 2006, 24(2):462-471.
19. Balduino A, Hurtado SP, Frazao P, Takiya CM, Alves LM, Nasciutti LE, El-Cheikh MC, Borojevic R: Bone marrow subendosteal microenvironment harbours functionally distinct haemosupportive stromal cell populations. *Cell Tissue Res* 2005, 319(2):255-266.
20. Kassis I, Vaknin-Dembinsky A, Karussis D: Bone marrow mesenchymal stem cells: agents of immunomodulation and neuroprotection. *Curr Stem Cell Res Ther* 2011, 6(1):63-68.
21. Chamberlain G, Fox J, Ashton B, Middleton J: Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 2007, 25(11):2739-2749.
22. Herzog EL, Chai L, Krause DS: Plasticity of marrow-derived stem cells. *Blood* 2003, 102(10):3483-3493.
23. Tuli R, Tuli S, Nandi S, Wang ML, Alexander PG, Haleem-Smith H, Hozack WJ, Manner PA, Danielson KG, Tuan RS: Characterization of multipotential mesenchymal progenitor cells derived from human trabecular bone. *Stem Cells* 2003, 21(6):681-693.
24. Dezawa M, Ishikawa H, Hoshino M, Itokazu Y, Nabeshima Y: Potential of bone marrow stromal cells in applications for neuro-degenerative, neuro-traumatic and muscle degenerative diseases. *Curr Neuropharmacol* 2005, 3(4):257-266.
25. Mahay D, Terenghi G, Shawcross SG: Schwann cell mediated trophic effects by differentiated mesenchymal stem cells. *Exp Cell Res* 2008, 314(14):2692-2701.