

Oriented Microstructure in Neural Tissue Engineering: A Review

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Abstract

Regeneration of neural injuries by formation of new axons and myelination to improve quality of patient life is undeniable difficulty in the world in which scientists examined different strategies from ancient times. Recently, 3D tissue engineering scaffolds simulated original extracellular matrix (ECM) and provide desirable substrate for cellular attachment, proliferation and differentiation. However, similarity of scaffold's materials to ECM contaminant is effective in achieving better results. Investigations demonstrated that oriented fibers, pores and unidirectional channels or conduits act as cell guidance and showed significant effect on cellular differentiation and axonal reconstruction. Between all the methods of scaffold fabrication, freeze casting provides lamellar type and controlled pores that are necessary in neural tissue engineering. In brief, designing scaffolds with oriented structure such as freeze casting with unidirectional solidification and seeding an appropriate cell before implantation improve repair process of neural damages.

Keywords: Nervous system; Tissue engineering; Scaffolds; Oriented-microstructure

Introduction

Nerve injury is one of the main concerns in the world due to lots of restriction in regeneration process such as slow rate of axonal regeneration (1-2 mm/day) and there is no exact method to increase this speed so regeneration process takes place in period of 12-18 months [1]. Therefore, finding an appropriate strategy to retrieval its functionality is serious topic in the world. The main purpose of nerve regeneration is restoration axons and nerve fibers [2], but higher density of intracellular environment in central nervous system (CNS), lower rate of cell proliferation, respiratory complications, arrhythmias and blood clots provide complexity in repairing CNS [3]. In other word, there are some circumstances to meet mentioned aim, i.e., swelling of the nerve cell body within a few days after nerve injury, movement of core toward perikaryon, chromatolysis [4-6]. Injury of the peripheral nerve systems (PNS) can reduce function of tissues or organs and deficits sensory-motor performance. Studies indicated that size of nerve gap, neuroma and scar tissue formation restricted nerve regeneration [1,7]. Reconstruction of damaged tissue by grafting have variety of difficulties such as disease transmission, allergic reaction, etc., tissue engineering scaffolds developed to solve problems [8] and their unidirectional microstructure acts as cellular guidance. Regeneration of peripheral nerve defects may require conduits, grafts or tissue engineering scaffolds. In fact, nerve conduits or cellular scaffolds are proper option when repair of 2 ends of nerve is not possible and the size of gap in less than 1 cm. therefore, constructs implanted in defect site to aim of sensory-motor regeneration and increase chance of target reinnervation [1,7]. Usage of scaffolds promotes the maintenance of the cells and neurotrophic molecules and guide axonal extension and targeted regeneration process. Character of tissue engineering scaffolds can control cell loading, differentiation and growth factor secretion. Ability of PNS and CNS to promote regeneration process is different with each other. Schwann cells in PNS providing required neurotrophic factors but presence of myelin inhibitors, activation of anti-apoptotic proteins and suppression of apoptotic pathways in CNS controlled the way of regeneration [3].

Cell Therapy

In cell therapy strategies, damaged tissue and death cells purged by

starting phagocytosis to provide a proper environment to regeneration of axons, reconstruction of myelin sheath and restoration of sensory and motor function [9]. During this process cells migrate to defect site, replacing lost cells due to injury and after secrete adherent molecules providing neural attachment sites [3]. There are two strategies in cell therapy: (1) Cell injection and (2) scaffolds. In spite of the fact that cell injection is minimal invasive technique, improper environment in defect site decreases cellular shelf life; hence, scaffolds introduce desired substrate for cellular attachment, proliferation and differentiation and are able to carry off required growth factors [10]. Pretense of cell with porous scaffolds or conduits is desirable due to secretion of growth factors in site of implantation and increase restoration power. As instance, Schwann cells are able to secrete growth factors, adherent molecules and produce ECM for nerve growth and induce more axonal reconstruction in defect site that direction of axonal growth will be controlled by unidirectional pores in microstructure of scaffolds. Other investigations indicated that adult-derived progenitor cells can differentiate to oligodendrocytes after implantation and enhance myelination [11]. Moreover, transplantation of embryonic stem cells can retrieval locomotors performance [12].

Variety of synthetic or natural materials especially ECM contaminant are useful to support axonal nucleation and spreading. Paino et al. represented that filling the neural conduit with Schwann cells incorporated in collagen gels caused restoration response and myelination after implantation in thoracic side of spinal cord injury [13]. Enhancing axonal spreading after culturing Schwann cells on poly hydroxyl butyrate channels confirmed the positive collaboration of cells and scaffolds in healing process that achieved by Kalbermatten et al. [14]. Chew et al. implanted composite fibers fabricated with

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Received September 27, 2016; **Accepted** November 01, 2016; **Published** November 10, 2016

Citation: Ghorbani F, Zamanian A (2016) Oriented Microstructure in Neural Tissue Engineering: A Review. J Tissue Sci Eng 7: 182. doi:10.4172/2157-7552.1000182

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poly ϵ -caprolactone and ethyl ethylene phosphate in 15 mm sciatic nerve defect and observed nucleation of axons and their orientation in direction of fibers that resulted sensorimotor improvement. Better results obtained in presence of BDNF [15]. Furthermore, Zamani et al. demonstrated that drawn oriented PLGA fibers and the amount of its pulling effect of neural cells spreading and these fibers introduced more linear cells compared with oriented PLGA fibers [16]. Arulmoli et al. indicated that Human neural stem/progenitor cells (hNSPCs) are able to treat neural lesions in CNS owing to secret required growth factor in healing process and they proved that pre-culture of biomaterial scaffolds can reduce cellular death due to transplantation [17].

Topology of Neural Tissue Engineering Scaffolds

Oriented microstructure of pores and fibers or unidirectional channels effect on guiding cells, i.e., mesenchymal stem cells, oligodendrocytes, neurons. Based on researches, materials, size and morphology of scaffolds, type and age of neurons and surface adhesion influence on stimulation of neurites growth and neural repair [18]. One of the most principal methods to fabrication of interconnected porous scaffolds with unidirectional channels is freeze casting technique that provides noteworthy improvement in physico-mechanical features, controlling pore size distribution in accordance with cell size and preventing foreign body reactions, designing elongated pores to guide cellular proliferation and differentiation [19-24]. So, it is expected that these types of scaffolds prepare a suitable morphology in neural regeneration. As can be seen in Figure 1, Answer: In freeze casting method the solution poured into poly tetra fluoro ethylene mold (nonconductor mold) and solvent crystals formed in vertical direction along the freezing direction with certain ratio that controlled by a proportional-integral-derivative controller and thermocouple after injection of liquid nitrogen into the tank. The frozen solvent acts as a binder and prevent from remodeling the structure. After that, frozen samples transferred to freeze dryer to sublimate ice crystals and produced anisotropic scaffolds [25-29].

The resultant samples indicated anisotropic behavior due to controlling direction and speed of solidification that investigated in other studies [24,30-32]. Figure 2 represented lamellar type microstructure of freeze casting compared with randomly oriented pores in freeze drying matrixes. Biodegradation behavior of scaffolds with unidirectional microstructure compared with randomly oriented one illustrated in Figure 3 and as can be seen, oriented pores reduced biodegradation rate due to production of more stable walls and enhancement of strength

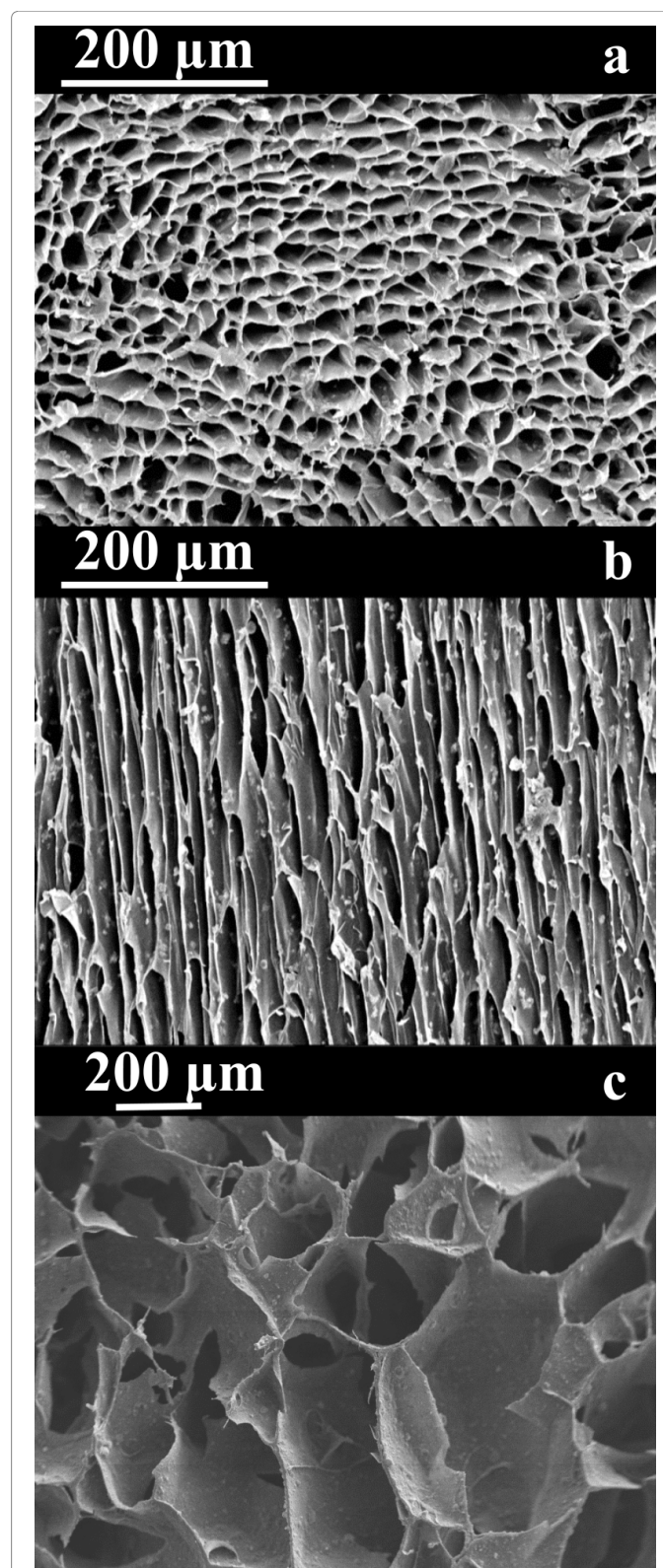
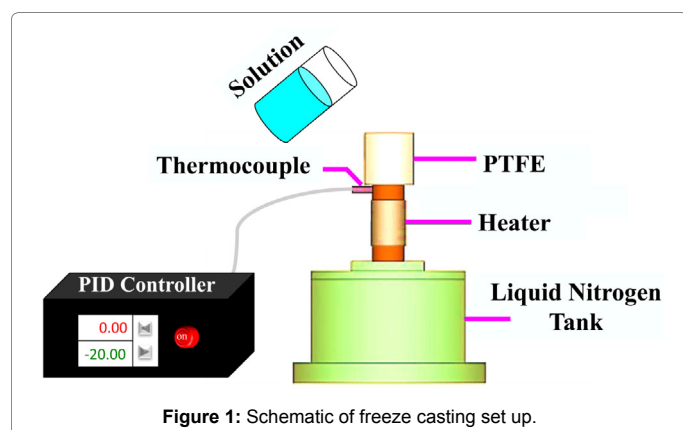


Figure 2: SEM micrographs of freeze casting scaffolds in perpendicular (a), parallel (b) direction and freeze drying scaffolds (c). Figure represented that direction of pores follows unidirectional solidification in freeze casting matrix compared with randomly oriented pores in freeze drying one.

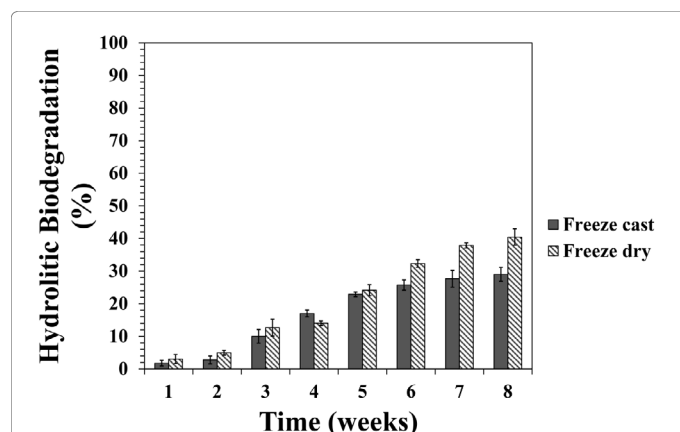


Figure 3: Hydrolytic biodegradation rate of PLGA scaffolds that fabricated by both freeze casting and freeze drying techniques. Figure indicated that oriented microstructure in freeze casting matrix reduced biodegradation rate as a function of time [33].

[33]. Madaghiele et al. cultured Schwann cells on freeze casting collagen scaffolds with oriented pores. Results indicated that cellular penetration and proliferation followed linear pattern [34].

Formation of new axons in direction of pores in unidirectional poly lactic acid (PLA) matrix after implantation in spinal cord defect compared with lack of axons in randomly oriented PLA microstructure illustrated by Cai et al. [35]. In other investigation by Stokols et al. unidirectional freeze dried agarose scaffolds indicated axonal penetration Schwann cells webbing and vascularization in which all of them improved after usage of Brain-derived neurotrophic factor (BDNF) [36].

Future Aspects

In spite of the fact that a large number of studies focused on neural regeneration issue, there are no certain therapies owing to lack of information about exact molecular mechanisms of nerve regeneration. Many investigations have been shown neural regeneration in animal studies but they cannot find a proper correlation with human defects and healing. Moreover, *in vivo* studies regenerate small neural lesions but in human defects growing represents in long distance and this event decrease ability of regeneration that followed by Schwann cells atrophy. To overcome all the mentioned problems, we should design an appropriate substrate to increase axonal outgrowth and prevent atrophy in the denervated Schwann cells [37] so freeze casting scaffolds that contained neural growth factor and culturing desired cells on the surface can be notable structure to regenerate defects.

Conclusion

Tissue engineering scaffolds introduced a proper substrate to simulate ECM and support biological behavior and acquisition better regeneration. Owing to special requirements of neural tissues in healing process, oriented microstructure of scaffolds discussed to guide cells in order to formation of myelinated axons. Therefore, freeze casting scaffolds with unidirectional channels introduced as proper substrate to repair defects.

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