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MINI-REVIEW

Application of Electrospun Nanofibrous PHBV Scaffold in Neural Graft and Regeneration: A Mini-Review

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ABSTRACT

Among the synthetic polymers, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) microbial polyester is one of the biocompatible and biodegradable copolymers in the nanomedicine scope. PHBV has key points and suitable properties to support cellular adhesion, proliferation and differentiation of nanofibers. Nanofibers are noticeably employed in order to enhance the performance of biomaterials, and could be effectively considered in this scope. Electrospinning is one of the well-known and practical methods that extremely employed in the construction of nanofibrous scaffolds for biomedical application and recently PHBV has exploited in nerve graft and regenerative medicine. PHBV composites nanofibrous scaffolds are able to be applied as promising materials in many fields, such as; wound healing and dressing, tissue engineering, targeted drug delivery systems, functional carries, biosensors or nano-biosensors and so on. In this mini-review, we attempt to provide a more detailed overview of the recent advances of PHBV electrospun nanofibers application in neural graft and regeneration.

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INTRODUCTION

Biodegradable and biocompatible artificial polymers illustrate several advantages over other materials for enhancing scaffolds in tissue engineering. These materials, due to their remarkable mechanical properties and degradation kinetics could be employed in a variety of applications. The synthetic polymers are also employed into different shapes with desired morphological features to conduct the tissue in-growth [1].

Tissue repair by autologous cell/tissue transplantation is employed as a promising technique for tissue regeneration [2]. In addition, the tissue engineering can demonstrate a promoting

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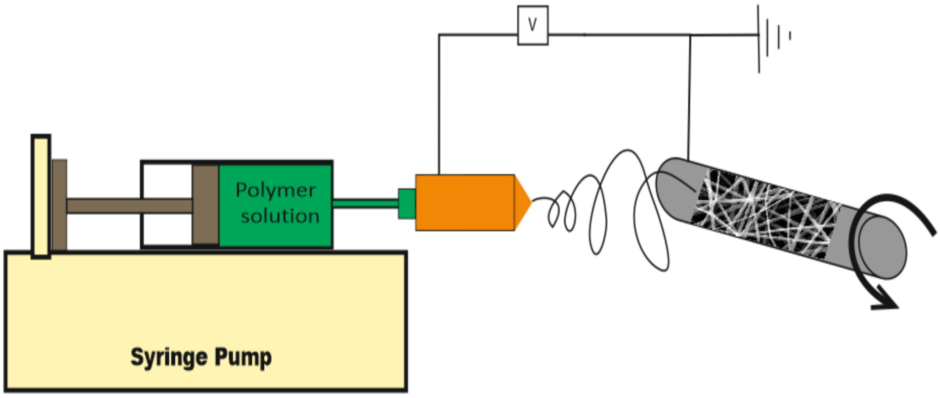

Fig.1. schematic representation of the electrospinning process

interdisciplinary field that applies the principles of biological, chemical, and engineering sciences [3]. The tissue engineering strategy usually involves the separation of healthy cells from a patient based on their expansion *in vitro*. The prolonged cells are then seeded onto a three-dimensional (3D) biodegradable scaffold that supplies structural support. The scaffold regularly degrades with time to be replaced by a newly mature tissue from the seeded cells [4].

Biomaterial, as a fundamental part of the tissue engineering field, plays a significant role in providing 3D synthetic frameworks (commonly referred to as scaffolds, matrices, or constructs) for cell attachment, proliferation, and ingrowth that eventually leads to the new tissue formation. Furthermore, the fabrication of biomaterial-based 3D scaffolds has been promoted by several modern approaches [4].

In recent years, tissues engineering nanofiber-based scaffolding systems are being devised [2]. The growth of nanofibers has developed the capacity of scaffolds fabrication that can potentially mimic the architecture of natural extra-cellular matrices of the normal human tissue at the nanometer scale. The high surface area to volume ratio of the nanofibers merged with their micro-porous structure favours' cell adhesion, proliferation, migration, and differentiation, in a way that all of which are extremely preferred properties for this field.

In this mini-review, we are going to provide an overview over nanofibrous PHBV scaffolds in neural graft and regeneration. In this regard, in section 2, we explain electrospinning process in brief. In section 3, nanofiber Scaffolds for Neural tissue engineering were defined. The Comparison of the polymers used in nerve tissue regeneration was summarized in section 4. And finally, application of PHVB nanofibers in neural tissue engineering was discussed.

Electrospinning process

Electrospinning is one of the well-known methods that extremely used in the fabrication of nanofibrous scaffolds for the biomedical application [5]. Electrospinning is also defined as a process to produce ultra-fine fibers. This approach is based on the application of an electric field that draws polymer solution or melts from the nozzle to the collector. The solution is kept at the tip of a capillary tube via its surface tension and electrical potential. Mutual charge repulsion in the polymer solution can cause a force that is directly opposite to the surface tension of the polymer solution. An augment in electrical potential leads to the formation of a conical structure called as Taylor cone and for further overcoming the surface tension forces to form a jet that is ejected from the tip of the Taylor cone. It forms randomly oriented nanofibers that can be collected on a stationary metallic collector. With this technique, it is possible to control thickness and porosity of nanofibers. The diameter of resulted fibers would vary several to tens of nanometers [6]. Fig. 1 shows the schematic representation of the electrospinning apparatus.

Nanofiber Scaffolds for Neural tissue engineering

Most of the irreparable disabilities are due to traumatic damages in central nervous system (CNS) namely the brain or spinal cord because of the CNS tissue inability to regenerate itself. However, a very limited number of studies have been done in this area.

The main strategy to regenerate the neural tissues

Table 1. The comparison between commonly of the studied polymers

Polymers	Polymer type	Fibers diameter	Type of fiber	Field of study	Ref.
PCL[1]	Synthetic	559±300 nm	Aligned fibers	Peripheral nerve regeneration	(12)
PGA[2]	Synthetic	80- 300 nm	Aligned and random fibers	Peripheral nerve regeneration	(13)
PLLA[3]	Synthetic	53-350 nm	Aligned & Random fibers	Central nervous system (CNS)	(9, 14)
PLGA[4]	Synthetic	1.27 mm	Random and aligned microfibers, conduits and films	Central nervous system (CNS)	(15) (7)
PLGA-PANi[5]	Synthetic	200-400 nm	Random fibers	-	(16)
PCL/PLGA[6]	Synthetic	280 nm to 8 μm	Aligned conduits	Peripheral nerve regeneration	(17)
Cs/PGA[7]	Natural/ Synthetic	4.5 mm	Aligned conduits	Peripheral nerve regeneration	(8, 18)
collagen/PCL[8] (3:1)	Natural/ Synthetic	541±164 nm	Aligned fibers	Peripheral nerve regeneration	(12)
PHBV[9]	Natural	50-500 nm	Random fibers	Central nervous system (CNS)	(11, 19)

[1]Poly(caprolactone), [2]poly(glycolic acid), [3]Poly L-Lactic acid, [4]Poly(lactic-co-glycolic acid), [5]Poly(lactic-co-glycolic acid)/Polyaniline, [6]Poly(caprolactone)/Poly(lactic-co-glycolic acid), [7]Chitosan/poly(glycolic acid), [8]Collagen/Poly(caprolactone), [9]Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

would be utilizing the polymeric scaffolds for cell transplantation. The scaffolds are usually made of synthetic or natural polymers that are produced by electrospinning technique. Polymeric fibers made with this approach could mimic the ECM of the normal structure of the nerve tissue and induce the tissue regeneration naturally [7].

On the other hand, peripheral nerve damages lead to severe disabilities in patients due to loss of neural control in the body. In extensive nerve deficits preferred treatment is autologous nerve graft, but at least it may result in sacrificing other nerves. The other main challenge is the problems of the second operation for nerve harvesting.

Investigated treatment strategies have utilized the synthetic scaffold to bridge nerve gap, local and controlled application of nerve growth factors, or biodegradable nerve guide seeded with cultures of Schwann cells (SCs) [6]. In particular, the scaffold must guide axonal regeneration. It is necessary to facilitate neural cells attachment and migration (such as for Schwann cells) to provide a successful nerve tissue engineering. Electrospun fibers are mostly used scaffolds for this purpose. In this respect, the scaffolds have been utilized almost in conduit forms [6, 8, 9].

Comparing the polymers used in nerve tissue regeneration

Recently Biodegradable synthetic polymers such as poly(glycolic acid), poly(lactic acid) and their copolymers, poly(p-dioxanone), and copolymers of trimethylene carbonate, Poly (hydroxybutyrate valerate) (PHBV) and polyglycolide have been extremely utilized in several clinical studies [10]. Among these polymers, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) microbial polyester has been highlighted as a biocompatible and biodegradable copolymer. Cellular growth, adhesion and benefits from controllable degradation are the remarkable proprieties of PHBV applications [11]. In the present study, we focus on the multiple applications of PHBV nanofibers in the neural regeneration fields.

Table 1. shows a comparison between commonly investigated polymers to this purpose in the summary. These polymers were used lonely or in a blend with the others.

PHBV nanofibers in neural tissue engineering

In a study by Yucel *et al.* (2010) which employs fibrous structures for nerve regeneration, they have fabricated a type of neural conduit comprised of aligned microfibers (for wrapping neural stem cells (NSCs)) and a micropatterned film containing astrocytes directed along the microgrooves (to support the NSCs). The random and aligned microfiber mats of PHBV and PLGA blend (ratio of 1:1, w/w) by electrospinning in Yucel's and his colleagues' study. The results have shown that the undifferentiated neural stem cells (NSCs) and also, differentiated astrocytes were directed according to the orientation of the microgrooves and the microfibers. The prepared structures demonstrated the ability to support the growth and proliferation of the cells in a conduit form and also in co-culture [20].

Furthermore, a new study had reported nerve defects repair by utilizing the polymeric neural tubes [21]. Biazar and his colleagues (2013) fabricated a nanofibrous electrospun PHBV sciatic nerve conduit. The prepared PHBV conduit has shown high mechanical capabilities sufficient to support the sciatic regeneration. Four months' follow-up in rats represented that in the scaffolds containing Schwann cells, the nerve trunk was efficiently regenerated in association with nerve myelination. Also, the gastrocnemius muscle cells on the functional side have shown uniformity in structure and size [22]. They also have prepared a chitosan–crosslinked electrospun nanofibrous PHBV nerve conduit. The *in vivo* study of the conduits was carried out by grafting into a 10 mm gap in the sciatic nerves of rats. The results obtained revealed that in the electrospun scaffold, the sciatic nerve trunk was reconstructed while nerve continuity was restored and myelination of nerve fibers occurred [23]. The researchers have reported a tubular, nanofibrous biodegradable polymeric nerve guidance conduit with gelatin to employ in the regeneration of the function of hurt nerve tissues. The gelatin-modified nanofibrous PHBV nerve tube implanted into rat sciatic nerve injury across the 30 mm long defect. The gelatin-modified nanofibrous also illustrate the appropriate physical, mechanical, and structural properties [24].

Collagen is one a well-known natural and fundamental component of extracellular matrix in nearly every tissue, such as bone, skin, tendon, ligament, and so on. The diameters of electrospun nanofibrous mats based on collagen fiber bundles is estimated between 50 and 500 nm [9].

Investigations have confirmed that the collagen-coated nanofibers show a better hydrophilicity than the uncoated ones., Moreover, cellular assays have shown the improved adhesion, growth and viability in the collagen-coated nanofibers than the uncoated nanofibers. As a result, the mentioned nanofibers can be noticeably employed for tissue engineering application [25].

Prabhakaran *et al.* (2013) have reported the electrospinning of random and aligned PHBV and composite PHBV/collagen nanofibers with diameters ranging from 386–472 nm and 205–266 nm, respectively. They have investigated the applicability of the resultant aligned nanofibrous scaffolds for nerve tissue engineering. For this purpose, the viability and neurite extension of the PC12 nerve cells on the electrospun fibers were studied. The results have illustrated a superior proliferation rate of neural cells on aligned PHBV/Coll 50:50 nanofibers in comparison with neat PHBV and PHBV/Coll 75:25 scaffolds. Also, alignment of the PHBV/Coll nanofibers provided a platform for the nerve cells to orientate along the direction of the fibers as well help the formation of elongated cell morphology. In this condition, cells showed a bipolar neurite extension that is requisite for nerve regeneration. The obtained results reveal that aligned PHBV/Coll scaffolds are more promising for neural tissue engineering than the random ones [26].

In a similar study, Masaeli et al. (2013) have fabricated blend electrospun scaffolds of Poly (3-hydroxybutyrate) (PHB) and PHBV in different compositions for the myelinic membrane regeneration. They have also prepared the random and aligned nanofibrous scaffolds of PHB and PHBV with and without collagen type I through electrospinning method. This study has evaluated the effects of mixed composition, fiber alignment and also, collagen presence in the scaffolds on the functions of SCs. The results illustrated the advantage of the aligned PHB/PHBV/collagen fibers in supplying SCs with a bipolar morphology and alignment along the fiber direction, over the random fibers. The presence of collagen within nanofibers not only increased proliferation of the SCs on day 14 but also, increased NGF secretion on day 6 and GDNF gene expression on day 7. The authors have concluded that the aligned PHB/PHBV electrospun nanofibers could define a potential application as scaffolds for nerve tissue engineering at the presence of collagen type I in the nanofibers to improve cell differentiation [27].

CONCLUSIONS

In this mini-review, the recent studies on the applications of the PHBV electrospun nanofibers in nerve graft and regeneration were considered. Due to the biocompatibility and biodegradability of the PHBV and unique properties of electrospun nanofibers, new studies tend to utilize novel composite scaffolds made of PHBV nanofibers to induce and promote the regeneration of the impaired neural tissues. The reports notably suggest that neat and also blended or surface-modified PHBV electrospun scaffolds would efficiently be utilized to support nerve cell functions including adhesion, proliferation, migration and differentiation. Therefore, PHBV-based electrospun scaffolds are among the most promising structures in neural regeneration.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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