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Molecular self-assembly guides the fabrication of peptide nanofiber scaffolds for nerve repair

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Abstract

Peptide nanostructures formed through molecular self-assembly are increasingly important for material science and regenerative medicine. Peptide self-assembly allows the design and fabrication of supramolecular architectures at nanoscale. In the β-sheet system, ionic self-complementary peptides and peptide amphiphiles (PAs) have been extensively developed to form cylindrical nanofibers and subsequent 3D biomaterial scaffolds, which have demonstrated the potential to repair nerve. In addition, modification with peptide epitopes (i.e. functional motifs) and incorporation of molecular signals are beneficial to the bioactivity of peptide nanofiber scaffolds, and these two methods contribute favorably to the improvement of cell function and tissue regeneration in neural tissue engineering. This review would focus on the design, fabrication and properties of these two peptide-based biomaterial scaffolds, as well as their application in nerve repair.

Key words: molecular self-assembly, ionic self-complementary peptide, peptide amphiphile, nanofiber scaffold, nerve repair.

1. Introduction

It is well known that central nervous system (CNS) and peripheral nervous systems (PNS) play indispensable roles in the regulation of physiological processes in human body.[1-3] Nerve injury is ubiquitous in clinical medicine, and it is mainly caused by trauma and degenerative diseases.[4-6] Nerve injury can lead to the formation of gaps or cavities in nervous system, the loss of sensory or motor function, and neuropathic pains. However, nerve tissue has very limited regenerative ability.[7, 8] PNS neurons

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have relatively better capability of regeneration than CNS neurons, because there are permissive environmental cues including neurotrophic factors and growth-supporting excelluar matrices (ECMs) following PNS injury.[9] This is also evidenced by that CNS neurons show increased regenerative capacity when they are transplanted to the microenvironment of PNS neurons.[10, 11] Generally, it is very difficult to bridge gaps or cavities of nervous system or to recover nerve function by means of the intrinsic ability of nerve regeneration.[7, 8]

Clinical intervention is necessary for nerve defects. There are two principal paths to treat nerve defects, involving systemic pharmacological treatments (i.e. neuroprotective drugs) in order to constrain side effects (e.g. ischemia, free radical release, and inflammation),[12-14] and promotion to self-regeneration by transplanting cells and active agents (e.g. growth factors and drugs) to the lesion site based on the strategy of tissue engineering.[15, 16] Tissue engineering has developed into an interdisciplinary field merged by life sciences, physical sciences and engineering.[17, 18] It has been found to have the potential in large fields including congenital defects, diseases, trauma and aging.[19, 20]

In tissue engineering, material scaffolds are required not only to provide physical support, but also to have good bioactivity in order to promote cellular behaviors and tissue regeneration.[21-24] A novel biomaterial scaffold, peptide nanofiber scaffold is fabricated through peptide self-assembly, and its microstructure highly mimics the natural ECM.[25] Synthetic peptide nanofiber scaffolds are derived from natural amino acids, and have the intrinsic property of biological self-recognition and good biocompability.[25, 26] Incorporation of molecular signals (e.g. grow factors, chemokine and cytokine)[27, 28] and modification with peptide epitopes (i.e. functional motifs)[18, 29, 30] are feasible within peptide nanofiber scaffolds with the aim of enhancing cell function and tissue regeneration. Furthermore, the degradation products of peptide nanofiber scaffolds are naturally nontoxic amino acids.[30] With these features, peptide nanofiber scaffolds that are derived from ionic self-complementary peptides and peptide amphiphiles (PAs) have emerged as a promising and exciting biomaterial for nerve repair.[31-35]

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2. The obstacles to nerve regeneration

PNS has an intrinsic but limited regenerative ability, and nerve conduit is required to bridge the relatively long nerve gap (e.g. > 6 mm in mouses and > 15 mm in rats) after the nerve injury.[36] After the injury, the distal portion of nerve has the risk of the atrophy of Schwann cells and the loss of a Schwann cell basal lamina tube.[37] Spontaneous deterioration of the distal portion and proximal ends at the nerve stump, has become ubiquitous in severed peripheral nerve injury.[22] Following the transected nerve injury, Wallerian degeneration would occur throughout the distal stump of transected nerves and within a small zone distal to the proximal stump, and lead to the disintegration of axoplasmic microtubules and neurofilaments.[38] In addition, the proximal end of injured nerve has the propensity to form neuroma and scar tissue which would prevent nerve regrowth.[37] By contrast, CNS has less ability to regenerate,[39] because there is the inhibitory environment consisting of chondroitin sulfate proteoglycans, myelin-associated inhibitors, and other barriers after CNS injury.[13, 40, 41]

Following the nerve injury such as spinal cord injury (SCI), ischemic events can inhibit the delivery of oxygen and glucose, and lead to neuronal cell death, axon damage and demyelination.[42-44] Subsequently, there are many other events including glial activation, release of inflammatory factors and cytokines, scar formation and cystic cavitation. They are regarded as important barriers to axon regrowth,[39, 45-48] and they are also thought to be secondary injury capable to cause post-traumatic neural degeneration and the increase in tissue loss.[13, 49, 50] Especially, glial scar formation, mainly comprised of reactive astrocytes and proteoglycans, is regarded as a mechanical and chemical barrier to nerve regeneration and functional recovery.[51]

Inflammatory responses are considered to be the major factor to trigger secondary injury and have the time scale ranging from hours to months following the nerve injury.[14, 52] After the injury, inflammatory cells (e.g. neutrophils, macrophages, monocytes, and lymphocytes) can be recruited to the lesion site through the damaged blood vessels.[53] These inflammatory cells can yield proteolytic enzymes which contribute to the degradation of ECM proteins and blood-brain barrier.[48, 54] They can

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also secret various pro-inflammatory cytokines and chemokines such as interleukin (IL)-α, interferon (INF)-γ, tumour necrosis factor (TNF)-α, reactive oxygen species, oxidative enzymes, and metalloproteinases, which are able to aggravate secondary damage and glial scar formation.[48, 53, 54] Meanwhile, these inflammatory reactions have some beneficial events including the clearance of hemorrhagic and necrotic tissue, the reduced lesion size, as well as the release of trophic factors and cytokines such interleukin (IL)-10 and transforming growth factor (TGF)-β that have favorable influence on nerve protection and axonal regeneration (Fig. 1).[54, 55] Following SCI, macrophages can gradually infiltrate the wound site. Furthermore, macrophages can lead to the prompt removal of inhibitory debris after PNS injury.[56] In general, these inflammatory reactions are complicated, and it is very difficult and important to control inflammation following nerve injury.

3. Nerve grafts

Nerve transplantation is increasingly important to treat nerve defects.[57, 58] Nerve autografts are generally accepted as the golden standard for bridging nerve gaps, but are limited by the shortage of donor grafts, the donor site morbidity, the potential loss of function at donor sites as well as the requirement of multiple surgeries.[59-61] Nerve allografts and xenografts have the possibility to cause a strong immune response after the transplantation.[8, 62] Tissue engineered-grafts are considered as the alternatives to these nerve grafts, and have become increasingly significant for nerve transplantation.[26, 63] With the assistance of nerve guidance channels, it is possible to repair nerve gap of 4 cm in PNS.[64, 65]

Many material scaffolds are extensively developed to serve as nerve conduits for nerve repair, including synthetic substances (e.g. aliphatic polyesters, polyurethanes, polyphosphoesters, and piezoelectric polymers) and natural substances (e.g. decellularized scaffolds, polysaccharides and collagen).[58, 63] Merely a few of them are approved by FDA for nerve regeneration, including polyglycolic acid (PGA),[66, 67] Type-I collagen,[68, 69] polylactide-caprolactone (PLCL),[70] and polyvinyl alcohol (PVA).[63] However, there are still some limitations. Examples include that PGA has a high rate of degradation, and there is the batch-to-batch variability during the process

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of manufacturing collagen.[63] In addition, the relative rigidity and inflexibility of PLCL increases the complexity of suturing, while PVA has the risk of nerve compression because of its non-biodegradation.[63, 71]

There is a great need for novel tissue engineered-grafts beneficial to neural repair, and stable symbiosis of biomaterial scaffolds, cells and signal molecules is fundamental to tissue engineering.[26] Ideal biomaterial scaffolds for nerve regeneration should satisfy the following requirements: 1) good biocompatibility to effectively reduce immune rejection, 2) appropriate porosity for cell immobilization, 3) promotion to vascularization, 4) the delivery of signal molecules, 5) appropriate mechanical strength and pliability, 6) non-toxic degradation products.[22, 59, 61, 72, 73]

4. Peptide-based biomaterial scaffolds

4.1 Molecular self-assembly

Molecular self-assembly has emerged as a promising field in constructing nanoscale materials due to its operational simplicity and capability for producing diverse nanostructures.[18, 74, 75] This dynamic process is a spontaneous assembly of molecules into well-organized and stable aggregates.[25] Although the noncovalent bonds between these molecules are relatively week in isolation, collectively they can form strong molecular forces capable to improve the stability of supramolecular architectures. These noncovalent bonds include: 1) hydrogen bonds, 2) electrostatic interactions, 3) hydrophobic interactions, 4) van der Waals forces and 5) water-mediated hydrogen bonds.[18, 25] Chemical complementarity and structural compatibility are fundamental to molecular self-assembly.[25] Indeed, molecular self-assembly has been widely used in the formation of natural substances such as DNA double helix and ribosomes. Here, peptide self-assembly guides the design and fabrication of nanofiber scaffolds, as evidenced by self-assembly of ionic self-complementary peptide d-EAK16 consisting of alanine, glutamic acid and lysine into nanofiber scaffolds (Fig. 2).[76]

4.2 Peptide-based biomaterials

Peptides are thought to be valuable building blocks for constructing nanostructures, and have the intrinsic properties of self-recognition and good

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biocompatibility.[29] Some peptides have demonstrated extraordinary propensity to assemble into β-sheet structures and 3D nanostructures by means of intermolecular hydrogen bonding and others.[25, 29] The design of peptide-based materials with controllable structural features at the nanoscale can be finely regulated by chemical design versatility of peptides and peptide-formed secondary structures.[77]

The two most common peptide secondary structures are β-sheet and α-helix.[78] Recently, collagen-mimicking peptides have achieved an increasing focus because of their biophysical and biochemical properties.[79-81] In the β-sheet system, many ionic self-complementary peptides are designed and fabricated, including EAK16-II, RAD16-I, and RAD16-II.[82, 83] Other β-sheet peptides include PAs,[84-86] self-assembling β-hairpins peptides,[87, 88] β-sheet tapes,[89, 90] ABA-block copolymer,[91, 92] various dipeptide and Fmoc-conjugates.[93-97] They readily undergo self-assembly into nanofibers and 3D biomaterial scaffolds by means of intermolecular hydrogen bonding and others.[29] Generally, β-sheet system plays a predominant role in the design and fabrication of peptide-based biomaterial and has constructed biomaterial scaffolds capable to support 3D cell culture and tissue engineering.[93, 95] Peptides or proteins used to fabricate functional biomaterials have been extensively reviewed elsewhere.[29] This review would focus on the utilization of ionic self-complementary peptides and PAs to create 3D biomaterial scaffolds that are favorable to nerve regeneration.

4.2.1 Ionic self-complementary peptides

Since the protein Zuotin (i.e. EAK16-II) was found to be able to spontaneously assemble into nanofibers and 3D nanostructure, ionic self-complementary peptides have been extensively developed to construct biomaterial scaffolds.[18, 82] These self-assembling peptides have alternating hydrophobic sides (e.g. alanine, valine, leucine, isoleucine, and phenylalanine), and hydrophilic sides including positively charged amino acid (e.g. lysine, arginine, histidine) and negatively charged amino acids (e.g. aspartic acids and glutamic acids).[18, 25] The hydrophilic surface of the molecules with charged amino acid residues assists in classifying complementary ionic sides into several moduli, such as modulus I, II, III, IV and mixed moduli:

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modulus I, -+ - + - + - +; modulus II, - - + + - - + + ; modulus III,---+++; and modulus IV,----++++ (Fig. 2).[18, 25, 76, 98-101] The design of charge orientation in reverse orientations can produce entirely different molecules with distinct molecular behaviors.[18, 25, 102]

These ionic self-complementary peptides have strong propensity to assemble into β-sheet structure and interwoven nanofibers, and consequently they can form biocompatible and stable hydrogels with extremely high water content, more than 99% in water (5–10 mg/ml, w/v).[99, 103] There are many factors that influence structural features of the final assemblies, and these factors include individual molecular building blocks, molecular chemistry, assembling environment (e.g. pH, ion strength, and temperature), and assembly kinetics.[25, 77, 101, 104] However, the mechanisms regulating self-assembly of peptides into nanofiber scaffolds remain elusive.

Amino acids are classified into D- and L-forms, but molecular evolution in nature has the preference for L-form amino acids. It will be very interesting and meaningful to explore this event. D-amino acids may contribute to the better stability of peptide bonds than L-amino acids, because proteases can degrade L-form peptide bonds but cannot degrade D-form peptide bonds.[82, 105, 106] Since peptide d-EAK16 was designed in 2007 and was published in 2008,[105, 106] more researches have focused on D-amino acids, as well as the hybrid of D-form and L-form of amino acids.[18, 76, 107] These also inspire the combination of L-form peptides and D-form peptides into hybrid biomaterials, and these biomaterial scaffolds may have the exceptional ability for medical application. Indeed, there have been enormous studies regarding ionic self-complementary peptides including RAD16-I, RAD16-II, EAK16-I, EAK16-II, and d-EAK16 (Fig. 3).[76, 101, 108]

4.2.2 Peptide amphiphiles (PAs)

The chemical structure of a representative PA molecule has four key structural features, including the hydrophobic domain (e.g. a long alkyl tail), a short peptide sequence capable of forming intermolecular hydrogen bonding, charged amino acids for the design of pH and salt-responsive nanostructures, as well as the hydrophobic alkyl tail (e.g. peptide epitopes) for the interactions with cells or proteins.[86, 109] Short

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peptide sequences immediately adjacent to the hydrophobic segment are crucial to produce intermolecular hydrogen bonding that induces the formation of β-sheet and cylindrical nanofibers.[84, 86] Additionally, peptide epitopes incorporated into PAs have favorable influence on cell function such as cell adhesion, proliferation and differentiation. Examples include RGD and IKVAV that have the ability to trigger cell adhesion.[86, 110-113] PAs-formed nanofiber scaffolds not only enable the encapsulation and delivery of signal molecules and hydrophobic drugs, but also facilitate cell culture and tissue regeneration (Fig. 4).[77]

4.3 Biological properties of peptide nanofiber scaffolds

4.3.1 Modification with functional motifs

A myriad of peptide epitopes (i.e. functional motifs) have been extensively developed to modify biomaterials scaffolds.[18, 29] Modification with peptide epitopes (e.g. RGD,[114] IKVAV,[115] YIGSR [116] and PHSRN [117]) is considered as a promising way to modulate cellular function within peptide nanofiber scaffolds. Short peptide epitope RGD is found in fibronectin and other ECM proteins, and can assist in inducing cell differentiation and migration through binding to $\alpha_5\beta_1$ integrin receptor.[118] Furthermore, peptide RGD can be combined with PHSRN to fabricate peptide PHSRNG6RGD which results in the improvement of cell binding, possibly due to its better similarity to functional structures of fibronectin.[119] It is believed laminin has an important role in regulating cell adhesion, migration, neurite outgrowth and angiogenesis, and its cell-binding domains consist of IKVAV capable to facilitate cell attachment, migration and neurite extension, as well as YIGSR capable to stimulate cell binding.[115, 116, 120-122] Conjugating these functional motifs to peptide nanofiber scaffolds has achieved some progress for neural cell adhesion, sprouting and neurite extension of neurons *in vitro*,[123, 124] as well as neural tissue regeneration *in vivo*.[114]

4.3.2 Controlled release of therapeutic agents

It is very important to incorporate therapeutic agents (e.g. drugs and growth factors) into peptide nanofiber scaffolds. Their sustained release is beneficial to constrain side effects of nerve injury and stimulate nerve regeneration. Controlled release of the drug dexamethasone has been feasible within PA nanofiber hydrogels,

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and it can effectively reduce the occurrence of inflammation and prevents the secondary injury to nerve tissue.[125] In addition, RADA16-I peptide nanofiber scaffolds not only allow the incorporation, store and sustained release of functional proteins (e.g. lysozyme, trypsin inhibitor, BSA, and IgG), but also have no influence on protein conformation and functionality based on secondary and tertiary structure analyses and biological assays (Fig. 5a).[27] Peptide nanofiber hydrogels formed by RADA16-I peptides can also be used for the sustained release of basic-fibroblast growth factor (βFGF), vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF), and this study has revealed that physical hinderances can prevent proteins mobility, while charges can trigger interactions between proteins and nanofibers (Fig. 5b).[28] Excitingly, human immunoglobulin (IgG) has sufficient activity after the sustained release of 3 months from the nanofiber hydrogel comprised of peptides RADA16-I and KLDL12 (Fig. 5c).[126]

5. Using peptide nanofiber scaffolds for nerve repair

There are several attractive and particular features that render ionic self-complementary peptides-formed and PAs-formed nanofiber scaffolds to be compelling biomaterial substrates for nerve repair: 1) good biocompability to reduce immune responses, 2) porous structure beneficial to cell infiltration and axon outgrowth, 3) modification with functional motifs and incorporation of growth-promoting molecules with the aim of stimulating cell function and neural tissue growth. Here, utilization of these biomaterial scaffolds for nerve repair would be discussed by 1) *in vitro* cell culture tests and 2) in *vivo* neural tissue regeneration (i.e. minimal inflammation, vasculature formation, axons regrowth and synaptic formation, as well as inhibition of neural scar formation).

5.1 *In vitro* cell culture

Self-assembly peptide nanofiber scaffolds have displayed the competence to reinforce neural cell activity (e.g. PC12 cell attachment and neurite outgrowth) by cell culture techniques.[31, 32] Both of RAD16-I and RAD16-II have tripeptide RAD that are similar to RGD motif, and they have been found to trigger neural cell function and neurite outgrowth though binding to some cell adhesion receptors.[32] In addition, NGF

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preprimed PC12 rat pheochromocytoma cells are cultured within RAD16-II peptide nanofiber hydrogel, resulting in extensively increased neurites formation following the contours of nanofiber scaffold, which indicates the importance of NGF delivery for cellular behaviors (Fig. 6a).[32] Controlled release of growth factors for neural cell culture requires the sustained release and released growth factors should have sufficient bioactivity.

Modification with functional motifs has important impact on cell function within peptide nanofiber scaffolds. IKVAV-containing PAs-formed matrices significantly benefit to differentiate neural progenitor cells (NPCs) into neuron-like cells and glial-like cells and no cytotoxicity is observed to NPCs (Fig. 6b,c).[33] Furthermore, peptides RADA16 are combined with peptides RADA16-IKVAV to form IKVAVmx hydrogel that serves as a substrate to seed neural stem cells (NSCs). The results suggest that IKVAVmx hydrogel has a better promotion to facilitate cellular proliferation, differentiation and migration than pure RADA16 nanofiber scaffolds.[127] Likewise, utilization of other functional motifs (e.g. FGL and bone marrow homing peptides) to modify peptide sequences can also increase the capability of adhesion and differentiation of neural cells.[103, 123]

Hybrid composites of self-assembly peptide nanofiber scaffolds and other materials have been developed in order to improve the mechanical stress for neural cell culture. Type I collagens are combined with IKVAV-presenting PA nanofibers in order to yield homogeneous nanofibers with the diameter of 20~30 nm and to subsequently assemble into nanofibrous scaffolds (IKVAV hybrid), in which epitope concentration can be altered through changing PA concentrations. And this approach provides the feasibility to study the optimal epitope concentration for cellular behaviors. Cerebellar cells, granule cells (GC) and purkinje cells (PC) cultured on these IKVAV hybrids result in good cell survival and function. Compared to the collagen substrate, GC density within IKVAV hybrid increases three-fold when PA concentration is more than or equal to 0.25 mg/ml, while the optimal PA concentration for PC culture is 0.25 mg/ml (Fig. 6d,e,f).[128]

5.2 *In vivo* neural tissue regeneration

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5.2.1 Minimal inflammation

Following nerve injury, inflammatory reactions can not only cause secondary injury to neural tissue, but also contribute to the formation of nerve scar tissue. Both of them are regarded as important factors for nerve regeneration failure. RADA16-I peptide nanofiber scaffolds are utilized to fill the nerve cavities of female Sprague-Dawley (SD) rats, and the results reveal minimal inflammation at the lesion site according to the quantities of macrophages, indicating the good biocompability of peptide nanofiber scaffolds for tissue repair (Fig. 7a,b).[129] Bone Marrow Homing Peptide 1 (BMHP1) functional motif (PFSSTKT) is conjugated with peptide RADA16-I to form BMHP1-self-assembly peptides (BMHP1-SAP) that are subsequently assembled into 3D interwoven matrices. Such biomaterial scaffolds are applied to treat SCI defects of female SD rats, resulting in minimal immune responses and inflammation as shown by negligible number of infiltrated macrophages (Fig. 7c).[34]

5.2.2 Vasculature formation

Since the role of new blood vessels is to provide nutrient, oxygen and to eliminate waste products for cells, vasculature formation is regarded as an essential part in tissue regeneration. NPCs and Schwann cells are seeded on RADA16-I peptide nanofiber scaffolds, and subsequently they are implanted to the transected spinal cord of rats. In addition to host cells migration and axon regrowth, the results also demonstrate the formation of many blood vessels in the implants.[130] Hybrid composite guidance channels comprised of RADA16-I-BMHP1 peptide nanofibers and electrospun poly(□-caprolactone)/poly(lactic-coglycolic acid) (PCL/PLGA) nanofibers, are combined with some cytokines such as BDNF, ciliary neurotrophic factor (CNTF), VEGF, and chondroithinaseABC. Then they are utilized to fill the cysts of a postcontusive and chronic SCI of rats. After six months, well-developed vascular network is yielded and well integrated with nerve fibers, neural and stromal cells, basal lamina and myelin, for the purpose of reconstructing an anatomical, structural, and histological structure for regenerative nerve (Fig. 7d).[131]

5.2.3 Axons regrowth and synaptic formation

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After the nerve injury, spinal cord axons have shown the initial regenerative response (e.g. axon regrowth and up-regulation of regeneration related genes), but lack an aligned substrate to direct the extending axons.[132, 133] It is believed that peptide nanofibers scaffolds can facilitate the regrowth and migration of neural cells, as well as the formation of lengthy axons aligned to the contours of scaffolds, indicating that peptide nanofiber scaffolds can serve as good substrates for axons extension.[25, 32] Two key elements are required for axons extension: 1) sufficient cell function such as migration, and 2) the delivery of molecular signals that direct cell migration towards target sites.

Nanofiber scaffolds formed by peptide RAD16-II are found to have the capability to stimulate extensive neurite outgrowth and functional synapse formation between the attached neurons *in vitro* (Fig. 7e).[32] While nanofiber scaffolds derived from peptide RADA16-I are used to repair a severed optic tract in the hamster, resulting in significantly regenerated axons for reconnecting defects, as well as the improvement of visually elicited orienting behavior (Fig. 7f).[31] These peptide nanofiber scaffolds also have the ability to induce axonal regeneration in young adult Syrian golden hamsters with chronic optic tract lesions.[134] In one study of treating the chronically injured spinal cord, IKVAV-PA nanofiber scaffolds demonstrate significantly increased serotonergic innervation than the treatment of non-bioactive PA nanofiber scaffolds, which suggests the importance of functional motif IKVAV in nerve repair *in vivo*.[35] In addition, peptide nanofiber scaffolds can serve as good substrates for cells. NSCs isolated from fetal spinal cord are incorporated within peptide nanofiber scaffolds. Subsequently, they are transplanted to the lesion of spinal cord hemisection in adult rats, leading to the robust formation of neural fibers crucial for the recovery of electrophysiological function.[135]

5.2.4 Inhibition of scar formation

Inhibition of scar formation is vital to the recovery of nerve function by preventing inflammation and glial reaction. One *in vivo* study has revealed that RADA16-I peptide nanofiber scaffolds can prevent inflammation and glial reaction when treating acutely traumatic brain, and are well integrated with host tissue (Fig.

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7g).[129] In addition, Schwann cells and RADA16-I hydrogel are combined to treat the spinal cord injury of rats, leading to significantly reduced astrogliosis.[136] IKVAV-PA nanofiber scaffolds have been revealed to inhibit glial differentiation and astrogliosis, and to induce neurite outgrowth as well as myelin sheath formation around neurons (Fig. 7h).[77] Their application in SCI repair also demonstrates inhibition of glial scar formation, and robust regeneration of motor fibers and sensory fibers that facilitate the behavioral improvement in animal models (Fig. 7i).[85, 137]

6. Conclusion and future perspectives

In summary, peptide nanofiber scaffolds formed by ionic self-complementary peptides and PAs have been found to be promising for nerve repair, as evidenced by tremendous studies regarding *in vitro* cell culture and *in vivo* neural tissue regeneration. Especially, their biological properties (e.g. modification with functional motifs and controlled release of therapeutic agents) contribute favorably to the improvement of nerve regeneration. It is believed that self-assembly peptide nanofiber scaffolds could produce more positive surprises in neural tissue engineering.

Although many studies have focused on the design, fabrication and properties of self-assembly peptides, it is still difficult to explain the mechanisms mediating self-assembly of peptides into interwoven nanofiber structures, and to completely control the microstructure at nanometer level. In addition, it is very difficult to control cell fates such as the differentiation of stem cells into the required lineage. Much more effort is needed to overcome these challenges.

Acknowledgement

D.M. Jiang was supported in part by China National ‘‘973 Project’’ and by the grant from the Natural Science Foundation Project of CQ CSTC (2009AB5080), and National Natural Science Foundation of China (NSFC, 81171685/H0604). We apologize to all the scientists whose work we could not cite due to space restrictions.

Conflict of interest

The authors declare no conflict of interest.

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Figures

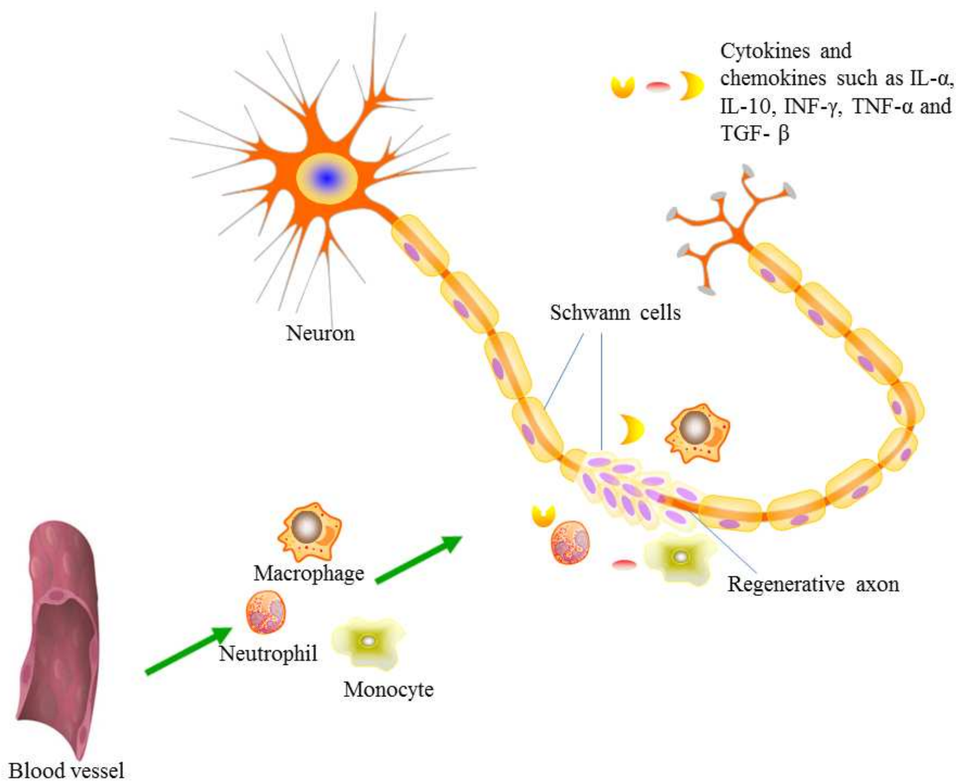

Figure 1

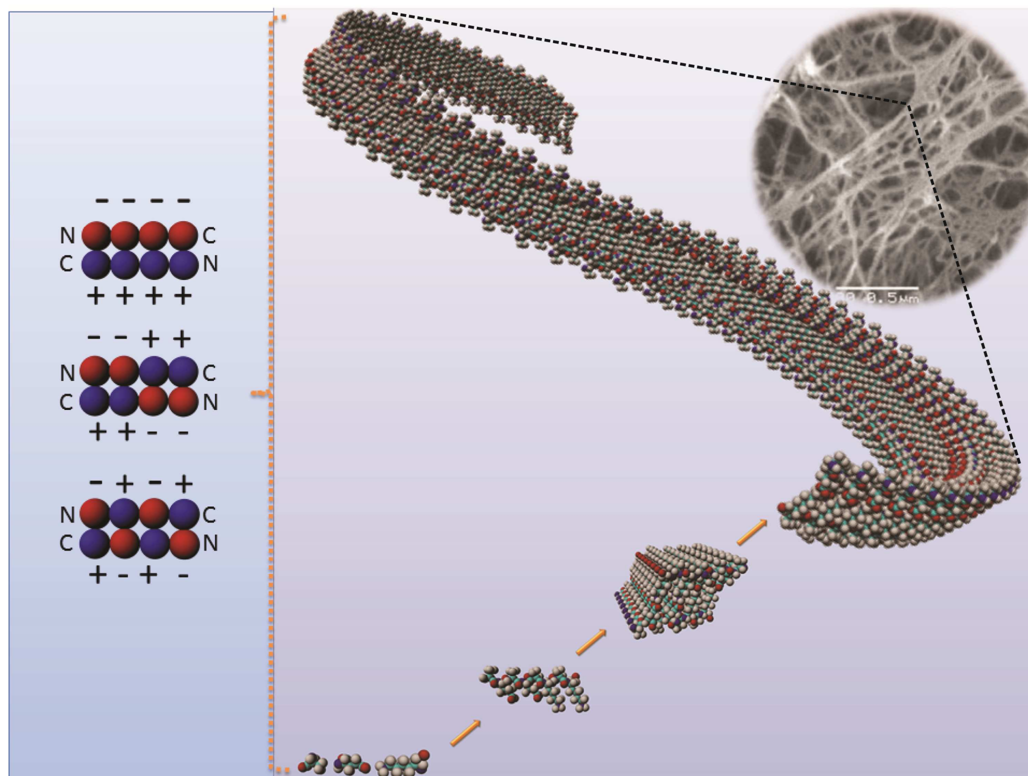

Figure 2

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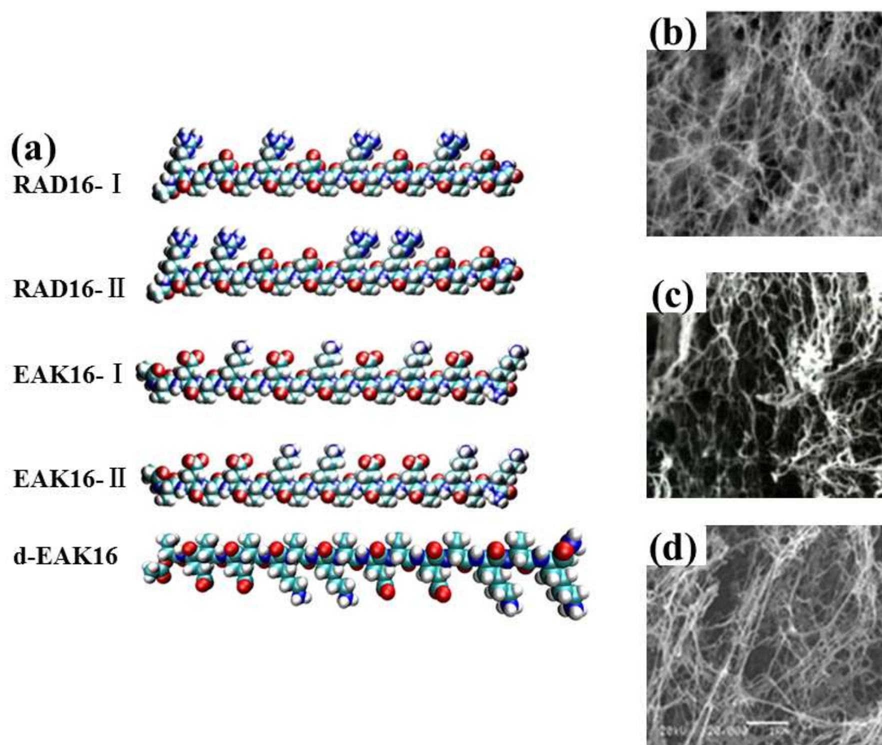

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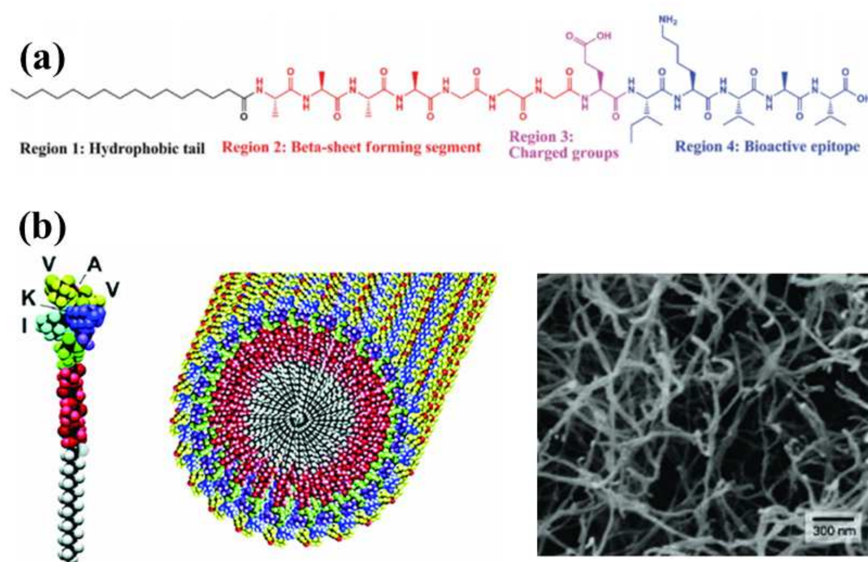

Figure 4

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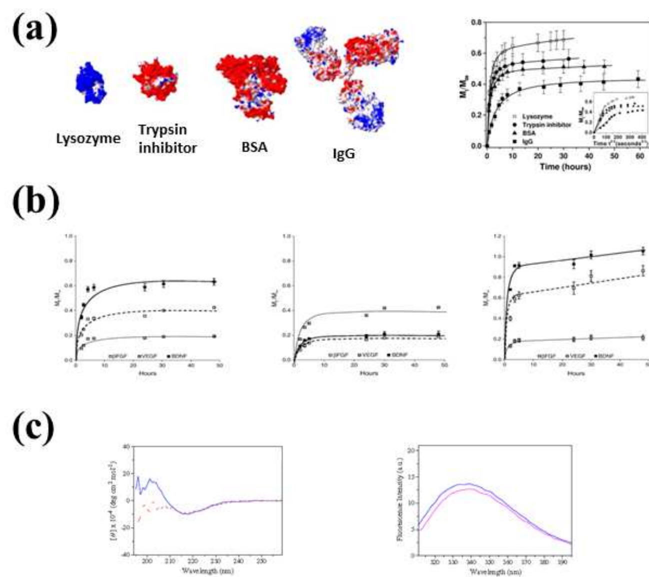

Figure 5

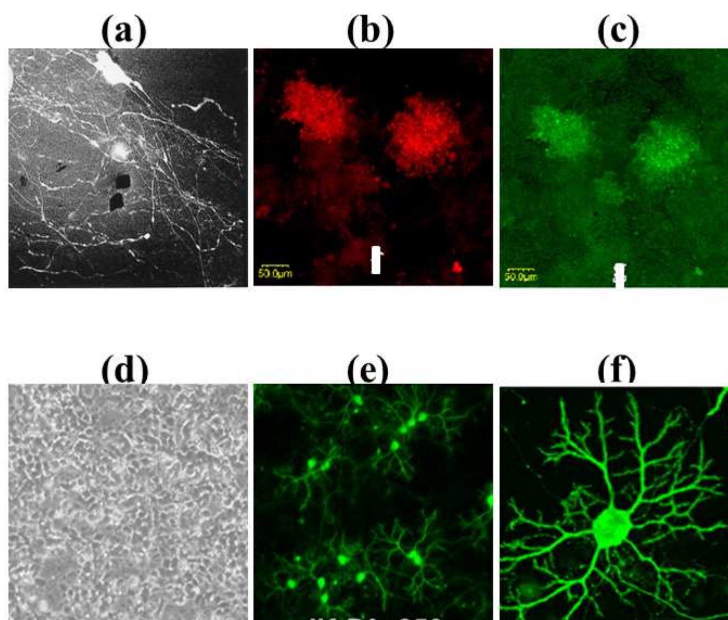

Figure 6

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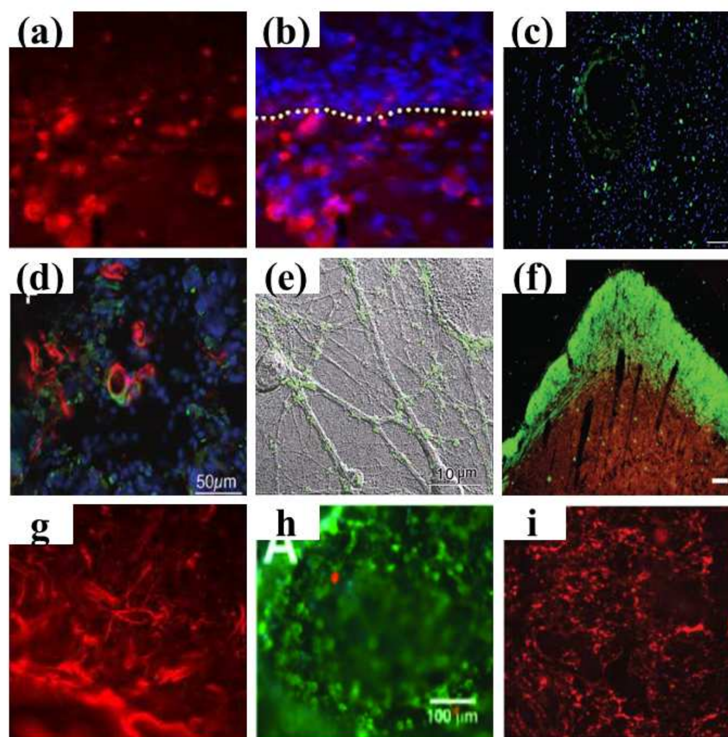

Figure 7

Figure legend

Fig. 1 Schematic representation of inflammatory responses following nerve injury: After the nerve injury, extrinsic inflammatory cells such as neutrophils, macrophages, and monocytes are recruited to the lesion site through the damaged blood vessel. Then inflammatory cells secrete proteolytic enzymes to degrade ECM proteins and others, as well as cytokines and chemokines (e.g. IL-α, INF-γ, TNF-α, IL-10 and TGF-β) to aggravate secondary damage or to facilitate nerve protection and axonal regeneration. Meanwhile, because the injured nerve has intrinsic but limited regenerative ability, Schwann cells are able to proliferate and migrate to the lesion site, and subsequently surround the regenerative axon in order to reconstruct the electrical circuit.

Fig. 2 Schematic representation of nanofiber scaffolds formed by ionic self-complementary peptides: self-assembly of peptide d-EAK16 consisting of alanine, glutamic acid and lysine can form nanofibers and 3D biomaterial scaffolds (SEM images of d-EAK16 peptide nanofiber scaffold from ref. [76]), and this process of peptide self-assembly is supported by ionic complementary interactions that are

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derived from + (blue) and – (red) charged amino acid residues (Modulus of charge arrangements: - - - -, + + + +; - - + +, + + - -; - + - +, + - +-). Adapted and reprinted with permission from ref. 76. Copyright 2011, Elsevier.

Fig. 3 (a) Molecular models of several self-assembling peptides, RAD16-I, RAD16-II, EAK16-I, EAK16-II, and d-EAK16.[76, 101, 108] (b) SEM image of RAD16-I nanofiber scaffold (PuraMatrix) with fiber diameter of ~10–50 nm and the pore size of ~10–200 nm.[108] (c) SEM image of EAK16-II nanofiber scaffold with the nanopores with ~5–200 nm in diameter.[101] (d) SEM image of d-EAK16 with fiber diameter of ~10 nm and pore size of ~20–500 nm.[76] Adapted and reprinted with permission from ref. 76, 101, 108. Ref. 76, Copyright 2011, Elsevier. Ref. 101, Copyright 2006, Royal Society of Chemistry. Ref. 108, Copyright 2008, Schneider et al.

Fig. 4 Schematic representation of self-assemble of PAs into nanofibers: (a) Chemical structure of PA with four key chemical entities. (b) Molecular model of an IKVAV-containing PA, their self-assembly into nanofibers, as well as SEM image of IKVAV nanofibers after adding cell media (DMEM) to PA aqueous solution [77]. Adapted and reprinted with permission from ref. 77. Copyright 2010, Wiley Periodicals, Inc.

Fig. 5 Controlled release of therapeutic agents from peptide nanofiber scaffolds: (a) Molecular models of lysozyme, trypsin inhibitor, BSA, and IgG (Color scheme for proteins and peptides: blue, positively charged; red, negatively charged; light blue, hydrophobic). The release profiles of lysozyme, trypsin inhibitor, BSA, and IgG from self-assembling peptide hydrogel in PBS (pH 7.4) at room temperature. (Inset) Protein release plotted as a function of the square root of time. The initial linear part of the plots represents simple diffusion of the proteins through the peptide hydrogel.[27] (b) The release profiles of βFGF, VEGF and BDNF through the RADA16-I hydrogels (left), the RADA16-DGE (Ac-RADARADARADARADAGGDGEA-CONH2 consisting of RADA16-I with two negatively charged amino acids added to its C-terminus) hydrogels (middle), and the RADA16-PFS (Ac-RADARADARADARADAGGPFSSTKT-CONH2 consisting of a RADA16-I segment with additional positively charged amino acids appended to the C-terminus)

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hydrogels (right) for up to 48 h.[28] (c) Spectroscopic examination of the human IgG in PBS (pH 7.4): released (broken line) IgG from peptide hydrogel is compared with native (solid line) IgG by Far-UV CD spectra and normalized fluorescence emission spectra. Excitation wavelength is 300 nm. Spectra are recorded at room temperature in 2-month post release samples.[126] Adapted and reprinted with permission from ref. 27, 28, 126. Ref. 27, Copyright 2009, National Academy of Sciences. Ref. 28, Copyright 2010, Elsevier. Ref. 126, Copyright 2012, Elsevier.

Fig. 6 Promotion to *in vitro* cell culture: (a) NGF preprimed PC12 cells seeded on self-assembly peptide scaffold result in extensive neurites formation.[32] NPCs are cultured within IKVAV-PA nanofiber scaffolds and have the ability to differentiate into (b) neurofilament (NF)-positive neuron-like cells with red fluorescence and (c) glial fibrillary acidic protein (GFAP)-positive glial-like cells with green fluorescence.[33] Hybrid matrix consisting of collagen (type I) and IKVAV-presenting PA nanofibers is used to culture cerebellar cells, GCs and PCs. (d) Freshly dissociated cerebellar cells cultured on the hybrid substrate comprised of collagen and IKVAV-PA (2 mg/ml) show good attachment and survival. (e) Purkinje cell (PC; calbindint+) and (f) its morphology (16 DIV) observed on the hybrid matrix consisting of collagen and IKVAV-PA (0.25 mg/ml).[128] Adapted and reprinted with permission from ref. 32, 33, 128. Ref. 32, Copyright 2000, National Academy of Sciences. Ref. 33, Copyright 2011, Taylor and Francis Ltd. Ref. 128, Copyright 2012, Elsevier.

Fig. 7 Promotion to *in vivo* neural tissue regeneration: Good biocompatibility between peptide nanofiber scaffolds and host tissue leads to minimal inflammation, (a,b) Immunocytochemical staining shows the cellular responses in the host tissue surrounding lesion sites, and there are very few ED1-positive macrophages that scatter in the graft (a) and at the boundaries of the defects (b).[129] (c) BMHP1-SAP scaffolds are used to treat SCI defects, resulting in negligible number of infiltrated macrophages. Cell nuclei (blue) are stained with DAPI.[34] (d) Hybrid matrix scaffolds comprised of peptide nanofibers and electrospun nanofibers are applied to treat chronic SCI of rats. Networks of capillaries and vessels (red, positive for VWF) are found adjacent to fibroblasts, and the latter are preferentially located in the tube

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walls.[131] (e) Primary rat hippocampal neurons cultured on peptide nanofiber scaffold can yield active synapses.[32] (f) Dark-field photo of optic tract axons that are stained by green fluorescence, shows the regeneration of axons through the parasagittal section in the brain of an 8-month-old hamster.[31] (g) Immunocytochemical staining shows the cellular responses in the host tissue surrounding lesion sites, and there are very few GFAP-positive astrocytes when using RADA16-I peptide nanofiber scaffolds to treat acutely traumatic brain.[129] (h) NPCs are cultured on IKVAV-PA gels at 1 day, and immunocytochemical staining demonstrates very few glial cells. Differentiated neurons are labeled for β-tubulin (in green) and differentiated astrocytes (glial cells) are labelled for GFAP (in orange). All cells are Hoechst stained (in blue).[77] (i) IKVAV-PA nanofibers can effectively reduce astrogliosis when repairing SCI, as evidenced by the representative confocal Z-stacks of injured areas stained with GFAP at 11 weeks (The lesion is defined as the area marked by dense infiltration).[85] Adapted and reprinted with permission from ref. 31, 32, 34, 77, 85, 129, 131. Ref. 31, Copyright 2006, National Academy of Sciences. Ref. 32, Copyright 2000, National Academy of Sciences. Ref. 34, Copyright 2011, American Chemical Society. Ref. 77, Copyright 2010, Wiley Periodicals, Inc. Ref. 85, Copyright 2008, Society for Neuroscience. Ref. 129, Copyright 2009, Elsevier. Ref. 131, Copyright 2011, American Chemical Society.

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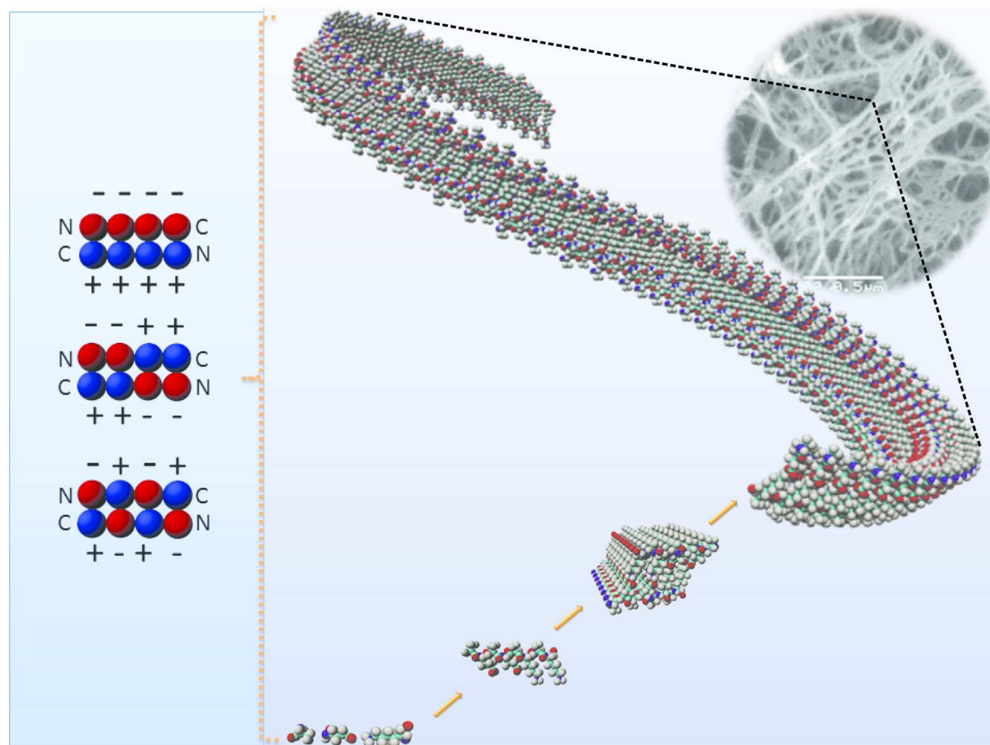

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The particular features render ionic self-complementary peptides-formed and peptide amphiphiles-formed nanofiber scaffolds to be compelling biomaterial substrates for nerve repair.

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