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Biomimetic nanofibrous scaffolds for neural tissue engineering and drug development

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Neural tissue engineering aims to develop functional substitutes for damaged tissues, creating many promising opportunities in regeneration medicine and drug discovery. Biomaterial scaffolds routinely provide nerve cells with a physical support for cell growth and regeneration, yielding 3D extracellular matrix to mimic the *in vivo* cellular microenvironment. Among the various types of cellular scaffolds for reconstruction, biomimetic nanofibrous scaffolds are recognized as appropriate candidates by precisely controlling morphology and shape. Here, we review the current techniques in fabricating biomimetic nanofibrous scaffolds for neural tissue engineering, and describe the impact of nanofiber components on the properties of scaffolds and their uses in therapeutic models and drug development. We also discuss the current challenges and future directions of applying 3D printing and microfluidic technologies in neural tissue engineering.

Introduction

The ability to reconstruct artificially functional 3D tissues or organs has been recognized as an important technology for animal-alternative drug screening and regenerative medicine [1–3]. Neural tissue engineering offers new therapeutic opportunities for regenerating the damaged nervous tissues in transplantation, and also creates *in vitro* 3D neural models for drug screening [4–6]. In these studies, biomaterial scaffolds are generally required to provide an artificial extracellular matrix (ECM) for the seeding and growth of nerve cells. Rather than simply mixing cells with biomaterials, an effective technique for neural tissue engineering often combines nano- and micro-technological strategies for designing and engineering complex tissues, and tailoring the properties of 3D biological scaffolds; this is crucial for a successful reconstruction to mimic the real cellular microenvironment and reproduce effective tissue functions [7,8]. Therefore, significant efforts have been devoted to promote effective organization and functional integration of the cells into biological scaffolds with closely resembled morphological and physiological features *in vivo* [9–11].

In recent years, many advanced strategies for neural tissue engineering have been developed, especially in fabricating biomimetic nanofibrous scaffolds used to mimic the ECM [12,13]. The current technologies have enabled precise control of the nanoscale morphologies and tune the biochemical properties of nanofiber biomaterials for tissue engineering [14]. In particular, among the materials used for neural tissue engineering, nanofibrous scaffolds have been widely used because of their high surface-area:volume ratio and close imitation of the natural ECMs [15–17]. At the same time, the development of nanofibers greatly extends the scope of fabricating biological scaffolds, and solves the problem of cell loss or neuropathy caused by nonphysiological local stress [13,18]. It is believed that these artificial biomaterials can serve as necessary tissue scaffolds for engineering functional neural tissues [19]. Nowadays, current technological development in biomimetic nanofibrous scaffolds has promoted many biomedical applications of neural tissue

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engineering [20,21]. Typically, it has evolved as an interdisciplinary technology that combines biology, engineering and material science, with the goal of developing neuroregenerative medicine for transplantation and 3D *in vitro* neural models for drug screening (Fig. 1) [9,22,23]. Besides nanofibrous scaffolds, an alternative choice to recapitulate the 3D aspects of neural connectivity or the microenvironment of the brain is *in vitro* bioengineering of 3D models that mimic native neural tissues. For instance, 3D brain-like cortical tissue formed from primary cortical neurons in modular 3D compartmentalized architectures was reported to be maintained for months *in vitro* [24]. To simplify the operation process, a unique, single-step bioacoustic levitational assembly technology was presented to implement brain bioengineering [25]. These bioengineering approaches accompanied with nanofibrous scaffolds constitute a broad toolbox for the fabrication of 3D architectures that recapitulate the physiological complexity of *in vivo* neural tissues.

In this review, we focus on discussing methods of fabricating nanofibrous scaffolds based on electrospinning and self-assembling, and describe the composites of nanofibrous scaffolds for neural tissue engineering. We start with a brief introduction to the recent development of biomimetic nanofibrous scaffolds in neural tissue engineering. Next, we give a detailed description of the strategies for the fabrication of electrospun and self-assembled nanofibrous scaffolds, classified by natural polymers, synthetic polymers and hybrid composites. Then, we present a discussion in the specific application domains, including drug development and nerve regeneration. The final section highlights the major challenges, emerging opportunities and perspectives for future developments.

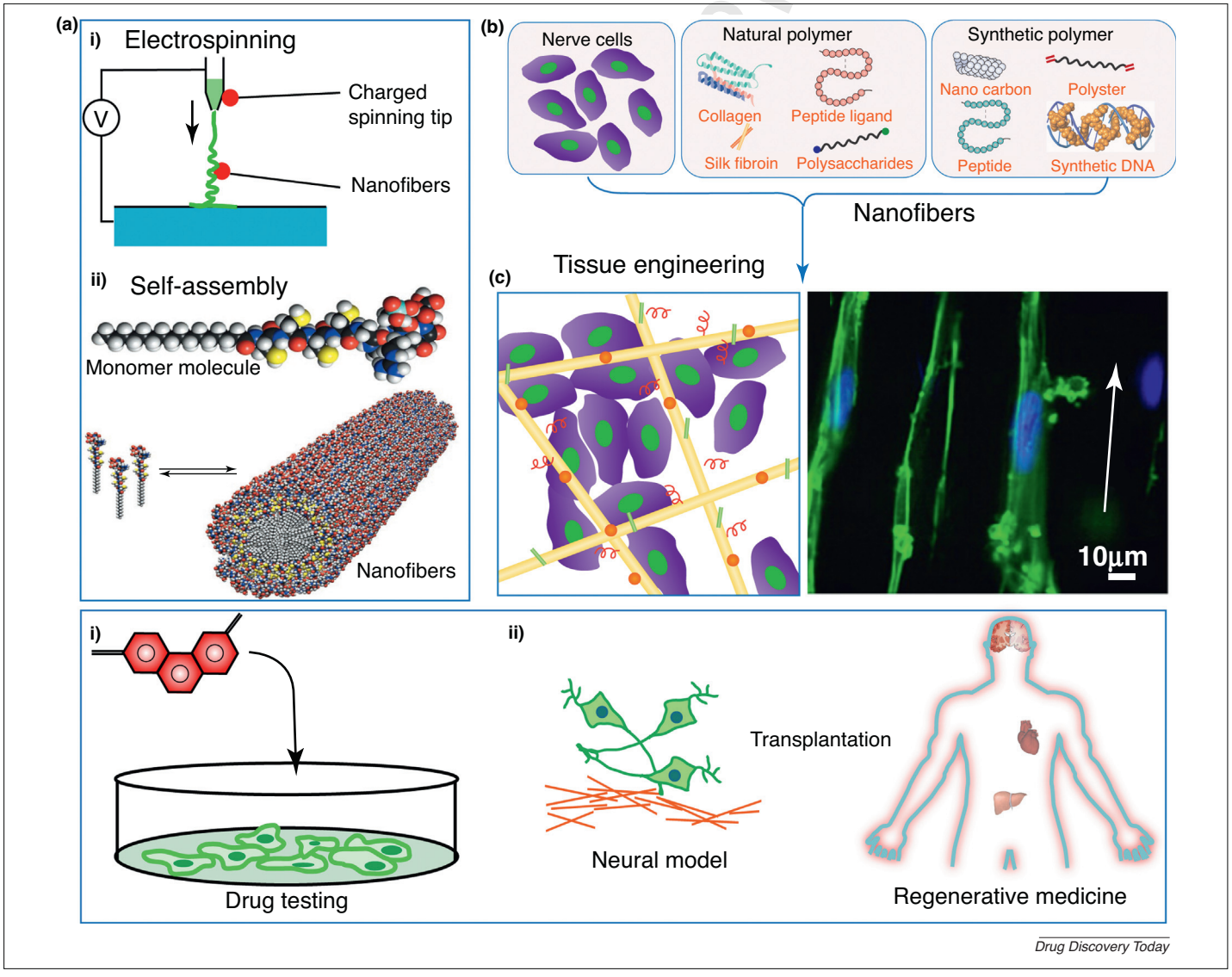

FIGURE 1

Fabrication of biomimetic nanofibrous scaffolds for neural tissue engineering. (a) Strategies of electrospinning and self-assembly used for nanofiber fabrication. Reprinted, with permission, from [22]. (b) A general pathway of engineering neural tissues with the use of nerve cells and nanofibers composed of natural polymers and synthetic polymers. **(c)** Schematic illustration and image of neural cells on nanofibrous scaffolds. Reprinted, with permission, from [9,23]. **(d)** Biomedical applications of neural tissue engineering in drug testing and regeneration medicine.

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Engineering technologies of nanofibrous scaffolds

Electrospun nanofibrous scaffolds

Electrospinning is derived from the electrospray process which continuously produces fibers with diameters ranging from nano- to micro-size using electrostatic forces [18,26]. It is advantageous in cost-saving and fabricating nanofibers with high porosity and interconnected fibrous networks mimicking ECM architectures [27,28]. Using this technique, it is flexible to fabricate nanofibrous scaffolds out of a wide range of materials including natural and synthetic polymers [29].

Natural polymer nanofibrous scaffolds

In general, natural polymers exhibit better biocompatibility and lower immunogenicity than synthetic polymers. For example, collagen is one of the main components of the ECMs and is frequently used in electrospun nanofibrous scaffolds. Gelatin is one kind of denatured collagen which is widely used as scaffolds for cell growth, migration and tissue engineering therapies owing to its good biocompatibility, low immunogenicity and tunable mechanical strength. Faghihi *et al*. reported the fabrication of 3D electrospun nanofibrous gelatin scaffolds to investigate the effects of retinoic acid (RA) and sonic Hedgehog on differentiation of human chorion-derived mesenchymal stem cells (C-MSCs) into motor-neuron-like cells [30]. Alternatively, silk fibroin is another natural protein often used in producing electrospun scaffolds because of its excellent oxygen–water permeability and biodegradability. Guo *et al*. successfully fabricated poly(lactic-*co*-glycolic acid)/multi-walled carbon nanotubes/silk fibroin nanofibrous Q7 scaffolds for neuronal differentiation [31]. They observed that catalpol, a natural active ingredient extracted from traditional Chinese medicine, can induce neuronal differentiation of human adipose-tissue-derived stem cells. Further, the combination of collagen and silk fibroin was employed to fabricate composite nanofibrous scaffolds for neural tissue engineering [32]. In this study, collagen and dragline silk proteins were homogeneously mixed to fabricate well-aligned composite nanofibers. The ultimate tensile strength and elasticity of the nanofibers can be increased by tuning silk percentage, and the improved stability in nanofibrous scaffolds can avoid the excessive fiber swelling and shape deformation in cell culture medium. As a result, such nanofibrous scaffolds were demonstrated to effectively support a normal proliferation and polarization of human *decidua parietalis* placental stem cells (hdpPSCs) at various levels (Fig. 2a).

Apart from protein-based nanofibrous scaffolds, polysaccharides, such as chitosan, dextran, alginate and cellulose, are also used as biomaterials for the fabrication of electrospun nanofibrous scaffolds in neural tissue engineering. Salati *et al*. reported a strategy for producing chitosan/gelatin-composited nanofibrous scaffolds using the electrospinning method [33]. The results showed that nanofibers made of 50% chitosan and 50% gelatin exhibited a good feasibility for neural tissue engineering, which was evaluated by the contact angle, porosity and biocompatibility with nerve cells. In addition, electrospun nanofibers of gelatin/cellulose-acetate composites were developed as artificial neural networks to mimic ECMs [34]. An optimized electrospinning procedure was performed to study the factors influencing the fiber diameter and quality, including applied voltage, gap distance, solvent composition and solution concentration. As a result, uniform bead-less nanofibers were obtained at the optimum conditions of 16.9 kV, 15.3 cm and 77.5 wt% of gelatin, 88.9 vol% of acetic acid and solution concentration of 20 wt%/vol%. Although the natural polymers provide an excellent matrix substance for cell adhesion and infiltration, the potential existing pathogens or undefined components would induce immunological and inflammatory responses. Meanwhile, undefined composition and batch-to-batch variation might lead to low reproducibility of experimental results.

Synthetic polymer nanofibrous scaffolds

In comparison with natural polymers, synthetic polymers are important alternatives for developing electrospun nanofibrous scaffolds, allowing functionalization and parameter adjusting on the biomaterial scaffolds for nerve cell culture. Among the various types of synthetic polymers, polyester is one of the most popular biomaterials for electrospun nanofibrous scaffolds. Vicentini *et al*. fabricated a kind of porous nanofibrous scaffold using poly(L-lactic acid) (PLLA) [19]. In this study, multi-walled carbon nanotubes (CNTs) were employed to promote neurite outgrowth. Uniform defect-free nanofibers were produced by optimizing blend preparation and deposition parameters. Meanwhile, 4-methoxyphenyl was used to covalently functionalize the CNTs, leading to a homogeneous dispersion of the nanofibers into the polymer matrixes and avoiding the aggregating tendency (Fig. 2b). As a result, we saw that neurites extended along the nanofiber direction; this proves the relevance of the tissue morphology with nanofibrous scaffolds when engineering complex tissue environments. In addition, graphene oxide (GO) was also employed to coat the PLLA nanofibers [35]. A significant promotion was observed on the proliferation and differentiation of rat pheochromocytoma cell line. Further, the researchers also found that the addition of nerve growth factor (NGF) into the nanofibrous scaffolds improved the neurite growth along the nanofibrous alignment, indicating a better performance in nerve regeneration. Therefore, the synthetic polymers own the obvious advantages in designing nanofibrous scaffolds with predefined biochemical properties.

Composite polymer nanofibrous scaffolds

As discussed above, natural polymers are advantageous in cell adhesion and infiltration, and the drawbacks are poor reproducibility and potential immunological responses; whereas the syn- Q8 thetic polymers lack active sites for cell recognition (Table 1). For these reasons, current technology has provided an optimal choice for engineering composite polymer nanofibrous scaffolds containing both types. For instance, Kuppan *et al*. demonstrated that cell adhesion and proliferation in the poly-(ε-caprolactone) (PCL)/gelatin nanofibers were more favorable than that in the pure PCL nanofibers [36]. In this study, the results showed an increase of α-actin, myosin heavy chain, collagen type I and elastin genes in human smooth muscle cells, which indicated that strong cell-matrix interactions exist in random and aligned PCL/gelatin nanofibrous scaffolds. Using this strategy, Wang *et al*. electrospun a kind of hybrid material by incorporating PLLA short nanofibers and thermoresponsive xyloglucan (XYLO) hydrogel into the nanofibrous scaffolds, and injected them into the injured brain of parkinsonian mice (Fig. 2c) [37]. Such composite nanofibrous scaffolds did not bring any deleterious impact on the host immune response; the survival of ventral midbrain grafts and reinnervation of the striatum were thus augmented. Moreover, the increased

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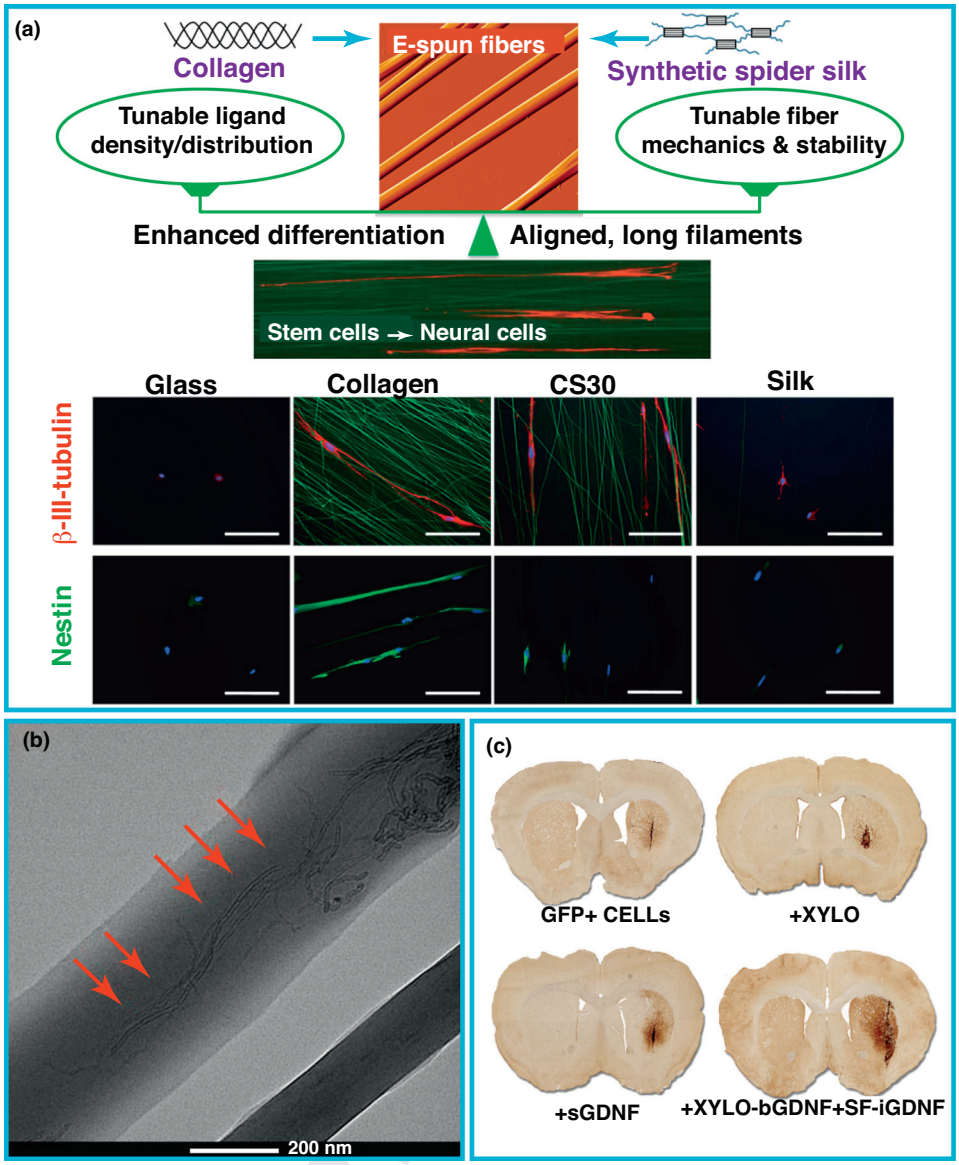

FIGURE 2

Q15 Electrospun polymer nanofibrous scaffolds. **(a)** Normal differentiation of hdpPSCs on the electrospun composite fibers composed of collagen and dragline silk proteins (CS30:30 wt% silk in collagen). The immunofluorescent images showed β-III-tubulin-positive cells (red) and nestin-positive cells (green). Cell nuclei were stained by DAPI (blue). The collagen morphology was stained by green. Scale bar: 100 μm. Reprinted, with permission, from [32]. (b) Transmission electron microscope (TEM) image of electrospun nanofibers functionalized with carbon nanotubes (CNTs). Red arrows indicate regions of CNTs aligned along the fiber's axis. Reprinted, with permission, from [19]. **(c)** Representative photomicrographs of a coronal view of the GFP+ grafts within the striatum. Images showed the grafts of GFP+ cells alone, GFP+ cells in xyloglucan (XYLO) scaffold and GFP+ cells in the presence of glial-derived neurotrophic factor (GDNF)+ scaffolds. Reprinted, with permission, from [37].

TABLE 1

The property comparisons of natural, synthetic and composite polymers

Polymer type	Biocompatibility	Immunogenicity	Mechanical properties	Applications	Refs
Natural polymer (gelatin, silk fibroin, collagen)	High	Low	Weak	Neural stem cells Differentiation; Neurogenesis	[30–32]
Synthetic polymers (PLLA, PGA, PCL, PHB)	Low	High	Strong	Neurite outgrowth; Neuronal differentiation	[19,80,81]
Polymer composites (PCL/gelatin, PLLA/XYLO, PLGA/gelatin, P(3HB-co-4HB)/cellulose acetate)	Middle	Middle	Tunable	Tissue repair and regeneration; Nerve cell culture	[21,36–38]

Q18 Abbreviations: PLLA, poly(L-lactic acid); PCL, poly-(ε-caprolactone); XYLO, xyloglucan; PHB, PGA, PGLA.

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number of green fluorescent protein (GFP) expression cells was realized by blending with glial-derived neurotrophic factor (GDNF).

Incorporation of nanomaterials into the composite polymers has also been developed to improve their properties. Mehrasa *et al*. reported an approach of embedding different amounts of mesoporous silica nanoparticles (MSNPs) into poly(D,L-lactide-*co*-glycolide) (PLGA)/gelatin nanofibers by the electrospinning method [38]. In this case, the incorporation of gelatin and MSNPs enhanced the hydrophilicity and decreased the porosity of the scaffolds. As a result, an improvement of nerve cell attachment and proliferation was observed on such hybrid nanofibrous scaffolds. Cai *et al*. fabricated an artificial biological membrane composed of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] and cellulose acetate using the electrospinning technique [21]. The results of *in vitro* degradation tests revealed that the biodegradation rates of the composite nanofibers were obviously higher than those of neat P(3HB-*co*-4HB). From the polymer point of view, these produced composite nanofibers combining specific characteristics of the respective constituents on the nanoscale are beneficial for cell adhesion, migration, proliferation and differentiation. These composite nanofibers thus serve as promising scaffold matrices for cell culture in neural tissue engineering.

Self-assembled nanofibrous scaffolds

Self-assembly is a process where molecules and components spontaneously organize into patterns or structures of nanofibers by noncovalent interactions and form soft, randomly oriented networks [13,20]. In particular, self-assembled nanofibrous scaffolds exhibit many benefits when applied in neural tissue engineering: (i) van der Waals forces, H-bonds and electrostatic forces are the driving force during the self-assembly process needing no human intervention; (ii) natural L-amino acids degraded from peptides are the building blocks of the self-assembled nanofibers used for cell growth and repair in the scaffolds; (iii) more *in-vivo*-like ECMs are provided because the sizes of them are one to two orders of magnitude smaller than typical electrospun fibers. Many types of biomaterials have been developed into self-assembled nanofibrous scaffolds [6,20,22]. In this section, emphasis is put on approaches and applications of self-assembled peptides (SAPeptides) obtained from naturally derived sources and synthetic routes.

Naturally derived peptide nanofibrous scaffolds

The nature of peptide motifs has inspired people to design the nanofibrous scaffolds, because the short peptides consisting of amino acids have been demonstrated to exhibit dynamic behaviors for self-assembly. For example, the peptides can self-assemble into α-helical, β-sheet or β-hairpin structures at the molecular structural level and form supramolecular structures for tissue engineering applications [39]. Typically, peptide RADA16-I (Ac-RADARADARADARADA-NH_2), the precursor of which was AEAK16-II (AEAEAKAKAEAEAKAK) discovered in the zuotin yeast proteins [40], can self-assemble into nanofibrous scaffolds for studying the reprogramming and maturation of human neurons. In this case, the human neurons differentiated from induced pluripotent stem cells (iPSCs) in the SAPeptide nanofibrous scaffolds showed high functional activity. At the same time, the RADA16-I scaffolds increased the survival of neurons and extended neurite length up to several hundred microns into the host brain tissue [41]. It is well known that isoleucine-lysine-valine-alanine-valine (IKVAV) promotes neurite outgrowth [42,43]. The peptide-containing IKVAV molecules can self-assemble into highly ordered nanofibrous hydrogel scaffolds to culture neural progenitor cells (NPCs). As a result, these NPCs in the 3D scaffolds had a higher neuronal differentiation rate than those cultured on the surface of the hydrogel. Thus, this self-assembled hydrogel can serve as an excellent biomaterial block for neural tissue engineering [44]. Cheng *et al*. linked a laminin-derived IKVAV motif onto the C terminus of RADA16 peptide to synthesize RADA16-IKVAV. These peptides self-assembled into nanofibrous morphology with a bilayer β-sheet structure [45]. This nanofibrous scaffold was demonstrated to exhibit similar mechanical stiffness to brain tissue. The modified IKVAV sequence played an important part in directing the neural stem cell (NSC) adhesion for neuronal differentiation.

Gelain *et al*. reported a self-assembly approach to construct LDLK12 (Ac-LDLKLDLKLDLK-NH_2) SAPeptide nanofibrous scaffolds for 3D culture of NSC-derived neural precursor cells (NPCs) [46]. They observed a high differentiation rate of human nerve cells on the functionalized LDLK12 SAPeptide nanofibrous scaffolds (Fig. 3a). The results indicated that the hydrogels functionalized with this SAPeptide could be used as a promising biomimetic scaffold for *in vitro* NSC differentiation and regenerative therapy of the injured nervous system. They further combined the electrospinning and self-assembly methods to attach the Ac-FAQ SAPeptides onto the PCL-PLGA nanofibers [47]. They obtained the two types of hybrid nanofibers with core/shell structures: PCL-PLGA@PCL-PLGA-Ac-FAQ and QPCL-PLGA@Ac-FA. Using these nanofibrous scaffolds, they demonstrated excellent viability of NSCs and inflammatory response of rodent spinal cords. In addition, a long motif of laminin, CQIK (CQAASIKVAV), was employed to mimic the conformation and active sites of native regions in the ECMs [48]. The neuroblastoma cells were cultured into the CQIK nanofibrous scaffolds with high cell viabilities. Such nanofibrous scaffolds are applicable for the recovery of spinal cord injury with an increased neurogenesis and a decreased astrogliosis. Therefore, the strategy of self-assembling peptides into nanofibrous scaffolds for mimicking native ECMs is a promising option for the reconstruction of the artificial neural tissues.

Synthetic peptide nanofibrous scaffolds

The development of synthetic peptides has created new opportunities to design and self-assemble the peptide nanofibers with desired functions and tailored biochemistry properties. Wang *et al*. obtained a novel hydrogel named FGLmx by incorporating RADA16 peptides and RADA16-FGL peptides together [6]. The FGLmx hydrogel showed excellent biocompatibility and bioactivity for the culture of spinal-cord-derived NSCs (SC-NSCs). The self-assembled nanofibers can promote SC-NSC proliferation and migration in the 3D scaffolds. To overcome the drawbacks of cell damage caused by RADA16-I, Sun *et al*. developed a strategy to conjugate the cell adhesion peptide RGD and neurite outgrowth peptide IKVAV with RADA16-I for self-assembly [49]. This nanofibrous scaffold provided an improved cell microenvironment for NPC/NSC neuron and astrocyte differentiation. Dong *et al*. used $K_2(QL)_6K_2$ (QL6) as a kind of multidomain peptide (MDP) to self-assemble nanofibers [50,51]. Because the QL6 peptides have

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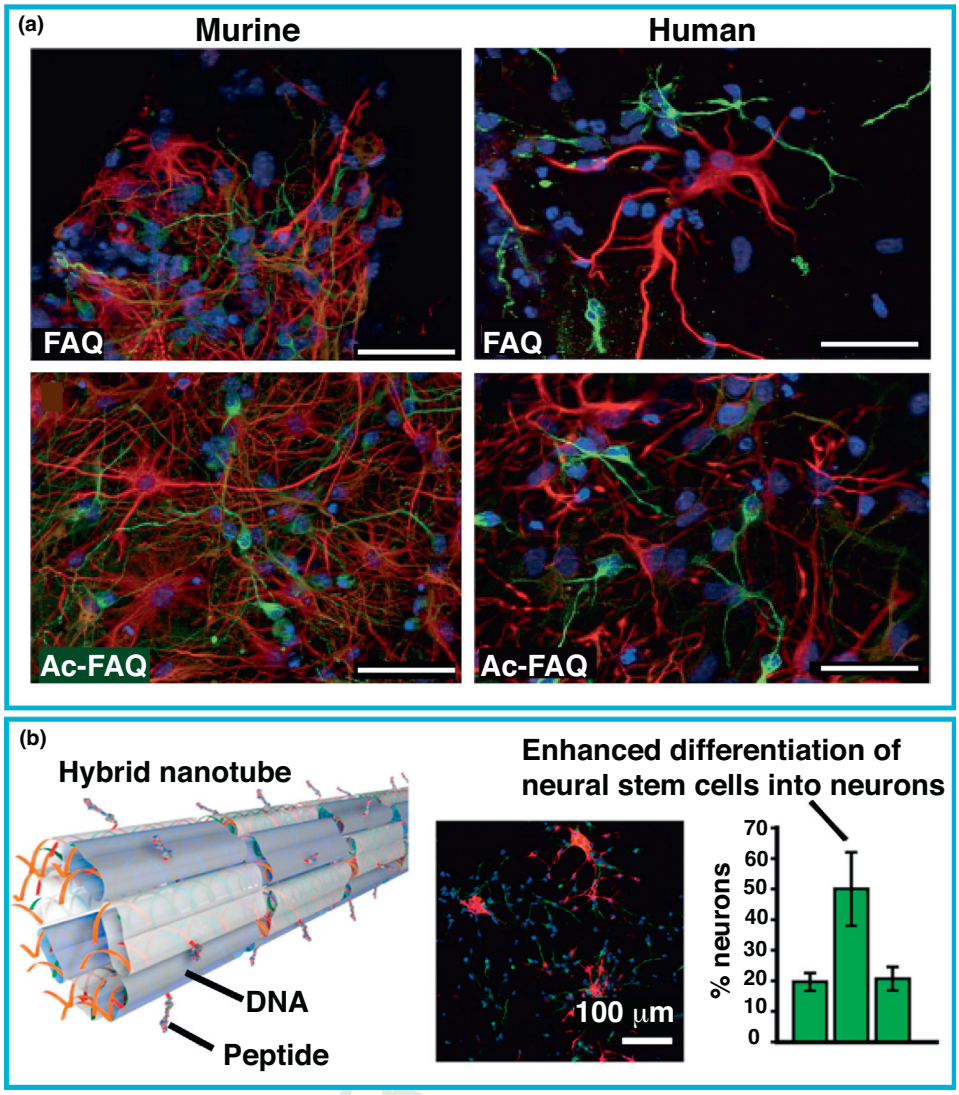

FIGURE 3

Self-assembled peptide nanofibrous scaffolds. **(a)** Neural differentiation of murine and human neural stem cells (NSCs) on the functionalized LDLK12 SAPeptide scaffolds (FAQ: NH_2-FAQRVPP-GGG-LDLKLDLKLDLK-$CONH_2$, Ac-FAQ: Ac-FAQRVPP-GGG-LDLKLDLKLDLK-$CONH_2$). βIII-Tubulin-positive neurons (green) and GFAP-positive astrocytes (red) were labeled. Nuclei were stained by DAPI (blue). Scale bars: 50 μm. Reprinted, with permission, from [46]. **(b)** DNA nanotube co-assembled cell adhesion peptide RGDs nanofibrous scaffolds guiding the differentiation of NSCs into neurons. In the confocal microscopy images, mitogen-activated protein (MAP)2 (green) indicated neurons, GFAP (red) indicated astrocytes and DAPI (blue) stained the nuclei. Reprinted, with permission, from [53].

a β-sheet conformation, they obtained a new structure of nanofibers with uniform diameter and controllable length without amorphous aggregates [51]. The β-sheet conformation of QL6 is stable enough and used as an ideal nanofibrous scaffold for tissue engineering. Further, Zweckberger *et al*. reported an enhanced NPC survival and differentiation by QL6 pretreatment [52]. Injection of QL6 into the injured spinal cord tissue led to reduced inflammation, tissue scarring and neurological deficits.

The utilization of programmable DNA blocks has also been recognized as a powerful strategy to create SAPeptide nanofibrous structures, with the benefits of better controllability and function tunability. For example, Stephanopoulos *et al*. reported an approach to co-assemble DNA nanotubes and cell adhesion peptide RGDs for differentiation of NSCs into neurons (Fig. 3b) [53]. Selective differentiation of the NSCs was achieved because of the morphology and mechanic property of the DNA substrate. In addition, inorganic material CNTs were also incorporated with the SAPeptides to control differentiation of human neural stem cells (hNSCs) [54]. Infrared (IR)-induced photothermal heating effect on CNTs enabled tuning the amount of bioactive ligands on the surface of nanofibers, precisely controlling integrin receptor clustering for differentiation of hNSCs into functional neurons. Thus, so far, such synthetic SAPeptide nanofibers have been found to have important applications in neural tissue engineering, mainly because they are easily synthesized, compatible with bioactive moieties and controllable in structure formation [10].

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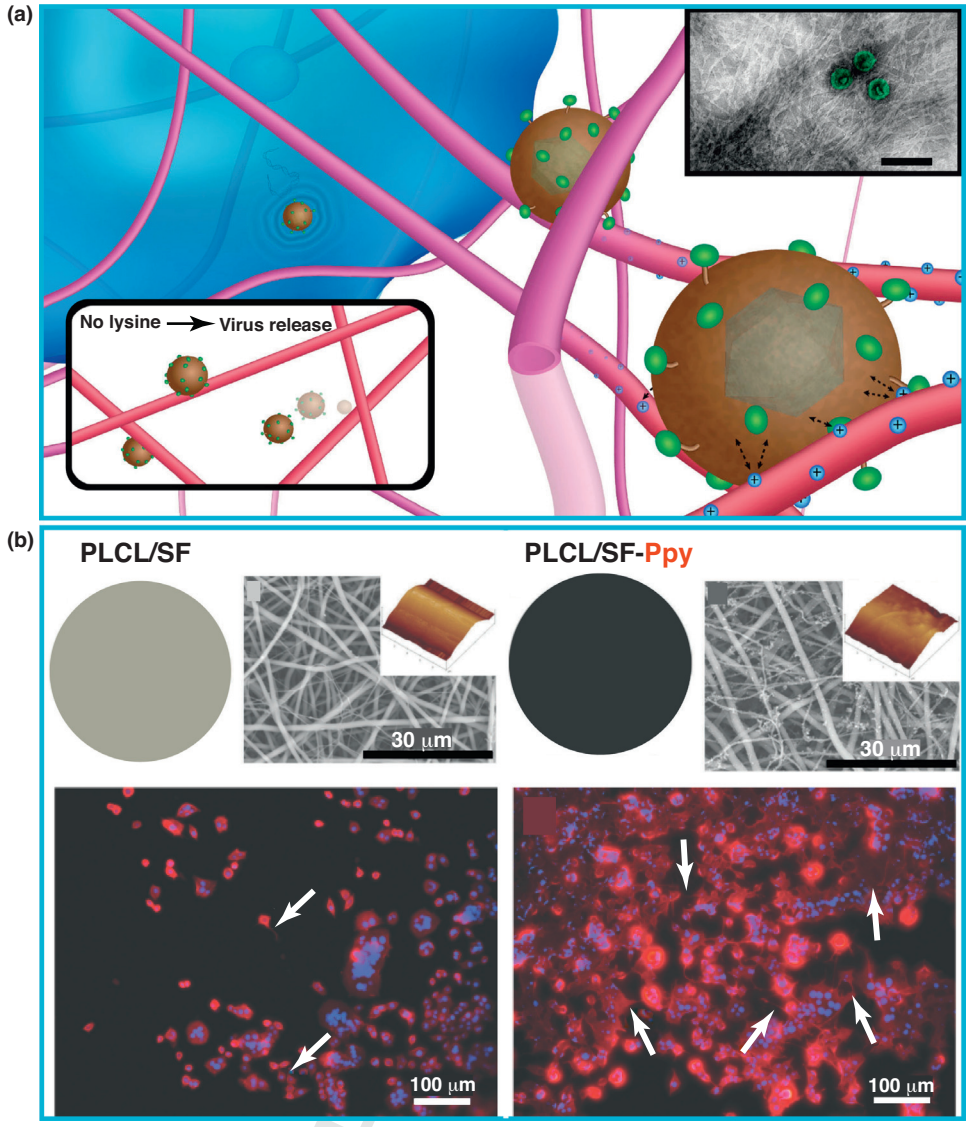

FIGURE 4

Nanofibrous scaffolds in neural drug development and nerve regeneration. **(a)** Principle of localized viral vector gene delivery vehicles on lentivirus/SAPeptide nanofibrous scaffolds. The K residue was functionalized to control the drug release. Reprinted, with permission, from [64]. **(b)** Conductive polymer incorporated nanofibrous scaffolds for neural tissue engineering. Scanning electron microscope (SEM) and atomic force microscope (AFM) images of poly(L-lactic acid-co-ε-caprolactone)/silk fibroin (PLCL/SF) nanofibers without and with polypyrrole (Ppy) incorporation were presented. Immunofluorescence images of nerve cells cultured on the PLCL/SF nanofibrous scaffolds without and with Ppy were shown, respectively. The cytoplasm and nucleus of nerve cells were stained with rhodamine-labeled β-tubulin III (red) and DAPI (blue), respectively. Reprinted, with permission, from [68].

Neural tissue engineering in biomedical applications

Alternative tissue models for drug screening

Cell-based drug screening is an essential procedure in drug development for disease therapy [55]. Reconstruction of effective neural tissue models is crucially important for drug screening and successful drug delivery in neural disease therapies. It should be noted that almost all the tissues are composed of functional cells and ECMs in 3D structures [56]. However, the conventional methods, which culture cells in a simple 2D plastic dish, poorly mimic *in vivo* tissues, leading to inefficient functions. In comparison, the animal tests for neural drug development are more effective but suffer the drawbacks of high cost and poor reproducibility. In the past, the development of neural tissue engineering has shown great promise for drug screening and discovery. They are low-cost, more-effective and, most importantly, can provide a direct investigation on 3D artificial neural models.

Currently developed technological strategies have opened a broad avenue for neural drug development through engineering neural tissues on biomimetic nanofibrous scaffolds. For example, using the electrospinning technique, biomimetic nanofibers made of ε-caprolactone/ethyl ethylene phosphate (PCLEEP) copolymers were synthesized for engineering an artificial 3D neural tissue model; two classical drugs, retinoic acid (RA) and brain-derived neurotrophic factor (BDNF), were applied to study the neural differentiation and synaptic transmission of stem cells [57–60]. Chew and colleagues studied the effects of drug release on the

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neural differentiation based on the electrospun nanofibrous scaffolds [61]. Their study demonstrated the feasibility of delivering two different drugs to enhance neuronal differentiation of stem cells on the electrospun nanofibers.

In an attempt to mimic the *in vivo* tissues for drug discovery, hydrogel nanofibrous scaffolds incorporated with drugs were developed to fabricate 3D neural tissue models [62]. Velasco and colleagues reported an artificial biological platform to study drug treatment in parkinsonian animal models [63]. They incorporated semaphorin 3C (Sema3C), a type of protein attracting dopamine neuron axons, into the PuraMatrix® SAPeptide nanofibers. After 4 weeks of culture, axonal extension of dopamine neurons was either induced from rat midbrain or differentiated from human embryonic stem cells because of slowly released Sema3C. The results indicated that the neural tissue engineering on nanofibrous scaffolds can serve as a tissue model for drug screening in parkinsonian treatment. Rodriguez *et al.* presented a concept of viral vector gene delivery in the nanofibrous scaffolds composed of *N*-fluorenylmethyloxycarbonyl SAPeptide hydrogels and laminin peptide sequence IKVAV [64]. They found that an addition of C-terminal lysine (K) residue into the hydrogel scaffolds can lead to an effective immobilization of lentiviral vector particles, because the additional amine side-chain obviously enhanced electrostatic interactions (Fig. 4a). The study suggests that it is possible to precisely control the properties of the nanofibrous scaffolds creating a more real 3D artificial neural tissue model for drug development.

Therapeutic models for nerve regeneration medicine

The limited number of donors and high cost are two major challenges in organ transplantations. Nowadays, the development of neural tissue engineering offers exciting opportunities for repairing injured nerves, mainly benefiting from current advanced fabrication technologies of biomimetic nanofibrous scaffolds. Paskiabi *et al.* used an electrospinning method to align the fibrous scaffolds and guide the growth direction of neurons to bridge the gap [65]. Vimal *et al.* also used an electrospinning set-up to produce electrospun fibers for the alignment degree control [66]. In particular, they fabricated three kinds of submicron fibers with different alignments for comparison, including nonalignment (NA), moderate alignment (MA) and high alignment (HA). The results showed that the HA fibers had an improved contact guidance to human astrocytoma cells (U373) compared with NA and MA fibers. Meanwhile, random (R) and aligned (A) poly (ε-caprolactone) (PCL)-nerve growth factor (NGF) and BSA nanofibrous scaffolds [(R/A)-PCL-NGF&BSA] were fabricated by an emulsion electrospinning technique [67]. The study found that an addition of BSA stabilizer led to a sustained release of NGF from the nanofibers for 28 days. Under the same case, neurites can grow along the fiber axis on the A-PCL-NGF&BSA scaffold. Most recently, Sun *et al.* used polypyrrole (Ppy) as a conductive polymer and incorporated the polymers into electrospun poly(L-lactic acid-co-ε-caprolactone)/silk fibroin (PLCL/SF) nanofibers [68]. Interestingly, the conductivity and hydrophilicity of the nanofibers were increased, and the nerve cell differentiation and axonal extension were promoted on the Ppy-coated nanofibers by electrical stimulation (Fig. 4b). This result demonstrated the possibility of such nanofibrous scaffolds for peripheral nerve repair and regeneration.

Toward nerve regeneration, the SAPeptide nanofibrous hydrogel composed of β-amino acids is an excellent material, because its stiffness is suitable for cell adherence and proliferation, and its self-healing property facilitates implantation with minimal postoperative damage [69]. For example, Shi *et al.* co-cultured human umbilical cord mesenchymal stem cells (hUC-MSCs) and activated astrocytes in RADA16-BDNF peptide scaffolds [70]. The constructed 3D cell microenvironment can promote neuron-like cell differentiation and neurite extension. The co-culture also enhanced BDNF secretion and thus further promoted neural differentiation of ectogenic hUC-MSCs and endogenic neurogenesis. In addition, the combination of electrospun nanofibers and SAPeptide hydrogels was proven to be effective in engineering a hybrid scaffold with topographical cues and biorecognition motifs. Nune *et al.* reported that the coating of RADA16 onto PLGA scaffolds efficiently promotes the proliferation and gene expression levels in Schwann cells [71].

Concluding remarks and future direction

Engineering 3D artificial tissues through the use of artificial ECM materials has exhibited great promise in drug development and regeneration medicine. In this paper, recent technological advances in fabricating biomimetic nanofibrous scaffolds and engineering 3D artificial neural tissues have been reviewed. In particular, we present a systemic discussion of current engineering technologies, biomaterial requirements and neural culture strategies for the reconstruction of highly artificial 3D nanofibrous scaffolds. In a promising development, the utilization of eletrospinning and the development of self-assembling strategies have proven valuable in neural tissue engineering. They are used to fabricate the biomimetic nanofibrous scaffolds with natural polymer, synthetic polymer and also hybrid polymer composites. One consideration is developing the biomaterials with cell compatibility, low cost and fewer immune responses. In addition, lots of important studies have been done to explore the applications of neural tissue engineering in drug discovery and therapeutic medicine. So far, the growing interest in neural tissue engineering focuses on mimicking complex neural microenvironments and reproducing effective tissue functions.

Until recently, we believed that significant progress has been achieved on 3D engineering of neural tissues; however, the use of all their complexity, functionality and variety is still a major challenge based on a simple electrospinning or self-assembling approach. For example, almost all tissues are composed of multiple types of cells located inside complex biochemistries and geometries of the ECMs; whereas electrospinning is limited in unidirectional alignment of nanofibers. 3D printing is able to construct more-sophisticated structures that better mimick *in vivo* microenvironments [72–74]. Moreover, microfluidic technologies have been increasingly emerging as a powerful tool for microscale tissue engineering and drug discovery [75–77]. In particular, organ-on-chips fabricated by microfluidic technologies have led to the design of more-complex neural tissues by the integration of multiple types of cells into a 3D tissue model, performing the drug screening with high-throughput and low-cost [78,79]. This is a broad avenue to fully realize the potential of this technique, and brings more scientific breakouts in the basic and applied biomedical applications. Future development could combine different

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techniques to meet the requirement of neural tissue engineering. We envision that the use of biomimetic nanofibrous scaffolds will become more prominent for tissue models in drug testing *in vitro* and organ transplantation in disease therapy.

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