Biomaterial and tissue-engineering strategies for the treatment of brain neurodegeneration

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Abstract

The incidence of neurodegenerative diseases is increasing due to changing age demographics and the incidence of sports-related traumatic brain injury is tending to increase over time. Currently approved medicines for neurodegenerative diseases only temporarily reduce the symptoms but cannot cure or delay disease progression. Cell transplantation strategies offer an alternative approach to facilitating central nervous system repair, but efficacy is limited by low in vivo survival rates of cells that are injected in suspension. Transplanting cells that are attached to or encapsulated within a suitable biomaterial construct has the advantage of enhancing cell survival in vivo. A variety of biomaterials have been used to make constructs in different types that included nanoparticles, nanotubes, microspheres, microscale fibrous scaffolds, as well as scaffolds made of gels and in the form of micro-columns. Among these, Tween 80-methoxy poly(ethylene glycol)-poly[(lactic-co-glycolic acid)] nanoparticles loaded with rhynchophylline had higher transport across a blood-brain barrier model and decreased cell death in an in vitro model of Alzheimer’s disease than rhynchophylline or untreated nanoparticles with rhynchophylline. In an in vitro model of Parkinson’s disease, trans-activating transcription biocomplexed with zwitterionic polymer poly(2-methacryloyloxyethyl phosphorylcholine) and protein-based nanoparticles loaded with non-Fe hemin had a similar protective ability as free non-Fe hemin. A positive effect on neuron survival in several in vivo models of Parkinson’s disease was associated with the use of biomaterial constructs such as trans-activating transcription biocomplexed with zwitterionic polymer poly(2-methacryloyloxyethyl phosphorylcholine) and protein-based nanoparticles loaded with non-Fe hemin, carbon nanotubes with olfactory bulb stem cells, poly[(lactic-co-glycolic acid)] microspheres with attached D-MIAm cells, ventral midbrain neurons mixed with short fibers of poly[(lactic-acid) scaffolds and reacted with xyloglucan with/without glial-derived neurotrophic factor, ventral midbrain neurons mixed with Fmoc-DIKVAV hydrogel with/without glial-derived neurotrophic factor. Further studies with in vivo models of Alzheimer’s disease and Parkinson’s disease are warranted especially using transplantation of cells in agarose micro-columns with an inner lumen filled with an appropriate extracellular matrix material.

Key Words: Alzheimer’s disease; biomaterial; cell transplantation; neurodegeneration; neurodegenerative disease; Parkinson’s disease; tissue-engineering; traumatic brain injury

Introduction

Aging is the main risk factor for neurodegeneration, with 30 million people estimated to be affected by neurodegenerative diseases worldwide (Vanni et al., 2020). It is difficult to understand the mechanism underlying the onset and propagation in these diseases due to different region-specific presentations and cell-cell communication, and which hamper the development of effective treatments. Regarding brain injury, there are more than 80 million people who have experienced a stroke (Lindsay et al., 2019) and 69 million individuals worldwide are estimated to sustain a traumatic brain injury each year, many being sports-related injuries (Theadem et al., 2020) or from road traffic accidents (Dewan et al., 2018).

The loss of functional neurons in the central nervous system (CNS) in neurodegenerative diseases and brain injuries causes substantial deterioration in the quality of life. They place a very high burden on families and health care systems (Peplow et al., 2021). A lack of innate cellular repair mechanisms in the CNS inhibits the restoration of the damaged brain. Moreover, a loss of axonal pathways frequently occurs in neurodegenerative diseases and brain injuries (Levin et al., 1983; Marshall et al., 1988; Cheng et al., 2010; Tallantyre et al., 2010), and natural regeneration of these long axon pathways is impaired by endogenous inhibition of axon growth and absence of directed guidance to far distant targets (Levin et al., 1983; Marshall et al., 1988; Curinga and Smith, 2008; Houghton and Strittmatter, 2009; Cheng et al., 2010; Tallantyre et al., 2010). The FDA-approved medicines for neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease only reduce the symptoms for a limited time and do not cure or delay the progression of the disease.

Strategies using cell replacement and promoting axonal outgrowth and guidance are currently being used to facilitate CNS repair. Stem cells obtained endogenously or delivered from exogenous sources are most commonly used in cell replacement strategies (Horner and Gage, 2000; Kim and de Vellis, 2009; Trueman et al., 2013). While some functional recovery has been shown in animal models using cell transplantation therapies, their efficacy has been limited by low in vivo survival rates of cells injected in suspension (Anderson et al., 2011; Kriks et al., 2011; Nakaji-Hirabayashi et al., 2013; Cordeiro et al., 2014; Yamauchi et al., 2015). Advantages of stem cell therapies include secretion of neuroprotective factors, providing glia to remyelinate denuded axons, and in some cases providing new neurons to discrete regions (Tate et al., 2002, 2004; Shear et al., 2004; Cummings et al., 2005; Orlacchio et al., 2010; Kim, 2011). While the transplantation of dissociated cells has received great attention to repair the brain, it cannot restore key anatomic features of damaged pathways, most notably long axon tracts. To restore long-distance axonal connections, studies typically aim to create a permissive environment of axonal outgrowth (Stichel et al., 1999; Bradbury et al., 2002; Mingorance et al., 2006; Tang et al., 2007) and/or promote the intrinsic capacity of axons to regenerate (Jain et al., 2004; Liu et al., 2010; Yip et al., 2010). These strategies most commonly involve biomaterial or cellular scaffolds to increase growth-promoting cues, decrease inhibitory factors, and/or augment the regenerative capacity of individual axons (Borisoff et al., 2003; Tsa et al., 2004; Moore et al., 2006; Filos et al., 2010; Silva et al., 2010). An ability to promote and guide axonal regeneration has been shown by these approaches (Tsa et al., 2004; Cai et al., 2005; Moore et al., 2006; Wen and Tresco, 2006a,b; Cullen et al., 2008; Kim et al., 2008; Silva et al., 2010), but do not address degeneration of source neuronal population(s). While the survival of transplanted neural cells and modest axonal outgrowth/guidance have been demonstrated, neuronal replacement together with targeted axonal regeneration to appropriate targets remains a major challenge.

Tissue engineering, using biomimetic scaffolds made of natural or synthetic material and enclosing cells, has the potential to regenerate damaged or lost tissues (Zamproni et al., 2021). The scaffold provides cell support and allows the exchange of oxygen, nutrients, growth factors, and cytokines between cells and medium. Such biomimetic scaffolds attempt to reproduce the...
conditions of the extracellular matrix (ECM), and need to possess a series of characteristics such as biodegradability and absence of an immune response by the host tissue, suitable mechanical properties, suitable porosity and permeability, and can be produced on a large scale in a reproducible way (Owen and Shoichet, 2010). In preparing for conventional transplantation procedure, cells must be first enzymatically detached from a 2D culture surface and dissociated into a single cell suspension, thereby disrupting cell-cell interactions and damaging any previously formed neuronal connections. Upon detachment from the ECM and neighboring cells, anchorage-dependant cells undergo apoptosis (Marchionini et al., 2003). Culturing cells within a transplantable 3D biomaterial scaffold has the added advantage of mimicking the ECM environment that maintains these cells and cell-cell interactions, leading adherent neuronal networks intact, during the transition to a potentially damaging in vivo location. A suitable 3D scaffold is able to mimic the in vivo microenvironment more closely than 2D culture (Barka et al., 2016).

Functionalized self-assembling peptides (SAPs) are a new class of biosynthetic materials with potential in the development of scaffolds for 3D cell cultures (Zhang et al., 2005; Zhang, 2008). SAPs have > 90% water content and are of peptide molecules that can break down into natural amino acids, which can potentially be used by the cells. Various SAPs have successfully been used for neural cell culture. An amphiphilic molecule containing the IKVAV (isoleucine-lysine-valine-alanine-valine) motif, an epitope derived from laminin, stimulated the differentiation of several progenitor cells into neurons (Silva et al., 2004). RADA16-like SAPs (the RADA motif refers to arginine, alanine, aspartic acid, alanine) are composed of natural amino acids that spontaneously self-assemble under physiologic conditions into antiparallel β-sheets forming nano- and micro-fibers and closely mimic the ECM architecture. Using pheochromocytoma 12 (PC12) cells, RADA16-I and RADA16-II induced neurite outgrowth and synapse formation (Holmes et al., 2000). RADA16-I enhanced the proliferation and differentiation of neural stem cells (NSCs) (Gelain et al., 2006), attracted migrating hippocampal neural cells, which are potential neuroprogenitors, at the interface between hippocampal slices and biomaterial (Semino et al., 2004), and promoted neurite outgrowth of a PC12 cell line (Li and Chau, 2010). RADA16-I is a 16-residue peptide composed of alternating hydrophilic arginine (R), hydrophobic alanine (A), and hydrophilic aspartic acid (D) units (RADARADARADARADA) and is the most used SAP for neural cell culture (Wang et al., 2019). RADA16-I can be synthesized commercially with high purity and can be custom-tailored to incorporate functional motifs for specific cell culture applications for neurons or other cell types. The aim of this review was to analyze the research literature describing recent biomaterial and tissue engineering strategies to promote neural repair and function in animal models of neurodegenerative disease and traumatic brain injury.

Biomaterial and Tissue Engineering Strategies to Promote Neuronal Survival and Function

We performed a PubMed search for original research articles published January 2009–April 2021 on the production and use of biomaterial and tissue-engineered constructs to promote neuronal survival and neurite outgrowth in vitro and in vivo studies. The steps involved in the review and its contents is shown in Figure 1. Of 15 articles selected for this review and the relevant findings are summarized in the following sections.

**Figure 1** Flow diagram to indicate how the review was performed and its contents.

**Performed PubMed searches using search terms: biomaterial 3D scaffold tissue engineering, neurodegeneration, treatment of neurodegenerative disease, and animal model**

**Chose original research articles published in the period January 2009–April 2021 excluding reviews, meta-analyses, and those written in a non-English language**

**Summarized research protocols and findings of individual in vitro and in vivo studies of biomaterial and tissue-engineered constructs with regard to neuronal survival and function – Tables 1 and 2**

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**Review**

**Table 1** Nanoparticles delivery and carbon nanotubes for cell engulfment

| Xu et al. (2020) | Prepared rhynchohpyline (RIN) loaded methoxy poly(ethylene glycol)-poly(lactic-co-glycolic acid) (mPEG-PLGA) nanoparticles (NPS). RIN is a major active tetracyclic oxindole alkaloid stem from traditional Chinese medicine Uncaria species, and has potential activites in the treatment of Alzheimer’s disease and stroke (Xu et al., 2014; Shaoy et al., 2015). T200 was added to the NPS solution and stirred slowly to facilitate T80-NPS-RIN formation. The mean particle size was 145 nm. T80-NPS-RIN had significantly higher transport across an in vitro brain-blood barrier model (endothelial barrier layer) compared to RIN or NPS-RIN, with drug accumulation increasing with time and reaching permeability saturation after 3 hours. In a biodistribution study using C57BL/6 mice divided into three groups (n = 5/group) and injected with 200 µL of D1D, NPS-D1D and T80-NPS-D1D solution via tail vein (D1D, 1,1-dioctadecyl-3,3,3’,3’-tetramethyldidecarboxyanine, 4-chlorobenzensulfonyl salt is a fluorescent dye), T80-NPS-D1D had significant distribution in the brain compared to free D1D and NPS-D1D. The D1D fluorescence sign in the brain suggested that the T80-NPS-D1D had penetrated the brain-blood barrier and was present in the mouse brain. The brain activity effect indicated that T80-NPS-D1D was essential for the delivery of drugs into the brain. An apoptosis assay of T80-NPS-RIN to protect mouse pheochromocytoma cells PC12 cells against Aβ42 induced apoptosis performed. In treatment with 6 x 10^7 M of Aβ42, T80-NPS-RIN, and T80-NPS-RIN (25 µM) for 24 hours. Exposure to 20 µM Aβ42, which could lead to 50–60% cell death, was used as an Alzheimer's disease model in vitro. After 4 hours of 20 µM Aβ42, the T80-NPS-RIN group at different concentrations markedly improved the cell death caused by the RIN group. Upon increasing the concentration of T80-NPS-RIN, a strong protective effect on PC12 cells was seen.

Zwitterionic polymer poly2-methacryloyloxyethyl phosphorylcholine capped protein-based nanoparticles (nBSA) were prepared by Wang et al. (2017). Non-Fe hemin (NFH)-loaded nanoparticles were made by loading the drug onto the nBSA (NFH-nBSA). HIV-1 trans-activating transcriptor (TAT) is a cell penetrating peptide that was bioconjugated with zwitterionic and protein-based nanoparticles and served as a carrier to increase brain-blood barrier permeability. The sizes of the TAT-modified nanoparticles were ~24 nm. NFH is a natural prototype iron chelator, and obtained by removing the iron core of hemin. An important factor involved in the loss of dopaminergic neurons in Parkinson’s disease is oxidative stress, and iron chelation therapy is an effective molecular strategy (Akbar et al., 2012; Yu et al., 2013; Weinreb et al., 2013). In the in vitro anti-Parkinson effect of NFH-loaded nanoparticles was examined using SH-SYSY cells (human neuroblastoma cell line). The cells were seeded at 1.0 × 10^5 cells/well in 200 µL medium and incubated for 24 hours, then the 10–80% confluent cells were treated with 100 µM MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 300 µM) per well for 1 hour. Then serial dilutions of free NFH, NFH-nBSA, and TAT-NFH-nBSA nanoparticles were added to each well. The cells were incubated for 24 hours before MTT assay. The cell viability of the TAT-NFH-nBSA nanoparticles against PD cells in each concentration of NFH. The NFH-nBSA group had little additional anti-Parkinson efficacy as the corresponding increase in MTT compared to free NFH and nBSA. The nBSA induced by MPTP for 24 hours was determined. NFH-nBSA treated cells did not show any significant decrease in ROS compared to untreated PD cells. TAT-NFH-nBSA nanoparticles caused a marked decrease in ROS compared to untreated PD cells. The nanoparticles were prepared using MPTP-treated C57BL/6 male mice, 10–11 weeks of age. The mice were divided into six groups (n = 5/group). After a 5-day continuous intraperitoneal injection of MPTP (15 mg/kg), the mice began to show Parkinson’s symptoms and a decrease of body weight, and treatment was then started. The mice in the different groups were injected via the tail vein with PBS, NFH-nBSA, and TAT-NFH-nBSA nanoparticles at an NFH dose of 1.2 mg/kg once every 3 days. Compared with the Parkinson group, the mice treated with TAT-NFH-nBSA nanoparticles had a greater increase in body weight. In the NFH-nBSA treated group, the body weight increased slowly especially during the last 10 days. The Parkinsonian symptoms were decreased by treatment with the TAT-NFH-nBSA nanoparticles for 12 days compared to the healthy group, with there being a greater increase in body weight improvement in two behavioral tests. TAT-5-NF-H-nBSA nanoparticles brought about significant behavioral improvement in Parkinsonian mice and were more effective than Tat-1-NF-H-nBSA and Tat-2-NF-H-nBSA nanoparticles. By qRT-PCR, the TAT-NF-H-nBSA group exhibited an increased mRNA level of tyrosine hydroxylase (TH) and dopamine transporter protein in the substantia nigra pars compacta (SNpc) compared to other groups. TH is the rate-limiting enzyme of dopamine biosynthesis in the brain and also a marker for dopaminergic neurons. The amount of dopamine transporter can indirectly reflect the function of dopaminergic neurons in the SNpc and thus dopamine transporter is considered a marker of dopaminergic neurons (Ike et al., 2019). In vivo toxicity evaluation of these four different drug delivery systems was performed on the Parkinsonian mice after treatment for 30 days. The delivery systems were injected via the tail vein once every 3 days for 24 days. No histopathological abnormalities or lesions in the heart, liver, spleen, lung, and kidney were caused by the injected nanoparticles.

Maraei et al. (2017) genetically engineered human olfactory bulb neural stem cells (OBNSCs) to overexpress green fluorescent protein (GFP). Upon reaching 80% confluence, the formed OBNSCs were collected and dissociated into single cells by accutase treatment. Multicellular carbon nanotubes (MCNTs) and 0.5–5 µg/mL trimethyltin chloride in saline at a dose of 6 mg/kg. At 4 weeks following trinitrophenylated carbon nanotubes (McNTs) were dispersed in PBS at a concentration up to 100 µg/mL. Neurodegeneration was induced in male Wistar rats, 220 ± 25 g, by intraperitoneal injection of 3% Trinitrophenylated carbon nanotubes (McNTs) at a dose of 6 mg/kg. For animals treated with McNTs, no significant subcutaneous injection of cyclosporine (10 mg/kg) was begun 1 day before grafting and finished on the day of euthanasia. The brain was dissected, and the hippocampus was fixed in 4% paraformaldehyde. The GFP-OBNCS/MCNTs survival rate was 94% ± 3% for 8 weeks. After the injection, no tumor formation was observed during 8 weeks. By 8 weeks post engraftment, GFP+ cells with morphological characteristics suggestive of mature neurons...
were recognized. During the 8-week time window, 60%, 17%, and 23% of the engrafted cells were differentiated into neurons, oligodendrocytes, and astrocytes, respectively. At 4 weeks post engraftment of GFP-BDNFs/CNTFs, the number of transplanted cells was maintained in normal tissues and repair of remnant of necrotic cells. By 8 weeks, restoration of the normal structure had occurred, including cytoplasmic layer cell thickness.

Microspheres and microscale scaffolds
In a study by Skop et al. (2019) chitosan microspheres were formed by electrospinning poly(dexamethasone) and polyethylene oxide (PAM) solution and subsequently diluted with cells at a ratio of 2:1 (i.e., the final 10% (w/v) ethylenediamine in isopropanol. Poly-D-lysine and 4-azidoaniline were electrospun as a 10% (w/v) polymer solution. Aligned PLLA scaffolds were produced by Yan et al. (2019). Bone marrow mesenchymal stem cells (BMSCs) were obtained from the bone marrow of all four limbs of male Wistar rats, 12 weeks of age ( body weight = 44) was prepared from the harvested cells by Trizol solution and subsequently diluted with a 1:9 volume of ethanol. BrdU-positive BMSCs were seen in and around the scaffold that did not express markers of neural differentiation. The number of neurons in the ipsilateral SN in 40% of the animals, suggesting that the neuroprotection of the nigrostriatal pathway occurred in addition to a repair mechanism due to fiber outgrowth. Transplantation of DI-MIAMI cells without PAMs-induced only a small, non-significant protection of the striatal dopaminergic fibers compared to sham-treated rats.

Functionalized composite scaffold
Collagen-chitosan porous scaffolds with a volume size of 3.0 × 3.0 × 2.0 mm were prepared by Yan et al. (2019). Bone marrow mesenchymal stem cells (BMsCs) were obtained from the bone marrow of all four limbs of male Wistar rats, 3 weeks of age, 100 g, and cultured using a complete bone marrow adherence method (Zhao et al., 2011). For the transplantation, significant wound repair for those rats that had received microspheres with cells was seen at both the gross level and the microscopic level. Microspheres were present within the lesion cavity adjacent to the host tissue but there appeared to be fewer spheres than seen at 3 days. GFP VZ NSCs had dispersed into the tissue and away from the microspheres compared with the 1-day time point, suggesting that the cells spanned several layers of the neocortex, and the lesion cavity was smaller in recipients that had received the VZ NSCs on microspheres versus controls that had received cells in the microspheres only. GFP VZ NSCs at the 1-day time point showed a total of differentiation toward mature neural cell types. Behavioral studies of rats showed improved recovery on the beam walking task using the VZ NSCs on the microspheres versus controls alone, but the behavioral recovery was not evident at 28 days post-transplantation. At 3 days post-transplantation, cells were seen surrounding the microspheres that did not express markers of neural cells. It was shown that they were neutrophils based on positive staining for Ly-6g and negative staining for Iba-1. By 2 weeks post-transplantation, there were no longer positive for Ly-6g or Iba-1. GFP VZ NSCs or GFP microspheres with cells were seen at both the gross and microscopic levels.

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a single cell VM suspension. The cell suspension (15 × 10^5 cells/µL) was diluted 1:2 in either Hank’s balanced salt solution, xyloglucan (±GDNF), or xyloglucan + PLLA short fibers (± gelial-derived neurotrophic factor, GDNF) to achieve a cell density of 5 × 10^5 cells/µL. GDNF was delivered to the cell preparation (Cells + soluble GDNF, 1 mg/injection), blended into the xyloglucan at the time of delivery with cells (Cells + XYLO-bGDNF, 1 mg/injection), delivered by immobilization of short fibers (Cells + XYLO + SF-GDNF) or a combination of blended and immobilized (Cells + XYLO-bGDNF + SF-GDNF). The various cell suspensions were stereotactically injected (2 µL, i.e., 10 × 10^5 cells) into the denervated host striatum at 10 weeks after transplantation, animals were euthanized and brains removed. At 3 weeks after transplantation, neither the presence of PLLA short fibers at the graft site induced elevated levels of reactive astrocytes (GFAP) nor microglia (CD11b) compared to cell implants alone. TH immunohistochemistry confirmed pronounced depletion of the host DA neurons following 4 to 5 weeks of GPF training in the grafts. The striatal morphology in the striatum of most (91%) of the mice. Quantification of GFAP cells revealed that there was no significant difference in cell proliferation rates between the cultured VM cells alone, whilst blended plus immobilization GDNF (XYLO-bGDNF + SFbGDNF) increased innervation by 3.1-fold. Self-assembling peptides as scaffolds. In a study by Rodriguez et al. (2018) N-fluorenylmethyloxycarbonyl (Fmoc)-DIKVAV hydrogels, containing the IKVAV motif from laminin, were prepared at 20 mg/mL with self-assembly occurring at pH 7.4. Donor tissue for transplantation was obtained from time-mated mice expressing green fluorescent protein (GFP) under the thyrosine hydroxylase promoter (TH-GFP). Embryos at E12.5 were collected, TH-GFP embryos were selected, their brains removed and VM dissected to isolate primary VM tissue. The VM tissue was dissolved in 0.1% DNase and 0.05% trypsin. Adult Swiss mice received unilateral microinjections of 6-OHDA 3 µg into the VM. At 3 weeks post lesioning, the mice were divided into three groups: Cells + Fmoc-DIKVAV + GDNF + Fmoc-DIKVAV + GFP (n = 7/group). Cells were implanted ectopically into the striatum. Cells were mixed at 1:1 ratio with Hank’s balanced salt solution or Fmoc-DIKVAV (±GDNF at 1 ng/µL) prior to implantation into the striatum. 2 µL (10 × 10^5 cells) was injected. After 10 weeks, mice were euthanized. The incorporation of GDNF within the Fmoc-DIKVAV hydrogel and its subsequent sustained delivery resulted in ~2-fold increase in the number of GFP+ cells surviving after 10 weeks compared with cells + DIKVAV-SAP alone. The volume of the cell bodies was measured as GFP fiber volume within the host striatum. When was reduced when cells were transplanted in the presence of Fmoc-DIKVAV alone with a corresponding increase in the cell density. When delivered with GDNF, however, the volume of innervation was comparable to cells transplanted alone. The larger increase in the volume of GFP+ cells demonstrated that the release of GDNF within the hydrogel was sufficient to overcome the restrictive growth with the hydrogel alone. The presence of Fmoc-DIKVAV and/or GDNF did not impair neurite extension and innervation of grafted VM cells into the host tissue. Francisco et al. (2016) encapsulated iPSCs infected with lentiviruses (termed iPSC-RN cells) and RAD16-1 (1:1 ratio) into GelMA-Cad hydrogels (average diameter 106.9 ± 2.5 µm). To induce neuronal induction, iPS-NCs were cultured in a medium supplemented with 10 ng/mL each of BDNF, GDNF, and NT3 and 2 µg/mL doxycycline, to induce NeuralF1 and EGF gene expression (iN cells). NODSCID IL2γc null mice, 20-35 g, were euthanized. On day 8 after initiating neuronal induction, dissociated GFP+ iN cells (10 × 10^6 cells/µL) or RADA16-encapsulated GFP+ iN cells (5 µL) were injected stereotactically into the striatum. Bilateral injections were made. At 3 weeks after transplantation, mice were euthanized. An immunohistochemical assay revealed that the injection of iPSC-RN cells alone resulted in a 2.7-fold increase in GDNF+ cell survival; however, the presence of GDNF on the short fibers (iN cells only) did not increase cell survival. Enhanced and prolonged GDNF delivery through a combination of both blending within the xyloglucan gel and tethering onto short fibers (XYLO-bGDNF + SF-GDNF) resulted in a 3.7-fold increase in GDNF+ cells compared to all other control groups. Only GDNF+ cells and not all other control groups were able to self-assemble in the presence of GDNF+ GAICAD grafts was examined. Only sustained GDNF delivery enhanced graft-derived striatal innervation. Blending GDNF into the xyloglucan gel (XYLO-bGDNF + SFbGDNF) increased the volume of cell body regions. Moreover, this technique produced long fiber innervation within the host striatum, which was better preserved than cells transplanted alone, suggesting that the prolonged release of GDNF from the SAPs allowed for more sustained and innervation of grafted VM cells into the host tissue.
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projecting unidirectional axonal tracts, as shown by TH and β-tubulin III immunostaining. As assessed by the length of the longest neurite in each micro-TENN, the axons projecting from the aggregates grew ~10X longer than axons growing within micro-TENNs. In vitro, astrocytes seeded within micro-TENNs showed increased neuritic outgrowth. The use of engineered neuronal aggregate specific protein components were critical factors in axonal extension. The mean neuronal aggregate length at 14 days in vitro was 1165 ± 212 µm, whereas the total tissue construct length was 4.5 ± 0.89 mm, where the dopaminergic aggregates in collagen was > 6 mm by 14 days in vitro which was suitable to span the nigrostriatal pathway in rats. The capacity of the micro-TENNs to release dopamine in vitro was examined. At 24 days in vitro, a dopamine receptor agonist used to elicit dopamine release in the striatum. As the dopaminergic aggregate within the micro-TENNs expressed TH in both the dopaminergic aggregate as well as the distal end of the micro-column containing the terminals of the axonal tracts. Following incubation in media containing L-DOPA, dopamine release could be elicited in both the dopaminergic aggregate and the astrocytes. At the 14 day time point, TH-positive processes from the nigrostriatal pathway synapse with striatal neurons in the brain, the ability of the tissue-engineered nigrostriatal pathway to synapse with a population of dopaminergic aggregate micro-TENNs was examined. These aggregates were everted generated and after 10 days in vitro, embryonic rat striatal aggregates were inserted into the vacant ends of the micro-columns. After 4 more days in vitro, immunocytochemistry was performed to assess potential synaptic interactions. Immunocytochemistry confirmed the presence of the appropriate neuronal subtypes in the two aggregate populations, specifically TH+ dopaminergic neurons and DARPP-32+ medium spiny striatal neurons. Confocal microscopy revealed unidirectional axonal-dendritic integration and processes involving the astrocytes and the dopaminergic neurons. Also, immunocytochemistry confirmed that the majority of the striatal (DARPP-32+) neurites were also MAP-2+, suggesting that these were dendrites. Dopaminergic micro-TENNs with an inner lumen could be used to provide neuronal precursors inserted to express the TH gene and grown for 14 days in vitro, after which time they were drawn into a custom needle and stereotactically microinjected to approximate the nigrostriatal pathway. Adult male Sprague-Dawley rats were euthanized after 15 minutes, 1 week or 1 month (n = 5 each). The tissue of animals that were euthanized after 15 minutes was labeled with the dopaminergic marker TH to confirm that the transplantation process itself did not harm the micro-TENN cytotype architecture, revealing surviving construct cells in the substantia nigra and maintenance of their axonal projections within the micro-column towards the striatum. At 1 week and 1 month time points, surviving GFP+ neurons and processes were observed within the micro-TENN lumen, which were easily identified spatially to the nigrostriatal axon terminal. Longitudinal projection of TH+ axons was present which confirmed that the micro-TENNs were mostly able to maintain their cytoarchitecture following longer-term transplantation into the brain. Micro-TENNs were also fabricated with TH+ aggregates derived from human fetal dopaminergic precursors on a substrate for system cells using a previously described protocol (Kris et al., 2011). At 14 days following plating in the micro-columns, it was found that these micro-TENNs displayed the correct cytoarchitecture of a discrete somatic zone with unidirectional axonal extension and a micro-TENN hydrogel parenchyma. These axonal extensions were > 4 mm in length at this time point.

Winter et al. (2016) fabricated micro-columns consisting of a thin molded cylinder composed of 3% agarose with a collagenous ECM on the interior to allow for astrocyte adhesion and growth. The hydrogel micro-columns had OD 798 µm–2 mm and ID 180–500 µm, trimmed to 5 mm in most cases, and consisted of a core (OD 500–990 µm and ID 180–500 µm) composed of an agarose ECM hydrogel molded into a cylinder through which axons could grow. The outer hydrogel structure consisted of 1–4% agarose and laminin-collagen to 2.5 cm. High-density micro-column seeding led to extensive astrocyte-collagen contraction along the length of the micro-column, resulting in continuous, dense 3D bundles of aligned bi-polar astrocytes measuring up to ~4 mm in length. The axial integrity and stability of the longitudinally aligned astrocyte bundles were assessed by physically removing them from the hydrogel columns. The bundles of aligned bi-polar astrocytes were maintained despite gripping the end and applying ≥ 2 mm of deformation to bundles without the physical support provided by the hydrogel scaffold. These bundles showed flexibility and maintenance of general alignment despite being physically manipulated. This demonstrated the durability and strength of the bundled astrocyte-collagen constructs and indicated their versatility to be used as a neuronal support out of the hydrogel encapsulation. To assess the ability of the aligned astrocyte constructs to support neuronal survival and neurite outgrowth, neuronal astrocyte constructs were grown for 14 days in vitro. The co-seeded neurons survived and associated closely with bundles of longitudinally aligned astrocytes. Immunocytochemistry and confocal microscopy showed that at 4 days in vitro, neurons cocultured with astrocytes were attached to and extended neurites directly along the longitudinal bundles of aligned astrocytes. These growth patterns were in marked contrast to those observed on a 2D polystyrene surface, as neuronal adhesion and neurite outgrowth were only colocalized with astrocytes in some cases, and somewhat less aligned than the aligned astrocyte constructs. These results demonstrate the ability of the aligned astrocyte micro-constructs to support neuron adhesion and survival as well as to provide structural and soluble cues to facilitate neurite extension directly along the aligned astrocyte soma and neurites. In an earlier study, Struzyna et al. (2015) manufactured micro-TENNs composed of an agarose ECM hydrogel molded into a cylinder through which axons could grow. The outer hydrogel structure consisted of 1–4% agarose and had OD 500–990 µm and ID 180–500 µm. The inner column was filled with gelatin (4% type I collagene), collagen (1.0 mg/mL; mouse laminin 1.0 mg/mL), or fibrin (salmon fibrin, 1.0 mg/mL/ fibrinogen with 0.5 U/mL thrombin). The micro-columns were incubated at 37°C for 30 minutes, cut to 4–5 mm length, and sterilized under UV light. DRG (dorsal root ganglion) neurons obtained from Sprague-Dawley rats were suspended in the medium containing collagen 1.0 mg/mL; mouse laminin 1.0 mg/mL, or fibrin (salmon fibrin, 1.0 mg/mL/fibrinogen with 0.5 U/mL thrombin). The micro-columns were placed in a humidified tissue culture incubator at 37°C for 50–75 minutes to allow cells to attach. Bipolar micro-TENNs were created by seeding neuronal populations on both ends of the micro-columns, with the extension of neurites through the interior of the tube. This strategy encouraged DRG neuronal soma of the bidirectional constructs to form dense ganglia, predominantly restricted to the extremities. These ganglia projected long neurites into the interior, which, given sufficient time in vitro, overlapped and grew along each other. The neurites primarily extended along the border between the ECM internal core and the agarose walls of the micro-column. The length of neurite penetration into the ECM core was measured by immunocytochemistry, with the length measured from the base of the ganglion to the last cell body. More than 32% of the neurites extended over 42 days in vitro. At 7 days in vitro, neurites extended 1.85 ± 0.62 mm into the micro-column interior. At 21 days in vitro, neurites penetrated 4.90 ± 1.25 mm, and by 42 days, axonal projections from bidirectional constructs were 10 times longer than the cell body. The micro-columns were filled with a collagen ECM alone in the micro-column interior did not adequately support cortical neuron survival and axon extension, resulting in almost complete neuronal death. However, both a salmon-derived fibrin matrix (1 mg/mL) and a laminin-collagen mixture (1 mg/mL LN and 1 mg/mL Col) supported the health and outgrowth of primary cortical neurons within the micro-columns. The effects were assessed of micro-column agarose concentration (1–4%; affecting hydrogel stiffness and pore size) and dimensions (OD/ID combination; affecting wall thickness and thus diffusional distances) on neuronal viability and neurite outgrowth and health (with laminin-collagen as the inner core ECM). Neuronal viability did not vary across the range of agarose concentrations or dimensions that were evaluated. The constructs of micro-TENNs fabricated with 3–4% agarose micro-columns maintained healthier neurites than those fabricated with 1–2% agarose. Neurite health did vary with the variance in the dimensions of the micro-columns. Cortical neurons were induced to form dense 3D clusters consisting of discrete neuronal populations spanned by long fasciculated axonal tracts within the micro-columns. Based on the number of neurons seeded and the intact construct, both low- and high-density micro-TENNs were generated. Immunocytochemistry and confocal microscopy revealed the presence of robust fasciculated and nonfasciculated axons (β-tubulin-III-) that were observed projecting between neuronal clusters (MAP-2). Immunoreactivity for TH was observed throughout the length of the micro-columns, demonstrating the ability to form neuronal networks at various spatial scales. Immunocytochemical analysis demonstrated a negligible astrocytic population within the micro-TENNs (near absence of GFAP+ cells/processes), which is consistent with the ability of the closely aligned astrocytes to support neuronal health but metabolically limiting for glial proliferation. A 4% micro-TENN (7–10 days in vitro for either DRG neuron or cortical cerebral micro-TENNs) was injected into the cortex of adult male Sprague-Dawley rats (n =...
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12). Histological examination and confocal microscopy revealed DRG neuron micro-TENNPs survived within the micro-column at 3 days postimplantation (the only time point evaluated for DRG neuron micro-TENNPs). The DRG neurons remained in a tight cluster with numerous axons projecting through the micro-column and along the cortical-thalamic axis. Micro-TENNPs generated using cerebral cortical neurons were also microinjected into naive rats along the cortical-thalamic axis. These pre-formed micro-TENNPs contained GFP® cortical neurons to permit the identification of transplanted neurons/neurites in vivo. At both 7 and 28 days postimplantation, histological and confocal assessment demonstrated surviving GFP® micro-TENN neurons contained within the micro-column interior in both the cerebral cortex and the thalamus. These transplanted neuronal clusters exhibited a healthy morphology and maintained a neurite-bearing cytoarchitecture. Immunohistochemistry revealed that the GFP® longitudinal projections within the micro-TENNs consisted of aligned axons. Moreover, immunohistochemistry for the presynaptic protein synapsin revealed numerous synapses involving the neurons and neurites within the micro-TENNs, suggesting multiple synaptic relays along these projections. At the micro-column ends, histological examination showed that the GFP® micro-TENN neurons extended numerous neurites into the host cortex. Interestingly, these neurites were predominantly projecting laterally from the micro-TENN extremity (at 90° to the cortical-thalamic axis). Neurites from micro-TENN neurons penetrated deep into the host cortex, generally of the order of 50–150 µm, but in some cases reached length up to several hundred microns. Moreover, using confocal microscopy, putative dendritic spines along GFP® neurites projecting from the micro-TENNs were identified. Immunohistochemistry suggested that these neurites projecting from the micro-TENNs formed synapses with host neurons in the cerebral cortex.

The main findings regarding the ability of nano- and micro-sized constructs and scaffolds and hydrogels to promote neuronal survival and neurite outgrowth are summarized in Tables 1 and 2.

Discussion

Neurodegenerative diseases are increasing in prevalence in many countries and the medications currently approved by the FDA only bring about a temporary reduction of symptoms in patients with Alzheimer’s or Parkinson’s disease. Moreover, they do not slow or halt the progression of the disease, and often have undesirable side effects. Cell transplantation strategies offer temporary reduction of symptoms in patients with Alzheimer’s or Parkinson’s disease, and traumatic brain injury (Tables 1 and 2). Among the in vitro studies, Tween 80-treated mPEG-PLGA nanoparticles loaded with rhynchophylline had higher transport across a blood-brain barrier model and decreased PC12 cell death in a model of Alzheimer’s disease than untreated or untreated nanoparticles with rhynchophylline (Xu et al., 2020). Using a model of Parkinson’s disease, TAT bioconjugated with zwitterionic polymer poly(2-methacyloxyethyl phosphorylcholine) and protein-based nanoparticles loaded with non-Fc hemin had similar protective ability against MPTP-treated SH-SYSY cells as free non-Fc hemin, and significantly decreased the level of reactive oxygen species in cells compared to untreated cells (Wang et al., 2017). Neuronal survival was examined in several studies using hydrogels. Cortical glutaminergic neurons within gelatin methacrylate gel with N-cadherin mimic peptide exhibited high viability, high neurite length and width, and expressed both synaptophysin and PSD-95 (O’Grady et al., 2020). Using micro-columns consisting of an outer agarose gel cylinder and an inner lumen filled with ECM (collagen, fibronectin, laminin-collagen) and dopaminergic neuron aggregates produced from mesencephalic neurons isolated from the ventral midbrain of rats (embryonic day 14) inserted into the ends of the micro-columns, long projecting unidirectional axonal tracts were produced that were 10–150% longer than analogous axons extending within micro-columns seeded with dissociated cells (Struzyna et al., 2018). In an earlier study using dorsal root ganglia neurons (embryonic day 16) and cerebral cortical neurons from rats (embryonic day 18) that were microinjected at both ends of the micro-columns, long axonal tracts projecting through the interior of the micro-column were generated. A collagen ECM alone in the micro-column interior did not adequately support cortical neuron survival and axon extension, resulting in a complete neuronal network. However, inclusion of a laminin-rich matrix and a laminin-collagen mixture supported the viability and outgrowth of cortical neurons within the micro-columns (Struzyna et al., 2015). Micro-columns consisting of an agarose cylinder with collagen ECM on the interior and neurites from astrocytes from rat cortex that were inserted into the micro-columns (embryonic day 0–1) produced a continuously aligned astrocyte network along the entire length of the micro-column. Cerebral cortical neurons from rats (embryonic day 18) inserted at the openings of both ends of the micro-columns following astrocyte seeding became attached to and also extended neurites directly along the longitudinal bundles of aligned astrocytes, showing that the aligned astrocyte constructs supported neuronal survival and neurite outgrowth (Winter et al., 2016).

Table 1: Nano- and micro-sized biomaterial and tissue-engineered constructs to promote neuronal survival and neurite outgrowth in vitro and in vivo studies

| Studies | Construct | Neurorestorative effects of constructs |
|---------|-----------|---------------------------------------|
| Nanoparticles delivery and carbon nanotubes for cell engraftment | Maret et al., 2017 | Multilayered carbon nanotubes (MWCNT) OD ~50–60 µm, length ~10–20 µm mixed with human OBNSCs neurones. | CNT/OBNSCs mixture injected at CA1 of the hippocampus in TMT-treated Wistar rats and after 8 wk morphological criteria suggested effective engraftment of cells. Engrafted cells differentiated into neurons, astrocytes, and neurites. |
| Wang et al., 2017 | TAT-NF-NbSA nanoparticles, size ~24 nm | TAT-NF-NbSA nanoparticles had similar cell viability compared to the TAT-MP-treated SH-SYSY cells in vitro as free NFM and decreased the ROS level in cells compared to untreated cells. |
| Xu et al., 2020 | T80-mPEG-PLGA nanoparticles (NPS)-RIN, mean size 145 nm | Higher transport across BBB in vivo model than RIN or NPS-RIN. Decreased PC12 cell death in vitro at A57-100 compared to RIN. |
| Microspheres and microscaffolds | Delcroix et al., 2011 | PAMs consisting of PGA microspheres, 60 µm diameter, coated with laminin and PDL, and release NT3 and used to attach human DI-MIAMI cells (generated from cells of a 3yr-old male cadaver) | 6-OHDA injected stereotactically into female Sprague–Dawley rats, 12 wk of age, to induce unilateral lesion of the nigrostriatal system. At 2 wk post lesion, DI-MIAMI cells or attached PAMs were injected into lesioned striatum. Transplantation of DI-MIAMI cells in combination with PAMs protected dopaminergic neurons in lesioned striatum, and also in ipsilateral substantia nigra in 40% of animals. Amphetamine-induced rotational behavior was significantly decreased by transplantation of DI-MIAMI with PAMs compared to sham rats or cells alone. |
| Carlson et al., 2015 | Microscaffolds 100 µm square created from pTEC fibrous microspun substrate fiber diameter 3.12 µm to which were downgraded to human in CNS cells. | Scaffolding supported or dissociated IN cells were injected into the striatum of male NOD-SCID IL2 Ryc mice, 20–35 g. At 3 wk post-implantation, there was a 38-fold improvement in an average survival rate of injected scaffold-seeded IN cells compared to injected dissociated IN cells. The scaffold-seeded cells, compared with pure IN, had longer neurite lengths, comparable to those of pure IN cells. | V2 INPs on microspheres injected into lesion cavity at 7 d after CCI in male Sprague Dawley rats, 2 mon of age; at 2 wk post-transplantation, lesion cavity was smaller than in controls that received cells or microspheres only. |

6-OHDA; 6-Hydroxydopamine; Aβ25–35; amyloid; BBB; blood-brain barrier; CCI; controlled cortical impact; DAT: dopaminergic transporter; DI-MIAMI: dopaminergic narrow-isolated adult multilineage inducible cell; pTEC: polycrystalline polyurethane ethyl ester carbons; IN: induced neural cell; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NT3: neurotrophin-3; OB: olfactory bulb; PC12: PC12 cell; PAM: photolytically cross-linked microcarriers; PDL: polyl-lysyl-diphenylalanine; RIN: rhynchophylline; ROS: reactive oxygen species; TAT-NF-NbSA: HIV-1 trans-activating transacting; non-Fc-human EPO-se serum albumine; TH: tyrosine hydroxylase; T80-mPEG-PLGA: 80–mPEG-PLGA; V80: polyethylene glycol-polylactic-co-glycolid acid; VZ: ventricular zone.
Table 2 | Scaffolds and hydrogel constructs to promote neuronal survival and neurite outgrowth in in vitro and in vivo studies

| Studies | Construct | Neurorestorative effects of constructs |
|---------|-----------|--------------------------------------|
| Functionalized composite scaffolds | | |
| Wang et al., 2016 | PLLA scaffolds cut into short fibers 2–10 mm in length, aminated, and PDL and 4-azido-aniline covalently bound and reacted with xyloglucan under UV light | 6-OHDA injected into SNpc of female adult Swiss mice and at 3 wk after lesioning mice received an intrastitial injection of single-cell suspension of VM from TH-GFP mice (embryonic day 12.5). The cell suspension was diluted in either medium, xyloglucan (± GDNF), or xyloglucan + PLLA short fibers (± GDNF). Inclusion of GDNF within donor cell preparation or the gel increased GFP cell survival. Blending GDNF into xyloglucan gel or blended plus immobilization GDNF increased striatal innervation. |
| Yan et al., 2019 | Collagen-chitosan scaffolds 3 mm × 3 mm × 2 mm with BMSCs from male Wistar rats at 3 wk of age | At 72 h after CCI in male Wistar rats, 12 wk of age, a scaffold seeded with BMSCs was placed in lesion cavity in the left hemisphere; on d 3 and d10 mNSS score was decreased compared to the sham group and GFAP+ and a few NSE+ BrdU-labeled BMSCs were seen in the cortex and striatum. |
| Self-assembling peptides (SAPs) as scaffolds | | |
| Francis et al., 2016 | iPSC-RN cells encapsulated within RADA16.1 microspheres, average diameter 107 µm and neuronal induction (IN) cells encapsulated within RADA16.1 microspheres | On d 8 after initiating neuronal induction, dissociated GFP IN cells or RADA16.1-encapsulated GFP IN cells were injected bilaterally into the striatum of NOD-SCID IL2Rγc null mice, 20–35 g. At 3 wk after transplantation, encapsulation of INs within SARKP microspheres increased survival in vivo compared to INs in suspension. Scaffold-encapsulated neurons extended neurites into the host brain tissue. |
| Rodriguez et al., 2018 | Fmoc-DIKVAV hydrogels were prepared with self-assembly occurring at pH 7.4 | 6-OHDA injected unilaterally into VM of adult Swiss mice. At 3 wk post-lesioning, primary VM cells isolated from embryos at E12.5 were mixed with Fmoc-DIKVAV ± GDNF and implanted ectopically into the striatum. Incorporation of GDNF within Fmoc-DIKVAV hydrogel resulted in ~2-fold increase in GFP cell survival after 10 wk. |
| Micro-tissue engineered neural constructs | | |
| Struzna et al., 2015 | Micro-column composed of an agarose cylinder OD 398 µm × 500–900 µm, ID 180–500 µm, 4–35 mm length and inner column filled with an ECM. Primary DRG neurons (embryonic day 16) and cerebral cortical neurons (embryonic day 18) were obtained from Sprague-Dawley rats and the cell suspension was delivered to one or both ends of micro-columns. | DRG neuronal somata of bidirectional constructs formed dense ganglia, mostly restricted to the extremities, these ganglia projected long neurites into the interior and primarily extended between the ECM internal core and the agarose walls of the micro-columns. By 42 d in vitro, axonal projections from bidirectional neurons formed constructs measuring up to 2 cm. A collagen ECM alone in the micro-column interior did not adequately support cortical neuron survival and axon extension. A salmon-derived fibrin matrix and a laminin-collagen mixture supported the growth and development of primary cortical neurons within the micro-columns. |
| Winter et al., 2016 | Hydrogel micro-column consisting of agarose cylinder with collagen type I ECM interior, OD 798 µm–2 mm, ID 180 µm–1 mm, 5 mm length. Primary cortical astrocytes from post-natal day 0–5 Sprague-Dawley rats were seeded into the micro-column. Cortical neurons isolated from cerebral cortices of Sprague-Dawley rats (embryonic day 18) were seeded at both ends of micro-columns at 40 min following astrocyte seeding. | Dopaminergic neuron aggregates were inserted into the ends of agarose micro-columns which in vitro produced long projecting unidirectional axonal tracts. The axons projecting from the aggregates grew ~10 × longer than analogous axons extending within micro-columns seeded with dissociated cells. The total micro-TENN length (neuronal aggregate + axon length) using dopaminergic aggregates in collagen was >6 mm by 3.4 d in vitro which was suitable to span the nigrostriatal pathway in rats. Dopaminergic neuron aggregates were created from VM mesencephalic neurons of Sprague-Dawley rats (embryonic day 14) and inserted into micro-column with inner lumen containing collagen 1 (in some instances transduced to express GFP) and grown for 3 wk before they were stereotactically injected to approximate the nigrostriatal pathway in adult male Sprague-Dawley rats. At 1 wk and 1 mol, surviving GFP+ neurons and axons were present within micro-TENN lumen, and were identified spanning the nigrostriatal pathway. Labeling sections with β-tubulin III and TH revealed the preservation of a robust neuronal and axonal population. |
| Struzna et al., 2018 | Micro-column consisting of an agarose cylinder OD 398 µm, ID 160 µm, 6–12 mm length, with ECM cocktail added to inner lumen. Dopaminergic neuron aggregates were inserted into the ends of the agarose micro-columns. | Neurons embedded in GeMa-Cad and Matrigel exhibited high viability while those embedded in GeMa-Cram or GeMa died within 4 d. On day 10, relative to Matrigel, neurons had higher average neurite length and width. At 21 d, neurons embedded in GeMa-Cad expressed both synaptophysin and PSD-95. |
| O’Grady et al., 2020 | Scrambled to make GeMa-Sram or N-Cadherin mimic to make GeMa-Cad hydrogels were prepared. Human iPSCs were differentiated into cortical glutaminergic neurons and single-cell suspensions were encapsulated into Matrigel, GeMa-Cram, GeMa-Cad, or GeMa. | 6-OHDA: 6-Hydroxodopamine; BMSC: bone marrow stem cell; BrdU: bromodeoxyuridine; CCI: controlled cortical impact; DRG: dorsal root ganglion; ECM: extracellular matrix; Fmoc-DIKVAV: N-fluorenylmethyloxycarbonyl-DIKVAV; GDNF: glial-derived neurotrophic factor; GeMa: gelatin methacrylate; GFAP: glial fibrillary acidic protein; GFP: green fluorescent protein; iNS: induced pluripotent stem cells infected with lentiviruses expressing retronectin; mNSS: modified neurological severity score; NSE: neuron-specific enolase; PDL: poly-D-lysine; PLLA: poly-(lactic acid); SAPS: self-assembling peptide nanofiber scaffold; SNpc: substantia nigra pars compacta; TENV: tissue-engineered neural networks; TH: tyrosine hydroxylase; VM: ventral midbrain. |

Other among the in vivo studies reviewed were five that had used models of Parkinson’s disease and two that had created a traumatic brain injury. TAT bioconjugated with zwitterionic polymer poly(2-hexylmethacryloyloxyethyl phosphorylcholine) and protein-based nanoparticles loaded with non-Fe hemin injected intravenously in MPTP-Treated C57BL/6 mice induced a reversal of Parkinsonian symptoms. At a molar ratio of 5:1 for TAT to BSA, the nanoparticles loaded with non-Fe hemin caused an increase in mRNA level of tyrosine hydroxylase and dopamine transporter in the substantia nigra (Wang et al., 2017). Carbon nanoparticles with olfactory bulb neural stem cells were injected at CA1 of the hippocampus in trimethyltin chloride-treated adult male Wistar rats and received daily immunosuppressive treatment. |
The CA1 neurons and oligodendrocytes had increased in number at 4 weeks posttransplantation, and by 8 weeks the normal structure of the CA1 was regained with the restoration of pyramidal cell layer thickness (Maraei et al., 2017). 6-OHDA was injected stereotaxically in adult female Sprague-Dawley rats to induce unilateral lesioning of the nigrostriatal system at 2 weeks post-injury. MI-MAMI cells or cells attached to PLGA microspheres crosslinked with heparin were injected into the lesion cavity at 7 days after cortical impact to check there are little or no off-target effects with using functionalized nanoparticles and tumor formation does not occur with stem cell transplantation. However, it appears that none of these trials are utilizing tissue-engineered scaffolds to improve the viability of cells transplanted into the brains of adult animals.

In conclusion, cell transplantation strategies using biomaterial constructs or systemic administration of functionalized nanoparticles have the potential to become an effective therapeutic modality for patients with neurodegenerative diseases or traumatic brain injury. Future studies are warranted in animal models of Parkinson's disease and Alzheimer's disease using various types of cells or substances obtained from Chinese herbs that could be of potential benefit to clinical trials. The use of micro-columns to transplant cell or substances obtained from Chinese herbs that could be of potential benefit to clinical trials. The use of micro-columns to transplant cell

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