Journal of
Stem Cell Research & Therapy

FSY Wong and ACY Lo, J Stem Cell Res Ther 2015, 5:2
DOI: 10.4172/2157-7633.1000267

Review Article Open Access

Collagen-Based Scaffolds for Cell Therapies in the Injured Brain

Francisca SY Wong[1]* and Amy CY Lo[1,2,*]

[1]*Department of Ophthalmology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China*
[2]*Research Centre of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China*

Abstract

Current therapies for treating brain injuries, including stroke and traumatic brain injury, are designed to modify symptoms pharmacologically but do not promote full structural and functional regeneration of the brain. Integrating the merits of cell, biomaterial scaffolds and pharmaceutical therapies, biodegradable scaffold-facilitated cell therapy is a promising multifaceted approach to modify the local injury environment, and also to promote transplanted cell replacement and endogenous regeneration mechanisms. Collagen is an attractive candidate for providing a temporary supportive matrix for cell and drug delivery to the brain. With good biocompatibility, design flexibility and biomimetic properties of the natural extracellular matrix, collagen-based cell transplantation has demonstrated therapeutic potentials in preclinical studies of brain injury. In this review, we will discuss the properties and design considerations of collagen-based scaffolds for brain tissue engineering. Also, the current developments of collagen-based scaffolds in conveying different sources of cells, such as mesenchymal stem cells, neural stem/progenitor cells, embryonic stem cells, induced pluripotent stem cells and derivatives, to the brain will be illustrated. Advanced understanding in the interactions between collagen, cells and the local injury environment has greatly expanded the potential of these biomimetic systems to repair and regenerate the brain.

Keywords: Acquired brain injury; Collagen; Scaffold-facilitated cell transplantation; Embryonic stem cells; Mesenchymal stem cells; Neural stem/progenitor cells; Tissue engineering

Introduction

Acquired brain injury, including stroke and traumatic brain injury (TBI), inflicts traumatic, ischemic or hemorrhagic brain insults and is the major contributor to death and persistent disabilities worldwide [1,2]. Currently, most therapies for brain injury treatments are designed to pharmacologically modify disease symptoms but do not promote neural tissue repair or restoration of severed neural networking. There is a great need to develop interventions that are able to address multiple pathological events post-injury and promote fuller structural and functional restoration. Biodegradable scaffold-facilitated cell therapy is a promising approach to modify the injury environment, promote cell replacement, stimulate endogenous regeneration mechanisms and provide a temporary scaffold to guide the behavior of transplant and endogenous cells. Collagen-based scaffold is an attractive candidate for cell delivery in the brain due to its biocompatibility and ability to provide a three-dimensional (3D) environment that mimics the natural extracellular matrix (ECM). In this review, we will first describe the tissue response of the brain towards injuries and the objectives of collagen-based cell therapy. Then, properties and design considerations of collagen-based scaffolds will be illustrated, followed by discussion on the current developments of these scaffolds in conveying different sources of cells. Lastly, conclusions and perspectives are presented for future work in collagen-based cell transplantation for treating brain disorder.

Post-injury Response of the Brain

After initial brain insult, a series of secondary injury events including inflammation, edema, excitotoxicity and increased free radicals follows causing further neuronal death [3-5] (Figure 1). This leads to expansion of cell death zone in the surrounding brain tissues and formation of glial scars mediated by activated astrocytes and microglia. Although glial scars can minimize secondary damage around the zone of necrotic cell death, clean debris at the injury site and protect the uninjured tissue, they contain axonal growth inhibitory factors such as chondroitin sulfate proteoglycan and myelin-associated glycoproteins that limit neuroregeneration. On the other hand, due to the presence of endogenous neural progenitor cells (NPCs), the adult brain has plasticity and potential to partially restore its function in response to stroke and trauma [6]. These cells can proliferate and migrate to the injury regions, express growth factors and potentially replace loss tissue by differentiating into neurons and glial cells [5,7]. However, these endogenous mechanisms are often inadequate to fully restore loss structures and functions. Moreover, the loss of ECM in the cystic cavities within the brain parenchyma fails to provide the scaffold needed for neuroblasts migration [8]. This limits tissue regeneration within the lesion. Strategies that support regeneration or replacement of the loss tissue as well as external neurotrophic and neuroregenerative stimulations are needed.

Scaffold-facilitated Cell Therapy for Brain Injuries

Scaffold-facilitated cell therapy offers a multifaceted approach to tackle the complex injury events following brain injuries. It integrates the merits of cell, biomaterial scaffolds and neuroactive factor therapy. Cells are able to respond dynamically to the local temporal and spatial cues, interact continuously with the surrounding tissue and act on multiple mechanisms simultaneously. However, transplanted cell survival is often hindered by the lack of oxygen, nutrition, trophic factors and structural support at the site of injury [9-12]. Also, post-injury excitotoxicity and inflammatory mechanisms, immunological rejection

***Corresponding author:** Amy CY Lo, Department of Ophthalmology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, Tel: +852-2831-5363; Fax: +852-2817-0491; E-Mail: amylo@hku.hk

Francisca SY Wong, Department of Ophthalmology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, Tel: +852-2831-5367; Fax: +852-2817-0491; E-mail: franwong@hku.hk

Received January 21, 2015; **Accepted** February 24, 2015; **Published** February 26, 2015

Citation: FSY Wong, ACY Lo (2015) Collagen-Based Scaffolds for Cell Therapies in the Injured Brain. J Stem Cell Res Ther 5: 267. doi:10.4172/2157-7633.1000267

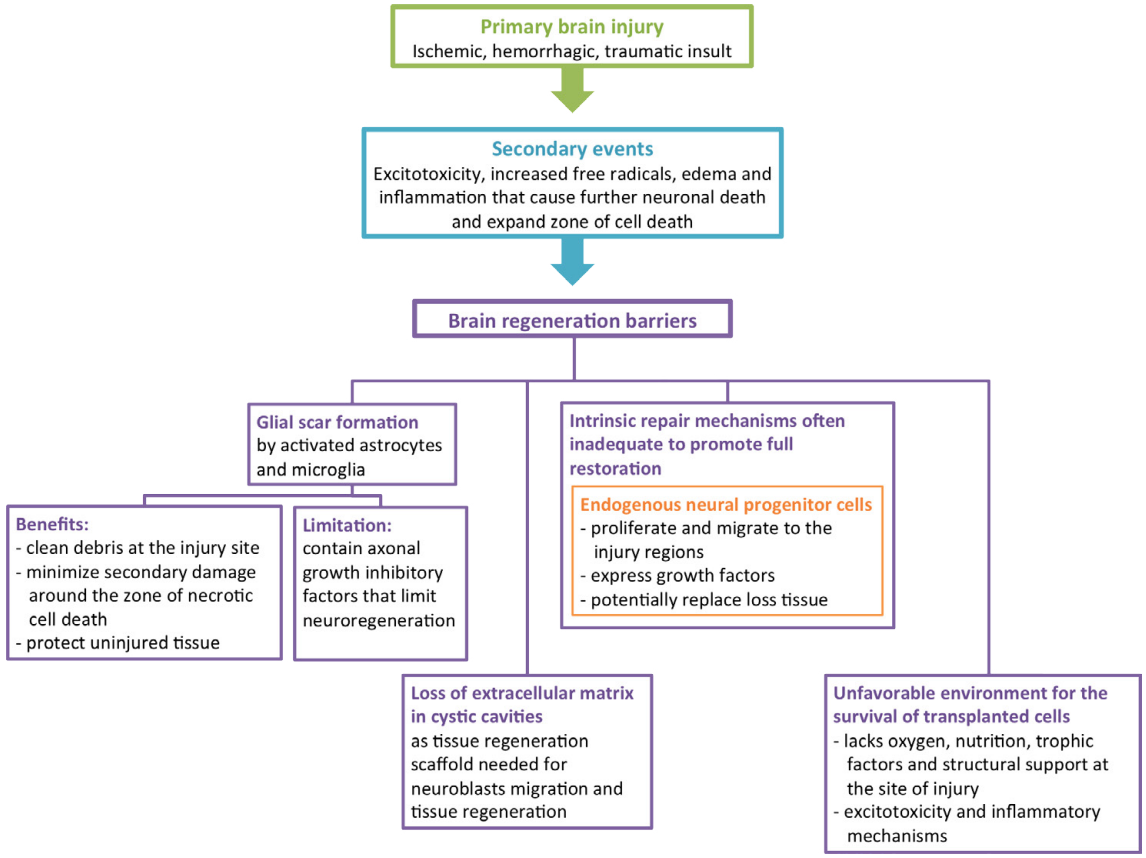

Figure 1: Response of brain tissue to injury and regeneration barriers.

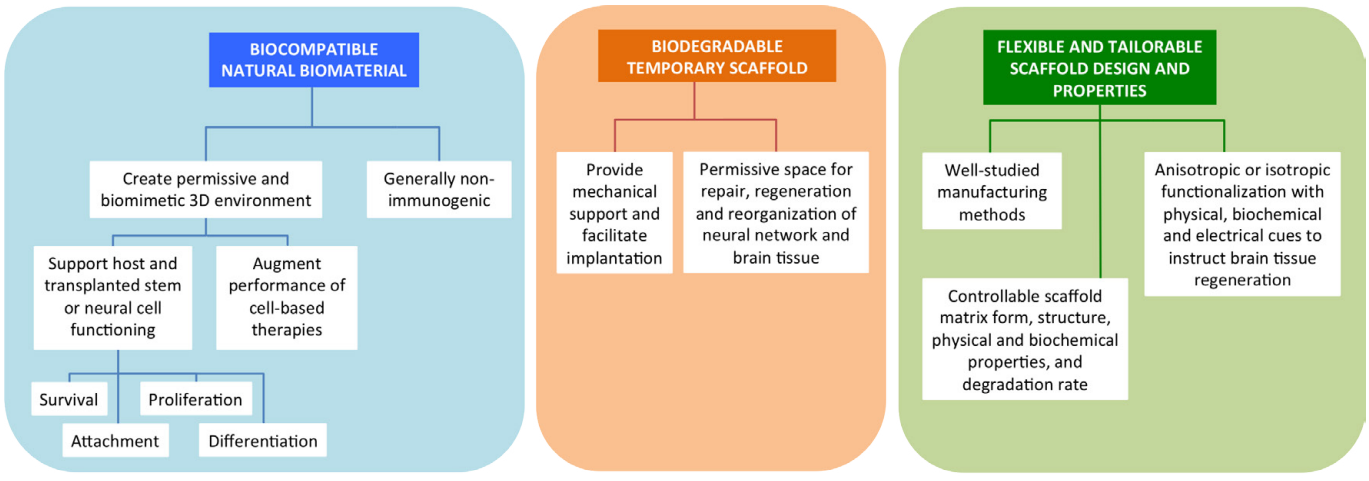

Figure 2: Advantages of collagen scaffolds for the injured brain.

and mechanical damages cause transplanted cell loss. Biomaterials are therefore applied to promote cell survival and functioning, facilitate implantation and augment performance of cell-based therapies in the central nervous system (CNS) and peripheral nervous system (PNS) [13]. Since the ultimate goals of cell-based therapies for the injured brain are cell replacement and tissue regeneration, biodegradable materials are more attractive than non-biodegradable ones for cell transplantation purposes.

Collagen is one of the most extensively used ECM components in neural tissue engineering (Figure 2). Collagen constitutes the greatest quantity of the total proteins in the human body, and is the major composition of ECM. Collagen-based scaffolds offer a permissive, generally non-immunogenic, biocompatible, biodegradable and biomimetic 3D environment for cell survival, attachment, proliferation, and differentiation. It has been widely applied in clinical and preclinical studies of neural injury and degenerative conditions of the CNS and

PNS [14-34]. For brain injury applications, collagen scaffolds have been applied as cell carriers for implantation in animal studies or as culture platforms to study interactions between various stem cells or neural cells in 3D biomimetic environments. Collagen scaffolds can be produced by top-down or bottom-up approach [35]. Top-down approach comprises of decellularizing extracellular collagenous tissues while preserving its native architecture. This type of scaffold consists not only of collagen but also a combination of structural and functional molecules present in the ECM, including proteins, proteoglycans, glycosaminoglycans, glycoproteins and other small molecules. Since decellularized ECM scaffolds can be sourced from a variety of tissues and mammals, there exist significant differences in structure, composition and characteristics between these scaffolds. For bottom-up approach, scaffolds are produced by reconstitution of collagen molecules into their native fibrillar structure. These molecules are obtained from broken down collagenous tissues. In this review, we will focus on the *in vitro* and *in vivo* cell delivery applications of the latter type of collagen scaffolds.

Collagen-based Scaffolds

Structure and types of collagen used for brain tissue engineering

Up to date, 29 different genetic types of collagen have been identified. All collagens in the family are modular proteins consisting of three polypeptide α chain with at least one stretch of triple helix. The non-triple helical regions can either be short or large structural domains [36]. Homotrimers or heterotrimers formed from 25 different conformations of α chain give rise to different types of collagen with varying size, function and tissue distribution. Based on their structure and supramolecular organization, collagen can be classified into fibril-forming collagens, fibril-associated collagens, network-forming collagens, anchoring fibrils, transmembrane collagens, basement membrane collagens and others with unique functions [36-38].

Fibril-forming collagens include type I, II, III, V, XI, XXIV, and XXVII. Type I collagen is the most abundant and well-studied collagen, accounting for 25% of the dry protein in mammals and forming more than 90% of the organic mass of bone [37]. It is also the major collagen of tendons, skin, ligaments, cornea, and many interstitial connective tissues with the exception of very few tissues such as hyaline cartilage, brain, and vitreous body. It functions to provide structural strength within tissues and to form a framework for other ECM components to interact [39]. Type I scaffold serves as a golden standard in tissue engineering [40]. Although the ECM of CNS tissue contains relatively little fibrous collagen and the brain normally expresses type IV collagen only, collagen type I is a better scaffold candidate than type IV for brain applications. Collagen type IV is the main ECM secreted by fibroblasts following trauma and has shown to induce scar formation and inhibit axonal regeneration [41]. In contrast, collagen type I has been applied as scaffolds or fillers of non-biodegradable implants for cell delivery in animal models of TBI, Huntington's disease (HD), Alzheimer's disease (AD) and Parkinson's disease (PD) with good biocompatibility [14-16,25-30]. Most reports of scaffolds for brain injury applications employed type I collagen.

Cell-collagen interactions

Collagen is an excellent attachment substrate for cells. Cells can bind to collagen directly or indirectly. Various cell surface collagen binding proteins or collagen receptors are able to recognize specific peptide sequence of collagen molecules, such as GPO (Gly-Pro-Hyp) and GFO (Gly-Phe-Hyp) motifs, and form direct interactions [40]. Indirect cell-collagen interactions can be formed in the presence of molecules that carry integrin and collagen binding motifs, such as the RGD (Arg-Gly-Asp) motif or similar sequences. Fibronectin, decorin and laminin are mediators of indirect collagen-cell interaction.

Fibril-forming collagen can self-assemble into fibrils at neutral pH and physiological temperature [42]. Fibrils formed *in vitro* have similar morphologies and characteristic "D" periodicity with those observed *in vivo* [43,44]. In the presence of cells, spontaneous collagen hydrogel contraction occurs, contracting the gel and yielding scaffolds with higher density, increased strength and stiffness [35]. Collagen degradation *in vivo* is mediated by human collagenases secreted from cells, mainly matrix metalloproteinase. Different collagenases have different rates of collagen hydrolysis. The extent of changes in mechanical and degradation properties of collagen scaffolds differs based on various factors, such as cell type, cell density, collagen concentration and culture conditions [35].

Scaffold design considerations

Various designs of collagen scaffolds have been explored for brain tissue engineering. Common forms include hydrogel, fibrous scaffolds, and porous scaffolds with foam, sponge and dehydrated matrix. Depending on the form of scaffold used, cells, neuroactive agents and other biomaterials can be incorporated during or after scaffold fabrication. Manufacturing techniques of collagen scaffolds are discussed in [45].

Stem cells and neural cells are highly responsive to physical, biochemical and electrical cues present in their microenvironment during growth, development and regeneration stages. To maximize the therapeutic outcomes, properties of collagen-based scaffolds should be optimized to recreate the spatial and temporal presentation of these cues. Features that are biologically relevant can be readily incorporated into the scaffolds. Functionalization of collagen scaffolds can potentially modulate the local injury environment and influence the function of transplanted and endogenous cells. Local modulations encourage cell survival and tissue regeneration by neutralizing local inhibitory signals, reducing inflammation and slowing scar formation. In response to the cues, cell functions such as attachment, migration, proliferation, gene expressions, differentiation and growth *in vivo* can be better controlled. Depending on the intended application of the implants, these signals can be applied in an isotropic or anisotropic manner.

Physical properties of a collagen-based scaffold, including their mechanical and topographical characteristics, have great influences on cell behavior, fate and functioning [46,47]. Examples of mechanical cues are stiffness [48], porosity [49], viscoelasticity, and topographical features include surface patterning, alignment, geometry and structure [46,50-52]. Scaffolds should be designed to have comparable mechanical strength with brain tissue. This enables the implant to resist physiological loads and mechanical stresses from neighboring tissues without collapsing or losing its shape [53]. Before degradation, the temporary scaffold should provide mechanical support in the lesion, allowing the survival, proliferation and differentiation of the transplanted cells and initial native tissue synthesis, which may take up to several weeks [12]. Also, culturing seeded scaffolds *in vitro* in bioreactors are applied to enhance cell survival and differentiation in some studies [54].

Collagen scaffolds can also be modified through incorporating soluble and insoluble biochemical cues. Soluble biochemical cues include diffusible signals such as neurotrophic factors, cytokines and

drugs that influence cell behavior and modulate the host environment [55]. Modifying collagen scaffolds with insoluble factors like other ECM molecules [16,56], immobilized neurotrophic factors or drugs [57], and engineered materials with biomimetic or mechanical features [52,58,59] can potentially improve mechanical strength and provide prolonged drug delivery and stimulation to cells. However, it is important to ensure that the immobilization process does not affect the efficacy and bioactivity of the drug [60].

Moreover, electrical stimulation can be applied to influence cell behavior. Electroactive conducting polymers like carbon nanotubes help improving cell survival, proliferation, differentiation as well as neurite extension, axonal regeneration and functional recovery [48,61,62].

In short, collagen-based scaffolds offer a flexible platform for creating interactive and instructive cell-carrying scaffolds.

Cell-based Therapy with Collagen-based Scaffolds

Cell transplantation offers the potential to treat brain injuries, largely because it can exert multiple mechanisms of treatment in a sustained fashion and transplanted cells may interact with the local environment [1]. Much focus have been placed on the transplantation of stems cells, which have the ability to renew themselves continuously and can differentiate into many cell types [63]. Mesenchymal stem cells (MSCs) and neural stem/progenitor cells (NSPCs) are the most studied cell types in collagen-based cell delivery in animal models (Table 1). *In vitro* cell culture systems for embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and other neural cells have been developed and investigated to gain better understanding of the cell-matrix interactions for potential application in brain tissue engineering. Functionalization with physical, biochemical or electrical cues are applied in some studies to increase the therapeutic efficacies of these systems.

Mesenchymal Stem Cells (MSCs)

MSCs are multipotent stem cells that are often utilized in collagen-based brain tissue engineering. Besides differentiating into mesenchymal tissues, MSCs have shown greater plasticity in transdifferentiating into several lineage pathways, yielding epithelial, endothelial, and neuronal cells [64,65]. MSCs are procured from various stem cell niches in adult and neonatal tissue [66-68]. Sources of adult tissue include bone marrow, which is the primary source, adipose tissue, peripheral blood and tooth pulp. As for neonatal tissue, MSCs are obtained from birth-associated tissues such as amnion, placenta, umbilical cord, and fresh or banked human umbilical cord blood. In the following discussion, we would use MSC as a general acronym of these cell populations.

MSC is an attractive cell candidate because it is relatively easy to obtain, expand, and manipulate *in vitro* [69]. MSCs are less immunogenic; they can be used as an autologous or allogeneic cell source [1], with low tumorigenicity and fewer ethical problems than ESCs and stem cells of a fetal origin [11,65,70]. Based on the expression of a complex set of factors, MSCs support neuroprotection and neuroregeneration of the brain through multiple mechanisms [1,63,71]. These factors include soluble signals, such as cytokines and growth factors, and insoluble factors like ECM components. Upon MSC transplantation, reduction in inflammation, immunomodulation, protection of surrounding brain cells, and also stimulation of host angiogenesis, neurogenesis and synaptogenesis can be achieved. In addition, a low percentage of MSCs may transdifferentiate into neural cells *in vivo* and replace loss neural tissue [72,73]. Several phase I/II clinical trials associated with brain injuries, including stroke, cerebral palsy and pediatric TBI, using genetically modified, autologous or allogeneic bone marrow stromal stem cells, or umbilical cord derived cells have been conducted [63]. Safety of MSC application for human brain is demonstrated in clinical trials involving TBI [74] and PD [75].

Collagen-based scaffolds magnify the therapeutic effects of MSCs in culture conditions and animal models. Using positron emission tomography imaging, Guan et al. showed that after 12 hours of surgery, collagen scaffold-facilitated delivery of human MSCs (hMSCs) into the lesion of TBI rats resulted in better cell retention and minimized diffusion to non-specific organs when compared to direct injection

Cell type	Origin	Animal and disease model	Functionalization(s) or special feature(s)	Results	Ref.
i. MSC	Human	TBI rat		Better MSC migration, survival and concentration. Enhanced tPA and VEGF levels and reduced Nogo-A and neurocan levels in host tissue. Higher axonal, synaptic and vascular density at the boundary zone. Reduced lesion volume and improved spatial learning and sensorimotor function.	[76-80]
	Human	TBI rat		Improved MSC retention with minimized diffusion to non-specific organs. Enhanced MSC transdifferentiation into neural lineage with neurite outgrowth. Improved brain metabolism, spatial learning and sensorimotor function.	[11]
	Rat	TBI rat		Absence of gliosis within scaffold. Enhanced MSC transdifferentiation into nerve and vascular endothelial cells.	[73]
	Human	PBI rat	*In situ* gelling scaffold	Better retention of brain tissue and improved motor functions. MSCs migrated into the surrounding tissue while activated native NSPCs and astrocytes infiltrated into the retained tissue.	[12]
	Rat	Healthy rat	- *In situ* gelling scaffold - Collagen and poly(ethylene glycol) ether tetrasuccinimidyl glutarate scaffold with GDNF over-expressing MSCs	GDNF detectable surround the graft site with diminished microglia and astrocytes recruitments in the brain.	[59]
ii. NSPC	Rat	PBI rat		NSPCs migrated into surrounding brain and differentiated into astrocytes, endothelial cells, oligodendrocytes and possibly macrophages.	[88]
	Mouse	TBI mouse	Collagen and laminin/fibronectin scaffold	Improved cognitive function. Enhanced NSPC survival and distribution. Collagen-laminin carriers showed better results than the fibronectin group.	[16]

GDNF: Glial Cell-derived Neurotrophic Factor; TBI: Traumatic Brain Injury; tPA: Tissue Plasminogen Activator; PBI: Penetrating Brain Injury; MSCs: Mesenchymal Stem Cells; NSPCs: Neural Stem/Progenitor Cells; VEGF: Vascular Endothelial Growth Factor.

Table 1: Collagen-based cell transplantation applied in animal models of brain injury.

[11]. *In vitro* gene expression analysis revealed that in the presence of collagen scaffolds, expressions of angiogenesis, neurogenesis, and signal transduction genes, including VEGFA, TGFB2, NOTCH4, MDK, BCL2, and BIRC5, were upregulated in hMSCs [76]. TBI rats receiving collagen-MSC implants seven days post-injury showed better spatial learning and motor-sensory function [76,77]. Analysis of the brain tissues at the lesion boundary zone of these animals further revealed the therapeutic effects of MSC-collagen exerted on the surrounding brain tissue. In the presence of collagen scaffolds, hMSCs enhanced the activity of tissue plasminogen activator (tPA) released by CNS neurons and endothelial one week post-implantation [77]. tPA functions to activate plasmin, which is involved in the activation of various neurotrophic factors in the CNS and in synaptic remodeling. Also, compared to transplanting hMSCs alone, cell-collagen group showed a higher vascular endothelial growth factor (VEGF) expression in astrocytes [76] and lower levels of growth-inhibitory molecules, including Nogo-A expression in oligodendrocytes [78] and neurocan from reactive astrocytes [79], at the lesion boundary zone. Homing response of MSCs was observed one week after implantation with cell migration towards the boundary zone of the collagen scaffold [11,77]. Enhanced axonal density [78] and synaptic density [79] in the lesion boundary zone in TBI rats indicated that collagen was able to enhance the effect of hMSCs on axonal plasticity. However, the lesion volume was not significantly reduced after one week of implantation, which indicated that more time was probably needed to achieve tissue reconstruction [76]. After one month of implantation, rodents with cell-collagen implants showed higher MSC migration, survival and concentration in the lesion boundary zone than collagen-alone or intracerebral/intravenous injected hMSC-alone controls [80]. Moreover, reduced lesion volume along with higher vascular density in the lesion boundary zone and hippocampus were reported [11,14,80]. Guan et al. also reported improved neurite outgrowth and brain metabolism in cell-collagen implanted TBI rats than controls with cells or scaffolds alone [11]. Nakata et al. showed there was an absence of gliosis after one month of implantation when TBI rats were treated with collagen-implants, either with or without MSCs [73]. Improved spatial learning and sensorimotor functional outcomes are reported in these studies [11,14,80].

Enhanced transdifferentiation of MSCs in collagen scaffolds has been reported. Neural differentiation and neurite outgrowth were detected in TBI rats receiving hMSCs one week after device implantation [11]. In another study, some of the collagen-delivered cells differentiated into nerve and vascular endothelial cells one month after implantation, a feature not observed in TBI rats receiving scaffolds alone [73]. However, some studies observed structural and functional rescue even when no neuronal differentiation was detected [12].

In order to minimize the invasiveness of the scaffold implantation surgery and maximize scaffold conformation to the irregular lesion, *in situ* gelation platforms for MSC delivery have been applied. Chen et al. injected hMSCs in liquefied collagen matrix into the injury tract of penetrating ballistic-like brain injury rats immediately after the injury induction and showed better retention of brain tissue along with improved motor functions [12]. Migration of transplanted MSCs into the subventricular zone and the corpus callosum, along with infiltration of native NPCs and astrocytes into the preserved brain tissue were identified. Also, endogenous NPCs in lesion boundary zone, the corpus callosum, and the thalamus were activated. In another study, Hoban et al. developed a modified *in situ* gelling scaffold with collagen and poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4S-StarPEG) to deliver rat MSCs with glial cell-derived neurotrophic factor (GDNF) overexpression [59]. Despite the poor survival of the cells, GDNF was detectable surrounding the graft site with diminished microglia and astrocytes recruitments in the host's brain. The system was well-tolerated and the scaffold gradually degraded *in vivo*.

To further influence the behavior of MSCs, collagen scaffolds are functionalized. With the use of collagen-based cell-encapsulating scaffolds made up of interfacial polyelectrolyte complexation fibers, Yok et al. were able to control the spatial distribution of transplanted cells [52]. In response to the topographical cues presented by the fiber, cells seeded on the fiber surfaces showed a lower proliferation rate than cells encapsulated within the fibers, yet they inclined to express neuronal-specific markers after prolonged culture. In another recent study, Lee et al. investigated the response of MSCs towards electrical and topographic cues presented on scaffolds in culture conditions [61]. Incorporating low concentrations of electrical-conducting carbon nanotubes stimulated the expressions of neural and synaptic markers in rat MSCs and upregulated the secretions of nerve growth factor and brain-derived neurotrophic factor.

In general, collagen-based delivery improves MSC survival and functionalities in animal models of brain injury, resulting in amplified functional improvements. Further modifications in collagen-based systems will be beneficial to expanding the potential of collagen-based cell therapies for brain tissue engineering.

Neural stem/progenitor cells (NSPCs)

NSPC is another attractive candidate for collagen-based brain tissue engineering. NSPCs are self-renewable multipotent stem cells that can differentiate into neuronal and glial cells in the CNS. NSPCs can be isolated from neurogenic regions of embryonic, fetal and adult brain, and also be derived from ESCs, iPSCs, MSCs or immortalized neural stem cells (NSCs) [81,82]. Although the use of adult-derived NSPCs does not share the ethical and practical concerns of the use of embryonic or fetal cells [83], it is difficult to secure adult human CNS tissues for preparation of adult NSCs. Transplanted NSPCs in neurological disorder models exhibit homing effects to the injured brain and encourage neural repair and regeneration through cell replacement, trophic support, immunomodulation and neuronal plasticity [84]. In Phase I/II clinical trials of brain tumors, PD, stable ischemic stroke, HD, Batten's disease and Pelizaeus-Merzbacher disease, autologous NSCs and fetal brain-derived human NSCs, either with or without cmycER immortilization, have been applied [63,84]. Compared to MSCs, there are fewer reports on the *in vivo* performance of NSPCs-collagen systems in brain.

Some studies investigated the response of various sources of NSPCs on collagen-scaffolds in two-dimensional (2D) culture conditions. When exposed to mitogen, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), Branvell et al. showed that embryonic mouse NSPCs have the highest proliferation rate whereas adult ones have the lowest [56]. On the other hand, postnatal NSPCs showed a faster differentiation process and a much higher differentiated neuronal percentage (70%) than both embryonic (26%) and adult (9%) NSPCs. This may be due to the ability of postnatal cells to adhere to collagen scaffold and establish paxillin-positive focal adhesions in an integrin-dependent manner unlike adult or embryonic mouse NSPCs [85].

As for 3D scaffolds, collagen provides transplanted cells with a biomimetic environment that supports cell survival and formation of functional synapses and neuronal networks. Bercu et al. showed that NSPCs survived longer in collagen scaffolds than 2D cultures for at least two months [8]. Embryonic rat hippocampal cells in collagen

showed a maintained neuronal phenotype with extensions of neuronal processes into the scaffold [49]. The scaffolds enabled the formation of a multi-level neuronal circuit with functional synapses. O'Conner et al. showed that collagen matrix supported the proliferation of embryonic rat NSPCs in the presence of bFGF and neural cell differentiation into neurons and astrocytes after bFGF withdrawal [86]. Also, embryonic rat NSPCs cultured in collagen matrix with bFGF were able to derive into functional neurons, which expressed neurotransmitters, neurotransmitter receptors, membrane excitability and synaptic activity, and formed functional synapses and neuronal networks in the scaffold [87]. However, *in vivo* applications of collagen-NSPC systems are sometimes limited by low survival of transplanted cell at initial stage of implantation [16,88]. Elias et al. implanted a collagen scaffold with adult rat hippocampal NSPCs seeded in its open pores one week post-injury [88]. Structural integrity of the scaffold was maintained while some NSPCs migrated into the surrounding brain and differentiated into astrocytes, endothelial cells, oligodendrocytes and possibly macrophages four weeks afterwards. However, low transplanted cell survival and differentiation were also reported, possibly due to cytotoxic initial injury environment and limited differentiation stimulation in brain regions that were generally non-neurogenic respectively.

To gain better control over the NSPC behaviors, some studies investigated the effects of scaffold functionalization. Compared to collagen-coated surfaces, Wang et al. demonstrated that postnatal rat NSPCs cultured on collagen nanoscaffolds showed a 30% increase in proliferation, in which electrospun aligned scaffolds performed better than randomly-oriented ones [50]. Such modulated proliferation was mediated by cell cycle progression through the b1 integrin/MAPK pathway. Besides controlling topographical properties, collagen can be modified biochemically to form composites and to present neuroactive factors. Some studies showed that the addition of ECM components such as hyaluronic acid (HA), laminin and fibronectin to collagen matrix enhanced NSPC survival and differentiation. Although addition of these ECM molecules altered the gelation temperature of the cell delivery systems, their gelling temperatures are still physiologically relevant [16,56]. Brannvall et al. showed that collagen-HA scaffolds supported the survival, proliferation and differentiation of mice NSPCs into neurons, astrocytes, and oligodendrocytes in proliferative culture with EGF and bFGF [56]. In another study, Tate et al. transplanted *in situ* gelling collagen-laminin or collagen-fibronectin systems with embryonic mice NSPCs into a TBI mice model one week post-injury [16]. Compared to the medium delivery control, collagen groups showed improved cognitive function after five weeks post-implantation and higher NSPC survival and distribution after eight weeks. Collagen-laminin carriers showed better results than the fibronectin group. To modify scaffolds with neuroactive factors, Ma et al. prepared genetically-modified bFGF with a fused collagen-binding polypeptide domain derived from mammalian collagenase, and applied it to a porous collagen sponge [57]. After seven days of culture, there was a significantly higher number of postnatal rat NSPCs in scaffolds with modified bFGF than those with native bFGF or phosphate-buffered saline control. Another study showed that extended release of neuroactive factors could be achieved with collagen-fibrin cell culture system. Lee et al. embedded VEGF-releasing fibrin gel and murine NSC line C17.2 into a collagen scaffold [55]. In contrast to control systems without VEGF or without fibrin, this system gradually released VEGF for at least three days in culture and supported better cell migration and proliferation [55].

In short, collagen-based delivery promotes NSPC functions including differentiation and formation of 3D neural networks. Since many of the experiments focused on *in vitro* investigation of NSPC-collagen relationships, further animal studies will be needed to understand how the system interacts with the injury environment, and whether functionalized scaffolds are biocompatible and can achieve better therapeutic effects. Strategies to improve the initial cell survival are also warranted.

Embryonic stem cells (ESCs)

ESCs are derived from the inner cell mass of the embryonic blastocyst. Human ESCs (hESCs) are pluripotent and can spontaneously differentiate to cells in endoderm, mesoderm and ectoderm lineages. It is an attractive cell source because it can be maintained indefinitely *in vitro* without loss of differentiation potential [64]. However, ESC applications for brain injuries are potentially plagued by differentiation issues, tumorgensis, genetic aberrations, inflammation and rejection, and ethical issues [89]. One major application of ESCs in neural studies is to generate neuronal cell lines for drug screening, mechanistic investigation, or therapeutic use [83].

3D collagen scaffold influences the *in vitro* behavior of ESCs. Collagen type I stimulated the self-renewal of mouse ESCs which may be mediated by Bmi-1 and its downstream pathways [90]. When cultured within collagen-based scaffolds and differentiating conditions, ESCs differentiated into cells of the neural lineage and expressed neuronal lineage markers [91,92]. Some studies suggest that microarchitecture of a cell matrix played a critical role in controlling the overall growth and differentiation pattern of hESCs. In collagen gels, rhesus monkey ESCs formed gland-like circular structures, whereas in collagen sponges, ESCs were scattered through the matrix or formed aggregates [91]. The differentiating neural tube-like structures showed an ependymal-like layer and neural structure with typical synapses [92]. Sridharan et al. demonstrated that ESCs in structureless and soft gelatin matrix differentiated in all three lineages, whereas in a collagen or a collagen-carbon nanotube (CNT) matrix with fibril structures, preferential development of ESCs into elongated cells with long filaments was observed [48]. In collagen-CNT, more than 90% of the cells differentiated to the ectodermal lineage at day 3, whereas for pure collagen, such differentiation took place at a later stage at day 6. These studies showed that despite having the same chemical makeup, different forms of collagen scaffold had varying influences on cells. Scaffold composition, architecture and designs are important factors when designing ESC-culture scaffolds. Further investigations are needed to optimize cell-material interaction to maximize therapeutic results.

Induced Pluripotent Stem Cells (iPSCs)

iPSCs and their derivatives have great potentials in repairing and regenerating the injured brain. By overexpressing a limited set of transgenes, such as Oct4, Sox2, c-Myc and KLF4 [93,94] or Oct4, Sox2, Nanog and Lin28 [95], adult somatic cells can be reprogrammed with pluripotency similar to ESCs. Compared to ESCs, the use and derivation process of iPSCs are ethically more advantageous. Although iPSCs retain epigenetic memory from the source tissue [96,97], they can differentiate into cells of all three embryonic germ layers, producing nearly identical progeny from neural, hepatic, and mesenchymal lineages [98]. As a result, patient-specific MSCs, NSPCs or other types of cells can be derived for autografting and potentially preventing immune rejection [99,100]. When applied to the brain, Wernig et al. showed that iPSC-derived NSPCs were able to migrate into various brain regions, differentiate into glia and neurons, and functionally integrate with the fetal mouse brain [101]. Also, the neurons derived promoted functional recovery in a rat PD model.

However, several obstacles associated with iPSCs applications for brain injury rescue are yet to be overcome. For example, many human iPSC establishment and induction protocols are time-consuming [99] and have difficulties fitting the short time frame required for brain injury treatments. Also, some protocols are challenged by the inability to generate adequate amounts of target cells for implantation [102]. Moreover, variations in isolation of iPSCs, differentiation and expansion of the derived cells exist between different iPSC cell lines [103]. For undifferentiated iPSCs, there exhibit risks associated with teratoma formation [104]. Also, abnormal gene expression in some of these undifferentiated cells may contribute to immune rejection by T-cell infiltration that led to massive necrosis in mice [105]. Optimizations in the protocols for reprogramming and differentiation of iPSCs are required to augment therapeutic efficiency and eliminate the risks of rejection and tumor formation.

Current applications of iPSCs for CNS rescue include the establishment of neurodegenerative disease-specific cellular models, drug screening platforms and autologous source for cell replacement [100,102,106]. A recent study showed the potential of collagen-based scaffolds in modulating the behaviors of iPSC-derived cells. Higher scaffold infiltration of iPSC-derived NSPCs was observed in collagen scaffolds with aligned and smaller pores, resulting from preparation under lower freezing temperatures [107]. Also, functionalizing these scaffolds with laminin coating showed a dose-dependent increase in cell proliferation and infiltration. Further exploration of the interaction between iPSCs and collagen scaffolds may shed light on the optimizing transplantation systems for the injured brain and cell fate control.

Other cell candidates

Besides stem cells, behavior and response of brain neurons and immune-related cells in collagen systems have been explored. East et al. showed that astrocytes cultured in aligned collagen scaffolds exhibited an enhanced neurite outgrowth [51]. Also, Yao et al. reported the growth and differentiation of oligodendrocyte progenitor cells into oligodendrocytes were supported in collagen microspheres under differentiation culture conditions [108]. To further functionalize collagen scaffolds, Sur et al. modified the matrix by adding nanofibers that displayed an adjustable density of laminin epitopes and hence bioactivity. This composite scaffold supported the survival and normal functions of Granule cells and Purkinje cells, two major neuronal subtypes of cerebellar cortex. By tuning the laminin epitope density, neuronal survival and morphology can be controlled [58]. Further animal studies will be required to evaluate the thereapeutic efficacy of these cell-collagen systems.

Conclusions

Collagen is a well-studied scaffold material that can potentially provide a flexible platform for drug and cell delivery for repair and regeneration of the injured brain. Not only can it be readily manufactured and fabricated into various forms with desirable macro- and micro-structure, internal architecture and mechanical properties, collagen scaffolds can also be functionalized isotrophically or anisotropically with instructive physical, biochemical and electrical cues to guide the repair and regeneration process. Collagen-based cell transplantation demonstrated therapeutic potentials in modulating cell behavior and promoting repair and regeneration in preclinical studies. These scaffolds showed good biocompatibility and multifaceted rescue mechanisms, including enhanced cell survival, proliferation, differentiation, migration and secretion of neuroactive factors, which can potentially modulate the injury environment, promote endogenous repair mechanisms and encourage tissue replacement and regeneration.

To further expand the potentials of collagen-based cell delivery, various issues have to be addressed. Firstly, despite many exciting ideas have been proposed to optimize, modify or functionalize collagen scaffolds for cell delivery, majority of studies evaluated their systems in culture conditions and in small animal models. Further application of these systems in larger animal models would be beneficial since they can better mimic the lesion size, complex pathophysiology of the injured human brain, and also the repair and regeneration mechanisms involved. Secondly, studies of longer durations are warranted for a more thorough understanding in how transplanted and host cells respond to the gradually biodegrading scaffold and mediate tissue reorganization. Thirdly, better guidance or control over transplanted and endogenous cell fates and functions in the scaffolds can potentially enhance cell survival and speed up graft integration, neural repair and regeneration process. Also, scaffold-facilitated or cell-based drug delivery of therapeutics, such as anti-inflammatory and growth factors, that modulate the early post-injury environment may encourage higher survival of transplanted cells at the site of injury. Further advancement and understanding in the various fields of study, including stem cell research and technologies, collagen-based scaffold manufacturing techniques, mechanisms of brain injury, repair and regeneration, and interactions between different cell candidates, and biomaterials and growth signals, would direct us to further optimize, refine and enrich the design of cell carrying collagen-based scaffolds as biomimetic tissue equivalents for the injured brain.

Acknowledgement

This research was supported by the grant from the Hong Kong Research Grants Council (GRF #HKU773613M)

References

1. Tate C, Case C (2012) Transplanted Mesenchymal Stem Cells Aid the Injured Brain through Trophic Support Mechanisms. Springer, Netherlands.
2. Feigin VL, Barker-Collo S, Krishnamurthi R, Theadom A, Starkey N (2010) Epidemiology of ischaemic stroke and traumatic brain injury. Best Pract Res Clin Anaesthesiol 24: 485-494. [PubMed]
3. Mitsios N, Gaffney J, Kumar P, Krupinski J, Kumar S, et al. (2006) Pathophysiology of acute ischaemic stroke: an analysis of common signalling mechanisms and identification of new molecular targets. Pathobiology 73: 159-175. [PubMed]
4. Werner C, Engelhard K (2007) Pathophysiology of traumatic brain injury. Br J Anaesth 99: 4-9.
5. Richardson RM, Sun D, Bullock MR (2007) Neurogenesis after traumatic brain injury. Neurosurg Clin N Am 18: 169-181. [PubMed]
6. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, et al. (1998) Neurogenesis in the adult human hippocampus. Nat Med 4: 1313-1317. [PubMed]
7. Kernie SG, Parent JM (2010) Forebrain neurogenesis after focal Ischemic and traumatic brain injury. Neurobiol Dis 37: 267-274. [PubMed]
8. Bercu MM, Arien-Zakay H, Stoler D, Lecht S, Lelkes PI, et al. (2013) Enhanced survival and neurite network formation of human umbilical cord blood neuronal progenitors in three-dimensional collagen constructs. J Mol Neurosci 51: 249-261. [PubMed]
9. Bakshi A, Keck CA, Koshkin VS, LeBold DG, Siman R, et al. (2005) Caspase-mediated cell death predominates following engraftment of neural progenitor cells into traumatically injured rat brain. Brain Res 1065: 8-19. [PubMed]
10. Williams AJ, Wei HH, Dave JR, Tortella FC (2007) Acute and delayed neuroinflammatory response following experimental penetrating ballistic brain injury in the rat. J Neuroinflammation 4: 17. [PubMed]
11. Guan J, Zhu Z, Zhao RC, Xiao Z, Wu C, et al. (2013) Transplantation of human

mesenchymal stem cells loaded on collagen scaffolds for the treatment of traumatic brain injury in rats. Biomaterials 34: 5937-5946. [PubMed]

12. Chen Z, Lu XC, Shear DA, Dave JR, Davis AR, et al. (2011) Synergism of human amnion-derived multipotent progenitor (AMP) cells and a collagen scaffold in promoting brain wound recovery: pre-clinical studies in an experimental model of penetrating ballistic-like brain injury. Brain Res 1368: 71-81. [PubMed]

13. Wong FS, Chan BP, Lo AC (2014) Carriers in cell-based therapies for neurological disorders. Int J Mol Sci 15: 10669-10723. [PubMed]

14. Lu D, Mahmood A, Qu C, Hong X, Kaplan D, et al. (2007) Collagen scaffolds populated with human marrow stromal cells reduce lesion volume and improve functional outcome after traumatic brain injury. Neurosurgery 61: 596-602. [PubMed]

15. Xiong Y, Qu C, Mahmood A, Liu Z, Ning R, et al. (2009) Delayed transplantation of human marrow stromal cell-seeded scaffolds increases transcallosal neural fiber length, angiogenesis, and hippocampal neuronal survival and improves functional outcome after traumatic brain injury in rats. Brain Res 1263: 183-191. [PubMed]

16. Tate CC, Shear DA, Tate MC, Archer DR, Stein DG, et al. (2009) Laminin and fibronectin scaffolds enhance neural stem cell transplantation into the injured brain. J Tissue Eng Regen Med 3: 208-217. [PubMed]

17. Keilhoff G, Stang F, Wolf G, Fansa H (2003) Bio-compatibility of type I/III collagen matrix for peripheral nerve reconstruction. Biomaterials 24: 2779-2787. [PubMed]

18. Udina E, Rodriguez FJ, Verdu E, Espejo M, Gold BG, et al. (2004) FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogeneic Schwann cells. Glia 47: 120-129. [PubMed]

19. Stang F, Fansa H, Wolf G, Reppin M, Keilhoff G (2005) Structural parameters of collagen nerve grafts influence peripheral nerve regeneration. Biomaterials 26: 3083-3091. [PubMed]

20. Phillips JB, Bunting SC, Hall SM, Brown RA (2005) Neural tissue engineering: a self-organizing collagen guidance conduit. Tissue Eng 11: 1611-1617. [PubMed]

21. Georgiou M, Bunting SC, Davies HA, Loughlin AJ, Golding JP, et al. (2013) Engineered neural tissue for peripheral nerve repair. Biomaterials 34: 7335-7343. [PubMed]

22. Cholas RH, Hsu HP, Spector M (2012) The reparative response to cross-linked collagen-based scaffolds in a rat spinal cord gap model. Biomaterials 33: 2050-2059. [PubMed]

23. Murakami T, Fujimoto Y, Yasunaga Y, Ishida O, Tanaka N, et al. (2003) Transplanted neuronal progenitor cells in a peripheral nerve gap promote nerve repair. Brain Res 974: 17-24. [PubMed]

24. Evans GR, Brandt K, Katz S, Chauvin P, Otto L, et al. (2002) Bioactive poly(L-lactic acid) conduits seeded with Schwann cells for peripheral nerve regeneration. Biomaterials 23: 841-848. [PubMed]

25. Emerich DF, Hammang JP, Baetge EE, Winn SR (1994) Implantation of polymer-encapsulated human nerve growth factor-secreting fibroblasts attenuates the behavioral and neuropathological consequences of quinolinic acid injections into rodent striatum. Exp Neurol 130: 141-150. [PubMed]

26. Emerich DF, Lindner MD, Winn SR, Chen EY, Frydel BR, et al. (1996) Implants of encapsulated human CNTF-producing fibroblasts prevent behavioral deficits and striatal degeneration in a rodent model of Huntington's disease. J Neurosci 16: 5168-5181. [PubMed]

27. Emerich DF, Winn SR, Hantraye PM, Peschanski M, Chen EY, et al. (1997) Protective effect of encapsulated cells producing neurotrophic factor CNTF in a monkey model of Huntington's disease. Nature 386: 395-399. [PubMed]

28. Emerich DF, Winn SR, Harper J, Hammang JP, Baetge EE, et al. (1994) Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman primate: rescue and sprouting of degenerating cholinergic basal forebrain neurons. J Comp Neurol 349: 148-164. [PubMed]

29. Kordower JH, Winn SR, Liu YT, Mufson EJ, Sladek JR Jr, et al. (1994) The aged monkey basal forebrain: rescue and sprouting of axotomized basal forebrain neurons after grafts of encapsulated cells secreting human nerve growth factor. Proc Natl Acad Sci USA 91: 10898-10902. [PubMed]

30. Emerich DF, Winn SR, Lindner MD (1996) Continued presence of intrastriatal but not intraventricular polymer-encapsulated PC12 cells is required for alleviation of behavioral deficits in Parkinsonian rodents. Cell Transplant 5: 589-596. [PubMed]

31. Deglon N, Heyd B, Tan SA, Joseph JM, Zurn AD, et al. (1996) Central nervous system delivery of recombinant ciliary neurotrophic factor by polymer encapsulated differentiated C2C12 myoblasts. Hum Gene Ther 7: 2135-2146. [PubMed]

32. Aebischer P, Schluep M, Deglon N, Joseph JM, Hirt L, et al. (1996) Intrathecal delivery of CNTF using encapsulated genetically modified xenogeneic cells in amyotrophic lateral sclerosis patients. Nat Med 2: 696-699. [PubMed]

33. Bloch J, Bachoud-Levi AC, Deglon N, Lefaucheur JP, Winkel L, et al. (2004) Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study. Hum Gene Ther 15: 968-975. [PubMed]

34. Bachoud-Levi AC, Deglon N, Nguyen JP, Bloch J, Bourdet C, et al. (2000) Neuroprotective gene therapy for Huntington's disease using a polymer encapsulated BHK cell line engineered to secrete human CNTF. Hum Gene Ther 11: 1723-179. [PubMed]

35. Abou Neel EA, Bozec L, Knowles JC, Syed O, Mudera V, et al. (2013) Collagen--emerging collagen based therapies hit the patient. Adv Drug Deliv Rev 65: 429-456. [PubMed]

36. Hulmes DJ (2002) Building collagen molecules, fibrils, and suprafibrillar structures. J Struct Biol 137: 2-10. [PubMed]

37. Gelse K, Poschl E, Aigner T (2003) Collagens--structure, function, and biosynthesis. Adv Drug Deliv Rev 55: 1531-1546. [PubMed]

38. Birk D, Brückner P (2011) Collagens, Suprastructures, and Collagen Fibril Assembly. Springer, Berlin Heidelberg.

39. Kielty CM, Grant ME (2003) The Collagen Family: Structure, Assembly, and Organization in the Extracellular Matrix. John Wiley & Sons, Inc.

40. Parenteau-Bareil R, Gauvin R, Berthod F (2010) Collagen-Based Biomaterials for Tissue Engineering Applications. Materials 3: 1863-1887.

41. Hermanns S, Klapka N, Muller HW (2001) The collagenous lesion scar--an obstacle for axonal regeneration in brain and spinal cord injury. Restor Neurol Neurosci 19: 139-148. [PubMed]

42. Silver FH, Freeman JW, Seehra GP (2003) Collagen self-assembly and the development of tendon mechanical properties. J Biomech 36: 1529-1553. [PubMed]

43. Dombi GW, Halsall HB (1985) Collagen fibril formation in the presence of sodium dodecyl sulphate. Biochem J 228: 551-556. [PubMed]

44. Parkinson J, Kadler KE, Brass A (1995) Simple physical model of collagen fibrillogenesis based on diffusion limited aggregation. J Mol Biol 247: 823-831. [PubMed]

45. Walters BD, Stegemann JP (2014) Strategies for directing the structure and function of three-dimensional collagen biomaterials across length scales. Acta Biomater 10: 1488-1501. [PubMed]

46. Clements IP, Munson JM, Bellamkonda RV (2013) Neuronal Tissue Engineering. Academic Press.

47. Delcroix GJ, Schiller PC, Benoit JP, Montero-Menei CN (2010) Adult cell therapy for brain neuronal damages and the role of tissue engineering. Biomaterials 31: 2105-2120. [PubMed]

48. Sridharan I, Kim T, Wang R (2009) Adapting collagen/CNT matrix in directing hESC differentiation. Biochem Biophys Res Commun 381: 508-512. [PubMed]

49. Xu T, Molnar P, Gregory C, Das M, Boland T, et al. (2009) Electrophysiological characterization of embryonic hippocampal neurons cultured in a 3D collagen hydrogel. Biomaterials 30: 4377-4383. [PubMed]

50. Wang Y, Yao M, Zhou J, Zheng W, Zhou C, et al. (2011) The promotion of neural progenitor cells proliferation by aligned and randomly oriented collagen nanofibers through beta1 integrin/MAPK signaling pathway. Biomaterials 32: 6737-6744. [PubMed]

51. East E, de Oliveira DB, Golding JP, Phillips JB (2010) Alignment of astrocytes increases neuronal growth in three-dimensional collagen gels and is maintained following plastic compression to form a spinal cord repair conduit. Tissue Eng Part A 16: 3173-3184. [PubMed]

52. Yow SZ, Quek CH, Yim EK, Lim CT, Leong KW (2009) Collagen-based fibrous scaffold for spatial organization of encapsulated and seeded human mesenchymal stem cells. Biomaterials 30: 1133-1142. [PubMed]

53. Gu X, Ding F, Yang Y, Liu J (2011) Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. Prog Neurobiol 93: 204-230. [PubMed]

54. Lin HJ, O'Shaughnessy TJ, Kelly J, Ma W (2004) Neural stem cell differentiation in a cell-collagen-bioreactor culture system. Brain Res Dev Brain Res 153: 163-173. [PubMed]

55. Lee YB, Polio S, Lee W, Dai G, Menon L, et al. (2010) Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. Exp Neurol 223: 645-652. [PubMed]

56. Brannvall K, Bergman K, Wallenquist U, Svahn S, Bowden T, et al. (2007) Enhanced neuronal differentiation in a three-dimensional collagen-hyaluronan matrix. J Neurosci Res 85: 2138-2146. [PubMed]

57. Ma F, Xiao Z, Chen B, Hou X, Han J, et al. (2014) Accelerating proliferation of neural stem/progenitor cells in collagen sponges immobilized with engineered basic fibroblast growth factor for nervous system tissue engineering. Biomacromolecules 15: 1062-1068. [PubMed]

58. Sur S, Pashuck ET, Guler MO, Ito M, Stupp SI, et al. (2012) A hybrid nanofiber matrix to control the survival and maturation of brain neurons. Biomaterials 33: 545-555. [PubMed]

59. Hoban DB, Newland B, Moloney TC, Howard L, Pandit A, et al. (2013) The reduction in immunogenicity of neurotrophin overexpressing stem cells after intra-striatal transplantation by encapsulation in an in situ gelling collagen hydrogel. Biomaterials 34: 9420-9429. [PubMed]

60. Willerth SM, Sakiyama-Elbert SE (2007) Approaches to neural tissue engineering using scaffolds for drug delivery. Adv Drug Deliv Rev 59: 325-338. [PubMed]

61. Lee JH, Lee JY, Yang SH, Lee EJ, Kim HW (2014) Carbon nanotube-collagen three-dimensional culture of mesenchymal stem cells promotes expression of neural phenotypes and secretion of neurotrophic factors. Acta Biomater 10: 4425-4436. [PubMed]

62. Ghasemi-Mobarakeh L, Prabhakaran MP, Morshed M, Nasr-Esfahani MH, Baharvand H, et al. (2011) Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering. J Tissue Eng Regen Med 5: e17-35. [PubMed]

63. Aboody K, Capela A, Niazi N, Stern JH, Temple S (2011) Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta stone. Neuron 70: 597-613. [PubMed]

64. Tuan RS, Boland G, Tuli R (2003) Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Res Ther 5: 32-45. [PubMed]

65. Glavaski-Joksimovic A, Bohn MC (2013) Mesenchymal stem cells and neuroregeneration in Parkinson's disease. Exp Neurol 247: 25-38. [PubMed]

66. Hass R, Kasper C, Bohm S, Jacobs R (2011) Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 9: 12. [PubMed]

67. Waddington RJ, Youde SJ, Lee CP, Sloan AJ (2009) Isolation of distinct progenitor stem cell populations from dental pulp. Cells Tissues Organs 189: 268-274. [PubMed]

68. Alhadlaq A, Mao JJ (2004) Mesenchymal stem cells: isolation and therapeutics. Stem Cells Dev 13: 436-448. [PubMed]

69. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98: 1076-1084. [PubMed]

70. Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, et al. (2010) Mesenchymal stem cells: Mechanisms of immunomodulation and homing. Cell Transplant 19: 667-679. [PubMed]

71. Parr AM, Tator CH, Keating A (2007) Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 40: 609-619. [PubMed]

72. Li Y, Chopp M (2009) Marrow stromal cell transplantation in stroke and traumatic brain injury. Neurosci Lett 456: 120-123. [PubMed]

73. Nakada A, Fukuda S, Ichihara S, Sato T, Itoi S, et al. (2009) Regeneration of central nervous tissue using a collagen scaffold and adipose-derived stromal cells. Cells Tissues Organs 190: 326-335. [PubMed]

74. Cox CS Jr, Baumgartner JE, Harting MT, Worth LL, Walker PA, et al. (2011) Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. Neurosurgery 68: 588-600. [PubMed]

75. Venkataramana NK, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, et al. (2010) Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. Transl Res 155: 62-70. [PubMed]

76. Qu C, Mahmood A, Liu XS, Xiong Y, Wang L, et al. (2011) The treatment of TBI with human marrow stromal cells impregnated into collagen scaffold: functional outcome and gene expression profile. Brain Res 1371: 129-139. [PubMed]

77. Mahmood A, Qu C, Ning R, Wu H, Goussev A, et al. (2011) Treatment of TBI with collagen scaffolds and human marrow stromal cells increases the expression of tissue plasminogen activator. J Neurotrauma 28: 1199-1207. [PubMed]

78. Mahmood A, Wu H, Qu C, Mahmood S, Xiong Y, et al. (2014) Down-regulation of Nogo-A by collagen scaffolds impregnated with bone marrow stromal cell treatment after traumatic brain injury promotes axonal regeneration in rats. Brain Res 1542: 41-48. [PubMed]

79. Mahmood A, Wu H, Qu C, Mahmood S, Xiong Y, et al. (2014) Suppression of neurocan and enhancement of axonal density in rats after treatment of traumatic brain injury with scaffolds impregnated with bone marrow stromal cells. J Neurosurg 120: 1147-1155. [PubMed]

80. Qu C, Xiong Y, Mahmood A, Kaplan DL, Goussev A, et al. (2009) Treatment of traumatic brain injury in mice with bone marrow stromal cell-impregnated collagen scaffolds. J Neurosurg 111: 658-665. [PubMed]

81. Kim SU, Lee HJ, Kim YB (2013) Neural stem cell-based treatment for neurodegenerative diseases. Neuropathology 33: 491-504. [PubMed]

82. Bithell A, Williams BP (2005) Neural stem cells and cell replacement therapy: making the right cells. Clin Sci (Lond) 108: 13-22. [PubMed]

83. Kalladka D, Muir KW (2014) Brain repair: cell therapy in stroke. Stem Cells Cloning 7: 31-44. [PubMed]

84. De Feo D, Merlini A, Laterza C, Martino G (2012) Neural stem cell transplantation in central nervous system disorders: from cell replacement to neuroprotection. Curr Opin Neurol 25: 322-333. [PubMed]

85. Bergstrom T, Holmqvist K, Tararuk T, Johansson S, Forsberg-Nilsson K (2014) Developmentally regulated collagen/integrin interactions confer adhesive properties to early postnatal neural stem cells. Biochim Biophys Acta 1840: 2526-2532. [PubMed]

86. O'Connor SM, Stenger DA, Shaffer KM, Maric D, Barker JL, et al. (2000) Primary neural precursor cell expansion, differentiation and cytosolic Ca(2+) response in three-dimensional collagen gel. J Neurosci Methods 102: 187-195. [PubMed]

87. Ma W, Fitzgerald W, Liu QY, O'Shaughnessy TJ, Maric D, et al. (2004) CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels. Exp Neurol 190: 276-288. [PubMed]

88. Elias PZ, Spector M (2012) Implantation of a collagen scaffold seeded with adult rat hippocampal progenitors in a rat model of penetrating brain injury. J Neurosci Methods 209: 199-211. [PubMed]

89. Li JY, Christophersen NS, Hall V, Soulet D, Brundin P (2008) Critical issues of clinical human embryonic stem cell therapy for brain repair. Trends Neurosci 31: 146-153. [PubMed]

90. Suh HN, Han HJ (2011) Collagen I regulates the self-renewal of mouse embryonic stem cells through alpha2beta1 integrin- and DDR1-dependent Bmi-1. J Cell Physiol 226: 3422-3432. [PubMed]

91. Chen SS, Revoltella RP, Papini S, Michelini M, Fitzgerald W, et al. (2003) Multilineage differentiation of rhesus monkey embryonic stem cells in three-dimensional culture systems. Stem cells 21: 281-295. [PubMed]

92. Baharvand H, Mehrjardi NZ, Hatami M, Kiani S, Rao M, et al. (2007) Neural differentiation from human embryonic stem cells in a defined adherent culture condition. Int J Dev Biol 51: 371-378. [PubMed]

93. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, et al. (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451: 141-146. [PubMed]

94. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861-872. [PubMed]

95. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318: 1917-1920. [PubMed]

96. Kim DS, Lee JS, Leem JW, Huh YJ, Kim JY, et al. (2010) Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. Stem Cell Rev 6: 270-281. [PubMed]

97. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, et al. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 5: 111-123. [PubMed]

98. Patterson M, Chan DN, Ha I, Case D, Cui Y, et al. (2012) Defining the nature of human pluripotent stem cell progeny. Cell Res 22: 178-193.

99. Matsui T, Akamatsu W, Nakamura M, Okano H (2014) Regeneration of the damaged central nervous system through reprogramming technology: basic concepts and potential application for cell replacement therapy. Exp Neurol 260: 12-18. [PubMed]

100. Gao A, Peng Y, Deng Y, Qing H (2013) Potential therapeutic applications of differentiated induced pluripotent stem cells (iPSCs) in the treatment of neurodegenerative diseases. Neuroscience 228: 47-59. [PubMed]

101. Wernig M, Zhao J-P, Pruszak J, Hedlund E, Fu D, et al. (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci USA 105: 5856-5861. [PubMed]

102. Mattis VB, Svendsen CN (2011) Induced pluripotent stem cells: a new revolution for clinical neurology? Lancet Neurol 10: 383-394. [PubMed]

103. Wang A, Tang Z, Park IH, Zhu Y, Patel S, et al. (2011) Induced pluripotent stem cells for neural tissue engineering. Biomaterials 32: 5023-5032. [PubMed]

104. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, et al. (2009) Variation in the safety of induced pluripotent stem cell lines. Nat Biotech 27: 743-745. [PubMed]

105. Zhao T, Zhang Z-N, Rong Z, Xu Y (2011) Immunogenicity of induced pluripotent stem cells. Nature 474: 212-215. [PubMed]

106. Jung Y-W, Hysolli E, Kim K-Y, Tanaka Y, Park I-H (2012) Human induced pluripotent stem cells and neurodegenerative disease: prospects for novel therapies. Curr Opin Neurol 25: 125-130. [PubMed]

107. Khayyatan F, Nemati S, Kiani S, Hojjati Emami S, et al. (2014) Behaviour of human induced pluripotent stem cell-derived neural progenitors on collagen scaffolds varied in freezing temperature and laminin concentration. Cell J 16: 53-62. [PubMed]

108. Yao L, Phan F, Li Y (2013) Collagen microsphere serving as a cell carrier supports oligodendrocyte progenitor cell growth and differentiation for neurite myelination in vitro. Stem Cell Res Ther 4: 109. [PubMed]