We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

18

Advances in the Combined Use of Adult Cell Therapy and Scaffolds for Brain Tissue Engineering

Elisa Garbayo[1], Gaëtan J.-R. Delcroix[2],
Paul C. Schiller[2] and Claudia N. Montero-Menei[3,4]
[1]*Pharmacy and Pharmaceutical Technology Department, University of Navarra, Pamplona,*
[2]*University of Miami, UMTB, Florida,*
[3]*LUNAM Université, Ingénierie de la Vectorisation Particulaire, F-49933 Angers,*
[4]*INSERM, U646, F-49933 Angers*
[1]*Spain*
[2]*USA*
[3,4]*France*

1. Introduction

No long-term effective treatments are currently available for brain neurological disorders such as focal and global cerebral ischemia, traumatic brain injury (TBI) and neurodegenerative disorders. Current treatments are primarily focused on limiting the damage and slowing the degeneration and, most of them are only symptomatic while function restoration is rarely achieved. This underlies the need for alternative therapies such as brain cell therapy that allows functional replacement of missing or damaged neurons by transplanting cells that may differentiate into the desired phenotype and integrate the host parenchyma, or alternatively rescue the affected neuronal population. Due to the high cell death that occurs during neurodegenerative processes, brain neurological disorders are an ideal target for cell-based therapies. Several teams tried to prevent the loss of neurons or to replace them, using neuronal precursors and lately, since stem cell discovery, embryonic stem (ES) cells, adult stem cells, tissue-derived stem cells or more recently induced pluripotent stem (iPS) cells (for review see (Abeliovich & Doege, 2009; Joyce et al., 2010; Chen & Xiao, 2011; Loewenbruck & Storch, 2011). These studies demonstrated the potential of cell therapy to repair the injured brain even if poor survival of grafted cells was reported (Brundin et al., 1985; Schierle et al., 1999; Brundin et al., 2000; Isacson et al., 2003; Olanow et al., 2003). In this book chapter, brain cell therapy studies performed with adult non-transformed cells that, by nature, allow the use of autologous tissues for transplantation and overcome the immunological, availability, as well as ethical concerns will be reported.

It is important to bear in mind that, for organs with a higher level of complexity such as the brain, cell therapy remains a challenging task. Fortunately, relatively recent medical, biological and technological advances in tissue engineering approaches allow functional

tissue and organ recovery by using the appropriate combination of three fundamental "tools" known as the tissue engineering triad: cells, engineered materials and signalling molecules (essentially growth factors). In practice, this is translated into a great versatility of delivery systems, which mimic the natural repairing environment of the brain tissue, creating tuneable and customized spatio-temporal gradients of signals guiding tissue regeneration. Bioactive scaffolds are likely to reinforce the success of cell replacement therapies by providing a microenvironment that facilitates the survival, proliferation, differentiation, and connectivity of transplanted and/or endogenous cells (Pettikiriarachchi et al., 2010). However, delivery of cells with scaffolds to the damaged brain still remains challenging due to practical limitations of delivery. The second part of this book chapter will provide an overview of what solutions tissue engineering may provide for adult cell therapy of the brain. Since the brain is a functionally complex organ, cell growth and differentiation alone are not enough in order to achieve its functional recovery. The correct re-establishment of the axonal connections and neuronal circuits is also necessary. In general, scaffolds for brain therapy should meet several essential requirements like being biocompatible, biodegradable, immunologically inert and able to support neurite outgrowth. The most common materials, fabrication methods and desirable properties of biomaterials used for brain protection repair and regeneration will be presented.

Brain tissue engineering has several limitations and many unanswered questions or concerns should be addressed before reaching the clinic. Moreover, with the current workflow, it takes a long time to select an effective brain tissue engineering strategy to translate into clinical studies. Additionally, recent developments in understanding the basic biology of brain tissue formation in physiological and pathological conditions have resulted in an explosion in the numbers of tissue engineering products that could be potential candidates for treating brain disorders. Screening platforms that bridge the gap between conventional tissue culture and animal models would help to improve understanding of cell-based therapies and optimize central nervous system (CNS) tissue engineering. In this regard, the usefulness of 3D brain organotypic cultures in CNS research as well as in the drug discovery process will be discussed in this book chapter.

2. Adult cell therapy for brain neuronal damages

2.1 Adult cells for cerebral ischemia and traumatic brain injury therapy

2.1.1 Adult stem cell therapy

Mesenchymal stem cells (MSCs) are the most widely investigated adult stem cells for brain cell therapy of cerebral ischemia and TBI (Table 1). Various animal models of ischemia have been used to investigate the therapeutic effects of MSCs on the lesioned brain and in all these models, bone marrow-derived MSCs resulted in an increased survival of neurons and most of the time in an improved cognitive function of the animals (reviewed in (van Velthoven et al. 2009)). In addition, MSCs have been described to reduce the thickness of the scar walls (Li et al., 2005) and may also favour angiogenesis (Chen et al., 2003; Chopp et al., 2008) as well as synaptogenesis (Chen & Chopp, 2006). Functional improvements may be observed whatever the implantation route, intravenously or intracerebrally, even if reduction in infarct volume is not always observed (Li et al., 2000). This underlines the capacity of MSCs to migrate towards lesions, as was already observed by other groups

(Chen et al., 2001) after IV injection, or after intracerebral graft in a lesioned rat brain (Sykova & Jendelova, 2007; Delcroix et al., 2009). Noteworthy, *in situ* neural or neuronal differentiation of transplanted MSCs has been described in several studies. For example, systemic or intracarotid artery administration of MSCs in rat models of ischemia improve neurogenesis and functional recovery, with the detection of neuronal or glial markers expressed by a fraction of MSCs in the brain (van Velthoven et al., 2009; Li et al., 2002; Esneault et al., 2008; Perasso et al., 2011). However, due to the low grafted cell survival and neural/neuronal differentiation, neurological benefits are often assumed to mainly derive from the increased production of growth factors and other paracrine factors from MSCs in the ischemic tissue (England, 2009; Yarygin et al., 2009). Factors secreted by the ischemic brain itself, such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO), are also thought to play a major role in brain protection from ischemia (Tang et al., 2006). MSCs combined with growth factors also provided functional effects and in some cases reduced the lesion volume, the number of apoptotic cells within the ischemic lesion and stimulated host repair responses as recruitment of host progenitor cells (Esneault et al., 2008; Rahnemai-Azar et al., 2011; van Velthoven et al., 2009). Modulation of inflammatory and immune response or production of neuroprotective chemokines such as fractalkine and monocyte chemoattractant protein-1 (MCP-1) by MSCs are others mechanisms that may be involved in neuronal protection during ischemia (Re & Przedborski, 2006; Ohtaki et al., 2008; Madrigal et al., 2009; Garbayo et al., 2011). Recruitment of host progenitor cells may also contribute to MSC-induced repair processes in response to chemokines [growth-related oncogene (GRO), MCP-1] secreted by the implanted cells (Rahnemai-Azar et al., 2011; Gordon et al., 2009). After a first clinical trial that underwent criticisms a few years ago (Bang et al., 2005; De Keyser, 2005), there is a growing number of phase I-II clinical trials to evaluate the safety and efficacy of MSCs or CD34+ cells delivered either by arterial infusion, IV or intracranial injection in the context of stroke (see clinicaltrials.gov for up to date informations). Moreover, two consecutive meetings have been held recently named the Stem cell Therapies as an Emerging Paradigm in Stroke (STEPS I & II), in order to provide guidelines for preclinical and clinical studies leading to the successful development of cell therapy for stroke (Broderick, 2009; Savitz et al. 2011).

In the context of TBI, MSCs may also improve functional benefits whatever the injection route. However, a maximum of 10 % implanted MSCs may transdifferentiate into neuronal cells *in vivo* so that, again, this mechanism was assumed not to be solely responsible for the functional benefits observed in TBI animal models (Li & Chopp, 2009). Growth factor production by MSCs stimulated by the lesioned brain (Chen et al., 2002) was certainly the main mechanism leading to functional recovery by promoting glial, neuronal and blood vascular remodelling (Li & Chopp, 2009; Richardson et al., 2007). However, despite the benefits obtained using this approach, no significant changes in the lesion volume were observed, a problem that tissue engineering alternatives may resolve (see section 3 of this chapter). Concerning adult neural stem cells (NSCs), very few studies were performed in the context of stroke, and none for TBI. It is however interesting to note that a fraction of adult NSCs, either derived from rat hippocampus (Zhang et al., 2011) or from human wisdom teeth (Yang, Chen et al., 2009), survived within the brain after transplantation and led to significant improvements in stroke. Another population of CD31-/CD146- cells isolated from dental pulp has also been used in stroke rat models, leading to migration and differentiation of the endogenous neuronal progenitor cells as well as vasculogenesis (Sugiyama et al., 2011).

Cells	Origins	Recipients	Scaffolds	Benefits	References
MSC	Mouse	MCAO mouse model		Functional benefits but no reduction in infarct volume	Li, Chopp et al. 2000
	Rat	MCAO rat model		IV transplantation: migration toward the lesion	Chen, Li et al. 2001
	Human	MCAO rat model		IV transplantation: importance of growth factor increase in the ischemic tissue	Li, Chen et al. 2002
	Rat	MCAO rat model		IV transplantation in combination with EPO: functional recovery and neurogenesis A fraction of MSCs with neuronal and glial markers	Esneault, Pacary et al. 2008
	Rat	MCAO rat model		IV transplantation: reduced thickness of the scar walls	Li, Chen et al. 2005
	Human	MCAO rat model		IV transplantation: promotion of angiogenesis	Chen, Zhang et al. 2003
	Human	tCCAO mouse model		Modulation of inflammatory and immune responses	Ohtaki, Ylostalo et al. 2008
	Mouse	2VO rat model		IV transplantation: increased number of pyramidal CA1 neurons	Perasso, Cogo et al. 2011
	Human	ACA rat model	FN-coated PLGA microspheres	Neuroprotection of CA1 hippocampal neurons with MIAMI cells: neuroprotection enhanced with microspheres	Garbayo, Raval et al. 2011
	Human	TBI rat model	Collagen cylinder	Scaffolds improved spatial learning, sensorimotor function & reduced the lesion volume	Lu, Mahmood et al. 2007
	Human	TBI rat model	Collagen cylinder	Delayed transplantation: increased angiogenesis in the injured cortex & transcallosal fiber length	Xiong, Qu et al. 2009
	Human	TBI mouse model	Collagen cylinder	Scaffolds improved spatial learning, reduced lesion volume & increased vascular density	Qu, Xiong et al. 2009
	Human	Human (autologous, stroke)		Proof of safety for MSCs use in human	Bang, Lee et al. 2005
NSC	Human From teeth	MCAO rat model		Functional benefits	Yang, Chen et al. 2009
	Rat hippocampi	MCAO rat model		Amelioration of neurological deficits Reduced total infarct volume	Zhang, Jin et al. 2011
CD31-/CD146-progenitor cells	Pig Teeth-derived	MCAO rat model		Migration and differentiation of the endogenous neuronal progenitor cells Vasculogenesis	Sugiyama, Iohara et al. 2011
iPS cells	Mouse Embryonic fibroblast-derived	MCAO rat model	Fibrin glue	Sub-dural injection reduced the infarct size compared to iPS cells directly injected in the parenchyma Functional improvements and no teratoma when sub-dural injection with fibrin glue is used	Chen, Chang et al. 2011
	Human Fibroblast-derived	MCAO rat model		Migration of the cells towards injured brain area *In situ* neuronal differentiation of iPS cells Improved sensorimotor function 4-16 days after grafting	Jiang, Lv et al. 2011

Abbreviations: ACA: asphyxial cardiac arrest; EPO: erythropoietin; FN: fibronectin; iPS: induced pluripotent stem; IV: intravenous; MCAO: middle cerebral artery occlusion; MIAMI: marrow isolated adult multilineage inducible; MSC: mesenchymal stem cell; NSC: neural stem cell; tCCAO: transient common carotid artery occlusion, UPDRS: unified Parkinson's disease rating scale.

Table 1. Adult and iPS cell therapies for cerebral ischemia and TBI

2.1.2 Adult derived pluripotent stem cell therapy

A growing interest is now observed in the scientific community for the iPS cells. These "embryonic-like" cells were primarily derived from adult fibroblasts by the expression of transcription factors (a combination of 4 factors within c-Myc, KLF4, LIN28, Nanog, Oct3/4 and Sox2) (Takahashi et al., 2007; Yu et al., 2007). Several applications are currently envisioned for these pluripotent cells, which share the same potential than ES cells, without the associated ethical problems. In addition to their applications for drug discovery and toxicity testing (Laustriat et al., 2010) these cells may be helpful for complex disease understanding. They are also attractive for cell therapy studies, due to the possibility to obtain a large amount of cells from various lineages and to perform autologous grafts. iPS have now been derived from several types of adult tissues, with variable efficiency, and always using refined protocols to avoid permanent genomic integration and the use of lentiviruses for transducing the cells (Okita et al., 2011; Narsinh, et al., 2011; Lee et al., 2009; Gonzalez et al., 2009; Zhou et al., 2009; Page et al., 2009; Yu et al., 2009). Indeed, uses of viral-based protocols may be at the origins of tumour formation and may also lead to further difficulties to differentiate the cells toward a given phenotype (Yu et al., 2009). The propensity of iPS to form teratomas may also be related to the tissue of origin of the cells (Miura et al., 2009). Despite their great interest, one has to keep in mind that further developments are still required to fully understand their reprogramming process (Nakagawa & Yamanaka, 2011) in order to provide a safe cell therapeutic product for the

clinic (Pera, 2011). In the context of ischemic stroke, an interesting study describes the use of iPS-derived neurons and astrocytes directly injected in the damaged cortex of a stroke animal model (Chen et al., 2011), which resulted in a reduced ischemic size with functional improvements. Another study describes the *in situ* migration and differentiation of human iPS cells in a similar context, with an improved sensorimotor function of the animals 4-16 days after grafting (Jiang et al. 2011).

2.2 Adult cells for neurodegenerative disorder therapy

Huntington's and Parkinson's diseases (HD and PD respectively) have been widely studied in cell therapy programs due to the relatively small area affected by the diseases, particularly at early stages (see for reviews (Lindvall & Kokaia, 2009; Loewenbruck & Storch, 2011)). In opposition, Alzheimer's disease (AD) would be more complex to treat due to the multiple sites of the brain affected in this disease. Moreover, no tissue engineering strategies have yet been described for AD so that we will only focus on HD and PD in the following (Table 2).

2.2.1 Adult cell therapy

An increasing number of studies performed with foetal tissue, ES cells or NSC grafts, mostly in quinolinic acid (QA)-induced animal models of the disease (Clelland et al., 2008) gave successful results leading to clinical trials with foetal-derived cells. However, cell therapy for HD, an incurable disease, is still not widely available in clinic due to ethical, logistical or safety concerns (Kelly et al., 2009). Adult cells have only been used in early studies with Sertoli cell grafts that protected the lesioned area in an animal model of HD; effects that may result from Sertoli cells trophic and anti-inflammatory potentials (Emerich, 2004).

One of the first cell therapy strategies to treat PD has been performed with adult cell types synthesizing dopamine or its precursor L-DOPA, in order to replenish the striatum level of dopamine (for review (Drucker-Colin & Verdugo-Diaz, 2004; Fernandez-Espejo et al., 2005). Autologous adrenal medulla tissue were grafted in the caudate nucleus of 2 young PD patients, but observed functional improvements were transitory and mainly due to trophic effects as only 1 % of the cells synthesized dopamine (Madrazo et al., 1987). Most importantly, very few cells were detected 1 or 2 years after transplantation (Hurtig et al., 1989). Other studies were performed using cultured chromaffin cell suspensions, but few surviving cells in long term studies did not validate this approach (Drucker-Colin et al., 1999). Human retinal pigment epithelium (hRPE) cells derived from the inner layer of the neural retina are isolated from human eye bank for transplantation purposes (reviewed in (Stover & Watts, 2008)). hRPE cells may be expanded, present a tyroxine hydroxylase activity (Pawelek & Korner, 1982), and produce L-DOPA, which is synthesized as a melanin precursor, so that these cells may supplement the oral administration of L-DOPA *in situ*. Moreover, it has been proposed that hRPE cells may be immune-privileged after transplantation due to their expression of Fas-ligand (Griffith et al., 1995; Jorgensen et al., 1998).

2.2.2 Adult stem cell therapy

Adult stem cells are expected to better differentiate and integrate the host brain compared to adult non-stem cells and are therefore widely investigated for their therapeutic potential in the context of neurodegenerative disorders. Whole bone marrow cells [i.e. hematopoietic

stem cells (HSCs) & MSCs] implanted into the bilateral lesioned striatum of HD rat models, reversed functional deficits such as working memory (Lescaudron et al., 2003) even if the cell population responsible for the beneficial effects remained unknown. Noteworthy, autologous grafts may not be an appropriate strategy for the treatment of HD as transplanted cells would also carry the mutant huntingtin gene responsible for the disease. More recently, transplantation of MSCs, either intracerebrally or intravenously, resulted in a decreased atrophy of lesioned rat striatum (Amin et al., 2008) and in some functional benefits (Edalatmanesh et al., 2009) even though only a fraction of cells (1 %) expressed neural phenotypes. Thus, it was suggested that MSCs, by producing growth factors, allowed surviving cells within the caudate nucleus to function more efficiently and to facilitate other compensatory responses (Dunbar et al., 2006). In this regard, another study demonstrated the importance of factors such as stem cell factor (SCF), produced *in situ* in the lesioned striatum, to promote the migration and engraftment of MSCs *via* SCF receptor c-kit (Bantubungi et al., 2008).

In the context of PD, several teams, including ours, reported the neuronal differentiation of human MSCs toward a dopaminergic phenotype *in vitro*, indicating that these cells may constitute an alternative dopamine secreting source of cells (Trzaska & Rameshwar, 2011; Barzilay et al., 2008; Trzaska et al., 2007; Tatard et al., 2007). An interesting study also reported no major differences between MSCs from normal patients compared to MSCs isolated from parkinsonian patients, which may be induced to produce up to 30 % of dopaminergic neurons *in vitro* (Zhang et al., 2008). Human MSCs, pre-induced towards a neuronal phenotype and transplanted in the totally dopaminergic deafferented striatum of rats, led to an improved functional recovery for up to 4 months *c*ompared to naïve hMSCs (Levy et al., 2008). A similar recovery has been observed in a rat partial lesion model of PD, in which dopaminergic fibers are spared in the striatum (Bouchez et al., 2008). Moreover, microdialysis demonstrated that part of the striatal pool of dopamine was restored upon MSCs transplantation. Naïve hMSCs or a subpopulation of hMSC pre-treated with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) also led to an efficient recovery after partial lesions, suggesting a paracrine restorative effect by MSCs. Indeed, hMSCs, that migrate toward lesions (Hellmann et al., 2006; Sadan et al., 2009; Delcroix et al., 2011) may induce a protective or restorative effect on the remaining neurons within the host brain, due to the secretion of a large panel of neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), which may also enhance endogenous neurogenesis (Levy et al., 2008; McCoy et al., 2008; Bahat-Stroomza et al., 2009; Sadan et al., 2009). MSCs may also modulate the host response to the lesion (Kim et al., 2008) and probably ultimately replace functional cells within the host brain as a few MSCs with neuronal-like morphology and markers were observed in a total lesion model (Levy et al., 2008). We are therefore starting to better understand the mechanisms of action of these cells in the context of PD, while other teams try, with interesting results, to find new ways to deliver the cells to the brain. Indeed recently, a non invasive intranasal delivery of MSC in an animal model of PD, with significant improvements in the functional outcome of the animals was reported (Danielyan et al., 2011). The growing amount of MSC therapy studies for PD is starting to set the ground for pre-clinical studies, and a trial is already underway to evaluate the safety and efficiency of intrastriatal grafts of autologous MSCs to treat PD (Jaslok Hospital and Research Centre, India, sources clinicaltrials.gov).

NSCs isolated from the adult brain have been evaluated for the treatment of PD and HD due to their potential to differentiate into neurons *in vivo* (Lois & Alvarez-Buylla, 1993), despite the problems encountered for their isolation. A study reported the transplantation of NSCs from adult rat SVZ in a rat model of HD, with NSC survival up to 8 weeks after grafting and migration throughout the brain. To a larger extent than MSCs, up to 15 % of adult NSCs differentiated into mature neurons with specific markers of striatal medium spiny projection neurons and interneurons (Vazey et al., 2006). In addition, priming these cells with lithium chloride resulted in an even further improved functional outcome of the grafted animals (Vazey & Connor, 2011). To treat PD, a clinical trial has been performed with adult NSCs (reviewed in (Arias-Carrion & Yuan, 2009)) and gave interesting proof of concept for the autologous use of these cells. NSCs were isolated from the patient's brain during the insertion of a thalamic stimulator and, after expansion and differentiation, a total of 6 millions cells, and among them GABAergic and dopaminergic cells, were grafted 9 months later into the patient's post-commissural putamen. An improvement in the Unified Parkinson's Disease Rating Scale (UPDRS) score was observed over the next 36 months, although results returned to baseline at 5 years post-operation (Levesque, 2009).

2.2.3 Adult cell-derived pluripotent stem cell therapy

As for several other disorders, iPS cell have been evaluated for PD cell therapy (Table 2). Dopaminergic neurons have efficiently been derived from iPS cells, and those cells have led to functional recovery in an animal model of PD (Wernig et al., 2008; Swistowski et al., 2010). In addition, it was recently demonstrated that iPS derived from patients suffering from idiopatic PD, after dopaminergic differentiation *in vitro*, led to an efficient functional recovery of the grafted animals (Hargus et al., 2010).

To conclude this section, it appears clearly that MSCs were, and still are, the most widely investigated adult cells for brain cell therapy. However, their neuronal differentiation potential remains very low or uncertain after transplantation, explaining the scientific interest of the recently discovered iPS cells. In addition, the poor cell survival and engraftment observed when using chromaffin cells, hRPE cells, MSCs and in general all kinds of transplanted cells, has called into question the efficacy of a cell therapy procedure. These issues may now be acknowledged by tissue engineering approaches, discussed in the following section.

3. Combined use of adult stem cells and scaffolds for cell delivery and regeneration of CNS disorders

It is now widely admitted that cell survival, differentiation, and more generally behaviour of cells *in vivo* may be greatly enhanced using adequate biomaterial supports. These supportive elements, called scaffolds, may be of various compositions and shapes and may improve cell behaviour due to the 3D environment as well as to the mechanical and signalling cues they provide to transplanted cells. In this regard, scaffolds may for example stimulate cell survival. These types of tissue engineering strategies for brain cell therapy have been primarily developed with neuronal cell lines (PC12 cells) or with cultured dorsal root ganglion neurons and foetal NSCs due to their availability, ease of expansion and their natural ability to integrate and differentiate within the brain. Benefits gained using these tissue engineering approaches are now being translated to adult cells such as MSCs in order to improve their survival, to guide their differentiation and integration within the host brain. In the following section, we describe the properties required for a scaffold in tissue

engineering applications within the brain, before reviewing the strategies used to improve brain cell therapy by combination with a tissue engineering approach. Improvement of classical scaffolds by means of a biomimetic approach as well as a novel strategy currently developed in our laboratory, the Pharmacologically Active Microcarriers (PAMs), will be presented.

	Cells	Origins	Recipients	Scaffolds	Benefits	References
HD	Sertoli		3-NP model		Possible modulation of local inflammation	Emerich 2004
	Choroid plexus	Rat	QA rat model	Alginate microcapsules	Selective neuroprotection, no evidence for sparing of striatal neurons	Borlongan, Thanos et al. 2008
	HSC+MSC	Rat	QA rat model		Reduction of working memory deficits	Lescaudron, Unni et al. 2003
	MSC	Rat	QA rat model		Decreased striatal atrophy	Amin, Reza et al. 2008
		Rat	QA rat model		Attraction of MSCs by SCF production in the striatum	Bantubungi, Blum et al. 2008
		Rat	QA rat model		IV transplantation: improved motor & cognitive performance	Edalatmanesh, Matin et al. 2009
	Adult NSC (SVZ)	Rat	QA rat model		Survival at 8 weeks, migration & 15 % of cells differentiated in mature neurons with marker of striatal medium spiny neurons	Vazey, Chen et al. 2006
		Rat	QA rat model		Lithium chloride priming of the NSCs resulted in accelerated functional outcome of the grafted animals Increased formation of projections from newly formed neurons in the damaged host striatum to the globus pallidus	Vazey and Connor 2011
PD	Adrenal chromaffin	Human	Human (autologous)		Functional improvements observed up to 10 months	Madrazo, Drucker-Colin et al. 1987
		Human	Human (autologous)		No cell detected after 1-2 years	Hurtig, Joyce et al. 1989
		Rat	6-OHDA rat model (unilateral)	Cytodex® Glass beads	Increased survival and functional benefits (8 months)	Cherksey, Sapirstein et al. 1996
		Rat	6-OHDA rat model (unilateral)	Cytodex® Glass beads	Increased survival and functional benefits (12 months)	Borlongan, Saporta et al. 1998
		Human	Human		Improved dopamine production with in vitro differentiation	Drucker-Colin, Verdugo-Diaz et al. 1999
	hRPE	Human	6-OHDA rat model (uni & bilateral)	Gelatine microcarriers (Spheramine®)	Functional recovery with chronic inflammation at late time-point	Flores, Cepeda et al. 2007
		Human	MPTP primate model	Gelatine microcarriers (Spheramine®)	Long term functional improvements an cell survival (18 months) No immunosuppression required	Doudet, Cornfeldt et al. 2004
		Human	Human	Gelatine microcarriers (Spheramine®)	Open-label study: good tolerability, sustained motor clinical improvement up to 6 months after grafting	Bakay, Raiser et al. 2004
		Human	Human	Gelatine microcarriers (Spheramine®)	Analysis of the brain of a patient who underwent the last trial performed with Spheramine® (phase II double-blind, randomized, multicenter, placebo-controlled clinical) with no significant functional improvements 6 months after grafting, only 0.036 % of surviving cells	Farag, Vinters et al. 2009
	MSC	Rat	6-OHDA rat model (unilateral & partial)		Adipose tissue-derived MSCs secreting BDNF, GDNF & NGF Functional recovery after transplantation in SN but no neuronal differentiation *in vivo*	McCoy, Martinez et al. 2008
		Human	6-OHDA rat model (unilateral & partial)		Induction of MSCs to secrete GDNF & BDNF Transplanted on the day of lesion, MSCs migrated toward the lesioned striatum and had a regenerative effect	Sadan, Bahat-Stromza et al. 2009
		Human	6-OHDA rat model (unilateral & total)		Improved functional recovery with differentiated cells (TH expression and DOPA secretion)	Levy, Bahat-Stroomza et al. 2008
		Human	6-OHDA rat model (unilateral & partial)		Trophic, restorative effect of MSCs	Bouchez, Sensebe et al. 2008
		Human	6-OHDA rat model (unilateral & partial)	PAMs with LM surface & releasing NT3	With PAMs: Increased MIAMI cells survival and differentiation *in vivo* Neuroprotection, neuroreparation of the nigrostriatal pathway Functional recovery	Delcroix, Garbayo et al. 2011
	MSC	Rat	6-OHDA rat model (unilateral & total)		Innovative intranasal delivery Migration of the cells throughout the brain 24 % of cells survival 4.5 months after grafting Improvement of motor function of the Parkinsonian forepaw	Danielyan, Schafer et al. 2011
	Adult NSC	Human	Human (autologous) Cortical and sub-cortical tissue-derived		After expansion and differentiation *in vitro*, NSCs improved UPDRS score over 36 months after grafting Back to baseline 5 years post-operation	Levesque 2009
	iPS cells	Mouse Fibroblast-derived	6-OHDA rat model (unilateral & partial)		Neuronal differentiation *in vivo* (TH-positive cells) Functional improvements 4 weeks after grafting	Wernig, Zhao et al. 2008
		Human Foetal-lung fibroblast-derived	6-OHDA rat model (unilateral & total)		Survival of iPSC-derived dopaminergic neurons *in vivo* Functional improvements 12 weeks after grafting	Swistowski, Peng et al. 2010
		PD patient Fibroblast-derived	6-OHDA rat model		Survival of iPS cell-derived DA neurons, with arborization Functional improvements 16 weeks after grafting Proof of concept for the autologous use of iPS cells	Hargus, Cooper et al. 2010

Abbreviations: AD: Alzheimer's disease; BDNF: brain-derived neurotrophic factor; GDNF: glial cell line derived neurotrophic factor; HD: Huntington's disease; hRPE: human retinal pigment epithelium; HSC: hematopoietic stem cell; iPS: induced pluripotent stem; IV: intravenous; LM: laminin; MIAMI: marrow isolated adult multilineage inducible; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSC: mesenchymal stem cell; NP: nitropropionic acid; NSC: neural stem cell; NT3: neurotrophin 3; OHDA: hydroxydopamine; PAMs: pharmacologically active microcarriers; PD: Parkinson's Disease; QA: quinolinic acid; SCF: stem cell factor; SN: substantia nigra; UPDRS: unified Parkinson's disease rating scale.

Table 2. Adult and iPS cell therapies for neurodegenerative disorders

3.1 Scaffold requirements for brain cell therapy

Implementation of tissue engineering in combination with adult cells for brain therapy is an emerging field and many requirements need to be taken into account in order to produce an appropriate tissue engineered product. The first consideration in scaffold tailoring for brain is size, but scaffolds must also be fully biodegradable and biocompatible, minimizing macrophage and microglial reaction, without inducing neurotoxicity. Scaffolds must be small enough to be easily implanted into the skull cavity in discrete and precise areas of the brain without perturbing the brain 3D organisation as observed with 30 µm microspheres (Veziers et al., 2001). Moreover, small-sized scaffolds render repeated implantations possible by stereotaxy, with no need for open-surgery (Menei et al., 2005). In this sense, microstructured and nanostructured scaffolds, produced by various techniques may be used (reviewed in (Seidlits et al., 2008)).

Scaffolds are based either on natural or on synthetic biomaterials used alone or in mixtures, providing scaffolds with different properties (see for review (Potter et al., 2008; Dalton & Mey, 2009). Scaffolds based on biodegradable gels encapsulating various molecules and cells have been studied, with e.g. polyethylene glycol (PEG) (Namba et al., 2009), diblock copolypeptide (Yang, Song et al., 2009) or hyaluronic acid (Wang & Spector, 2009) hydrogels. However, gel-based scaffold strategies most of the time require open-surgery, unless an *in situ* gelling process is used (Kim, 2009; Yang, Song et al., 2009) but no gel swelling must be observed. In opposition to gel-based scaffolds, particulate scaffolds, that may be constituted of aliphatic polyesters, including poly(lactic-co-glycolic acid) (PLGA), overcome this problem of delivery and have been intensively studied with embryonic cells and NSCs (Newman & McBurney, 2004; Bible et al., 2009). Cell-material interactions may be advantageously increased due to a large specific surface as with microsphere-shaped scaffolds or scaffolds based on nanofibrous technology (Valmikinathan et al., 2008; Cao et al., 2009).

The adverse host cell response, such as glial scar and inflammation, occurring after scaffold implantation have to be minimized (see for review (Fournier et al., 2003)) therefore rendering the biocompatibility and biodegradability of the biomaterials a crucial parameter (Vert, 2009; Yang, Song et al., 2009). For example, implantation of PLGA microspheres into the brain does not induce a specific astrocytic or macrophage/microglia reaction, which is similar to the one observed after control fluid injection. Moreover, PLGA microspheres fully degrade into CO_2 and H_2O (Menei et al., 2005). Scaffolds should be able to degrade with time, with degradation products that may also be eliminated by the host, allowing a full integration of transplanted cells into the brain. This criteria was not observed for synthetic poly(methylidene malonate 2.1.2) microspheres implanted into rat brains even if biocompatibility of the intact microspheres was satisfactory (Fournier et al., 2006). It is interesting to note that size of particles may also affect the extent of the host response. *In vitro*, small size phagocytable hydroxyapatite particles (1-30 µm) have been shown to induce a strong production of the inflammatory cytokines tumor necrosis factor-alpha (TNFα), interleukin 6 (IL6) and interleukin 10 (IL10) by human monocytes, the first cells recruited to the inflammation site, which may be correlated to a stronger host inflammatory response. On the other hand, this effect decreased for particles of more than 30 µm in diameter. Importantly, shape is also critical for the extent of the response, needle-shape being potentially more detrimental compared to spherical-shaped particles (Laquerriere et al., 2003).

Cell attachment to the biomaterial critically depends on its surface charges, cells being attracted to positive charged surface due to sialic acid residues on the cell membrane which

produce a net negative charge on the cell surface. Furthermore, the first step following implantation of a scaffold within the brain is its coverage by a non-specific layer of proteins, which may contribute to the inflammation process and biocompatibility problems (Fournier et al., 2003). Surface characteristics (charge, hydrophilicity and hydrophobicity) are therefore important points to consider when designing a new type of scaffold. Outer but also inner topography of scaffolds may affect cell behaviour once implanted into the brain. For example, the presence of pores and channels on the surface of synthetic poly-ε-caprolactone (PCL) scaffolds may enhance host astrocytic infiltration and affect host cell migration (Wong et al., 2008). Moreover, access to nutrients is a critical parameter for neuronal cells, which require large amounts of nutrients such as glucose. Therefore, if larger implants are used, vascularisation is required for cell survival. In this sense, porous scaffolds, or scaffolds that become porous after implantation during degradation, may alleviate vascularisation problems.

Finally, a very interesting study first described the effects of matrix elasticity to direct MSCs lineage specification. Soft type matrices mimicking brain being neurogenic while stiffer matrices appeared to be myogenic and furthermore osteogenic. The observed phenotypic specification was irreversible after several weeks in culture, therefore reflecting neuronal commitment of the MSCs cultivated on soft matrices (Engler et al., 2006). Noteworthy, new data from this team seem to indicate that stiffness variation, not just stiffness alone, can be an important regulator of MSCs behaviour (Tse & Engler, 2011). Again, this underlines the importance of the choice of the biomaterial for brain tissue engineering.

3.2 Scaffold design and manufacture

The following section reviews materials and fabrication methods used in the development of scaffolds to enhance brain tissue regeneration.

3.2.1 Materials

One of the first considerations when designing a scaffold for brain tissue engineering is the choice of the material. Some of the aspects that should be carefully taken into account are: 1) if the material maintains an appropriate shape after implantation, 2) if sterilization of the scaffold prior to implantation is possible, 3) if the scaffold is brain biocompatible to avoid abnormal immune responses, 4) if the material has the appropriate degradation rate for the desired application and 5) the ability to provide a controlled release of the drug, in case of materials encapsulating a therapeutic molecule such as growth factors. Materials used in the development of scaffolds for brain tissue engineering can be broadly divided into biodegradable and non-biodegradable. Due to the transient nature of the biodegradable polymers, they are preferred for a brain application because they do not require surgical removal when the treatment is finished. Depending on their nature they can be classified in natural and synthetic materials. Both of them used alone or in combination have been tested in clinical practice. Natural materials are very interesting because they contain sites for cell adhesion, allowing for cell infiltration. These natural materials also exhibit similar properties to the soft tissues they are replacing. However, since these materials are obtained from natural sources, they must be purified before use and it is difficult to control the homogeneity of product between batches. Synthetic materials in contrast, have a known composition and can be designed to minimize the immune response or the degradation rate.

Materials (natural and synthetic) used in brain tissue engineering applications, with their benefits and drawbacks will be discussed below in this section.

3.2.1.1 Natural materials

Collagen. Collagen, one of the most common extracellular matrix (ECM) proteins, has been extensively used as a potential scaffold for neural tissue. Collagen is an easily accessible material that can be isolated from mammals, including rats, bovines and humans. One advantage of collagen use is that the scaffold properties can be easily varied by using different concentrations of collagen or by covalently modifying the cell adhesion sites. However, immune response could arise if cross-species transplantation is used. In the CNS collagen has been mainly used as scaffolds for the treatment of TBI among others (Qu et al., 2009; Qu et al. 2011).

Alginate. Alginate is a natural anionic polysaccharide composed of D-mannuronic (M) and L-guluronic (G) residues in varying proportions. It is easily obtained from algae and can be cross-linked to form three dimensional scaffolds. Cross-linking and gel formation takes place when divalent cations, such as calcium, ionically bind carboxylic acid groups of blocks of guluronic residues between chains. They must undergo extensive purification to prevent immune responses after implantation. The main use for alginate in the area of brain tissue engineering is in encapsulating cells with the purpose of immune-isolation from the host. Its efficacy has been evaluated in models of CNS diseases like PD, stroke, ALS, spinal cord injury, TBI and HD among others (Orive et al., 2003; Grandoso et al., 2007; Orive et al., 2009; Purcell et al., 2009).

Fibrin. Fibrin is a protein involved in the clotting of blood. Similar to collagen, fibrin scaffolds contain sites for cell adhesion and the scaffold properties vary depending on the concentration of fibrin used. Fibrin matrix has favorable features as a scaffold, that would fit well in the fragile CNS tissue, including biocompatibility, biodegradability, binding capacity to the tissue, low risk of foreign body reaction, physiological flexibility, good plasticity. Fibrin scaffolds have been proposed as potentially suitable vehicle for cell transplantation therapy and, combined with bone marrow stroma cells, have been evaluated after cortical injury in rats (Yasuda et al., 2010).

Chitosan. Chitosan is a polysaccharide industrially derived from partial deacetylation of chitin, the major compound of exoskeletons in crustaceans. Chitosan has been reported to be suitable for preparation of particulate systems (micro and nanoparticles) for brain application due to its good biodegradability, biocompatibility, stability and low toxicity. Chitosan nanoparticles coated with polysorbate 80 have been proposed for brain targeting (Aktas et al., 2005). However, despite its desirable characteristic, its actual use is limited because of its poor solubility in water.

Other natural materials. Other examples of natural materials used in the fabrication of brain tissue scaffolds are dextran, fibronectin (FN), laminin (LM) or hyaluronic acid. An interesting approach used hyaluronic acid and collagen to develop a tridimensional biodegradable porous scaffold which is a sponge with an open porous structure and mechanical behaviour comparable to brain tissue (Wang & Spector, 2009). Dextran hydrogels have been investigated as drug delivery vehicles and as macroporous scaffolds However, the dextran non-cell-adhesive nature has limited its use in tissue engineering. To overcome this limitations, Levesque *et al*., proposed the use of macroporous scaffolds of methacrylated dextran (Dex-MA) copolymerized with aminoethyl methacrylate (AEMA) introducing primary amine groups for covalent immobilization of extracellular-matrix-

derived peptides for axonal guidance (Levesque & Shoichet, 2006). Recently, the use of bioactive scaffolds generated by cryogelation of dextran or gelatine linked to LM to create niche-like structures that promote the differentiation of stem cells was proposed (Jurga et al. 2011). LM and FN scaffolds have been proposed as appropriate extracellular-matrix based scaffolds that can be exploited to improve cell transplantation into the injured brain (Tate et al., 2009).

3.2.1.2 Synthetic materials

Poly(α-hydroxyacids). Poly (α-hydroxyacids) were found to be bioabsorbable and biocompatible in the 1960's. They are the most widely known, studied and used polymers for brain drug delivery due to its biodegradable properties, biocompatibility and the absence of significant toxicity (Athanasiou et al., 1996; Shive & Anderson, 1997). They have suitable mechanical properties for its use in scaffold fabrication. Poly (α-hydroxyacids) are approved by the Food and Drug Administration (FDA) for its use in humans and have been used extensively in medicine in a variety of applications. For instance, more than 100 different molecules have been incorporated in PLGA microspheres since their first application (Menei et al., 2005). Poly (α-hydroxyacids) are constituted of lactic and/or glycolic acid units and degrade *in vivo* by nonenzymatic hydrolysis to lactic and glycolic acids. These can be further metabolized or excreted via normal physiological pathways.

Polycaprolactone (PCL). PCL is a biodegradable polyester with minimal bio-reactivity that has been widely used in the biomedical field and in CNS applications in particular. PCL degrades very slowly by hydrolysis of ester bonds. PCL based matrices have been used for instances, as scaffolds for tissue regeneration after controlled cortical impact induced-TBI (Wong et al., 2008).

Others. Other examples of synthetic materials used in the fabrication of brain tissue scaffolds are poly (glycerol sebacate) (PGS), gelatin-siloxane (GS), poly (ethylene glycol)/poly (ethylene oxide), poly (ethylene-co-vinyl acetate) (EVA), poly (2-hydrixyethil methacrylate) (pHEMA) and poly (2-hydroxyethyl methacrylate-co-methyl methacrylate) (pHEMA-MMA).

3.2.2 Scaffold fabrication techniques

As brain tissue engineering progresses, the need of novel scaffold structures and fabrication techniques has become of great importance (See Walker et al., 2009; Subia, 2010). The most common scaffold production methods are revised below. After fabrication, all of these scaffolds are then characterized in terms of morphology, mechanical, bulk and surface properties.

Phase separation. Phase separation technique is based on temperature changes to separate a polymeric solution in two phases. When liquid-liquid phase is separated, it is quenched to form two solid phases. Finally, solvent is removed and porous scaffolds are obtained. Biological molecules can be incorporated to the polymeric solution to obtain drug-loaded scaffolds. Phase separation technique can be combined with other fabrication techniques like leaching or prototyping to create 3D-scaffolds with controlled pore morphology.

Particulate Leaching. Leaching is a reproducible technique to fabricate porous, sponge-like scaffolds with a desirable cellular structure for tissue engineering applications. Salt, wax or sugars known as porogens are used to create porous scaffolds. The fabrication process

involves casting the polymer mixed with a porogen into a desired shape, and then introducing a solvent that would dissolve the salt and leave the polymer intact, leaving a foam-like structure.

Electrospinning. Electrospinning is an efficient method to produce nano or micro-scaffolds comparable to the ECM fibers natively found in the tissue. In this fabrication method, a polymer solution is pumped through a syringe connected to a high voltage source. As a droplet forms at the needle tip, electrostatic repulsions form long fibers that are collected onto a grounded metal plate in the form of a nanofibrous mat. Due to the simplicity of this method, it has received considerable attention for use in tissue engineering and more than 200 polymers have been used for electrospinning. However, it is difficult to obtain 3D scaffolds with well defined pore structure and complex geometry using this technique and electrospinning is preferably used to produce thin 2D sheets.

Rapid prototyping. Rapid prototyping describes different manufacturing processes that allow automated fabrication using methods of material bonding or deposition. This fabrication method produces objects with geometry difficult to be created using the "traditional" machining methods of milling, turning, or drilling. Generally, files generated using standard computer aided design (CAD) software are used to produce the scaffolds.

Other techniques. Stereolithography uses a laser and a photosensitive liquid polymer solution to fabricate scaffolds. Three dimensional printing has been employed to fabricate porous scaffolds by inkjet printing liquid blinder droplets onto particulate matter.

3.3 Overview of strategies used to combine adult stem cells and scaffolds

The following section reviews primary studies that investigated the potential of different combinations of cells and scaffolds to improve the cell therapy benefits for PD, HD, cerebral ischemia and TBI. Studies on hRPE cells in combination with gelatine-based scaffolds, a device that underwent a phase II clinical trial for the treatment of PD will also be described. Several strategies are now focusing on improving cell interactions with the biomaterials by modifying its surface using biological molecules, mainly derived from ECM, to better regulate grafted cell behaviour. After briefly describing the molecular mechanisms of cell-ECM molecule interactions and its effect on cell behaviour, we will present some studies using such "biomimetic scaffolds". Finally, the PAMs developed in our laboratory, that constitute a more advanced approach combining a bioactive surface with the controlled delivery of a growth factor will be presented.

3.3.1 Gel-based scaffolds and cell encapsulating technology

By contributing to maintain brain's integrity as well as by favouring the integration of host cells inside the marginal cavities, gel-based scaffolds, without cells, may be advantageous to repair the brain after an ischemic stroke (Yamashita et al., 2009). Moreover, differentiated iPS injected together with a fibrin glue under the dura mater enhanced the effect of these iPS injected alone, which led to a reduced infarct volume as well as to functional recovery after an ischemic stroke; thereby underlining the importance of a support for the cells (Chen & Xiao, 2011).

In the context of TBI, degradable collagen scaffolds did not reduce the lesion size nor did they improve functional recovery, unless if seeded with hMSCs (Lu et al., 2007). Indeed, four days after TBI, transplantation of a cylindrical collagen scaffold seeded with hMSCs in the lesion cavity induced a reduction in the lesion volume, together with an improved

spatial learning and sensorimotor function of the animals. Recently, the same team reported that delayed transplantation of these complexes (7 days after TBI) further enhanced spatial learning and sensorimotor function, and induced angiogenesis in the injured cortex as well as transcallosal fiber length (Qu et al., 2009; Xiong et al., 2009). In experimental models of TBI, MSCs are usually injected adjacent to the lesion within the parenchyma to avoid injection within the lesion cavity. An advantage of this gel-based strategy was the possible use of 3 fold more MSCs compared to parenchymal injection, therefore increasing the regenerative potential. However, the major limitation was the need for open-surgery to implant the device. This issue may potentially be addressed using an *in situ* gelling process (Perale et al., 2011), even if the possible expansion and ensuing damage to the brain parenchyma of the solidifying gel has to be taken in consideration. Nevertheless, all these studies demonstrate the benefits of a 3D support which are probably improving cell survival, even if the underlying mechanisms are still not always fully understood.

3.3.2 Cell adhesion on particulate scaffolds

As previously described, the major problems encountered when grafting chromaffin cells in the context of PD was the poor cell survival and the absence of long term effects *in vivo* (Drucker-Colin & Verdugo-Diaz, 2004). Two studies gave the proof of concept that particulate scaffolds may enhance survival of cells adhered onto collagen-coated dextran (Cytodex 3®) or glass bead microcarriers into the brain of hemi-parkinsonian rats. The pivotal finding of these studies is that adult rat adrenal chromaffin cells implanted in the brain after attachment to microcarriers retain their ability for a prolonged period (8-12 months) to correct a striatal dopamine deficit as judged by their efficacy in reducing apomorphine-induced rotation (Cherksey et al., 1996; Borlongan et al., 1998). Even if the underlying mechanisms were not studied, it is noteworthy that no inflammation was detected when implanted into the striatum. During the same period, these scaffolds were used for transplanting human FVM cells in similar rat model of PD (Saporta et al., 1997) and the Cytodex® microcarriers allowed an increased cell survival without immunosuppression. This effect was thought to result from the presence of a protective astrocytic cloak around the cell/microcarrier complexes. These data were the first to demonstrate the need for cell attachment to a 3D complex to improve grafted cell survival, and it also underlined the immunomodulatory benefits that may be gained from the use of a tissue engineering strategy.

A similar strategy combines cultured hRPE cells attached to biocompatible, non biodegradable cross-linked porcine gelatine microcarriers, with a mean diameter of 100 µm. Several preliminary studies in parkinsonian rat models (unilateral and bilateral lesions) proved the efficacy of these grafted complexes, named Spheramine® (Watts et al., 2003). An increased survival of hRPE cells, without immunosuppression, and long term functional improvements were observed, although chronic inflammation was reported at later time-points (5 months) (Flores et al., 2007). These microcarriers have also been implanted in the brain of hemi-parkinsonian monkeys and resulted in long term cell survival and functional improvements at 18 months (Doudet et al., 2004). As expected, hRPE cells unattached to microcarriers did not survive well in the brain, and did not produce a lasting therapeutic effect in various PD animal models. These encouraging results led to an open-label clinical study that included 6 patients with advanced PD receiving 325,000 hRPE cells attached to microcarriers and demonstrated a good tolerability to Spheramine®. Moreover, at 6 months

post-operation, the mean UPDRS-M (off) score improved to 34 % from the pre-operation baseline. Half of the patients also demonstrated a reduced Dyskinesia Rating Scale scores (Bakay et al., 2004). The success of this strategy finally led to a phase II double-blind, randomized, multicenter, placebo-controlled (sham surgery) study to evaluate safety, tolerability, and efficacy of Spheramine® implanted bilaterally into the postcommissural putamen of patients with advanced PD, even if the mechanisms underlying the improved survival of hRPE cells upon attachment to gelatin are not yet well elucidated (Stover & Watts, 2008). Unfortunately, preliminary results of the phase II clinical trial seem to indicate that the study failed to demonstrate the efficacy of Spheramine® beyond a remarkable placebo effect. A reason for that may be the lack of long term survival of the cells in the human brain, as only around 0.036 % of cells survival was observed in the brain of a patient who died 6 months after surgery (Farag et al., 2009).

Adult rat choroid plexus cells have the potential to secrete a wide range of growth factors, and have been used as candidate cells for HD cell therapy (Borlongan et al., 2008). In this study, choroid plexus cells were encapsulated in alginate beads to improve cell viability and to prevent host rejection. Transplantation of these cells into rat striatum resulted in an encouraging neuroprotection when a QA lesion was performed 3 days after cell transplantation, even if no evidence was provided concerning the specific sparing of GABAergic medium spiny projection neurons, especially sensitive to degeneration in the context of HD.

3.3.3 Molecular mechanisms of cell adhesion to scaffolds

The ECM contains adherent glycoproteins, glycosaminoglycans and ions. ECM proteins, such as collagen, FN, LM, tenascin and proteoglycans, interact with each other forming a supportive scaffold for the cells within the tissue (see for review (Bosman & Stamenkovic, 2003)). Composition and proportion of its constituents vary depending on the type of tissue, thereby giving different mechanical, chemical or signalling cues to the surrounding cells. We will here focus on FN and LM because of their astonishing variety of effects on cells as well as because of their wide use for brain tissue engineering applications. FN and LM structures have been described a few decades ago and are constituted of an assembly of large polypeptides, all of them having specific interaction sites with other ECM molecules or cell surfaces (Hynes & Yamada, 1982; Engvall & Wewer, 1996; Powell & Kleinman, 1997). LM and FN interact with cells *via* the integrin family of receptors, therefore allowing cell attachment to the matrix and further signal transduction. Integrins are a family of proteins constituted of an α subunit and a β subunit which assemble into distinct integrin receptors having specific binding affinities with ECM molecules (Barczyk et al., 2009). These interactions result in highly complex downstream signalling pathways that originate at the focal adhesion sites, where a variety of proteins (e.g. Ilk, Fak, Src) interact with the integrin tails on their cytoplasmic ends, thereby regulating cell survival, proliferation and differentiation (Hynes, 2002; Chen, 2010).

Potential roles of ECM molecules for brain repair are now widely admitted. Endogenous levels of FN and LM increase in a TBI context *in vivo*, thereby suggesting a reparative role of these molecules (Tate et al., 2007). Tissue repair benefits have also been obtained by transplanting LM-based hyaluronic gel scaffolds, without cells, in a rat model of cortical lesion (Hou et al., 2005). A recent study demonstrated an enhanced survival *in vitro* of human MSCs within a PEG hydrogel modified with RGD peptides, a motif involved in

ECM-cell interactions. In this study, RGD peptides attached to the scaffold provided the required adhesion sites to maintain MSC survival, while soluble peptides resulted in a strong decrease of cell viability. Noteworthy, the presence of a glycine spacer between the RGD peptides and the gel further improved MSC survival, therefore underlining the importance of the presentation context of the peptide within the gel (Salinas & Anseth, 2008). Another team described the effect of FN and LM-derived peptides (RGDSP and IKVAV, respectively) using a PEG-hydrogel array to screen the effect of these ECM molecules on hMSCs viability. Their strategy allowed to also analyse the consequences of the hydrogel used, for example, the combination of the IKVAV peptides with degradable gels resulted in decreased sustained viability compared to non-degradable gels (Jongpaiboonkit et al., 2008). A recent study described the single-cell encapsulation of hMSCs in FN- and fibrinogen-containing hydrogel capsules that rescue the cells from apoptosis induced by loss of anchorage, while providing an increased cell metabolic activity in culture. Effects that were certainly mediated *via* the MAPK/ERK signalling cascade and upstream integrin/ECM molecule interactions (Karoubi et al., 2009). Using a subpopulation of hMSCs, we recently showed in a rat model of global ischemia, that marrow-isolated adult multilineage inducible (MIAMI) cells (D'Ippolito et al., 2004) adhered onto PLGA microcarriers with a FN biomimetic surface enhanced the neuroprotection effect observed with the cells alone (Garbayo et al., 2011). This effect may be attributed to an increased survival of the cells on these carriers, or the increased production of neuroprotective mediators by these cells or both.

ECM molecules may also affect proliferation as well as life span of cells, this being the rationale for expanding MSCs on ECM molecules in several *in vitro* protocols (see for example (D'Ippolito et al., 2004; Matsubara et al., 2004)). Modification of PLCL (poly(L-lactic acid)-co-poly-(ε-caprolactone)) nanofibrous scaffolds with collagen I resulted in an increased proliferation of human MSCs and neuronal differentiation after exposure to an induction media, compared to standard PLCL scaffolds (Prabhakaran et al., 2009). The benefits of collagen I was assumed to result from its high cell adhesion properties, thought to be required to ensure a proper neuronal differentiation. In our laboratory, we observed an increased proliferation of the human stromal MIAMI cells expanded on FN compared to standard culture substrates (Delcroix et al., 2011). Extensive crosstalk takes place between integrin and growth factor receptor signalling pathways to stimulate progression through the G1 phase of the cell cycle, as mitogenic signalling may be weak and transient in the absence of integrin-mediated cell adhesion for cells with an anchorage dependent growth, such as MSCs (see (Danen & Yamada, 2001) for review).

Neural precursor differentiation, by enhancing neurite outgrowth *in vitro* or *in vivo* is certainly one of the first and most studied effects of LM and its derived bioactive peptides (Rogers et al., 1983; Grimpe et al., 2002). Accumulating evidence support a bioactive signalling role of LM in the morphological and molecular induction of MSCs toward a neuronal lineage (Qian & Saltzman, 2004; Ho et al., 2006; Delcroix et al., 2011). In our laboratory, the enhanced expression of β3-Tubulin and NFM when human mesenchymal stromal MIAMI cells were differentiated on a LM substrate was also accompanied with an increased cell length and a decreased proliferation rate (Delcroix et al., 2011). In this regard, a study reported the increased length of neurite-like extensions of MSCs, when incorporated within a collagen gel containing FN and LM, an effect at least partly mediated *via* the FAK pathway (Lee et al., 2011). Specific fragments of LM, e.g. the peptides IKVAV & YIGSR play

crucial signalling roles in neurite outgrowth and cell adhesion. Accordingly, studies also aim at functionalizing scaffolds with similar peptide fragments to reproduce the effect of the native ECM molecules, but also to isolate one of its specific effects (Powell & Kleinman, 1997; Orive et al., 2009). Some observations of how ECM components control MSC behaviour are summarized in Table 3.

Effects	ECM component or derived peptides	Observations	References
Survival	*RGD*	PEG hydrogel RGD improved hMSCs survival *in vitro* Improvement enhanced with the use of a glycine spacer between RGD and the gel	Salinas and Anseth 2008
	RGDSP & IKVAV	PEG-hydrogel Both peptides improved hMSCs survival Combination of IKVAV with degradable gels decreased the benefits compared to non-degradable gels	Jongpaiboonkit, King et al. 2008
	FN & fibrinogen	Single-cell encapsulation of hMSCs in FN- or fibrinogen-containing hydrogel capsules Enhanced survival of cells + increased cell metabolic activity in vitro Validation of the concept in vivo (uninjured hindlimb model in rats)	Karoubi, Ormiston et al. 2009
Proliferation	*Collagen I*	PLCL/collagen I nanofibrous scaffolds Increased proliferation of hMSCs *vs* standard PLCL scaffolds Effect due to adequate cell adhesion properties of collagen I Allow neuronal differentiation of hMSCs when exposed to the appropriate inducers	Prabhakaran, Venugopal et al. 2009
	FN	FN substrate Increased proliferation of MIAMI cells *in vitro*	Delcroix, Garbayo et al. 2011
Neuronal differentiation	*LM*	LM substrate Induction of nestin expression by MSCs *in vitro*	Ho, Yu et al. 2006
		LM substrate Morphological changes in hMSCs during neuronal differentiation *in vitro* Increased percentage of cells with secondary and tertiary branching on LM *vs* FN or PDL	Qian and Saltzman 2004
		LM substrate Increased cell length and expression of neuronal proteins (β3-Tubulin and NFM) during differentiation of MIAMI cells *in vitro vs* FN or glass	Delcroix, Garbayo et al. 2011
		Collagen gel enriched with FN and LM Increased neurite length of MSCs incorporated within the gel Effect mediated *via* the FAK pathway	Lee, Yu et al. 2011

Abbreviations: ECM: extracellular matrix molecules; FN: fibronectin; LM: laminin; MIAMI: marrow isolated adult multilineage inducible; MSC: mesenchymal stem cell; NFM: neurofilament medium; PDL: poly-D-lysine; PEG: polyethyleneglycol; PLCL: poly(L-lactic acid)-co-poly-(e-caprolactone).

Table 3. Control of MSC behaviour by ECM components

3.3.4 The pharmacologically active microcarriers (PAMs): a tool to combine the biomimetic approach and the controlled release of a growth factor

In addition to the biomimetic approach, another way to improve the efficiency of cell grafts is to deliver a growth factor by the transplanted scaffolds, further affecting the fate of both transplanted and host cells. Interestingly, the potential of microsphere-hydrogel scaffolds to deliver 2 growth factors at specific rates has been described (Burdick et al., 2006), but has not yet led to *in vivo* studies. For reviews, see (Tatard et al., 2005; Delcroix, Schiller et al., 2010). Our group has formulated PLGA microspheres which deliver therapeutic proteins in a sustained and controlled manner. The use of these growth factor delivery vectors for neuroprotection or for the repair of the nigro-striatal dopaminergic system has been successfully validated in animal models of AD or of PD, respectively (Pean et al., 2000; Jollivet et al., 2004; Menei et al., 2005). In this sense, we developed the PAMs that combine these two approaches and may be easily injected in the desired tissue (Figure 1). These PAMs are biodegradable and biocompatible PLGA microspheres conveying cells on their surface, therefore providing an adequate 3D microenvironment *in vivo*. Moreover, the controlled delivery of a trophic factor in combination with a biomimetic surface act synergistically to stimulate the survival and/or differentiation of the grafted cells toward a specific phenotype, therefore enhancing their engraftment after their complete degradation (Tatard et al., 2005). Finally, it should be noted that the delivered molecule may also affect the host microenvironment allowing the integration of the grafted cells and/or stimulating the lesioned brain repair capacities.

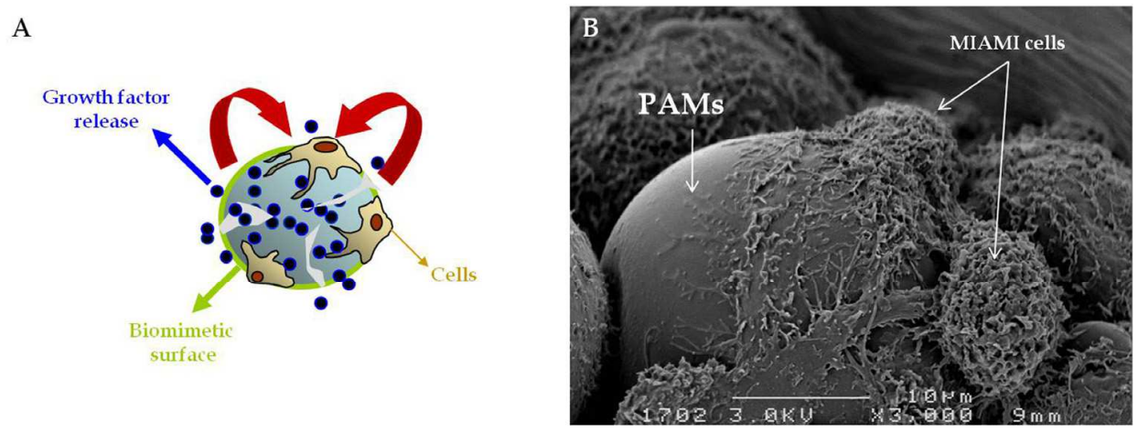


Fig. 1. A) Schematic representation of PAMs. These PAMs are PLGA microspheres with a biomimetic surface that convey cells and release a growth factor in a controlled manner. B) Scanning electron microscopy image of PAMs conveying MIAMI cells on their surface

The proof of concept of this unique and simple device of cell and protein-delivery in neuroprotection and tissue repair for the treatment of neurological disorders has first been validated in a PD rat model using a neuronal cell line (PC12 cells) transported by nerve growth factor (NGF)-releasing PAMs. (Tatard et al., 2004). The efficacy of PAMs for cell therapy of PD in a clinical paradigm was then demonstrated using GDNF-releasing PAMs, conveying a small number of embryonic ventral mesencephalon dopaminergic cells (Tatard et al., 2007). We next implemented the PAMs transporting MIAMI cells for PD adult stem cell therapy. MIAMI cells, may differentiate toward neuronal-like cells in a neurotrophin 3 (NT3) dependent manner (Tatard et al., 2007), especially when pre-treated with EGF and bFGF (Delcroix, Curtis et al., 2010). Moreover, a LM surface enhances the neuronal differentiation (Delcroix et al., 2011). We designed PAMs made of 60 µm PLGA microspheres encapsulating NT3 and covered with a LM biomimetic surface. After adhesion of dopaminergic-induced (DI)-MIAMI cells, the PAM/cell complexes were grafted in the partially dopaminergic-deafferented striatum of rats and led to a strong reduction of the amphetamine-induced rotational behaviour together with the protection/repair of the nigrostriatal pathway. These effects were correlated with the increased survival of DI-MIAMI cells which differentiated towards dopaminergic-like cells and may also secrete growth factors (Delcroix et al., 2011). We thus showed that combining growth factors, cell adhesion molecules and an adapted 3D structure in the same polymeric scaffold allows the synthesis of an adaptable and very efficient system that can deliver stem cells and give them appropriate cues allowing better stem cell survival, differentiation and integration into the host tissues after implantation. After deeper characterization of the underlying mechanisms, this tissue engineering strategy may ultimately set the ground for pre-clinical studies with non-human primates to increase the efficiency of MSC therapy of the brain.

4. Unanswered questions in tissue engineering for targeting CNS disorders using conventional tissue culture and animal models

Although brain tissue engineering has shown a certain level of therapeutic benefits, there are still many unanswered questions and concerns that need to be addressed. For instance,

one general problem found in cell therapy is the difficulty to reproduce results between laboratories. Differences on the source, method of preparation, differentiation status and age of the stem cells used may be the cause. Moreover, with the current workflow it takes a long time to select an effective stem cell strategy to translate into clinical studies. The main steps to follow include the selection of the source of cells (MSC, NSC, ESC, iPS), the choice of the culture media, the state of stem cell cycle (undifferentiated *vs* differentiated), the mode of culture, the form of growth (neurosphere *vs* monolayer for instance), the study of substrate variations (FN, LN, matrigel among others) and the use of different animal models to test each therapeutic variation. Moreover, very little is known about the exact mechanisms by which stem cells may repair damaged tissues. It is not yet totally understood if stem cells may directly replace lost cells *via* their differentiation potential or if the beneficial effect could be due to their paracrine secretions or their immune regulatory functions, or if it is due to a combination of these effects. An additional and very troublesome problem is the difficulty to track the location and activity of stem cells once they are grafted into the animal. Several strategies have been proposed to follow the grafted cells *in vivo*. One of the most commonly used is to label the cells with fluorescent markers like quantum dots, organic dyes or fluorescent proteins among others. Other authors have grafted sex mismatched stem cells to identify them by fluorescent *in situ* hibridation (FISH) on the X and Y chromosomes. The injection of human cells into rodent animals or *vice versa* to use species-specific antibodies for its identification is another approach.

In general, it is difficult to understand stem cell mechanisms of action and fate using conventional tissue culture or animal models due to the limitations of both systems. *In vitro* models are a good high throughput screening tool but they do not realistically mimic the *in vivo* situation. Moreover, the 3D integration of grafted cells into the host tissue cannot be studied using tissue culture models. On the other hand, it is clear that animal models are essential in proof-of-concept principle experiments and in establishing the preclinical safety and efficacy data required before human clinical trials. However, most of them are expensive, laborious and time consuming to be useful as screening tests. Generally, it can be stated that as a system becomes more complex, the throughput in terms of screening capacity decreases (Sundstrom et al., 2005). Moreover, the real-time monitoring of grafted cells using animal models is not easy. A screening platform in-between *in vitro* and *in vivo* models is required to improve understanding of stem cells. A possible option would be the use of brain organotypic slice culture for optimization of CNS cell therapies. These organotypic cultures mimic *in vivo* models of brain diseases better than cell cultures, they are cost-effective and easier to optimize, use and manipulate than rodent and primate CNS disease models. The usefulness of brain organotypic slices in CNS research as well as in the drug discovery process has been increasing in recent years and will be discussed in the next section.

5. Organotypic 3D culture models: novel platforms for optimization of CNS cell therapy and tissue engineering

Since their introduction, organotypic cultures of rat brain slices have become a useful tool to study drug effects. Brain organotypic slices are *ex-vivo* cultures that bridge the gap between *in vitro* and *in vivo* models. Slices are easy to prepare and they preserve the tissue

architecture of the brain regions that they originated from, allowing interaction of multiple cell types like neurons, astrocytes and microglia and maintaining neuronal activities. This 3D environment has tremendous importance to evaluate the efficacy of tissue engineering approaches since most of the cells require cues from a truly 3D environment to form relevant physiological tissue structures *in vitro*. This 3D provides external mechanical inputs and cell adhesion parameters, which affects intracellular signalling. Importantly, these slices permit direct treatment or injection of drugs, virus, cells, microscaffolds... making them ideal for screening (Cho et al., 2007).

5.1 Preparation of organotypic cultures of CNS tissue

Organotypic brain slice culture was developed by inspiration on explant cultures techniques of various anatomical origins. Since then, several methods have been developed to prepare and maintain slices alive in long-term culture. The roller-tube technique based on the use of roller tubes was first described in 1981 (Gahwiler, 1981). However, most studies now use the membrane interface method described in 1991 (Stoppini et al., 1991) which provides an easier access to the slice culture. The principle of the membrane interface method is to maintain brain slices on a porous membrane filter at the interface between medium and a humidified atmosphere. The medium provides adequate nutrition to tissues through the membrane *via* capillary action. Rats and mice are the most common donor sources, but also rabbits, pigs and human fetuses have been used. Most organotypic brain slice cultures have been derived from neonatal (P0-P10) animals, but recently also adolescent or adult rats have been used and even human postmortem brain tissue slices have been kept alive for a few weeks in culture (Noraberg et al., 2005). Brain slices are relatively easy to prepare (Figure 2).

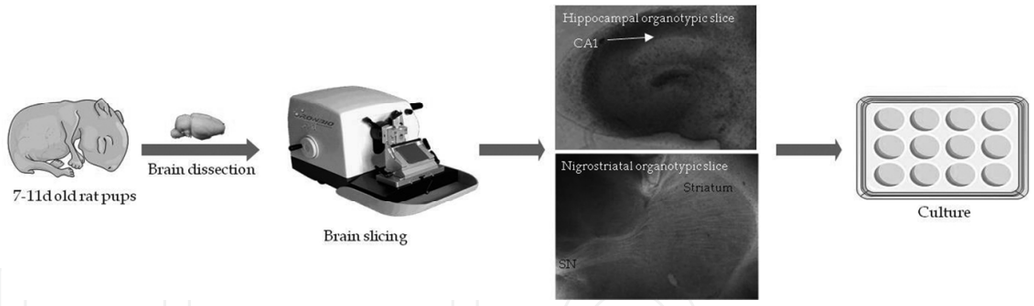


Fig. 2. Organotypic preparation; 7 to 11 days old rats are commonly used to prepare brain organotypic cultures. First the brain is dissected free of skull. Second, slicing is performed with a vibrating microtome. Finally, slices are maintained in cultured using the membrane interface method. Representative bright field images of hippocampal and nigrostriatal organotypics are shown in the figure

Briefly, the brain is dissected free of the skull and placed into a saline solution whose composition resembles that of the cerebro-spinal fluid and is thus often referred to as artificial cerebro-spinal fluid (ACSF). Slicing is performed with a vibrating microtome at high amplitude and very slow speed. Slice thickness varies according to specimen and the type of experiment, from 150 to 400 µm (Lossi et al., 2009). Over the years, slice culture systems have been successfully established from a variety of brain regions including hippocampus (far more frequently), striatum, cortex, spinal cord and cerebellum. Furthermore, a number of tissue slice co-cultures have been developed, which allow the

assessment of inter-neural responses across brain regions. Moreover, when organotypic slices are exposed to certain toxic conditions (oxygen-glucose deprivation, neurotoxins, glutamate-mediated excitotoxicity) they develop many pathophysiological features found in brain disorders and consequently, brain organotypic slices can be used as ex-vivo models of CNS diseases. Nowadays, organotypic models for global cerebral ischemia, ischemic stroke, Alzheimer's disease, PD, HD, TBI, epilepisa and amyotrophic lateral sclerosis have been described (For review see (Noraberg et al., 2005; Sundstrom et al., 2005; Cho et al., 2007; Cimarosti & Henley, 2008; Lossi et al., 2009)). Among them, hippocampal organotypic slices exposed to oxygen-glucose deprivation, a model for global cerebral ischemia is the most commonly used.

5.2 Applications

Organotypic slices have been commonly used as models to investigate mechanism and treatment strategies for neurodegenerative disorders. Interestingly, the utility of brain slices to test CNS cell therapy efficacy has recently started to be investigated. Organotypic cultures are a very useful tool for screening of candidate stem cells for a specific pathology. They also could be used to track survival, differentiation, proliferation and migration of the transplanted cells with or without microscaffolds or to study graft and host interactions (Figure 3). Stem cell mechanisms of neuroprotection or paracrine secretions of MSCs could be also studied using brain slices. Charriere *et al.,*(Charriere et al. 2010) analyzed the interactions between bone marrow stromal cells and hippocampal slice cultures to clarify putative cross-interactions between MSCs and the CNS that could explain the molecular mechanism of stem cells. The dopaminergic differentiation of ES in a PD organotypic model and the effect of morphogenetic proteins such as LM on the differentiation of the cells has also been studied (Kearns et al., 2006). It was also shown that olfactory ensheathing cells (OEC) when co-cultured with the auditory brain stem slice culture not only promoted neurite outgrowth from the cochlear nucleus region of the brain stem slice but also supported the OEC indicating positive interactions between both (Jiao et al. 2010). Recently, our group examined the potential of MIAMI cells injected into the hippocampus to prevent neuronal damage induced by global ischemia using rat hippocampal slices exposed to oxygen-glucose deprivation. We showed that MIAMI cells prevented neuronal damage. MIAMI cell therapeutic value was significantly increased when delivering the cells complexed with FN-coated biomimetic microcarriers probably by increasing stem cell survival and paracrine secretion of pro-survival and/or anti-inflammatory molecules as concluded from survival, differentiation and gene expression analysis (Garbayo et al., 2011). Furthermore, brain organotypic slices can be used to perform tissue biocompatiblity studies of scaffolds prepared for brain tissue engineering application and to determine how changes in the composition or in the functionalization of the scaffold could compromise their brain biocompatibility. In this context, Kristensen *et al*., have used organotypic brain slice cultures to assess silicon-based arrays biocompatibility (Kristensen et al., 2001). Other exciting application could be to use them to determine what scaffold composition best promotes proper adhesion and proliferation of the stem cells. Recently, in various pathologies it has been reported that multiple growth factor delivery is more effective than single growth factor administration since it mimics better the natural microenvironments of tissue formation and repair (Richardson et al., 2001; Barrientos et al., 2008). The difficulties of this approach are principally to find the optimised growth factor ratio, each factor at a

physiological dose and in a specific spatiotemporal pattern. Brain organotypic slices could be used to determine proper growth factor cocktail for a specific brain damage and to control the relationship between growth factor concentration gradient and timing.

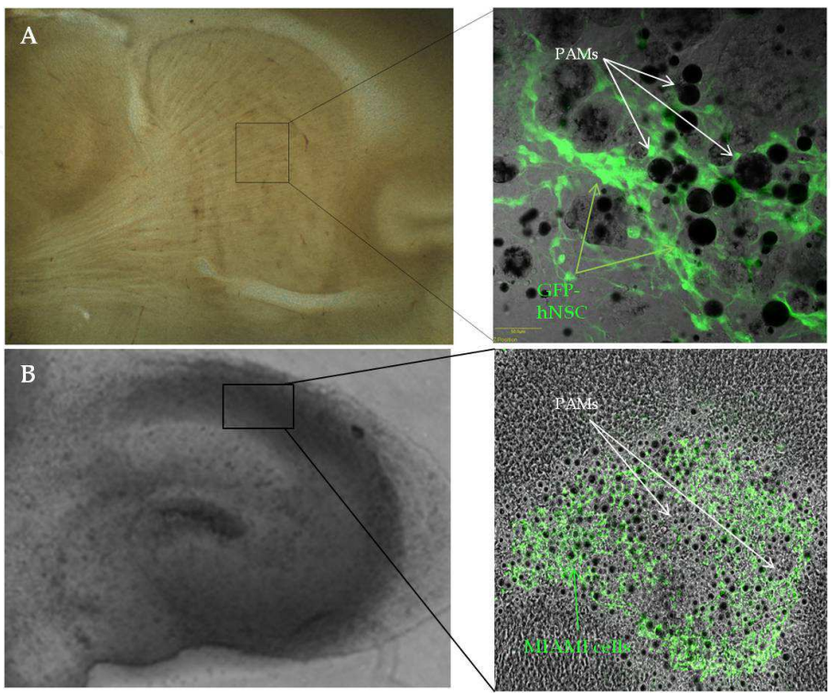


Fig. 3. A) Nigrostriatal organotypic culture with GFP-hNSC forming complexes with PAMs injected into the striatum. GFP-hNSC cells were kindly provided by Dr Martinez-Serrano. B) Hippocampal organotypic culture with MIAMI cells forming complexes with PAMs injected into the CA1 hippocampus

An innovative approach using modified organotypic slices derived from a transgenic, mutant, null, gain-of-function, loss-of function, knock-in, or knockout animals was recently proposed (Li & Loudon, 2008). They discuss the possibility of using modified organotypic slice cultures to understand how implanted cells interact with resident cellular matrix and injured residential cells to predict their *in vivo* behaviour (Li & Loudon, 2008). Organotypic cultures could also be used to evaluate how temporal expression levels of stem cell chemokine receptors can be quantitatively related with their migration capacity toward brain tumor- or lesion producing -signal ligand SDF-1. The foundation of this experimental platform is to establish a system that mimics *in vivo* properties, first to maintain stem cells in a quiescent state, and then induce stem cells to produce targeting molecule cytokine receptors and matrix remodeling enzymes. Li & Loudon showed that migration of stem cells was enhanced by an intermediate concentration of SDF-1 gradient but inhibited by higher concentrations, with no stimulation at low concentrations (Li & Loudon, 2008).

5.3 Limitations of organotypics

Organotypic slices are not without limitations. The most limiting features are: 1) currently brain slices are only produced from juvenile donor animals as it is known that young tissue has more neuronal plasticity and are more resistant than older tissue. Recent reports have

used older donors to prepare brain organotypic slices but although it is possible, a low yield was observed and slices remained viable for only 3 to 4 weeks. 2) Automation is a challenge since a skilled operator is required for the production of the slices. 3) Not all brain areas are amenable to culture being the most appropriate the regions with a lamellar structure that can be aligned parallel to the plan of slicing. 4) Organotypics do not have a functional vascular compartment (for review see Sundstrom et al., 2005).

6. Conclusions

Although the ideal brain scaffold that satisfies all the requirements does not exist yet, the past several years have seen considerable progress in this field of study. For instance, materials of many types have been used to create brain scaffolds capable of providing sustained delivery of signalling molecules and an adequate 3D support for transplanted cells, thereby increasing cell survival and even guiding cell differentiation and fate *in vivo* Scaffold surface characteristics have also been modified to better mimic the natural brain environment, to control cell attachment, growth and differentiation or for specific uses. A key-point to remember is that most regenerative technologies in the future will probably be combinatorial, including biocompatible scaffolds, stem cells and the various factors necessary for their survival and function. This might be especially true in order to implement iPS cells therapy in the future, to control their proliferation and differentiation potential. Continual progress in the design and fabrication of future scaffolds is required to improve current delivery platforms since there is a need for development of custom matrices either tailored for purpose, or for the individual patient. Recent developments in understanding the basic biology of brain tissue formation in physiological and pathologic conditions have resulted in an explosion in the numbers of bio-engineered and tissue engineering products that could be potential candidates for treating brain disorders. A major challenge for the pharmaceutical industry is to find useful tools for screening since a bottleneck exists between the number of compounds that are interesting and the relatively limited *in vitro* and *in vivo* existing methods. Animal models are still the main choice for such studies but over the past years, brain organotypic cultures have begun to emerge as useful tools for screening of new neuroprotective and neuroregenerative approaches. In the current chapter, the combination of adult stem cells with microscopic scaffolds has been revised. Recently, research on the application of nanotechnology in stem cell and tissue engineering research has gained much attention in the scientific community. A significant and exciting area of research is the use of nanoparticles to engineer different patterned topographies of scaffolds that mimic the ECM, to study their effects on stem cells.

7. References

Abeliovich, A. & Doege, C. A. (2009). Reprogramming therapeutics: iPS cell prospects for neurodegenerative disease. *Neuron,* Vol.61, No.3, pp. 337-339.

Aktas, Y., Yemisci, M., Andrieux, K., Gursoy, R. N., Alonso, M. J., Fernandez-Megia, E., Novoa-Carballal, R., Quinoa, E., Riguera, R., Sargon, M. F., Celik, H. H., Demir, A. S., Hincal, A. A., Dalkara, T., Capan, Y. & Couvreur, P. (2005). Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26. *Bioconjug Chem,* Vol.16, No.6, pp. 1503-1511.

Amin, E. M., Reza, B. A., Morteza, B. R., Maryam, M. M., Ali, M. & Zeinab, N. (2008). Microanatomical evidences for potential of mesenchymal stem cells in amelioration of striatal degeneration. *Neurol Res,* Vol.30, No.10, pp. 1086-1090.

Arias-Carrion, O. & Yuan, T. F. (2009). Autologous neural stem cell transplantation: A new treatment option for Parkinson's disease? *Med Hypotheses*, Vol.73, No.5, pp. 757-759.

Athanasiou, K. A., Niederauer, G. G. & Agrawal, C. M. (1996). Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials,* Vol.17, No.2, pp. 93-102.

Bahat-Stroomza, M., Barhum, Y., Levy, Y. S., Karpov, O., Bulvik, S., Melamed, E. & Offen, D. (2009). Induction of adult human bone marrow mesenchymal stromal cells into functional astrocyte-like cells: potential for restorative treatment in Parkinson's disease. *J Mol Neurosci,* Vol.39, No.1-2, pp. 199-210.

Bakay, R. A., Raiser, C. D., Stover, N. P., Subramanian, T., Cornfeldt, M. L., Schweikert, A. W., Allen, R. C. & Watts, R. (2004). Implantation of Spheramine in advanced Parkinson's disease (PD). *Front Biosci,* Vol.9, pp. 592-602.

Bang, O. Y., Lee, J. S., Lee, P. H. & Lee, G. (2005). Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol,* Vol.57, No.6, pp. 874-882.

Bantubungi, K., Blum, D., Cuvelier, L., Wislet-Gendebien, S., Rogister, B., Brouillet, E. & Schiffmann, S. N. (2008). Stem cell factor and mesenchymal and neural stem cell transplantation in a rat model of Huntington's disease. *Mol Cell Neurosci,* Vol.37, No.3, pp. 454-470.

Barczyk, M., Carracedo, S. & Gullberg, D. (2009). Integrins. *Cell Tissue Res*, Vol.339, No.1, pp. 269-280.

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H. & Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. *Wound Repair Regen,* Vol.16, No.5, pp. 585-601.

Barzilay, R., Kan, I., Ben-Zur, T., Bulvik, S., Melamed, E. & Offen, D. (2008). Induction of human mesenchymal stem cells into dopamine-producing cells with different differentiation protocols. *Stem Cells Dev,* Vol.17, No.3, pp. 547-554.

Bible, E., Chau, D. Y., Alexander, M. R., Price, J., Shakesheff, K. M. & Modo, M. (2009). The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles. *Biomaterials*, Vol.30, No.16, pp. 2985-2994.

Borlongan, C. V., Saporta, S. & Sanberg, P. R. (1998). Intrastriatal transplantation of rat adrenal chromaffin cells seeded on microcarrier beads promote long-term functional recovery in hemiparkinsonian rats. *Exp Neurol,* Vol.151, No.2, pp. 203-214.

Borlongan, C. V., Thanos, C. G., Skinner, S. J., Geaney, M. & Emerich, D. F. (2008). Transplants of encapsulated rat choroid plexus cells exert neuroprotection in a rodent model of Huntington's disease. *Cell Transplant,* Vol.16, No.10, pp. 987-992.

Bosman, F. T. & Stamenkovic, I. (2003). Functional structure and composition of the extracellular matrix. *J Pathol,* Vol.200, No.4, pp. 423-428.

Bouchez, G., Sensebe, L., Vourc'h, P., Garreau, L., Bodard, S., Rico, A., Guilloteau, D., Charbord, P., Besnard, J. C. & Chalon, S. (2008). Partial recovery of dopaminergic pathway after graft of adult mesenchymal stem cells in a rat model of Parkinson's disease. *Neurochem Int*, Vol.52, No.7, pp. 1332-1342.

Broderick, J. P. (2009). Endovascular therapy for acute ischemic stroke. *Stroke,* Vol.40, No.3 Suppl, pp. S103-106.

Brundin, P., Barbin, G., Isacson, O., Mallat, M., Chamak, B., Prochiantz, A., Gage, F. H. & Bjorklund, A. (1985). Survival of intracerebrally grafted rat dopamine neurons previously cultured in vitro. *Neurosci Lett,* Vol.61, No.1-2, pp. 79-84.

Brundin, P., Karlsson, J., Emgard, M., Schierle, G. S., Hansson, O., Petersen, A. & Castilho, R. F. (2000). Improving the survival of grafted dopaminergic neurons: a review over current approaches. *Cell Transplant,* Vol.9, No.2, pp. 179-195.

Burdick, J. A., Ward, M., Liang, E., Young, M. J. & Langer, R. (2006). Stimulation of neurite outgrowth by neurotrophins delivered from degradable hydrogels. *Biomaterials,* Vol.27, No.3, pp. 452-459.

Cao, H., Liu, T. & Chew, S. Y. (2009). The application of nanofibrous scaffolds in neural tissue engineering. *Adv Drug Deliv Rev,* Vol.61, No.12, pp. 1055-1064.

Cimarosti, H. & Henley, J. M. (2008). Investigating the mechanisms underlying neuronal death in ischemia using in vitro oxygen-glucose deprivation: potential involvement of protein SUMOylation. *Neuroscientist,* Vol.14, No.6, pp. 626-636.

Clelland, C. D., Barker, R. A. & Watts, C. (2008). Cell therapy in Huntington disease. *Neurosurg Focus,* Vol.24, No.3-4, pp. E9.

Charriere, K., Risold, P. Y. & Fellmann, D. (2010). In vitro interactions between bone marrow stromal cells and hippocampal slice cultures. *C R Biol,* Vol.333, No.8, pp. 582-590.

Chen, C. & Xiao, S. F. (2011). Induced pluripotent stem cells and neurodegenerative diseases. *Neurosci Bull,* Vol.27, No.2, pp. 107-114.

Chen, J. & Chopp, M. (2006). Neurorestorative treatment of stroke: cell and pharmacological approaches. *NeuroRx,* Vol.3, No.4, pp. 466-473.

Chen, J., Li, Y., Wang, L., Lu, M., Zhang, X. & Chopp, M. (2001). Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *J Neurol Sci,* Vol.189, No.1-2, pp. 49-57.

Chen, J., Zhang, Z. G., Li, Y., Wang, L., Xu, Y. X., Gautam, S. C., Lu, M., Zhu, Z. & Chopp, M. (2003). Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res,* Vol.92, No.6, pp. 692-699.

Chen, S. J., Chang, C. M., Tsai, S. K., Chang, Y. L., Chou, S. J., Huang, S. S., Tai, L. K., Chen, Y. C., Ku, H. H., Li, H. Y. & Chiou, S. H. (2011). Functional improvement of focal cerebral ischemia injury by subdural transplantation of induced pluripotent stem cells with fibrin glue. *Stem Cells Dev,* Vol.19, No.11, pp. 1757-1767.

Chen, X., Katakowski, M., Li, Y., Lu, D., Wang, L., Zhang, L., Chen, J., Xu, Y., Gautam, S., Mahmood, A. & Chopp, M. (2002). Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. *J Neurosci Res,* Vol.69, No.5, pp. 687-691.

Chen, X. D. (2010). Extracellular matrix provides an optimal niche for the maintenance and propagation of mesenchymal stem cells. *Birth Defects Res C Embryo Today,* Vol.90, No.1, pp. 45-54.

Cherksey, B. D., Sapirstein, V. S. & Geraci, A. L. (1996). Adrenal chromaffin cells on microcarriers exhibit enhanced long-term functional effects when implanted into the mammalian brain. *Neuroscience,* Vol.75, No.2, pp. 657-664.

Cho, S., Wood, A. & Bowlby, M. R. (2007). Brain slices as models for neurodegenerative disease and screening platforms to identify novel therapeutics. *Curr Neuropharmacol,* Vol.5, No.1, pp. 19-33.

Chopp, M., Li, Y. & Zhang, J. (2008). Plasticity and remodeling of brain. *J Neurol Sci,* Vol.265, No.1-2, pp. 97-101.

D'Ippolito, G., Diabira, S., Howard, G. A., Menei, P., Roos, B. A. & Schiller, P. C. (2004). Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. *J Cell Sci,* Vol.117, No.Pt 14, pp. 2971-2981.

Dalton, P. D. & Mey, J. (2009). Neural interactions with materials. *Front Biosci,* Vol.14, pp. 769-795.

Danen, E. H. & Yamada, K. M. (2001). Fibronectin, integrins, and growth control. *J Cell Physiol,* Vol.189, No.1, pp. 1-13.

Danielyan, L., Schafer, R., von Ameln-Mayerhofer, A., Bernhard, F., Verleysdonk, S., Buadze, M., Lourhmati, A., Klopfer, T., Schaumann, F., Schmid, B., Koehle, C., Proksch, B., Weissert, R., Reichardt, H. M., van den Brandt, J., Buniatian, G. H., Schwab, M., Gleiter, C. H. & Frey Ii, W. H. (2011). Therapeutic Efficacy of Intranasally Delivered Mesenchymal Stem Cells in a Rat Model of Parkinson Disease. *Rejuvenation Res*, Vol.14, No.1, pp. 3-16.

De Keyser, J. (2005). Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol,* Vol.58, No.4, pp. 653-654; author reply 654-655.

Delcroix, G. J., Curtis, K. M., Schiller, P. C. & Montero-Menei, C. N. (2010). EGF and bFGF pre-treatment enhances neural specification and the response to neuronal commitment of MIAMI cells. *Differentiation,* Vol.80, No.4-5, pp. 213-227.

Delcroix, G. J., Garbayo, E., Sindji, L., Thomas, O., Vanpouille-Box, C., Schiller, P. C. & Montero-Menei, C. N. (2011). The therapeutic potential of human multipotent mesenchymal stromal cells combined with pharmacologically active microcarriers transplanted in hemi-parkinsonian rats. *Biomaterials,* Vol.32, No.6, pp. 1560-1573.

Delcroix, G. J., Jacquart, M., Lemaire, L., Sindji, L., Franconi, F., Le Jeune, J. J. & Montero-Menei, C. N. (2009). Mesenchymal and neural stem cells labeled with HEDP-coated SPIO nanoparticles: in vitro characterization and migration potential in rat brain. *Brain Res,* Vol.1255, pp. 18-31.

Delcroix, G. J. R., Schiller, P. C., Benoit, J.-P. & Montero-Menei, C. N. (2010). Adult cell therapy for brain neuronal damages and the role of tissue engineering. *Biomaterials,* Vol.31, No.8, pp. 2105-2120.

Doudet, D. J., Cornfeldt, M. L., Honey, C. R., Schweikert, A. W. & Allen, R. C. (2004). PET imaging of implanted human retinal pigment epithelial cells in the MPTP-induced primate model of Parkinson's disease. *Exp Neurol,* Vol.189, No.2, pp. 361-368.

Drucker-Colin, R. & Verdugo-Diaz, L. (2004). Cell transplantation for Parkinson's disease: present status. *Cell Mol Neurobiol,* Vol.24, No.3, pp. 301-316.

Drucker-Colin, R., Verdugo-Diaz, L., Morgado-Valle, C., Solis-Maldonado, G., Ondarza, R., Boll, C., Miranda, G., Wang, G. J. & Volkow, N. (1999). Transplant of cultured neuron-like differentiated chromaffin cells in a Parkinson's disease patient. A preliminary report. *Arch Med Res,* Vol.30, No.1, pp. 33-39.

Dunbar, G. L., Sandstrom, M. I., Rossignol, J. & Lescaudron, L. (2006). Neurotrophic enhancers as therapy for behavioral deficits in rodent models of Huntington's

disease: use of gangliosides, substituted pyrimidines, and mesenchymal stem cells. *Behav Cogn Neurosci Rev,* Vol.5, No.2, pp. 63-79.

Edalatmanesh, M. A., Matin, M. M., Neshati, Z., Bahrami, A. R. & Kheirabadi, M. (2009). Systemic transplantation of mesenchymal stem cells can reduce cognitive and motor deficits in rats with unilateral lesions of the neostriatum. *Neurol Res*, Vol. 32, No.2, pp. 166-172.

Emerich, D. F. (2004). Sertoli cell grafts for Huntington's disease. An opinion. *Neurotox Res,* Vol.5, No.8, pp. 567.

England, T. (2009). Stem cells for enhancing recovery after stroke: a review. *International journal of stroke,* Vol.4, pp. 101-110.

Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell,* Vol.126, No.4, pp. 677-689.

Engvall, E. & Wewer, U. M. (1996). Domains of laminin. *J Cell Biochem,* Vol.61, No.4, pp. 493-501.

Esneault, E., Pacary, E., Eddi, D., Freret, T., Tixier, E., Toutain, J., Touzani, O., Schumann-Bard, P., Petit, E., Roussel, S. & Bernaudin, M. (2008). Combined therapeutic strategy using erythropoietin and mesenchymal stem cells potentiates neurogenesis after transient focal cerebral ischemia in rats. *J Cereb Blood Flow Metab*, Vol. 28, No.9, pp. 1552-1563.

Farag, E. S., Vinters, H. V. & Bronstein, J. (2009). Pathologic findings in retinal pigment epithelial cell implantation for Parkinson disease. *Neurology,* Vol.73, No.14, pp. 1095-1102.

Fernandez-Espejo, E., Armengol, J. A., Flores, J. A., Galan-Rodriguez, B. & Ramiro, S. (2005). Cells of the sympathoadrenal lineage: biological properties as donor tissue for cell-replacement therapies for Parkinson's disease. *Brain Res Brain Res Rev,* Vol.49, No.2, pp. 343-354.

Flores, J., Cepeda, I. L., Cornfeldt, M. L., O'Kusky, J. R. & Doudet, D. J. (2007). Characterization and survival of long-term implants of human retinal pigment epithelial cells attached to gelatin microcarriers in a model of Parkinson disease. *J Neuropathol Exp Neurol,* Vol.66, No.7, pp. 585-596.

Fournier, E., Passirani, C., Colin, N., Sagodira, S., Menei, P., Benoit, J. P. & Montero-Menei, C. N. (2006). The brain tissue response to biodegradable poly(methylidene malonate 2.1.2)-based microspheres in the rat. *Biomaterials,* Vol.27, No.28, pp. 4963-4974.

Fournier, E., Passirani, C., Montero-Menei, C. N. & Benoit, J. P. (2003). Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility. *Biomaterials,* Vol.24, No.19, pp. 3311-3331.

Gahwiler, B. H. (1981). Organotypic monolayer cultures of nervous tissue. *J Neurosci Methods,* Vol.4, No.4, pp. 329-342.

Garbayo, E., Raval, A. P., Curtis, K. M., Della-Morte, D., Gomez, L. A., D'Ippolito, G., Reiner, T., Perez-Stable, C., Howard, G. A., Perez-Pinzon, M. A., Montero-Menei, C. N. & Schiller, P. C. (2011). Neuroprotective Properties of Marrow-Isolated Adult Multilineage Inducible Cells in Rat Hippocampus Following Global Cerebral Ischemia Are Enhanced When Complexed to Biomimetic Microcarriers. *J Neurochem*, In press

Gonzalez, F., Barragan Monasterio, M., Tiscornia, G., Montserrat Pulido, N., Vassena, R., Batlle Morera, L., Rodriguez Piza, I. & Izpisua Belmonte, J. C. (2009). Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. *Proc Natl Acad Sci U S A,* Vol.106, No.22, pp. 8918-8922.

Gordon, R. J., McGregor, A. L. & Connor, B. (2009). Chemokines direct neural progenitor cell migration following striatal cell loss. *Mol Cell Neurosci,* Vol.41, No.2, pp. 219-232.

Grandoso, L., Ponce, S., Manuel, I., Arrue, A., Ruiz-Ortega, J. A., Ulibarri, I., Orive, G., Hernandez, R. M., Rodriguez, A., Rodriguez-Puertas, R., Zumarraga, M., Linazasoro, G., Pedraz, J. L. & Ugedo, L. (2007). Long-term survival of encapsulated GDNF secreting cells implanted within the striatum of parkinsonized rats. *Int J Pharm,* Vol.343, No.1-2, pp. 69-78.

Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R. & Ferguson, T. A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science,* Vol.270, No.5239, pp. 1189-1192.

Grimpe, B., Dong, S., Doller, C., Temple, K., Malouf, A.T. & Silver, J. (2002). The critical role of basement membrane-independent laminin gamma 1 chain during axon regeneration in the CNS. *J Neurosci*, Vol.22, No.8, pp. 3144-60.

Hargus, G., Cooper, O., Deleidi, M., Levy, A., Lee, K., Marlow, E., Yow, A., Soldner, F., Hockemeyer, D., Hallett, P. J., Osborn, T., Jaenisch, R. & Isacson, O. (2010). Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc Natl Acad Sci U S A*, Vol. 107, No.36, pp. 15921-15926.

Hellmann, M. A., Panet, H., Barhum, Y., Melamed, E. & Offen, D. (2006). Increased survival and migration of engrafted mesenchymal bone marrow stem cells in 6-hydroxydopamine-lesioned rodents. *Neurosci Lett,* Vol.395, No.2, pp. 124-128.

Ho, M., Yu, D., Davidsion, M. C. & Silva, G. A. (2006). Comparison of standard surface chemistries for culturing mesenchymal stem cells prior to neural differentiation. *Biomaterials,* Vol.27, No.24, pp. 4333-4339.

Hou, S., Xu, Q., Tian, W., Cui, F., Cai, Q., Ma, J. & Lee, I. S. (2005). The repair of brain lesion by implantation of hyaluronic acid hydrogels modified with laminin. *J Neurosci Methods,* Vol.148, No.1, pp. 60-70.

Hurtig, H., Joyce, J., Sladek, J. R., Jr. & Trojanowski, J. Q. (1989). Postmortem analysis of adrenal-medulla-to-caudate autograft in a patient with Parkinson's disease. *Ann Neurol,* Vol.25, No.6, pp. 607-614.

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell,* Vol.110, No.6, pp. 673-687.

Hynes, R. O. & Yamada, K. M. (1982). Fibronectins: multifunctional modular glycoproteins. *J Cell Biol,* Vol.95, No.2 Pt 1, pp. 369-377.

Isacson, O., Bjorklund, L. M. & Schumacher, J. M. (2003). Toward full restoration of synaptic and terminal function of the dopaminergic system in Parkinson's disease by stem cells. *Ann Neurol,* Vol.53 Suppl 3, pp. S135-146; discussion S146-138.

Jiang, M., Lv, L., Ji, H., Yang, X., Zhu, W., Cai, L., Gu, X., Chai, C., Huang, S., Sun, J. & Dong, Q. (2011). Induction of pluripotent stem cells transplantation therapy for ischemic stroke. *Mol Cell Biochem*, In press.

Jiao, Y., Novozhilova, E., Karlen, A., Muhr, J. & Olivius, P. (2010). Olfactory ensheathing cells promote neurite outgrowth from co-cultured brain stem slice. *Exp Neurol*, In press.

Jollivet, C., Aubert-Pouessel, A., Clavreul, A., Venier-Julienne, M. C., Remy, S., Montero-Menei, C. N., Benoit, J. P. & Menei, P. (2004). Striatal implantation of GDNF releasing biodegradable microspheres promotes recovery of motor function in a partial model of Parkinson's disease. *Biomaterials,* Vol.25, No.5, pp. 933-942.

Jongpaiboonkit, L., King, W. J. & Murphy, W. L. (2008). Screening for 3D Environments That Support Human Mesenchymal Stem Cell Viability Using Hydrogel Arrays. *Tissue Eng Part A*, Vol. 15, No.2, pp. 343-353.

Jorgensen, A., Wiencke, A. K., la Cour, M., Kaestel, C. G., Madsen, H. O., Hamann, S., Lui, G. M., Scherfig, E., Prause, J. U., Svejgaard, A., Odum, N., Nissen, M. H. & Ropke, C. (1998). Human retinal pigment epithelial cell-induced apoptosis in activated T cells. *Invest Ophthalmol Vis Sci,* Vol.39, No.9, pp. 1590-1599.

Joyce, N., Annett, G., Wirthlin, L., Olson, S., Bauer, G. & Nolta, J. A. (2010). Mesenchymal stem cells for the treatment of neurodegenerative disease. *Regen Med,* Vol.5, No.6, pp. 933-946.

Jurga, M., Dainiak, M. B., Sarnowska, A., Jablonska, A., Tripathi, A., Plieva, F. M., Savina, I. N., Strojek, L., Jungvid, H., Kumar, A., Lukomska, B., Domanska-Janik, K., Forraz, N. & McGuckin, C. P. (2011). The performance of laminin-containing cryogel scaffolds in neural tissue regeneration. *Biomaterials,* Vol.32, No.13, pp. 3423-3434.

Karoubi, G., Ormiston, M. L., Stewart, D. J. & Courtman, D. W. (2009). Single-cell hydrogel encapsulation for enhanced survival of human marrow stromal cells. *Biomaterials*, Vol.30, No.29, pp. 5445-5455.

Kearns, S. M., Scheffler, B., Goetz, A. K., Lin, D. D., Baker, H. D., Roper, S. N., Mandel, R. J. & Steindler, D. A. (2006). A method for a more complete in vitro Parkinson's model: slice culture bioassay for modeling maintenance and repair of the nigrostriatal circuit. *J Neurosci Methods,* Vol.157, No.1, pp. 1-9.

Kelly, C. M., Dunnett, S. B. & Rosser, A. E. (2009). Medium spiny neurons for transplantation in Huntington's disease. *Biochem Soc Trans,* Vol.37, No.Pt 1, pp. 323-328.

Kim, M. (2009). Electrostatic Crosslinked In Situ-Forming In Vivo Scaffold For Rat Bone Marrow Mesenchymal Stem Cells. *Tissue Eng Part A*, Vol.15, No.10, pp. 3201-9

Kim, Y. J., Park, H. J., Lee, G., Bang, O. Y., Ahn, Y. H., Joe, E., Kim, H. O. & Lee, P. H. (2008). Neuroprotective effects of human mesenchymal stem cells on dopaminergic neurons through anti-inflammatory action. *Glia*, Vol. 57, No.1, pp. 13-23.

Kristensen, B. W., Noraberg, J., Thiebaud, P., Koudelka-Hep, M. & Zimmer, J. (2001). Biocompatibility of silicon-based arrays of electrodes coupled to organotypic hippocampal brain slice cultures. *Brain Res,* Vol.896, No.1-2, pp. 1-17.

Laquerriere, P., Grandjean-Laquerriere, A., Jallot, E., Balossier, G., Frayssinet, P. & Guenounou, M. (2003). Importance of hydroxyapatite particles characteristics on cytokines production by human monocytes in vitro. *Biomaterials,* Vol.24, No.16, pp. 2739-2747.

Laustriat, D., Gide, J. & Peschanski, M. (2010). Human pluripotent stem cells in drug discovery and predictive toxicology. *Biochem Soc Trans,* Vol.38, No.4, pp. 1051-1057.

Lee, H., Park, J., Forget, B. G. & Gaines, P. (2009). Induced pluripotent stem cells in regenerative medicine: an argument for continued research on human embryonic stem cells. *Regen Med,* Vol.4, No.5, pp. 759-769.

Lee, J. H., Yu, H. S., Lee, G. S., Ji, A., Hyun, J. K. & Kim, H. W. (2011). Collagen gel three-dimensional matrices combined with adhesive proteins stimulate neuronal differentiation of mesenchymal stem cells. *J R Soc Interface*, In press.

Lescaudron, L., Unni, D. & Dunbar, G. L. (2003). Autologous adult bone marrow stem cell transplantation in an animal model of huntington's disease: behavioral and morphological outcomes. *Int J Neurosci,* Vol.113, No.7, pp. 945-956.

Levesque, M. F. (2009). Therapeutic Microinjection of Autologous Adult Human Neural Stem Cells and Differentiated Neurons for Parkinson's Disease: Five-Year Post-Operative Outcome. *The Open Stem Cell Journal,* Vol.1, pp. 20-29.

Levesque, S. G. & Shoichet, M. S. (2006). Synthesis of cell-adhesive dextran hydrogels and macroporous scaffolds. *Biomaterials,* Vol.27, No.30, pp. 5277-5285.

Levy, Y. S., Bahat-Stroomza, M., Barzilay, R., Burshtein, A., Bulvik, S., Barhum, Y., Panet, H., Melamed, E. & Offen, D. (2008). Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease. *Cytotherapy,* Vol.10, No.4, pp. 340-352.

Li, S. C. & Loudon, W. G. (2008). A novel and generalizable organotypic slice platform to evaluate stem cell potential for targeting pediatric brain tumors. *Cancer Cell Int,* Vol.8, pp. 9.

Li, Y., Chen, J., Chen, X. G., Wang, L., Gautam, S. C., Xu, Y. X., Katakowski, M., Zhang, L. J., Lu, M., Janakiraman, N. & Chopp, M. (2002). Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology,* Vol.59, No.4, pp. 514-523.

Li, Y., Chen, J., Zhang, C. L., Wang, L., Lu, D., Katakowski, M., Gao, Q., Shen, L. H., Zhang, J., Lu, M. & Chopp, M. (2005). Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. *Glia,* Vol.49, No.3, pp. 407-417.

Li, Y. & Chopp, M. (2009). Marrow stromal cell transplantation in stroke and traumatic brain injury. *Neurosci Lett,* Vol.456, No.3, pp. 120-123.

Li, Y., Chopp, M., Chen, J., Wang, L., Gautam, S. C., Xu, Y. X. & Zhang, Z. (2000). Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. *J Cereb Blood Flow Metab,* Vol.20, No.9, pp. 1311-1319.

Lindvall, O. & Kokaia, Z. (2009). Prospects of stem cell therapy for replacing dopamine neurons in Parkinson's disease. *Trends Pharmacol Sci,* Vol.30, No.5, pp. 260-267.

Loewenbruck, K. & Storch, A. (2011). Stem cell-based therapies in Parkinson's disease: future hope or current treatment option? *J Neurol*, In press.

Lois, C. & Alvarez-Buylla, A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci U S A,* Vol.90, No.5, pp. 2074-2077.

Lossi, L., Alasia, S., Salio, C. & Merighi, A. (2009). Cell death and proliferation in acute slices and organotypic cultures of mammalian CNS. *Prog Neurobiol,* Vol.88, No.4, pp. 221-245.

Lu, D., Mahmood, A., Qu, C., Hong, X., Kaplan, D. & Chopp, M. (2007). Collagen scaffolds populated with human marrow stromal cells reduce lesion volume and improve functional outcome after traumatic brain injury. *Neurosurgery,* Vol.61, No.3, pp. 596-602; discussion 602-593.

Madrazo, I., Drucker-Colin, R., Diaz, V., Martinez-Mata, J., Torres, C. & Becerril, J. J. (1987). Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med,* Vol.316, No.14, pp. 831-834.

Madrigal, J. L., Leza, J. C., Polak, P., Kalinin, S. & Feinstein, D. L. (2009). Astrocyte-derived MCP-1 mediates neuroprotective effects of noradrenaline. *J Neurosci,* Vol.29, No.1, pp. 263-267.

Matsubara, T., Tsutsumi, S., Pan, H., Hiraoka, H., Oda, R., Nishimura, M., Kawaguchi, H., Nakamura, K. & Kato, Y. (2004). A new technique to expand human mesenchymal stem cells using basement membrane extracellular matrix. *Biochem Biophys Res Commun,* Vol.313, No.3, pp. 503-508.

McCoy, M. K., Martinez, T. N., Ruhn, K. A., Wrage, P. C., Keefer, E. W., Botterman, B. R., Tansey, K. E. & Tansey, M. G. (2008). Autologous transplants of Adipose-Derived Adult Stromal (ADAS) cells afford dopaminergic neuroprotection in a model of Parkinson's disease. *Exp Neurol,* Vol.210, No.1, pp. 14-29.

Menei, P., Montero-Menei, C., Venier, M. C. & Benoit, J. P. (2005). Drug delivery into the brain using poly(lactide-co-glycolide) microspheres. *Expert Opin Drug Deliv,* Vol.2, No.2, pp. 363-376.

Miura, K., Okada, Y., Aoi, T., Okada, A., Takahashi, K., Okita, K., Nakagawa, M., Koyanagi, M., Tanabe, K., Ohnuki, M., Ogawa, D., Ikeda, E., Okano, H. & Yamanaka, S. (2009). Variation in the safety of induced pluripotent stem cell lines. *Nat Biotechnol*, Vol.7, No.8, pp. 743-5.

Nakagawa, M. & Yamanaka, S. (2011). Reprogramming of somatic cells to pluripotency. *Adv Exp Med Biol,* Vol.695, pp. 215-224.

Namba, R. M., Cole, A. A., Bjugstad, K. B. & Mahoney, M. J. (2009). Development of porous PEG hydrogels that enable efficient, uniform cell-seeding and permit early neural process extension. *Acta Biomater*, Vol.5, No.6, pp. 1884-1897.

Narsinh KH, Jia F, Robbins RC, Kay MA, Longaker MT, Wu JC. (2011) Generation of adult human induced pluripotent stem cells using nonviral minicircle DNA vectors. *Nat Proc.* Vol.6 pp.78-88

Newman, K. D. & McBurney, M. W. (2004). Poly(d,l lactic-co-glycolic acid) microspheres as biodegradable microcarriers for pluripotent stem cells. *Biomaterials,* Vol.25, No.26, pp. 5763-5771.

Noraberg, J., Poulsen, F. R., Blaabjerg, M., Kristensen, B. W., Bonde, C., Montero, M., Meyer, M., Gramsbergen, J. B. & Zimmer, J. (2005). Organotypic hippocampal slice cultures for studies of brain damage, neuroprotection and neurorepair. *Curr Drug Targets CNS Neurol Disord,* Vol.4, No.4, pp. 435-452.

Ohtaki, H., Ylostalo, J. H., Foraker, J. E., Robinson, A. P., Reger, R. L., Shioda, S. & Prockop, D. J. (2008). Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. *Proc Natl Acad Sci U S A,* Vol.105, No.38, pp. 14638-14643.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K. I., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H. & Yamanaka, S. (2011). A more efficient method to generate integration-free human iPS cells. *Nat Methods*, In press.

Olanow, C. W., Schapira, A. H. & Agid, Y. (2003). Neuroprotection for Parkinson's disease: prospects and promises. *Ann Neurol,* Vol.53 Suppl 3, pp. S1-2.

Orive, G., De Castro, M., Kong, H. J., Hernandez, R. M., Ponce, S., Mooney, D. J. & Pedraz, J. L. (2009). Bioactive cell-hydrogel microcapsules for cell-based drug delivery. *J Control Release,* Vol.135, No.3, pp. 203-210.

Orive, G., Gascon, A. R., Hernandez, R. M., Igartua, M. & Luis Pedraz, J. (2003). Cell microencapsulation technology for biomedical purposes: novel insights and challenges. *Trends Pharmacol Sci,* Vol.24, No.5, pp. 207-210.

Page, R. L., Ambady, S., Holmes, W. F., Vilner, L., Kole, D., Kashpur, O., Huntress, V., Vojtic, I., Whitton, H. & Dominko, T. (2009). Induction of Stem Cell Gene Expression in Adult Human Fibroblasts without Transgenes. *Cloning Stem Cells*, pp.

Pawelek, J. M. & Korner, A. M. (1982). The biosynthesis of mammalian melanin. *Am Sci,* Vol.70, No.2, pp. 136-145.

Pean, J. M., Menei, P., Morel, O., Montero-Menei, C. N. & Benoit, J. P. (2000). Intraseptal implantation of NGF-releasing microspheres promote the survival of axotomized cholinergic neurons. *Biomaterials,* Vol.21, No.20, pp. 2097-2101.

Pera, M. F. (2011). Stem cells: The dark side of induced pluripotency. *Nature,* Vol.471, No.7336, pp. 46-47.

Perale, G., Giordano, C., Bianco, F., Rossi, F., Tunesi, M., Daniele, F., Crivelli, F., Matteoli, M. & Masi, M. (2011). Hydrogel for cell housing in the brain and in the spinal cord. *Int J Artif Organs*, Vol.34, No.3, pp. 46-7

Perasso, L., Cogo, C. E., Giunti, D., Gandolfo, C., Ruggeri, P., Uccelli, A. & Balestrino, M. (2011). Systemic administration of mesenchymal stem cells increases neuron survival after global cerebral ischemia in vivo (2VO). *Neural Plast,* Vol.2010, pp. 534925.

Pettikiriarachchi, J. T. S., Parish, C.L., Shoichet, M.S., Forsythe, J.S. & Nisbet D.R. (2010). Biomaterials for brain tissue engineering. *Australian Journal of Chemistry,* Vol.63, No.8, pp. 1143-1154.

Potter, W., Kalil, R. E. & Kao, W. J. (2008). Biomimetic material systems for neural progenitor cell-based therapy. *Front Biosci,* Vol.13, pp. 806-821.

Powell, S. K. & Kleinman, H. K. (1997). Neuronal laminins and their cellular receptors. *Int J Biochem Cell Biol,* Vol.29, No.3, pp. 401-414.

Prabhakaran, M. P., Venugopal, J. R. & Ramakrishna, S. (2009). Mesenchymal stem cell differentiation to neuronal cells on electrospun nanofibrous substrates for nerve tissue engineering. *Biomaterials*, Vol.30, No.28, pp. 4996-5003.

Purcell, E. K., Singh, A. & Kipke, D. R. (2009). Alginate composition effects on a neural stem cell-seeded scaffold. *Tissue Eng Part C Methods,* Vol.15, No.4, pp. 541-550.

Qian, L. & Saltzman, W. M. (2004). Improving the expansion and neuronal differentiation of mesenchymal stem cells through culture surface modification. *Biomaterials,* Vol.25, No.7-8, pp. 1331-1337.

Qu, C., Mahmood, A., Liu, X. S., Xiong, Y., Wang, L., Wu, H., Li, B., Zhang, Z. G., Kaplan, D. L. & Chopp, M. (2011). The treatment of TBI with human marrow stromal cells impregnated into collagen scaffold: functional outcome and gene expression profile. *Brain Res,* Vol.1371, pp. 129-139.

Qu, C., Xiong, Y., Mahmood, A., Kaplan, D. L., Goussev, A., Ning, R. & Chopp, M. (2009). Treatment of traumatic brain injury in mice with bone marrow stromal cell-impregnated collagen scaffolds. *J Neurosurg*, Vol. 111, No.4, pp.658-55.

Rahnemai-Azar, A., D'Ippolito, G., Gomez, L. A., Reiner, T., Vazquez-Padron, R. I., Perez-Stable, C., Roos, B. A., Pham, S. M. & Schiller, P. C. (2011). Human marrow-isolated adult multilineage-inducible (MIAMI) cells protect against peripheral vascular ischemia in a mouse model. *Cytotherapy,* Vol.13, No.2, pp. 179-192.

Rogers, S. L., Letourneau, P.C., Palm, S.L., McCarthy, J. & Furcht L.T. (1983). Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. *Dev Biol,* Vol. 98, No.1, pp. 212-220.

Re, D. B. & Przedborski, S. (2006). Fractalkine: moving from chemotaxis to neuroprotection. *Nat Neurosci,* Vol.9, No.7, pp. 859-861.

Richardson, R. M., Sun, D. & Bullock, M. R. (2007). Neurogenesis after traumatic brain injury. *Neurosurg Clin N Am,* Vol.18, No.1, pp. 169-181, xi.

Richardson, T. P., Peters, M. C., Ennett, A. B. & Mooney, D. J. (2001). Polymeric system for dual growth factor delivery. *Nat Biotechnol,* Vol.19, No.11, pp. 1029-1034.

Sadan, O., Bahat-Stromza, M., Barhum, Y., Levy, Y. S., Pisnevsky, A., Peretz, H., Bar Ilan, A., Bulvik, S., Shemesh, N., Krepel, D., Cohen, Y., Melamed, E. & Offen, D. (2009). Protective effects of neurotrophic factors secreting cells in a 6OHDA rat model of Parkinson disease. *Stem Cells Dev*, Vol.18, No.8, pp. 1179-1190

Salinas, C. N. & Anseth, K. S. (2008). The influence of the RGD peptide motif and its contextual presentation in PEG gels on human mesenchymal stem cell viability. *J Tissue Eng Regen Med*, Vol.2, No.5, pp. 296-304

Saporta, S., Borlongan, C., Moore, J., Mejia-Millan, E., Jones, S. L., Bonness, P., Randall, T. S., Allen, R. C., Freeman, T. B. & Sanberg, P. R. (1997). Microcarrier enhanced survival of human and rat fetal ventral mesencephalon cells implanted in the rat striatum. *Cell Transplant,* Vol.6, No.6, pp. 579-584.

Savitz, S. I., Chopp, M., Deans, R., Carmichael, S. T., Phinney, D. & Wechsler, L. (2011). Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) II. *Stroke,* Vol.42, No.3, pp. 825-829.

Schierle, G. S., Hansson, O., Leist, M., Nicotera, P., Widner, H. & Brundin, P. (1999). Caspase inhibition reduces apoptosis and increases survival of nigral transplants. *Nat Med,* Vol.5, No.1, pp. 97-100.

Seidlits, S. K., Lee, J. Y. & Schmidt, C. E. (2008). Nanostructured scaffolds for neural applications. *Nanomed,* Vol.3, No.2, pp. 183-199.

Shive, M. S. & Anderson, J. M. (1997). Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev,* Vol.28, No.1, pp. 5-24.

Stoppini, L., Buchs, P. A. & Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *J Neurosci Methods,* Vol.37, No.2, pp. 173-182.

Stover, N. P. & Watts, R. L. (2008). Spheramine for treatment of Parkinson's disease. *Neurotherapeutics,* Vol.5, No.2, pp. 252-259.

Subia, B., Kundu, J. & Kundu, S.C. (2010). Biomaterial scaffold fabrication techniques for potential tissue engineering applications., InTech.

Sugiyama, M., Iohara, K., Wakita, H., Hattori, H., Ueda, M., Matsushita, K. & Nakashima, M. (2011). Dental Pulp Derived CD31-/CD146- Side Population Stem/Progenitor

Cells Enhance Recovery of Focal Cerebral Ischemia in Rats. *Tissue Eng Part A*, In press.

Sundstrom, L., Morrison, B., 3rd, Bradley, M. & Pringle, A. (2005). Organotypic cultures as tools for functional screening in the CNS. *Drug Discov Today,* Vol.10, No.14, pp. 993-1000.

Swistowski, A., Peng, J., Liu, Q., Mali, P., Rao, M. S., Cheng, L. & Zeng, X. (2010). Efficient Generation of Functional Dopaminergic Neurons from Human Induced pluripotent Stem Cells under Defined Conditions. *Stem Cells*, Vol.28, No.10, pp. 1893-1904.

Sykova, E. & Jendelova, P. (2007). In vivo tracking of stem cells in brain and spinal cord injury. *Prog Brain Res,* Vol.161, pp. 367-383.

Takahashi, K., Okita, K., Nakagawa, M. & Yamanaka, S. (2007). Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc,* Vol.2, No.12, pp. 3081-3089.

Tang, Y., Pacary, E., Freret, T., Divoux, D., Petit, E., Schumann-Bard, P. & Bernaudin, M. (2006). Effect of hypoxic preconditioning on brain genomic response before and following ischemia in the adult mouse: identification of potential neuroprotective candidates for stroke. *Neurobiol Dis,* Vol.21, No.1, pp. 18-28.

Tatard, V. M., D'Ippolito, G., Diabira, S., Valeyev, A., Hackman, J., McCarthy, M., Bouckenooghe, T., Menei, P., Montero-Menei, C. N. & Schiller, P. C. (2007). Neurotrophin-directed differentiation of human adult marrow stromal cells to dopaminergic-like neurons. *Bone,* Vol.40, No.2, pp. 360-373.

Tatard, V. M., Menei, P., Benoit, J. P. & Montero-Menei, C. N. (2005). Combining polymeric devices and stem cells for the treatment of neurological disorders: a promising therapeutic approach. *Curr Drug Targets,* Vol.6, No.1, pp. 81-96.

Tatard, V. M., Sindji, L., Branton, J. G., Aubert-Pouessel, A., Colleau, J., Benoit, J. P. & Montero-Menei, C. N. (2007). Pharmacologically active microcarriers releasing glial cell line - derived neurotrophic factor: Survival and differentiation of embryonic dopaminergic neurons after grafting in hemiparkinsonian rats. *Biomaterials,* Vol.28, No.11, pp. 1978-1988.

Tatard, V. M., Venier-Julienne, M. C., Benoit, J. P., Menei, P. & Montero-Menei, C. N. (2004). In vivo evaluation of pharmacologically active microcarriers releasing nerve growth factor and conveying PC12 cells. *Cell Transplant,* Vol.13, No.5, pp. 573-583.

Tatard, V. M., Venier-Julienne, M. C., Saulnier, P., Prechter, E., Benoit, J. P., Menei, P. & Montero-Menei, C. N. (2005). Pharmacologically active microcarriers: a tool for cell therapy. *Biomaterials,* Vol.26, No.17, pp. 3727-3737.

Tate, C. C., Shear, D. A., Tate, M. C., Archer, D. R., Stein, D. G. & LaPlaca, M. C. (2009). Laminin and fibronectin scaffolds enhance neural stem cell transplantation into the injured brain. *J Tissue Eng Regen Med,* Vol.3, No.3, pp. 208-217.

Tate, C. C., Tate, M. C. & LaPlaca, M. C. (2007). Fibronectin and laminin increase in the mouse brain after controlled cortical impact injury. *J Neurotrauma,* Vol.24, No.1, pp. 226-230.

Trzaska, K. A., Kuzhikandathil, E. V. & Rameshwar, P. (2007). Specification of a dopaminergic phenotype from adult human mesenchymal stem cells. *Stem Cells,* Vol.25, No.11, pp. 2797-2808.

Trzaska, K. A. & Rameshwar, P. (2011). Dopaminergic Neuronal Differentiation Protocol for Human Mesenchymal Stem Cells. *Methods Mol Biol,* Vol.698, pp. 295-303.

Tse, J. R. & Engler, A. J. (2011). Stiffness gradients mimicking in vivo tissue variation regulate mesenchymal stem cell fate. *PLoS ONE,* Vol.6, No.1, pp. e15978.

Valmikinathan, C. M., Tian, J., Wang, J. & Yu, X. (2008). Novel nanofibrous spiral scaffolds for neural tissue engineering. *J Neural Eng,* Vol.5, No.4, pp. 422-432.

van Velthoven, C. T., Kavelaars, A., van Bel, F. & Heijnen, C. J. (2009). Regeneration of the ischemic brain by engineered stem cells: fuelling endogenous repair processes. *Brain Res Rev,* Vol.61, No.1, pp. 1-13.

Vazey, E. M. & Connor, B. (2011). Differential fate and functional outcome of lithium chloride primed adult neural progenitor cell transplants in a rat model of Huntington disease. *Stem Cell Res Ther,* Vol.1, No.5, pp. 41.

Vazey, E. M., Chen, K., Hughes, S. M. & Connor, B. (2006). Transplanted adult neural progenitor cells survive, differentiate and reduce motor function impairment in a rodent model of Huntington's disease. *Exp Neurol,* Vol.199, No.2, pp. 384-396.

Vert, M. (2009). Degradable and bioresorbable polymers in surgery and in pharmacology: beliefs and facts. *J Mater Sci Mater Med,* Vol.20, No.2, pp. 437-446.

Veziers, J., Lesourd, M., Jollivet, C., Montero-Menei, C., Benoit, J. P. & Menei, P. (2001). Analysis of brain biocompatibility of drug-releasing biodegradable microspheres by scanning and transmission electron microscopy. *J Neurosurg,* Vol.95, No.3, pp. 489-494.

Walker, P. A., Aroom, K. R., Jimenez, F., Shah, S. K., Harting, M. T., Gill, B. S. & Cox, C. S., Jr. (2009). Advances in progenitor cell therapy using scaffolding constructs for central nervous system injury. *Stem Cell Rev,* Vol.5, No.3, pp. 283-300.

Wang, T. W. & Spector, M. (2009). Development of hyaluronic acid-based scaffolds for brain tissue engineering. *Acta Biomater,* Vol.5, No.7, pp. 2371-2384.

Watts, R. L., Raiser, C. D., Stover, N. P., Cornfeldt, M. L., Schweikert, A. W., Allen, R. C., Subramanian, T., Doudet, D., Honey, C. R. & Bakay, R. A. (2003). Stereotaxic intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson's disease. *J Neural Transm Suppl*, No.65, pp. 215-227.

Wernig, M., Zhao, J. P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Isacson, O. & Jaenisch, R. (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 U S A,* Vol.105, No.15, pp. 5856-5861.

Wong, D. Y., Krebsbach, P. H. & Hollister, S. J. (2008). Brain cortex regeneration affected by scaffold architectures. *J Neurosurg,* Vol.109, No.4, pp. 715-722.

Xiong, Y., Qu, C., Mahmood, A., Liu, Z., Ning, R., Li, Y., Kaplan, D. L., Schallert, T. & Chopp, M. (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*, Vol. 1263, pp. 183-191.

Yamashita, T., Deguchi, K., Sehara, Y., Lukic-Panin, V., Zhang, H., Kamiya, T. & Abe, K. (2009). Therapeutic strategy for ischemic stroke. *Neurochem Res,* Vol.34, No.4, pp. 707-710.

Yang, C. Y., Song, B., Ao, Y., Nowak, A. P., Abelowitz, R. B., Korsak, R. A., Havton, L. A.,Deming, T. J. & Sofroniew, M. V. (2009). Biocompatibility of amphiphilic diblock

copolypeptide hydrogels in the central nervous system. *Biomaterials*, Vol. 30, No.15, pp. 2881-2898.

Yang, K. L., Chen, M. F., Liao, C. H., Pang, C. Y. & Lin, P. Y. (2009). A simple and efficient method for generating Nurr1-positive neuronal stem cells from human wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. *Cytotherapy*, Vol. 11, No.5, pp. 1-12.

Yarygin, K. N., Kholodenko, I. V., Konieva, A. A., Burunova, V. V., Tairova, R. T., Gubsky, L. V., Cheglakov, I. B., Pirogov, Y. A., Yarygin, V. N. & Skvortsova, V. I. (2009). Mechanisms of positive effects of transplantation of human placental mesenchymal stem cells on recovery of rats after experimental ischemic stroke. *Bull Exp Biol Med,* Vol.148, No.6, pp. 862-868.

Yasuda, H., Kuroda, S., Shichinohe, H., Kamei, S., Kawamura, R. & Iwasaki, Y. (2010). Effect of biodegradable fibrin scaffold on survival, migration, and differentiation of transplanted bone marrow stromal cells after cortical injury in rats. *J Neurosurg,* Vol.112, No.2, pp. 336-344.

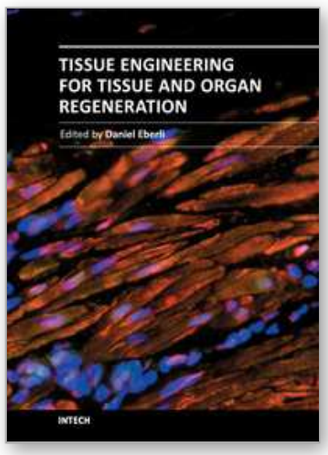
Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, II & Thomson, J. A. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. *Science,* Vol.324, No.5928, pp. 797-801.

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., Slukvin, II & Thomson, J. A. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science,* Vol.318, No.5858, pp. 1917-1920.

Zhang, Z., Jin, D., Yang, Z., Shen, B. & Liu, M. (2011). Effects of 17beta-estradiol pre-treated adult neural stem cells on neuronal differentiation and neurological recovery in rats with cerebral ischemia. *Brain Inj,* Vol.25, No.2, pp. 227-236.

Zhang, Z., Wang, X. & Wang, S. (2008). Isolation and characterization of mesenchymal stem cells derived from bone marrow of patients with Parkinson's disease. *In Vitro Cell Dev Biol Anim,* Vol.44, No.5-6, pp. 169-177.

Zhou, H., Wu, S., Joo, J. Y., Zhu, S., Han, D. W., Lin, T., Trauger, S., Bien, G., Yao, S., Zhu, Y., Siuzdak, G., Scholer, H. R., Duan, L. & Ding, S. (2009). Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell,* Vol.4, No.5, pp. 381-384.



Tissue Engineering for Tissue and Organ Regeneration

Edited by Prof. Daniel Eberli

ISBN 978-953-307-688-1
Hard cover, 454 pages
Publisher InTech
Published online 17, August, 2011
Published in print edition August, 2011

Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Elisa Garbayo, Gaëtan J.-R. Delcroix, Paul C. Schiller and Claudia N. Montero-Menei (2011). Advances in the Combined Use of Adult Cell Therapy and Scaffolds for Brain Tissue Engineering, Tissue Engineering for Tissue and Organ Regeneration, Prof. Daniel Eberli (Ed.), ISBN: 978-953-307-688-1, InTech, Available from: http://www.intechopen.com/books/tissue-engineering-for-tissue-and-organ-regeneration/advances-in-the-combined-use-of-adult-cell-therapy-and-scaffolds-for-brain-tissue-engineering

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen