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Review: Preclinical studies on placenta-derived cells and amniotic membrane: An update

O. Parolini*, M. Caruso

Centro di Ricerca E. Menni, Fondazione Poliambulanza-Istituto Ospedaliero, Via Bissolati 57, I-25124 Brescia, Italy

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ABSTRACT

Recent years have seen considerable advances in our knowledge of the biology and properties of stem/progenitor cells isolated from placental tissues. This has encouraged researchers to address the potential effects of these cells in animal models of different diseases, resulting in increasing expectations regarding their possible utility for cell-based therapeutic applications. This rapidly evolving research field is also enriched by studies aimed at expanding the use of the whole amniotic membrane (AM), a well-known surgical material, for pathological conditions other than those tested so far and for which clinical applications already exist.

In this review, we provide an update on studies that have been performed with placenta-derived cells and fragments of the entire AM to validate their potential clinical applications in a variety of diseases, in particular those associated with degenerative processes induced by inflammatory and fibrotic mechanisms. We also offer, as far as possible, insight into the interpretation and suggested mechanisms to explain the most important outcomes achieved to date.

1. Introduction

The placenta is generally recognized for important functions such as nutrition, respiration and excretion as well as maintenance of fetomaternal tolerance. The components of this organ include the fetal membranes, umbilical cord and trophoblast. In recent years, besides the use of fragments of the entire amniotic membrane (AM) as a surgical material [1,2], much attention has been given to the different cell types that can be isolated from the placenta. Progress in understanding the biology and properties of these cells has encouraged researchers to explore their potential effects in animal models of different diseases, in the hope of future clinical applications [3,4].

1.1. Placenta-derived cells

Several researchers have reported the isolation and characterization, from human and animal placentas, of cell populations with the properties of stem/progenitor cells [3]. Considering the current lack of standardization regarding the isolation and cultivation of placental cells applied in different laboratories and given that, in many cases, there is no precise description of the placental regions from which the cells are isolated, it is often quite difficult to compare results reported by different groups. This is further complicated by the fact that some groups work with freshly isolated cells, while others work with cultured and expanded cells, opening the possibility that the culture conditions themselves may give rise to selection of different cell populations. This scenario is further confounded by the different developmental origins of cells isolated from human and non-human placentas. Indeed, it is important to bear in mind that, whilst the placentas of eutherian mammals share common physiological and functional features, there are remarkable differences in terms of macroscopic and microscopic structure. Generally, classification of the placental types encountered in mammals is based on two main characteristics: *i)* the shape of the placenta and the distribution of contact sites between the fetal membranes and endometrium (diffuse, cotyledonary, zonary or discoid); *ii)* the number of tissue layers intervening between the maternal and fetal blood (epitheliochorial, endotheliochorial, and hemochorial). Moreover, differences exist between animals with a similar type of placentation (*e.g.* human and mouse) [5,6]. These differences should be kept in mind when selecting isolation protocols for placental cells, and especially when adapting protocols from one species to another, in order to ensure that cells are derived from the same anatomical region, thereby avoiding misinterpretations, as highlighted in some recent reports [6].

In this review, when discussing cells isolated from the human amniotic membrane, we will adopt the nomenclature of Parolini

* Corresponding author. Tel.: +39 0302455754; fax: +39 0302455704.
E-mail addresses: ornella.parolini@tin.it, parolini-ornella@poliambulanza.it (O. Parolini).

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et al. [3]: human amniotic epithelial cells (hAEC) and human amniotic mesenchymal stromal cells (hAMSC), to indicate cells isolated from the epithelial and mesenchymal layers of the amniotic membrane, respectively, although it should also be noted that these cell populations are very heterogeneous. When describing results obtained with cells isolated from other placental regions, or cells which are defined by a specific phenotype, as well as with cells isolated from non-human placentas, we will maintain the nomenclature chosen by the authors of the studies to which we refer.

Here we will not discuss cells isolated from umbilical cord and umbilical cord blood (UCB), for which we refer readers to other reviews specifically dedicated to these cells (*e.g.* [7]).

Aside from their relative ease of isolation and the lack of ethical concerns for their procurement, three main characteristics of placenta-derived cells make them viable candidates for cell-based therapeutic approaches: *i)* their absent or low immunogenicity and immunomodulatory properties (*e.g.* fetal membrane-derived cells fail to induce an allogeneic T-cell response, actively suppress T-cell proliferation induced by alloantigens or by a mitogenic stimulus, block differentiation and maturation of monocytes into dendritic cells) which suggest their utility in allogeneic transplantation settings [3,4]; *ii)* their multilineage differentiation capacity *in vitro*, even across germinal boundaries outside of their specific lineage [3], suggesting their utility in tissue regeneration approaches; and *iii)* their ability to successfully engraft and survive long-term in various organs and tissues, *e.g.* after transplantation into neonatal animals [8] and after in utero transplantation into pregnant rats [9]. Notably, human microchimerism has been detected in host organs without evidence of inflammation or rejection, indicating active tolerance of the cells [8]. Besides these important properties, it is noteworthy that placenta-derived cells can secrete a number of factors involved in various pathophysiological events, such as cytokines which have immunomodulatory and anti-inflammatory effects [10], as well as angiogenic factors associated with wound healing [10,11], growth factors related to cell proliferation and differentiation [10–14], and anti-apoptotic and anti-oxidative factors [15].

1.2. Amniotic membrane

Amniotic membrane (AM) displays anti-inflammatory properties, anti-bacterial properties and wound protection, and anti-fibroblastic and epithelialization effects (for reviews see [1,2]). These properties, combined with the absent/low immunogenicity of AM-derived cells, has led to the use of AM as a dressing in the clinic, *e.g.* to promote healing of burned skin and leg ulcers, as well as to treat a continuously widening spectrum of ophthalmic disorders [1,2]. In this review we will not include these well-known clinical applications of AM, but focus instead on recent studies where the use of fragments of the entire AM has been proposed for the treatment of other pathological conditions in preclinical settings.

This paper reviews and summarizes the majority of preclinical studies which have been performed using cells isolated from placental tissues or using fragments of the entire AM, both in allogeneic and xenogeneic settings, and offers, as far as possible, insight into the interpretation and suggested mechanisms to explain the most significant results achieved.

2. Preclinical studies

2.1. Neurological diseases

Since 1996, when the group of Sakuragawa [16] suggested that hAEC may act as progenitors for neurons and glial cells, due to their expression of markers for both cell types, accumulating evidence has provoked researchers to employ direct transplantation *in vivo* of placental cells for the treatment of neurological disorders which affect both the brain and spinal cord.

As it was believed that differentiation of cells toward the neurogenic lineage was the first necessity for their application *in vivo,* several authors investigated the ability of placental cells to differentiate *in vitro* into neural-like and astrocytic-like cells. Indeed, placenta-derived cells, under particular culture conditions, express neuronal and glial markers [17–19]. Amniotic epithelial cells (AECs) derived from both human and non-human placentas can synthesize and release neurotransmitters and neurotrophic factors [13].

2.1.1. Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive death of nigral dopaminergic (DA) neurons.

The majority of studies that assess the ability of placental cells to treat this disorder have been performed with hAEC in xenogeneic transplantation settings. Intriguingly, in all of these studies, despite expectations, the authors conclude that the beneficial effects conferred by hAEC are most likely related to the bioactive molecules secreted by transplanted cells, which may act by paracrine mechanisms on surrounding host tissues, rather than their ability to differentiate toward the neurogenic lineage.

Specifically, Kakishita et al. [14,20] showed that transplantation of hAEC into an immunosuppressed rat model of PD counteracted the degeneration of nigral DA neurons [20] and afforded significant functional recovery [14], likely by means of DA and other diffusible molecules released by the transplanted cells (Table 1). A study [21] in non-immunosuppressed rats extended these results by providing evidence of a significant functional recovery, which was associated with a significant increase in DA and DOPAC (3,4-dihydroxyphenylacetic acid) levels in the striatum and of DA levels in the cerebrospinal fluid of the hAEC-treated group compared to the control group (Table 1).

Intra-striatal xenogeneic transplantation of hAEC also conferred benefit in a mouse model of PD [22]. These authors did not find neurons derived from hAEC in the mouse brains and suggested that transplanted cells could increase brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) levels in the striatum, promoting survival of DA neurons and endogenous neurogenesis (Table 1).

2.1.2. Stroke

Ischemic stroke results from a transient or permanent reduction in cerebral blood flow, causing cell death within minutes. Cell therapy has been investigated as a neurorestorative treatment for this disorder [23]. Okawa and colleagues [24] showed that rat-derived AECs infused into the ischemic brains of adult gerbils could migrate into the CA1 pyramidal layer, survive and be transformed into neuronal-like cells and neural stem cells (Table 1). Subsequently [25], it was shown that hAEC transplantation into ischemic rats resulted in a significant improvement of behavioral dysfunction and reduction of infarct volume. When infected to overexpress GDNF, these cells reduced the deficit more rapidly and stably than non-infected hAEC [25]. Considering that some of the transplanted cells expressed the neuronal marker MAP-2 (microtubule associated protein 2) and the neuronal progenitor marker nestin, together with the astrocyte marker glial fibrillary acidic protein (GFAP), the authors suggested that the beneficial effects exerted by hAEC could be due in part to differentiation of these cells toward the neurogenic lineage *in vivo,* and in part to paracrine actions of the neurotrophic factors that they secrete (Table 1).

Table 1
Preclinical studies for neurological diseases.

Disease	Cells	Animal Model	Manipulation *in vitro*[a]	Cell Transplantation (dose/route/timing)	Detection Time[b]	Results *in vivo*	Suggested mechanisms		Ref
							Paracrine Mechanisms	Tissue-specific Differentiation	
Parkinson's Disease	hAEC	6-OHDA-treated rats	culture and infection with recombinant adenovirus.	2 implants of 4×10^4 cells into denervated striatum, 2 wks after 6-OHDA treatment.	2 wks	Improvement of neurobehavioral deficit. No overgrowth of the grafted tissue.	hAEC may produce DA and other diffusible molecules with trophic and beneficial activities on DA neurons.		[14]
			culture and labeling	supranigral injection of 4×10^4 cells, on the day of 6-OHDA treatment.	2 wks	Increase of nigral DA cell number. No overgrowth of the grafted tissue.			[20]
			culture	1×10^6 cells into the lateral cerebral ventricle, 4 wks after 6-OHDA treatment.	5 wks	Improvement of neurobehavioral deficit. Increase of DA and DOPAC levels.	hAEC may produce neurotrophic factors.		[21]
		MPTP-treated mice	culture and labeling	2×10^4 cells into the right striatum, 1 wk after MPTP-treatment.	4 wks	Improvement of neurobehavioral deficit. Enhanced neurogenesis in the SVZ. Preservation of DA neurons. Increase of BDNF and GDNF levels.	Transplantation of hAEC could increase BDNF and GDNF levels in striatum, with DA neuron survival and endogenous neurogenesis.		[22]
Stroke	rat AECs	gerbils with occlusion of bilateralcarotid artery	culture and labeling	implantation of 2×10^5 cells into the right dorsal hippocampus 1 wk after ischemic insult.	5 wks	Grafted cells migrated into the CA1 pyramidal layer and survived in a manner similar to CA1 pyramidal neurons.	Rat AECs could have trophic effects on damaged neurons and regenerate neuronal cells.	Rat AECs may differentiate into neuronal-like and neural stem cells.	[24]
	hAEC	rats with middle cerebral artery occlusion	culture and infection with recombinant lentivirus	8×10^5 cells into the right dorsolateral striatum, one day after stroke.	3 wks	Functional recovery, reduction of infarct area and of cell apoptosis. After transplantion, some hAEC expressed astrocytic and neuronal markers.	hAEC may release neurotrophic factors. Reduction of apoptosis of host cells.	hAEC may differentiate into astrocytic and neuronal-like cells.	[25]
	human fpMSC and mpMSC	rats with experimental stroke	2D culture and expansion in a 3D bioreactor	single and dual injection of 1×10^6 cells in the tail vein, 8h and 24h upon stroke onset.	N.D.	Functional recovery and reduction of infarct area. Improvement of astroglial reactivity. Best results with mpMSCs.	Release of soluble factors. Modulation of immunoreactions. Adjustment of astroglial reactivity.		[26]
Spinal Cord Injury	rat AECs	rats with laminectomy	culture and infection with recombinant retrovirus	transplantation, alone or with NSCs, into the injured spinal cord 7 days after SCI. Allogeneic transplantation.	5 wks	Hindlimb motor function improvement. Rat AECs promoted the survival and neural differentiation of co-transplanted NSCs, and bFGF enhanced this ability. Rat AECs supported survival of the host neurons.	Rat AECs could improve the local microenvironment of the injured spinal cord and promote the differentiation of NSCs into neuron-like cells.		[27]
	hAEC	monkeys with transection of the spinal cord.	culture and labeling	$10-12 \times 10^3$ cells per mm^3 into the transection cavity.	60 days	No formation of glial scar at the cut ends. hAEC graft was penetrated by the host axons.	Prevention of death in the axotomized neurons or neurotrophic effects by hAEC.		[28]
		rats with transection of the spinal cord	culture and labeling	piece of Gelfoam soaked in hAEC suspension (1×10^5 cells) into the injured site.	8 wks	Hindlimb motor function recovery. The atrophy was ameliorated and the size of injured neurons partially restored.	Release of neurotrophic factors by hAEC.		[29]

AECs: Amniotic Epithelial Cells; BDNF: Brain-Derived Neurotrophic Factor; bFGF: basic Fibroblast Growth Factor; DA: DopAmine; DOPAC: 3,4-dihydroxyphenylacetic acid; fpMSC: Chorionic Villi-derived MSCs; GDNF: Glial cell line-Derived Neurotrophic Factor; hAEC: human Amniotic Epithelial Cells; mpMSC: decidua-derived MSCs; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; N.D. Not Determined; NSCs: Neural Stem Cells; 6-OHDA: 6-hydroxydopamine; Ref: References; SVZ: SubVentricular Zone; wks: weeks.

[a] Manipulation of cells *in vitro* before transplantation.

[b] Time of detection of transplanted cells in host tissues.

Interestingly, Kranz and colleagues [26] have recently reported comparative results obtained after transplantation of MSCs (mesenchymal stromal/stem cells) isolated from decidua (maternal part of placenta, indicated as mpMSC) and from chorionic villi (fetal part of placenta, indicated as fpMSC) (Table 1). The authors observed that transplanted cells, mainly mpMSC, significantly increased functional recovery in treated rats with respect to control animals, with a reduced infarct ratio in mpMSC-treated subjects and an improvement in astroglial reactivity. To explain this, the authors suggested a paracrine action of the transplanted cells through modulation of peripheral and local immunoreactions, which is significant given that stroke outcome is strongly influenced by inflammatory reaction [23]. Furthermore, the authors also proposed that the transplanted cells could secrete soluble factors with anti-apoptotic, neurogenic and angiogenic effects.

2.1.3. Spinal cord injury

Spinal cord injury (SCI) produces progressive cell death, axonal degeneration, and functional loss in multiple motor, sensory, and autonomic systems [27]. AECs have been employed to treat this disease in both xenogeneic and allogeneic settings (Table 1). When transplanted into bonnet monkey and rat models of SCI, hAEC survived and integrated in the host spinal cord, without evoking immune response or formation of scars in lesion areas [28,29], with improvement in hind limb motor function observed in rats [29]. Although the underlying mechanisms remain to be elucidated, the transplanted cells might prevent degeneration of axotomized neurons and exert neurotrophic effects [28,29]. In an allogeneic setting, rat AECs modified to express the basic fibroblast growth factor (bFGF) gene and co-transplanted with neural stem cells (NSCs) into a rat model of SCI, promoted the survival and neural differentiation of co-transplanted cells and supported survival of host neurons, likely by improving the local microenvironment of the injured spinal cord [27].

2.2. Pancreatic disease

Cell-based therapy with hAEC has also been attempted in animal models of insulin-dependent diabetes mellitus (DM) (Table 2), a disease characterized by autoimmune destruction of pancreatic β-cells and lack of insulin production. Wei and colleagues [30] showed that hAEC were capable of normalizing blood glucose level in diabetic mice up to 1 month after injection. Given that transplanted human cells co-localized with human insulin secretion in the mouse tissues, the authors concluded that hAEC may differentiate into pancreatic β-cells *in vivo*.

Further insight into this concept came from Hou and colleagues [31] who again demonstrated the ability of hAEC to reverse hyperglycemia after transplantation into diabetic mice. Intriguingly, these positive results were obtained with hAEC which had been transplanted after induction to differentiate into functional insulin producing cells *in vitro*, therefore supporting the idea that differentiated hAEC may provide a source of β-cells for the treatment of insulin-dependent DM.

2.3. Muscle disease

Recently Kawamichi et al. [32] tested the possibility of using placental cells to treat Duchenne muscular dystrophy (DMD), one of the most prevalent types of muscular dystrophy, for which an effective treatment is currently unavailable (Table 2). DMD is an X-linked recessive genetic disease caused by a deficiency in dystrophin. These authors compared the *in vitro* myogenic differentiation abilities of placental cell populations of both maternal and fetal origin. Only hAMSC, CP (chorionic plate-derived cells), and VC (villous chorion-derived cells) were able to express muscle-specific genes during differentiation *in vitro* (suggesting differentiation into myotubes). When hAMSC were transplanted into a mouse model of DMD, myofibers in the muscle tissues expressed human dystrophin and laminin. The authors suggested that this acquisition of dystrophin expression could be attributed to two different mechanisms, namely, myogenic differentiation of transplanted cells and/or cell fusion of transplanted cells with host muscle cells.

Park and colleagues [33] recently explored the *in vivo* myogenic and angiogenic potential of various perivascular cell populations isolated from chorionic villi, after intramuscular injection of these cells into immunodeficient dystrophic mice. The authors studied pericytes (CD146+/CD34−/CD45−/CD56−) and non-pericytes, namely, endothelial (CD146−/CD34+/CD45−/CD56−) and non-vascular (CD146−/CD34−/CD45−/CD56−) cells. Both pericytes and endothelial cells (the latter to a far lesser extent), generated differentiated dystrophin-expressing myofibers in the host, while non-vascular cells gave rise only to limited numbers of separated single myofibers. Injection of all villi-derived cells, and to a greater extent injection of pericytes, promoted local angiogenesis, likely via secretion of angiogenic factors or by recruitment of endogenous endothelial cells (Table 2).

2.4. Vascular disease

Peripheral vascular disease (PVD) refers to diseases caused by a significant narrowing of arteries distal to the aortic arch, most often due to atherosclerosis and inflammatory processes. PVD can cause either acute or chronic ischemia, and the advanced stage is represented by critical limb ischemia (CLI). Ishikane et al. [34] investigated whether allogeneic injection of MSCs isolated from the fetal membranes of pregnant rats had therapeutic effects in a rat model of hind limb ischemia (Table 2). Three weeks after cell injection, they observed a significant improvement in blood perfusion and a higher capillary/muscle fiber ratio of ischemic muscle in transplanted groups compared with the control group. Few green fluorescent protein-positive allogeneic cells remained in the hind limb tissues, despite no evidence of endothelial differentiation or cellular fusion of transplanted cells with host cells. To explain this beneficial action, the authors suggested a paracrine mechanism whereby transplanted cells might act as a source of cytokines, which also exert angiogenic effects, and/or may have mobilized host stem/progenitor cells (*e.g.* endothelial progenitor cells) to the injured site to accelerate angiogenesis.

Prather et al. [35], using human placenta-derived mesenchymal-like stromal cells (named PLX-PAD for placental expanded-peripheral artery disease) expanded in a 3D bioreactor, have investigated the possibility of treating CLI in a mouse model (Table 2). Injection of PLX-PAD cells significantly improved blood flow, increased capillary density, reduced oxidative stress and endothelial damage, with a slight increase in limb function, likely through a hypothesized, though still unvalidated, paracrine secretion mechanism. Recently, data from clinical trials have shown that PLX-PAD cell therapy is safe and improves quality of life and efficacy measurements in patients with CLI (http://www.pluristem.com).

2.5. Pulmonary and liver fibrosis

Fibrosis is defined as the overgrowth, hardening, and/or scarring of various tissues. It is attributed to excess deposition of extracellular matrix and results from chronic inflammation and an uncontrolled repair process [36]. Treatment with placenta-derived cells has been tested for pulmonary and liver fibrosis.

Table 2
Preclinical studies for pancreatic, muscle and vascular diseases.

Disease	Specific Disease	Cells	Animal Model	Manipulation *in vitro*[a]	Cell Transplantation (dose/route/timing)	Detection Time[b]	Results *in vivo*	Suggested Mechanisms		Ref.
								ParacrineMechanisms	Tissue-specific Differentiation	
Pancreatic Disease	Type 1 DM	hAECh, AMSC	streptozotocin-induced diabetic SCID mice	culture	1×10^6 cells into the spleen	1 months	Decrease of blood glucose level and restoration of body weight. Human cells co-localized with human insulin secretion in the mouse tissues.		hAEC may differentiate into pancreatic β-cells *in vivo*.	[30]
		hAEC	streptozotocin-induced diabetic C57 mice	infection with recombinant lentivirus and differentiation into insulin-producing cells	$2–3 \times 10^6$ cells into left subrenal capsule	30 days	hAEC transplantation reversed hyperglycemia, restored body weight, and maintained euglycemia for 30 days.		Functional insulin-producing hAEC may provide a source of β-cells.	[31]
Muscle Disease	DMD	hAMSC, hAEC, UC, CP VC, DB	mdx/mdx scid/ scid mice	culture	2×10^7 injected into the right tibialis anterior muscle	4 wks	Human dystrophin expression in dystrophic muscle of mice transplanted with hAMSC.		Myogenic differentiation of transplanted cells and/or cell fusion of transplanted cells with host muscle cells.	[32]
		Chorionic villi-isolated cells: peryctes, endothelial, non-vascular cells	SCID/*mdx* mice	culture and sorting	2×10^4 injected into the gastrocnemius muscle	2 wks	Human dystrophin-expressing myofibers in dystrophic muscle of mice transplanted with all the three cell populations, but mainly after injection of percytes. All human cell-injected mouse muscles contained more blood vessels than control mouse muscles.	Transplanted cells promote local angiogenesis, likely via secretion of angiogenic factors or by recruiting endogenous endothelial cells.	Chorionic villi-derived pericytes and endothelial cells give rise to differentiated dystrophin-expressing myofibers.	[33]
Vascular Diseases	Hind Limb Ischemia	rat FM-MSCs	rat with resection of the left common iliac artery	culture and expansion	5×10^6 cells injected into the ischemic thigh muscle, one day after injury induction. Allogeneic transplantation.	3 wks	Improvement in blood perfusion and in capillary/ muscle fiber ratio of ischemic muscle. No evidence of endothelial differentiation or fusion of transplanted cells with host cells.	FM-MSCs maybe a source of cytokine cocktails with angiogenic effects. FM-MSC may mobilize host stem-progenitor cells to the injured site to accelerate angiogenesis.		[34]
	CLI	human PLX-PAD	mice with hind limb ischemia	2D culture and expansion in a 3D bioractor	intramuscular injection of 1×10^6 PLX-PAD cells/ mouse/50 μL, 5h post-ischemia	N.D.	Improvement in blood flow, capillary density, oxidative stress and limb function. Reduction of endothelial damage.	Likely trough paracrine secretion mechanisms: N.D.		[35]

CLI: Critical Limb Ischemia; CP: Chorionic Plate-derived cells; DB: Decidua Basalis-derived cells; DMD: Duchenne Muscular Dystrophy; FM: Fetal Membrane; hAEC: human Amniotic Epithelial Cells; hAMSC: human Amniotic Mesenchymal Stromal Cells; MSCs: Mesenchymal Stromal/Stem Cells; N.D.: Not Determined; PLX-PAD: Placental eXpanded-Peripheral Artery Disease cells; Ref: References; Type 1 DM: Diabetes Mellitus Type 1; UC: Umbilical Cord-derived cells; VC: Villous Chorion-derived cells; wks: weeks.

[a] Manipulation of cells *in vitro* before transplantation.

[b] Time of detection of transplanted cells in host tissues.

Lung fibrosis often represents the final stage of progression for many interstitial lung diseases, and is characterized by irreversible changes in alveolar architecture and loss of respiratory capacity.

Our own efforts have demonstrated that fetal membrane-derived cells, after both allogeneic (murine cells into mice) and xenogeneic [a mix of hAEC, hAMSC and human chorionic mesenchymal stromal cells (hCMSC) into mice] transplantation into bleomycin-challenged immunocompetent mice, by different delivery routes, significantly reduces the severity of lung fibrosis (Table 3) [37]. Concomitantly, we observed a decrease in neutrophil infiltration. Considering that these beneficial effects were observed despite a sparse presence of donor cells in host lungs, we hypothesized that these effects were not only related to engraftment and differentiation of the transplanted cells, but more to paracrine actions exerted by the soluble molecules they secreted. A different mechanism has been proposed by Moodley and colleagues [38], who suggest that the beneficial effects of hAEC on lung fibrosis could result from adoption of a lung phenotype *in vivo* by transplanted hAEC and by an overall anti-inflammatory effect of these cells that modifies the response of the mouse lung to injury (Table 3). Specifically, they demonstrated that primary hAEC express lung-associated markers *in vitro,* and when cultured in a particular differentiation medium, produce and release surfactant proteins and display lamellar bodies typical of type II pneumocytes. When transplanted into bleomycin-instilled immunocompromised mice it was found that transplanted hAEC became positive for surfactant protein expression in the lungs of injured mice, suggesting their differentiation into type II pneumocytes *in vivo*. hAEC injection was associated with reduced levels of pro-inflammatory cytokines [interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α] and of a pro-fibrogenic cytokine [transforming growth factor (TGF)-β] in the lungs. Moreover, hAEC transplantation reduced fibrosis and collagen deposition and induced a collagen-degrading environment (altered proteases levels) in the injured lungs.

Since Sakuragawa and colleagues [39] first demonstrated that albumin- and α-fetoprotein-producing hAEC are promising transgene carriers for allogeneic transplantation into liver, other work has encouraged the use of placental cells for restoring functionality of hepatic tissues. For example, human AM-derived cells have been shown to engraft in the liver [8] and differentiate toward the hepatogenic lineage *in vitro* and *in vivo* [3,17].

Liver fibrosis is a common outcome of a variety of chronic liver diseases following different insults, such as viral infection, alcoholism, chemical toxicity or metabolic and biliary disorders. Manuelpillai and colleagues [40] have recently investigated the effects of hAEC transplantation in a mouse model of CCl_4-induced liver fibrosis (Table 3). These authors found hAEC in host liver, as well as in the spleen, lung and heart of some animals. After hAEC transplantation, a significant decrease was observed for hepatocyte apoptosis, hepatic inflammation and fibrosis, with a reduction in the hepatic content of pro-inflammatory cytokines (TGF-β, IL-6 and TNF-α), an increased expression of the anti-inflammatory cytokine IL-10 and collagenases, as well as a reduction of the number of activated collagen producing hepatic stellate cells.

We have recently demonstrated the value of fragments of the entire AM for treatment of biliary fibrosis in a rat model of bile duct ligation (BDL) (Table 3) [41], providing evidence that fragments of human AM, when applied as a patch onto the rat liver surface, significantly reduce the severity and slow the progression of liver fibrosis. The exact mechanism whereby human AM patches exert these beneficial effects on BDL-induced liver fibrosis remains to be defined. Since no human cells were detected in AM-treated rat livers, it is conceivable that the effects exerted by AM patch application might not be due to a replacement mechanism whereby AM-derived cells present in the host liver differentiated toward the hepatogenic lineage, but rather to the release of soluble factors by cells of the AM patch with paracrine effects on host tissue.

2.6. Heart disease

Although the ability of placenta-derived cells to differentiate toward cardiomyocytes is still debated [3,4,42], some groups have tested the ability of these cells to treat cardiac diseases.

Fujimoto et al. [43] investigated the potential of syngeneic transplantation of rat AM-derived cells (ADCs) into a rat model of myocardial infarct (Table 4). Transplanted cells were found in the host cardiac tissues at all time points studied, and remarkably, reduction of myocardial scarring and prevention of myocardial thinning was observed. The authors suggested a two-fold explanation for these results: *i)* differentiation of transplanted cells into cardiomyocytes and/or into vascular endothelial and smooth muscles cells; *ii)* stimulation of angiogenesis and prevention of residential cardiomyocyte death by transplanted cells. A similar double-faceted explanation was offered by Ventura and colleagues [44] to explain the ameliorating effects observed after transplantation of human fetal membrane-derived MSCs, which had been pre-treated with a cardiogenic/vasculogenic agent, into infarcted rat hearts (Table 4).

Our recent investigation into the utility of human AM patching to repair cardiac ischemia in a rat model [42] demonstrated that fragments of the entire AM could significantly reduce postischemic cardiac dysfunction (Table 4). Indeed, when a fragment of human AM was applied to the left ventricle of rats that had undergone ischemia, these rats showed higher preservation of cardiac dimensions and improved cardiac contractile function in terms of higher left ventricular ejection fraction, fractional shortening, and wall thickening, over a two month follow-up period. As we did not detect human cells in the host tissues, we believe that the beneficial effects were due not to differentiation of the transplanted cells, but more likely to release of mediators by the AM-cells that promoted protection and regeneration of host tissues.

2.7. Use of placental cells and fragments of AM for tissue engineering

Given that placenta-derived MSCs, like those derived from other sources, are able to differentiate *in vitro* toward the chondrogenic lineage [3], the use of these cells has been investigated for repair/regeneration of cartilage defects *in vivo*, which is a frequent defect with limited self-healing. Zhang and colleagues [45] inserted human chorionic villi-derived MSCs, which had been pre-embedded in a collagen sponge and cultured for two weeks in chondrogenic medium, into the articular osteochondral defect of nude rats (Table 4). Six weeks after surgery, the original defect was covered with reparative tissue and the bottom part of this tissue showed hyaline cartilage appearance. However, the edge of the reparative tissue showed hypertrophic cartilage formation, suggesting that while placental cells could be used to regenerate/repair damaged cartilage, it is necessary to set up appropriate transplantation conditions to avoid side effects. Recently, Wei and colleagues [46] also demonstrated that hAMSC, when implanted with collagen scaffolds into the cartilage defects of nude rats, underwent characteristic morphological changes concurrently with deposition of collagen type II, suggesting their differentiation into chondrocytes *in vivo* (Table 4).

Recently Ismail et al. [47] investigated the possible utility of fragments of the entire human AM as a bile duct substitute to repair three types of bile duct damage in a dog model, in the presence or absence of a vascularized support, with beneficial outcomes (Table 4).

Table 3
Preclinical studies for lung and liver diseases.

Disease	Specific Disease	Cells or Fragments of AM	Animal Model	Manipulation *in vitro*[a]	Cell Transplantation (route/dose/timing)	Detection Time[b]	Results *in vivo*	Suggested Mechanisms		Ref.
								Paracrine Mechanisms	Tissue-specific Differentiation	
		hAEC + hAMSC + hCMSC, murine FM-cells	bleomycin-challenged immunocompetent mice	freshly isolated and cryopreserved cells	intraperitoneal (4×10^6) or intrajugular (1×10^6) injection of cells, 15 min after bleomycin instillation.	14 days	Reduction in severity of lung fibrosis with a decrease in neutrophil infiltration. Low presence of transplanted cells in host lungs.	Paracrine actions on host tissues by the bioactive molecules secreted by transplanted cells.		[37]
Pulmonary Disorder	Lung Fibrosis	hAEC	bleomycin-challenged SCID mice	culture	1×10^6 hAEC into the tail vein, 24h after bleomycin instillation.	2 wks	hAEC became positive for surfactant protein expression *in vivo*. Reduction of inflammation and fibrosis. Reduction of several inflammatory and fibrotic cytokines. Altered protease levels in the injured lung.	Reduction in inflammation and cytokine expression maybe important for limiting damage and subsequent scarring in the lung. Changes in protease levels would constitute a pro-degradative environment for breakdown of deposited collagen.	The adoption of a lung phenotype *in vivo* by hAEC would assist in restituting alveolar epithelium.	[38]
	Hepatic Fibrosis	hAEC	mice with CCl4-induced fibrosis	culture	2×10^6 cells via the tail vein, midway through CCl4 treatment	2 wks	Human albumin was detected in murine sera. Reduction of serum ALT. Reduction of IL-6 and TNF-α and increase of IL-10 in the liver. Reduction of apoptotic hepatocytes and of α−SMA-positive cells. Less fibrotic areas and reduction of hepatic collagen content. Reduction of hepatic TGF-β levels. Increase of MMP-2 level.	Reduction in inflammation and cytokine expression maybe important for limiting damage and subsequent scarring in the liver.	hAEC xenografts survive and continue to secrete albumin in the host serum.	[40]
Liver Diseases	Biliary Fibrosis	Fragments of human AM	rat with Bile Duct Ligation (BDL)		AM fragment placed as a patch onto the liver surface immediately after BDL	not found	Rats treated with a fragment of AM showed confined fibrosis at the portal/periportal area with no signs of cirrhosis, and significant reduction in collagen deposition. The fragment of AM was able to significantly slow the gradual progression of the ductular reaction and reduce, at all time points, the area occupied by activated myofibroblasts.	Release of soluble factors by cells of the human AM patch		[41]

α-SMA: alpha-Smooth Muscle Actin; ALT: Alanine AminoTransferase; AM: Amniotic Membrane; FM: Fetal Membrane; hAEC: human Amniotic Epithelial Cells; hAMSC: human Amniotic Mesecnhymal Stromal Cells; hCMSC; human Chorionic Mesenchymal Stromal Cells; IL-: InterLeukin-; MMP-2: Matrix MetalloProteinase-2; Ref: References; TGF-β: Transforming growth factor-beta; TNF-α: Tumor Necrosis Factor-alpha; wks: weeks.

[a] Manipulation of cells *in vitro* before transplantation.

[b] Time of detection of transplanted cells in host tissues.

Table 4
Preclinical studies for other pathological conditions.

Disease	Specific Disease	Cells or Fragments of AM	Animal Model	Manipulation *in vitro*[a]	Cell Transplantation (dose/route/timing)	Detection Time[b]	Results *in vivo*	Suggested Mechanisms		Ref
								Paracrine Mechanisms	Tissue-specific Differentiation	
Cardiac Disease	Cardiac Ischemia	Fragments of human AM	rat with left ventricular infarction		AM fragment applied on the left ventricle immediately after ischemia induction.	not found	Preservation of cardiac dimensions. Improvement of cardiac contractile function: higher left ventricle ejection fraction, fractional shortening, and wall thickening.	Cells of the AM fragment may release soluble factors to promote protection and regeneration of host tissues.		[42]
		rat ADCs		not specified	2×10^6 cells into the infarcted myocardium. Syngeneic transplantation.	6 wks	Reduction of myocardial scar and prevention of myocardial thinning.	Stimulation of angiogenesis and prevention of residential cardiomyocytes death.	Transplanted cells may differentiate toward the cardiomyogenic lineages and into vascular endothelial and smooth muscles.	[43]
		human FM-MSCs	rats with myocardial infarction	culture and expansion	1×10^6 cells, pre-treated or not with cardiogenic-vasculogenic agent, into the myocardium.	4 wks	Increase of capillary density, normalization of left ventricular function, and decrease in scar tissue.	Transplanted cells may supply angiogenic and anti-apoptotic factors.	Transplanted cells may differentiate into vascular cells.	[44]
Tissue Engineering	Osteochondral Defect	human chorionic villi-MSCs	nude rat with articular osteochondral defect	culture and differentiation	cell-loaded collagen sponge inserted into articular osteochondral defect.	6 wks	The original defect was covered with reparative tissue. Human β2-microglobulin was observed in the defect area.		Differentiated transplanted cells could produce a substantial cartilage matrix *in vivo*.	[45]
		hAMSC	nude rat with osteochondral defect	culture	1×10^5 cells embedded in pig collagen type I gel into cartilage defects.	2 months	The defects were covered with soft tissue 2 months after transplantation. Presence of human cells in the defect recipient area.		Transplanted cells may differentiate into collagen type II-producible chondrocytes *in vivo*.	[46]
	Common Bile Duct Injuries	Fragments of human AM	dog with three types of bile duct damage: ligated duct, avulsion injury and segmental loss		a double layer of fragments of AM alone or with a vascularized support.	N.D.	No leakage or restricture occurred in animals with ligated duct, avulsion injury treated with fragments of AM alone or AM + vascularized flap. Partial mucosal repair with creeping endothelium over the amniotic graft was observed at the graft site.	N.D.	N.D.	[47]
Enhancement of Engraftment of Hematopoietic cells	Bone Marrow cell defects	human placental cells	NOD/SCID mice sublethally irradiated	culture and expansion	5×10^4 CD34 + cells alone or with 4×10^4 placental cells.		Co-transplantation of placental cells enhances engraftment of UCB-derived CD34 + cells.	N.D.		[48]
		human PLX-I	NOD/SCID mice sublethally irradiated and chemotherapy treated	2D culture and expansion in a 3D bioractor	$50-100 \times 10^3$ UCB-derived CD34 + cells injected into the tail vein alone or with $0.5-1.0 \times 10^6$ PLX-I cells.		Increase in the percentage of human CD45 + cells rate in mice co-transplanted with PLX-I with respect to the control.	N.D.		[49]

ADCs: Amnion Derived Cells; AM: Amniotic Membrane; F.M. : Fetal Membrane; FM-MSCs: Fetal Membrane-derived MSCs; hAMSC: human Amniotic Mesenchymal Stromal Cells; MSCs: Mesenchymal Stromal/Stem Cells; N.D. Not Determined; PLX-1: Placental-derived and expanded mesenchymal-like stromal cells; Ref: References.

[a] Manipulation of cells *in vitro* before transplantation.

[b] Time of detection of transplanted cells in host tissues.

2.8. Enhancement of UCB-derived hematopoietic stem/progenitor cell (HSC) engraftment

In patients with damaged or defective bone marrow (BM), BM transplantation or intravenous infusion of BM-derived HSC has become a standard approach. However, as it is not always possible to find a suitable, matched donor, alternative strategies are being developed. One of these is the use of UCB-derived HSC. Although this approach is effective for pediatric patients, in adults it results in delayed engraftment due to limited cell doses that can be recovered from UCB. Therefore, strategies to enhance engraftment of UCB-derived HSC are needed. Recently, Hiwase and colleagues [48] demonstrated that co-transplantation of human placental MSCs into NOD/SCID mice enhanced engraftment of CD34+ cells isolated from human UCB, both when CD34+ cells were obtained from a single UCB and when these cells were isolated from two UCBs (Table 4). By using a dual labeling strategy of cell tracking, these authors demonstrated that the injected cells homed to the BM. Similar experiments in the same animal model, have been reported by Prather et al. [49] who used a higher dose of human placenta-derived mesenchymal-like stromal cells (named PLX-I by the authors) expanded in a 3D bioreactor (Table 4).

3. Conclusions

It is clear that the range of potential clinical applications of placenta-derived cells and of fragments of the entire AM is continuously widening and evolving.

Although, initially, differentiation of placenta-derived cells to specific lineages was considered the first necessity for their therapeutic application *in vivo*, with the idea that these cells should replace defective cells and regenerate damaged tissues, it is now emerging that the beneficial effects may more likely be due to secretion of bioactive molecules that could act on other cells and on the microenvironment which they occupy, promoting endogenous tissue repair or eliciting other beneficial effects (anti-inflammatory, anti-scarring, angiogenic, etc.) through paracrine actions. Nevertheless, the two mechanisms (tissue-specific differentiation versus paracrine actions) are not mutually exclusive and both can account for the observed improvements. Although many of the observations from preclinical models support the hypothesis of a paracrine mechanism of action of placental cells and fragments of the entire AM, in most cases this remains a hypothesis arising very often from purely descriptive results, with no molecular dissection of mechanisms, components or target cells of these mechanisms, and with many vital pieces of information still lacking. Therefore, elucidation of these mechanisms is paramount to future clinical applications.

It is conceivable that in the future, cell-free treatments based on the use of culture medium conditioned by placenta-derived cells, rather than treatment based on the use of cells per se, will represent a novel therapeutic strategy which could potentially complement or replace cell transplantation.

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Conflict of interest

The authors declare that they have no conflicts of interest of any kind with respect to any of the data/information reported in this review.

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References

[1] Parolini O, Soncini M, Evangelista M, Schmidt D. Amniotic membrane and amniotic fluid-derived cells: potential tools for regenerative medicine? Regen Med 2009;4:275–91.
[2] Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. Br J Ophthalmol 1999;83:399–402.
[3] Parolini O, Alviano F, Bagnara GP, Bilic G, Buhring HJ, Evangelista M, et al. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international workshop on placenta derived stem cells. Stem Cells 2008;26:300–11.
[4] Parolini O, Alviano F, Bergwerf I, Boraschi D, De Bari C, De Waele P, et al. Toward cell therapy using placenta-derived cells: disease mechanisms, cell biology, preclinical studies, and regulatory aspects at the round table. Stem Cells Dev 2010;19:143–54.
[5] Carter AM. Animal models of human placentation–a review. Placenta 2007;28 (Suppl. A):S41–7.
[6] Dobreva MP, Pereira PN, Deprest J, Zwijsen A. On the origin of amniotic stem cells: of mice and men. Int J Dev Biol 2010;54:761–77.
[7] Francese R, Fiorina P. Immunological and regenerative properties of cord blood stem cells. Clin Immunol 2010;136:309–22.
[8] Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, Lombardi G, et al. Engraftment potential of human amnion and chorion cells derived from term placenta. Transplantation 2004;78:1439–48.
[9] Chen CP, Liu SH, Huang JP, Aplin JD, Wu YH, Chen PC, et al. Engraftment potential of human placenta-derived mesenchymal stem cells after in utero transplantation in rats. Hum Reprod 2009;24:154–65.
[10] Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea 2000;19:348–52.
[11] Steed DL, Trumpower C, Duffy D, Smith C, Marshall V, Rupp R, et al. Amnion-derived cellular cytokine solution: a physiological combination of cytokines for wound healing. Eplasty 2008;8:e18.
[12] Koizumi NJ, Inatomi TJ, Sotozono CJ, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. Curr Eye Res 2000;20:173–7.
[13] Uchida S, Inanaga Y, Kobayashi M, Hurukawa S, Araie M, Sakuragawa N. Neurotrophic function of conditioned medium from human amniotic epithelial cells. J Neurosci Res 2000;62:585–90.
[14] Kakishita K, Elwan MA, Nakao N, Itakura T, Sakuragawa N. Human amniotic epithelial cells produce dopamine and survive after implantation into the striatum of a rat model of Parkinson's disease: a potential source of donor for transplantation therapy. Exp Neurol 2000;165:27–34.
[15] Liu SH, Huang JP, Lee RK, Huang MC, Wu YH, Chen CY, et al. Paracrine factors from human placental multipotent mesenchymal stromal cells protect endothelium from oxidative injury via STAT3 and manganese superoxide dismutase activation. Biol Reprod 2010;82:905–13.
[16] Sakuragawa N, Thangavel R, Mizuguchi M, Hirasawa M, Kamo I. Expression of markers for both neuronal and glial cells in human amniotic epithelial cells. Neurosci Lett 1996;209:9–12.
[17] Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells 2005;23:1549–59.
[18] Chen L, He DM, Zhang Y. The differentiation of human placenta-derived mesenchymal stem cells into dopaminergic cells in vitro. Cell Mol Biol Lett 2009;14:528–36.
[19] Portmann-Lanz CB, Schoeberlein A, Portmann R, Mohr S, Rollini P, Sager R, et al. Turning placenta into brain: placental mesenchymal stem cells differentiate into neurons and oligodendrocytes. Am J Obstet Gynecol 2010;202 (294):e291–4. e211.
[20] Kakishita K, Nakao N, Sakuragawa N, Itakura T. Implantation of human amniotic epithelial cells prevents the degeneration of nigral dopamine neurons in rats with 6-hydroxydopamine lesions. Brain Res 2003;980:48–56.
[21] Yang XX, Xue SR, Dong WL, Kong Y. Therapeutic effect of human amniotic epithelial cell transplantation into the lateral ventricle of hemiparkinsonian rats. Chin Med J (Engl) 2009;122:2449–54.
[22] Kong XY, Cai Z, Pan L, Zhang L, Shu J, Dong YL, et al. Transplantation of human amniotic cells exerts neuroprotection in MPTP-induced Parkinson disease mice. Brain Res 2008;1205:108–15.
[23] Yu SJ, Soncini M, Kaneko Y, Hess DC, Parolini O, Borlongan CV. Amnion: a potent graft source for cell therapy in stroke. Cell Transplant 2009;18:111–8.
[24] Okawa H, Okuda O, Arai H, Sakuragawa N, Sato K. Amniotic epithelial cells transform into neuron-like cells in the ischemic brain. Neuroreport 2001;12:4003–7.
[25] Liu T, Wu J, Huang Q, Hou Y, Jiang Z, Zang S, et al. Human amniotic epithelial cells ameliorate behavioral dysfunction and reduce infarct size in the rat middle cerebral artery occlusion model. Shock 2008;29:603–11.

[26] Kranz A, Wagner DC, Kamprad M, Scholz M, Schmidt UR, Nitzsche F, et al. Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in rats. Brain Res 2010;1315:128–36.
[27] Meng XT, Li C, Dong ZY, Liu JM, Li W, Liu Y, et al. Co-transplantation of bFGF-expressing amniotic epithelial cells and neural stem cells promotes functional recovery in spinal cord-injured rats. Cell Biol Int 2008;32:1546–58.
[28] Sankar V, Muthusamy R. Role of human amniotic epithelial cell transplantation in spinal cord injury repair research. Neuroscience 2003;118:11–7.
[29] Wu ZY, Hui GZ, Lu Y, Wu X, Guo LH. Transplantation of human amniotic epithelial cells improves hindlimb function in rats with spinal cord injury. Chin Med J (Engl) 2006;119:2101–7.
[30] Wei JP, Zhang TS, Kawa S, Aizawa T, Ota M, Akaike T, et al. Human amnion-isolated cells normalize blood glucose in streptozotocin-induced diabetic mice. Cell Transplant 2003;12:545–52.
[31] Hou Y, Huang Q, Liu T, Guo L. Human amnion epithelial cells can be induced to differentiate into functional insulin-producing cells. Acta Biochim Biophys Sin (Shanghai) 2008;40:830–9.
[32] Kawamichi Y, Cui CH, Toyoda M, Makino H, Horie A, Takahashi Y, et al. Cells of extraembryonic mesodermal origin confer human dystrophin in the mdx model of Duchenne muscular dystrophy. J Cell Physiol 2010;223:695–702.
[33] Park TS, Gavina M, Chen CW, Sun B, Teng PN, Huard J, et al. Placental Perivascular Cells for Human Muscle Regeneration. Stem Cells Dev. 2010 Nov 30. [Epub ahead of print].
[34] Ishikane S, Ohnishi S, Yamahara K, Sada M, Harada K, Mishima K, et al. Allogeneic injection of fetal membrane-derived mesenchymal stem cells induces therapeutic angiogenesis in a rat model of hind limb ischemia. Stem Cells 2008;26:2625–33.
[35] Prather WR, Toren A, Meiron M, Ofir R, Tschope C, Horwitz EM. The role of placental-derived adherent stromal cell (PLX-PAD) in the treatment of critical limb ischemia. Cytotherapy 2009;11:427–34.
[36] Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008;214:199–210.
[37] Cargnoni A, Gibelli L, Tosini A, Signoroni PB, Nassuato C, Arienti D, et al. Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. Cell Transplant 2009;18:405–22.
[38] Moodley Y, Ilancheran S, Samuel C, Vaghjiani V, Atienza D, Williams ED, et al. Human amnion epithelial cell transplantation abrogates lung fibrosis and augments repair. Am J Respir Crit Care Med 2010;182:643–51.
[39] Sakuragawa N, Enosawa S, Ishii T, Thangavel R, Tashiro T, Okuyama T, et al. Human amniotic epithelial cells are promising transgene carriers for allogeneic cell transplantation into liver. J Hum Genet 2000;45:171–6.
[40] Manuelpillai U, Tchongue J, Lourensz D, Vaghjiani V, Samuel CS, Liu A, et al. Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl_4-treated mice. Cell Transplant 2010;19(9):1157–68.
[41] Sant'anna LB, Cargnoni A, Ressel L, Vanosi G, Parolini O. Amniotic Membrane application reduces liver fibrosis in a Bile Duct Ligation rat model. Cell Transplant; 2010 Aug 18. [Epub ahead of print].
[42] Cargnoni A, Di Marcello M, Campagnol M, Nassuato C, Albertini A, Parolini O. Amniotic membrane patching promotes ischemic rat heart repair. Cell Transplant 2009;18:1147–59.
[43] Fujimoto KL, Miki T, Liu LJ, Hashizume R, Strom SC, Wagner WR, et al. Naive rat amnion-derived cell transplantation improved left ventricular function and reduced myocardial scar of postinfarcted heart. Cell Transplant 2009;18:477–86.
[44] Ventura C, Cantoni S, Bianchi F, Lionetti V, Cavallini C, Scarlata I, et al. Hyaluronan mixed esters of butyric and retinoic acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. J Biol Chem 2007;282:14243–52.
[45] Zhang X, Mitsuru A, Igura K, Takahashi K, Ichinose S, Yamaguchi S, et al. Mesenchymal progenitor cells derived from chorionic villi of human placenta for cartilage tissue engineering. Biochem Biophys Res Commun 2006;340:944–52.
[46] Wei JP, Nawata M, Wakitani S, Kametani K, Ota M, Toda A, et al. Human amniotic mesenchymal cells differentiate into chondrocytes. Cloning Stem Cells 2009;11:19–26.
[47] Ismail A, Ramsis R, Sherif A, Thabet A, El-Ghor H, Selim A. Use of human amniotic stem cells for common bile duct reconstruction: vascularized support of a free amnion graft. Med Sci Monit 2009;15:BR243–7.
[48] Hiwase SD, Dyson PG, To LB, Lewis ID. Cotransplantation of placental mesenchymal stromal cells enhances single and double cord blood engraftment in nonobese diabetic/severe combined immune deficient mice. Stem Cells 2009;27:2293–300.
[49] Prather WR, Toren A, Meiron M. Placental-derived and expanded mesenchymal stromal cells (PLX-I) to enhance the engraftment of hematopoietic stem cells derived from umbilical cord blood. Expert Opin Biol Ther 2008;8:1241–50.