

Chapter 2

Human Amniotic Fluid-Derived and Amniotic Membrane-Derived Stem Cells

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Abstract Application of amnion membrane with multiple bioactive biomaterial has over 100 years of history. Amnion membrane- and amniotic fluid-derived stem cells mainly included mesenchymal stem cells and epithelial cells. They have special morphology and express some of stem cell markers, different immunophenotype molecules, and germ layer original protein markers for identification. Amnion membrane-derived stem cells may be isolated and purified by using two digestive enzymes, with different adherence time and subculture. They may differentiate into kinds of function cells of three germ layers in in vitro and in vivo. Amniotic fluid-derived and amniotic mesenchymal stem cells not only have the power of proliferation and plasticity feature, but also have other functions, such as immunoregulation, angiogenic potential, and secretion. Amniotic epithelial cells seem to play a more effective role in neuronal damage. The immunoregulation of amniotic mesenchymal stem cells is emphasized on effects and the mechanism. The transplantation of amnion membrane- and amniotic fluid-derived stem cells, and engineered seed cells generate significant therapeutic actions on regeneration of tissue or organ injury and autoimmune diseases, etc. Although the safety and effectiveness still need further investigations, amnion membrane, amnion membrane- and amniotic fluid-derived stem cells have been shown a broad application prospect. The mesenchymal stem cells are considered as available sources of regenerative treatment. As adult mesenchymal stem cells, are generally derived from the mesoderm, such as bone marrow, umbilical cord blood, adipose, amnion, amniotic fluid, Wharton's jelly, and mobilizing peripheral blood. They have multipotent differentiation capacity and can be differentiated into various cell types, except for self-renewal. Many studies have demonstrated that the stem cells identified from amniotic membrane and amniotic fluid are shown to have advantages for many reasons, including the possibility of noninvasive isolation, low immunogenicity,

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abundant sources, anti-inflammatory and non-tumorigenicity properties, and minimal ethical problem (Ilancheran et al. in *Placenta*, 30:2–10, 2009; Wolbank et al. in *Tissue Eng*, 13:1173–83, 2007; Ilancheran et al. in *Biol Reprod*, 77:577–588, 2007; Wolbank et al. in *Tissue Eng Part A*, 15:1843–1854, 2009; Moodley et al. in *Am J Respir Crit Care Med*, 182:643–651, 2010).

Keywords Amnion membrane • Mesenchymal stem cell • Amniotic fluid • Stem cell

2.1 Amniotic Membrane and Amniotic Fluid

Amniotic membrane (AM) is a component of the placenta that originates in the extraembryonic tissue and has functions to protect the fetus during pregnancy with supplemental nutrients. AM is composed of three major layers (Sippel et al. 2001): a single epithelial layer, a thick basement membrane, and an avascular mesenchyme. Amniotic fluid (AF) contains a large of heterologous cell population from different tissues of all three germs, while mainly derived from AM, fetal skin, fetal digestive tract, respiratory tract, and urethra cast-off cells of the developing embryo, and so on. Currently, the treated human AM is widely used as biomaterial for clinical treatment (see Table 2.1) (Feng and Yu 2014). Because it has the ability

Table 2.1 Clinical application of amniotic membrane

Origin form	Application	Advantage	Defect
Fresh, cryopreserved, or freeze-dried AM, as surgical dressing	Eyelid and skin burn, wound, ulcer, skin coloboma	Powerful anti-inflammatory, anti-biosis, anti-infection, immunoregulation, and barrier function	Closely plying-up with tissue to replace with difficulty
Fresh AM, as tissue graft or surgical dressing	Corneal injury, tympanoplasty, vestibuloplasty, angioplasty, urinary tract reconstruction	Secrete active substance, keep long time for drug release	Activity is decreased after preservation
Fresh AM, as AM-derived cells or engineered tissue	Cell therapy (type 1 diabetes), engineered tissue as artificial skin, engineered osseous and blood vessel, spinal cord injury (preclinical animal studies)	Absorbability and plasticity, a host material of natural biological membrane structure is beneficial to cell adherence and growth	Potential ethical issue
Fresh or freeze-dried AM, as biomaterial, tissue graft, trestle, nerve conduit	Gastroschisis, post-natal sternal repair, myelomeningocele, Mayer-Rokitansky-Kuster-Hauser syndrome	Prevention of adhesion, abroad origin, low immunogenicity, no tumorigenicity for autograft or allograft	Need to eliminate pathogenic micro-organism infection and hereditary disease

to produce scarring reduction, and antiangiogenic and anti-inflammatory properties, the AM is generally known as tissue engineering material for therapy of serious burns, skin, and corneal transplantation (Luo et al. 2004; Fairbairn et al. 2014; Fan et al. 2006; McGhee and Patel 2011; Mi et al. 2012; Shimazaki et al. 2002). Many studies have demonstrated that a lot of amniotic mesenchymal stem cells (AMSCs) and amniotic epithelial cells (AECs) are derived from AM. They are what is called AM-derived MSCs and AM epithelial cells, and they have powerful self-renewal and pluripotency (Ochsenbein-Kölble et al. 2003; Miki and Strom 2006; In't Anker et al. 2003). AM also is an important source of stem cells.

AF exists in bag of waters. Along with fetal development, the bag of waters is gradually filled with AF. AF contains water, protein, carbohydrate, lipid, fetus urine, and electrolyte composition (Westgren et al. 1995). The volume and element all come up with unceasing changes following fetal development. Human AF was formed at 2 weeks after fertilization in the amniotic cavity of early gestation. During pregnancy, AF is secreted mainly as a result of active transport of sodium and chloride, which is accompanied by transport of water through the chorio-AM and embryo's skin, as well as some of protein molecules. The production of urine and respiratory fluid both contributes to the volume of AF. AF is important to keep the fetus safe, and it supports organ development. The average volume is increasing from 270 to 400 ml at week 16 and week 20 of pregnancy. From weeks 15 to 20 of pregnancy, AF cells are routinely used to evaluate karyotyping and they are genetic and molecular tested for prenatal diagnostic testing (Bocian 2007). The human AF has been proposed as a source of stem cells. One of the adherence and shuttle cells is termed human amniotic fluid-derived MSCs (AF-MSCs), which were more studied on biological characteristics and therapeutic uses. Furthermore, there are also multidirectional differentiation potential stem cells in AF. Many studies have identified that human AF has been proposed as a source of stem cells. The adherence and shuttle AF-MSCs are like bone marrow or AM-derived MSCs. AF-MSCs also are extensively studied on biological characteristic and therapeutic bases (Tsai et al. 2007; Zheng et al. 2008; Lovati et al. 2011; Zhou et al. 2014).

2.2 Isolation and Culture

AMSCs, AECs, and AF-derived stem cells (AFSCs) express some of stem cell protein makers, some of immune molecules and have biological function of stem cells. Many methods have been established that a lot of AMSCs, AECs, and AFSCs can be isolated and successfully cultured. These cells have stem cell characteristics and differential growth features.

2.2.1 AMSCs and AECs

AM usually was discarded after delivery. Human and the other animal AM collection can be obtained via normal delivery without the use of invasive methodologies. To prevent contamination and damage to the tissues, AM samples were collected immediately after parturition using sterilized surgical equipment. The collected placenta samples were stored at 4 °C and transported to the laboratory as quickly as possible. The AM is mechanically peeled away from placenta or allantois. The collected amniotic membrane is washed with 0.9 % normal saline three or four times under sterile conditions to remove debris and blood. After washing, the AM is minced with a surgical blade and scissors. The minced tissue was digested and gently shaken at 37 °C for approximately 3–4 h with collagenase type I or 20 min with 0.05 % trypsin–0.02 % EDTA-2Na for three times (Filioli Uranio et al. 2014; Miki et al. 2005). Amnion digests will be then filtered through a micron nylon mesh and centrifuged for the collection of AECs (see Fig. 2.1). These AECs will be washed and suspended in Dulbecco-modified essential medium (DMEM) with 10 % heat-inactivated fetal bovine serum (FBS). After digestion, the supernatant will be mixed with an equal volume of DMEM with 10 % FBS and centrifuged. The pellet will be resuspended with DMEM. Remaining amnion trypsin digests will be treated again. This process was repeated three times. AECs will be collected and pooled with the previous cell suspension. AECs will be cultured in low-glucose DMEM and then supplemented with 10 % heat-inactivated fetal bovine serum, 10 ng/ml human epidermal growth factor, 100 UI/ml penicillin, and 100 µg/ml streptomycin for culture. Additive may be included in nonessential amino acid, β-mercaptoethanol, and sodium pyruvate. Cell cultures are usually used at passages 2 and 5 for experiment (Zhao et al. 2012). Human AECs have a short life, but could be established the immortalized human AECs by introducing with viral oncogenes E6/E7 and with human telomerase reverse transcriptase.

The remaining fragments will be digested with 0.75–0.94 mg/ml collagenase II and 0.075 mg/ml or 20 mg/ml DNase I for 1–3 h at 37 °C with 200-rpm shaking (Yao et al. 2013a). Amnion fragments will be then removed, mobilized cells were passed through a 100–300-µm cell strainer and collected by centrifugation at 400 g for 10 min. As reported above, these cells are referred to as human AMSCs (see Fig. 2.1). AMSCs at different passages are more than freshly isolated cells that will be plated at a density of 5×10^5 cells/ml to 1×10^6 cells/ml. Upon reaching about 80 % confluence, adherent cells will be trypsinized and then subcultured at a density of 1×10^5 cells/ml until passages 10 (Han et al. 2008), at least passages 22–24. The cell samples will be washed in phosphate-buffered saline and centrifuged at 350 or 400 g. The cells will be cultured in L-DMEM containing 10 % fetal bovine serum or umbilical cord blood serum with basic fibroblast growth factor (bFGF) 4–10 ng/ml or umbilical cord blood serum supplemented

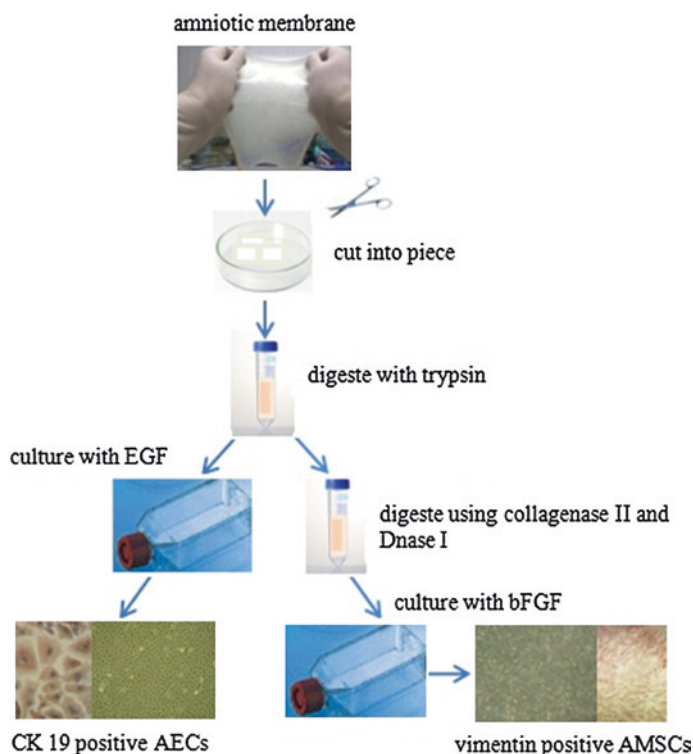


Fig. 2.1 The isolation, culture, and identification of AMSCs and AECs. The AM was peel off placenta, and then AMSCs and AECs were isolated by trypsin, collagenase II and DNase I digestion. Two kinds of diAM-derived stem cells were identified by immunohistochemical staining, morphology, and flow cytometry. AMSCs are fusiformis and vimentin positive, but AECs are like flagstone and CK19 positive

with 1 % penicillin (100 UI/ml), streptomycin (100 μ g/ml) or 0.25 mg/ml amphotericin B, with 2 mmol/L L-glutamine (Zhang et al. 2007). These cells cultured in a humidified atmosphere with 5 % CO₂. The culture medium will be changed every 3–5 days in primary culture and passaged after reaching 80 % confluency after trypsin digestion and centrifugation. The cell pellet was resuspended in complete culture medium. The cells will be cultured in a humidified atmosphere with 5 % CO₂. The basal culture medium was changed three times a week and passaged after reaching 80–90 % confluency. AMSCs were purified by differential adhesion and subculturing (Alviano et al. 2007). Literatures reported that AMSCs were cryopreserved after passage 3 in 50–90 % fetal bovine serum and dimethyl sulfoxide or protein-free cryopreservation for human mesenchymal stem cells (MSCs). These AMSCs were used for basic cytobiological and cell therapeutic researches.

2.2.2 AFSCs

Mesenchymal stem cells from amnion and AF are successfully isolated and cultured from human, as well as from the bovine. The isolation of AFSCs is a simpler process than isolation of AECs and AMSCs. After patients received detailed information, each participant gave her written informed consent. Two or three milliliters of amniotic fluid samples was obtained from 16- to 19-week pregnant women who underwent amniocentesis for fetal genetic determination in routine prenatal diagnosis (Tsai et al. 2004). Large numbers of AFSCs can be isolated by centrifuging the samples at 250–400 g for 10 min at room temperature and expanded in cultured condition. Cell samples will be used only when a normal karyotype was detected by the cytogenetic analysis. These cells proliferate rapidly with doubling times of 30–36 h and do not require supportive feeder layers for many passages, while maintaining chromosomal stability. AFSCs are cultured in serum-free culture medium or 10 % fetal bovine serum L-DMEM, or L-DMEM: F12 medium or α -minimal essential medium (α -MEM), supplemented 10 ng/ml EGF and 2 mmol/L L-glutamine in a humidified incubator at 37 °C with 5 % CO₂ (Ghaderi et al. 2011; Yang et al. 2013; Li et al. 2006). Culture medium was changed once every 3–5 days, suspension cells will be wiped off, and fibroblast-like colony will be scraped using cell scratcher. Suspension cells 4×10^4 cells/ml will be prepared and cultured by complete medium. Alternatively, AF-MSCs, a kind of shuttle cells of adherence, can be cultured in medium with 4–10 ng/ml bFGF until 70–80 % confluency in primary culture (Liu et al. 2009), and then, depurative cells will be continuously cultured by different adherence as passage 2 to 3. The AF-MSCs will be routinely subcultured every three days at 1:3 or 1:4 dilution and allowed to expand in complete medium. Cells usually will be maintained in culture for up to 4–8 passages and used for all the experiments. The pregnant metaphase AFSCs may be amplified at $1\text{--}5 \times 10^{12}$ cells/ml at the tenth generation from 20 to 40 ml AF. AF cell is not only used for prenatal diagnosis, but also as another source for stem cells of fetus. AFSCs also will be isolated and purified by immunomagnetic bead method and flow cytometry sorting. CD117-positive cells are one of sorting method by immunomagnetic bead method (Chen et al. 2009). MSCs from the amniochorionic membrane will be extracted using the markers CD34⁺, CD45⁺, CD73⁺, CD90⁺, CD105⁺, and CD29⁺ at the fluorescence-activated cell sorting analysis. The vitrification is a reliable and effective method for cryopreservation of human AF-MSCs (Moschidou et al. 2013).

Oxygen is a potent biochemical signaling molecule which exerts significant effects on the growth and development of mammalian cells. The state of hypoxia is cell-type dependent and affects critical cellular processes, such as proliferation, differentiation, adhesion, apoptosis, metabolism, extracellular matrix secretion, and growth factor expression. It has been demonstrated that hypoxic preconditioning of MSCs can reduce hypoxia-induced cell death, which is caused by the paracrine activity of MSCs. Hypoxia (1 or 5 % O₂) similarly not only increases the proliferation of AF-MSCs, but also maintains their constitutive characteristics

(surface marker expression and differentiation potentials). Notably, more paracrine factors, vascular endothelial growth factor, and transforming growth factor beta 1, will be secreted into hypoxic conditioned medium from AF-MSCs than normoxic conditioned medium (Jun et al. 2014).

These cells may be cryopreserved, and the cell viability of revived cells is higher. The surface makers and multidirectional differentiation potential also are not affected. Therefore, ASCs, AECs, and AFSCs are suitable for large-scale culture and reservation (Janz Fde et al. 2012). Compared to other stem cells, amniotic cells and AFSCs can be easily collected during routine prenatal testing, and the AM can also be obtained during cesarean section after birth. These isolation methods are noninvasive progress without destroying human embryos and thus avoid ethical controversy the most.

2.3 Identification and Characterization

Among extrafetal tissues, recently, AM appeared to be an important stem cell source in different species, and AM-MSCs have been isolated and characterized in different species, including the human, horse, sheep, dog, rat, and cat (Marcus et al. 2008). AECs have been found only in humans, horses, and sheep. Human MSCs from AM or AF represent a population of multipotent adherent cells able to be differentiated into many lineages. The AMSCs can differentiate into all three germ layers for ectodermal, mesodermal, and endodermal lineage cells. They are positively expressed mesenchymal markers, such as CD44, CD73, CD29, CD105, and CD90, and negatively expressed hematopoietic markers, as CD34, CD45, CD11b, CD19, and human leukocyte antigen (HLA)-A, HLA-B, and DR antigens (Kim et al. 2007) (see Table 2.2 and Fig. 2.2). In addition, the AM expresses

Table 2.2 Comparison of biomarker proteins with AM- and AF-derived stem cells

Antigens	Expression		
	ASCs	AECs	AF-MSCs
<i>Mesenchymal stem cell markers</i>			
CD13	+		+
CD44	++	±	++
CD73	++	+	+
CD71		–	
CD90	++	++	+
CD105	++	++	±
<i>Haematopoietic stem cell markers and immunoreactions moleculars</i>			
CD34	–	–	–
CD45	–	–	–

(continued)

Table 2.2 (continued)

Antigens	Expression		
	ASCs	AECs	AF-MSCs
CD133			
CD14		±	—
CD19		—	—
CD11b	—		
CD117	—		
HLA-DR	—	—	±
HLA-A	±	+	±
HLA-B	±	+	±
HLA-C	±	+	±
HLA-G		++	
CD40	±	+	
CD40L	±	±	
CD80	±	±	
CD86	±	±	
<i>Somatic stem cell markers</i>			
CD24		+	
CD29	++	++	+
CD49d	+	—	
CD49f		++	
CD271		+	
CD166	+		+
CD146			++
Vimentin	++	—	
CK19	—	++	—
E-cadherin	±	++	
Nestin		++	
<i>Stem cell markers</i>			
Oct4	+	++	+
SOX-2	+	++	++
SSEA-3	+	++	—
SSEA-4	+	++	
Nanog	+	++	++
c-myc	+		+
Klf4		++	++
TRA1-60	+	+	—
TRA1-81	+	++	—

— negative expression; ± low expression; + moderate expression; ++ or +++ high expression

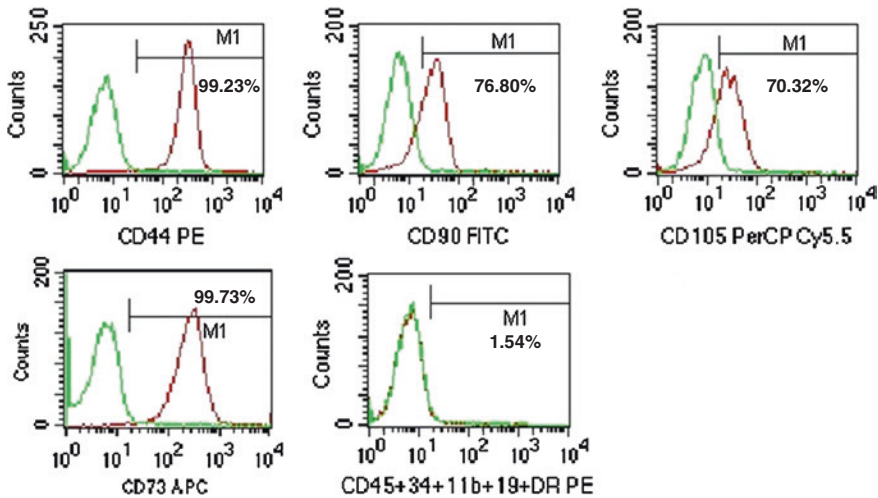


Fig. 2.2 Phenotypic analyses of human AMSCs by flow cytometry. Human AMSCs of passage 5 expressed positive CD44, CD90, CD150, and CD73, but negative CD45, CD34, CD11b, CD19, and HLA-DR by using human MSC identification kit from BD Biosciences

antiangiogenic and anti-inflammatory proteins. A lot of results show that the AMSCs are very important for advanced regenerative medicine, because inflammatory regulation and low immunogenicity remain indispensable factors, despite the pluripotent marker expression of AM-MSCs, such as Oct-4, Nanog, TRA-1-60, and TRA-1-81 (Miki et al. 2007; Yu et al. 2012). Human AECs have a low immunogenic profile and possess potent immunosuppressive properties and also have several characteristics similar to stem cells. They do not express CK-19 protein and do not form teratoma (Bilic et al. 2008). The cultured human AECs from P0 to P4 expressed and downregulated the stemness gene expression for Oct-4, Sox-2, Nanog3, FGF4, Rex-1, FZD-9, BST-1, and ABCG2. However, vimentin and nestin gene expression were upregulated (Simat et al. 2008).

Immunological rejection after therapy does not occur in the AM, and the cells are derived from AM and AF. For these reasons, AM and AM-derived stem cells might be useful sources for cell transplantation and tissue engineering for regenerative and autoimmune diseases with fewer ethical problems. After AMSCs are cryopreserved in 40 % FBS and 10 % DMSO with 50 % α -MEM culture medium, biological characteristics of revived AMSCs had no remarkable change, such as morphology, vitality, CD molecules, growth curve, cell cycles, and Oct 4 protein (Mann et al. 2013; Wang et al. 2012).

AF cells can be used as a source of fetal progenitor cells or otherwise discarded. Research results showed that AFSCs express embryonic stem cells-specific markers. AF- and AM-MSCs are same as bone marrow MSCs sharing similar morphological characteristics of the fibroblastoid shape. They possess the feature of adherence growth. After subculturing, the morphological change did not occur in

fitting culture conditions. The growth curves showed the AM, and AF-MSCs had a similar proliferative capacity at passage 5 and passage 10. These cells have kept the length and activity of telomerase. The surface markers, karyotype, cell cycle, and apoptosis all do not change between passage 5 and passage 25 in subculture. The use of AFSCs could minimize the ethical objections as well. AF-MSCs have easy isolation, a high renewal activity, and maintenance stability. Oct-4, Nanog, SSEA-4, and SOX2 are all important regulation molecules on pluripotency and self-renewal. AFSCs also express MSC makers: CD29, CD44, CD58, CD73, CD90, CD105, CD117, and CD166. They are positive for major histocompatibility complex I class (MHC I) molecular antigen HLA-A, HLA-B, and HLA-C, but were negative, or mildly positive, for MHC class II antigen HLA-DR. And CD34, CD45, ABCG2, C-MET, SSEA-1, SSEA-3, TRA-1-60, and TRA-1-80 expressions are negative (see Table 2.2) (Rossi et al. 2014; Chen et al. 2011). The protein markers of AM- and AF-derived stem cells are analysed by flow cytometry, immunocytochemistry and immunofluorescence staining.

AMSCs express some of moderate stemness markers (FGF-2, LIF, Nanog, etc.), important signal molecules for stem cell maintenance and Wnt and Notch self-renewal genes, CD44-specific mesenchymal original markers and higher cell adhesion molecules and cell cycle regulators, and low differentiation markers after cultured passage 4–5, but did not express TERT gene by gene chip test, etc. (Terai et al. 2014). Furthermore, AF-MSCs enable to be use of autologous cells obtained from patients' tissues. Moreover, they maintain genetic stability and offer advantages of non-tumorigenicity and low immunogenic activity. These findings show that AF-MSCs are being considered as potential sources of treatment with diseases.

2.4 Function Features

2.4.1 *Plasticity In Vivo*

AM- and AF-derived stem cells show high proliferative capacity in culture and multilineage differentiation potential. This multipotential differentiation capability of these stem cells can be utilized for giving rise to a variety of differentiated cell types for tissue repair and regeneration. MSCs, AECs, and AF-MSCs were seeded in special culture for differentiation studies (Miki and Strom 2006; Saito et al. 2012). When cultured in osteogenic medium, these stem cells displayed a significant increase in alkaline phosphatase activity and mRNA expression, Alizarin red S staining, and Runx2 mRNA expression (Kim et al. 2013). While maintaining in an adipogenic culture medium, these cells gave a time-dependent increase in PPAR γ and FABP4 mRNA expression, glycerol-3-phosphate dehydrogenase activity, and positive lipid droplets to Oil Red Oil staining (Vidane et al. 2014). These results confirm that they can differentiate toward osteogenic

and adipogenic phenotypes. Chondrogenic and neurogenic differentiations were assessed as previously reported. Chondrogenic was demonstrated by Alcian blue staining and neurogenic by conventional Nissl staining, which showed increasing ribosomes, and nerve cell shape was observed microscopically (Manochantr et al. 2010). Pleiotrophin is involved in the AEC-induced differentiation into dopaminergic neuron-like cells. Monolayer cultured human AMSC cell is differentiated into chondrocytes for the original cells of cartilage with transforming growth factor- β , dexamethasone, vitamin C, and insulin-transferrin-sodium selenite, which indicates that human amnion cells can be used as the seed cell of cartilage. Human AECs and AMCs have osteogenon characteristic when 3-glycerophosphate is added, which demonstrates that human amnion cells can be the resource of seed cells of bone tissue engineering.

The AM- and AF-MSCs were, respectively, seeded on plastic plates precoated with matrigel in L-DMEM containing FBS culture medium with 5-azacytidine. The differentiation capacities of AM- and AF-MSCs were detected to express GATA-4, cardiac troponin T, α -actin, Cx43, and Nkx2.5 genes, as myocardial genes after myocardial induction in vitro. Induced AMSCs and AECs all expressed desmin and α -actinin proteins after being treated with 5-azacytidine and bFGF or 5-azacytidine alone. Both human AMSCs and AECs possess the potential to differentiate into cardiomyocyte-like cells in vitro (Nagura et al. 2013; Bai et al. 2012; Han et al. 2011). And they might be candidate for cellular cardiomyoplasty for the treatment of heart failure caused by ischemic injury because adult cardiomyocytes do not regenerate. AF-MSCs also have the potential clinical application for myogenesis in cardiac regenerative therapy.

Combined approach of dexamethasone, hepatocyte growth factor, insulin-like growth factor, and other cytokines were used to induce the differentiation of human AECs and AF-MSCs into hepatocyte-like cells (Luo et al. 2011; Choi et al. 2013). The shuttle shape of AM- or AF-derived MSCs changed into polygon. The liver-like cells show changes on stem cell biomarker genes and liver cell special protein, the latter as the mRNA expression of alpha fetal protein, hepatocyte growth factor receptor the latter C-met, hepatocyte nuclear factor-3 β , cytokeratin-18, expression of hepatic microsomal enzyme in vitro and in vivo. The differentiated cells also developed hepatocyte-specific functions, i.e., they secreted albumin, absorbed indocyanine green, and stored glycogen (Liu et al. 2011; Tamagawa et al. 2007; Miki et al. 2009). In the near future, coculture without contact of human AMSCs and normal human liver cell line also can successfully induce AMSC differentiation into liver-like cells.

After induction in vitro, AMSC is differentiated into neural stem-like cells that expressed higher levels of the neural stem cell markers, Nestin, Sox2, and Musashi. Interestingly, the neurotrophic factors, brain-derived neurotrophic factor, nerve growth factor, neurotrophin 3, glial cell-derived neurotrophic factor, and ciliary neurotrophic factor were markedly upregulated (Yan et al. 2013). AF-MAC-induced functional dopaminergic neuron-like cells in vitro showed increased activity in regeneration of dopaminergic neuron-like cells, increased migration distances, and improvement of animal behavior in the Parkinson's disease rat model (Liu et al.

2011). Following transplantation in a rat traumatic brain injury model, AECs showed significant improvements on neurological function and brain tissue morphology. Human AMSCs and AECs not only expressed neuron-specific enolase, neurofilament, glial fibrillary acidic protein, β -tubulin-III, microtubule-associated protein, and neuronal nuclei, but also the level of dopamine is risen at mean value in medium with all-trans retinoic acid (Chang et al. 2010). Human and sheep AECs can transform the neuron-like cell or neurons by coculture with traumatic brain tissue extracts or in conditional medium. Though AEC proliferation is not significant, the AECs cultured by low cell density survive more easily. Human AECs were induced to differentiate into neurocytes by using chemical inducer all-trans retinoic acid and astragalus, but astragalus induction has a higher cell survival rate, and the expression of Notch1 signal molecules is inhibited during the induction (Zhu et al. 2013; Chen and Wang 2012).

Human AMSCs and AECs may be induced to differentiate into insulin-secreting cells in nicotinamide and N_2 supplement medium. The induced ratio of insulin-positive cells or islet-like cells was above 70 %. The contents of insulin were 328.47 and 331.60 μ IU/ml in the supernatant of cultured AMSCs and AECs, respectively (Peng et al. 2011; Zhao et al. 2012). This suggests that human-derived stem cells might become a new cell source of therapy for diabetes. The vascular endothelial cells were induced, when human AMSCs and AECs were cultured in DMEM with vascular endothelial growth factor and basic fibroblast growth factor. The CD34, CD54, and CD31 expressions of cultured AECs in induced medium with high and low glucoses showed no difference. However, the CD54 expression of AMSCs cultured in induced medium with high glucose was much lower than low-glucose DMEM. AMSCs have an angiogenic potential. These data suggest that human amniotic cells might become a seed cell of angiogenesis in tissue engineering and could become an alternative cell resource for repair of vascular injuries (Warrier et al. 2012). In addition, calcium-sensing receptor is a G-protein-coupled receptor able to bind calcium ions and plays a physiological role in regulating bone metabolism. Its agonist calcimimetics can prompt osteogenesis in AF-MSCs, perhaps being used in bone traumatic and degenerative damage (Di Tomo et al. 2013). AECs as an ideal stem cell resource for the cell replacement therapy, transplanted into the injured submandibular salivary gland in salivary gland dysfunction diseases. Notably, identification of GFP-labeled AFSCs and immunostaining with antihuman antigen-specific antibodies demonstrated that grafted human AFSCs survived and differentiated into granulosa cells during oocyte development in chemotherapy-induced sterility (Lai et al. 2013). Human AFSCs seem to be a good candidate for cell reprogramming in embryonic stem cell conditions with valproic acid administration, a transgene-free approach, and they are more efficiently reprogrammed to pluripotency than adult cells, as skin cells, except for differentiation into chondrocytes and lipoblast, etc. AF stem cell-induced pluripotent stem cells were able to form derivatives of the three embryonic germ layers, but also of the extraembryonic trophoblast lineage activating BMP signaling cascades and blocking of TGF- β /Activin/Nodal signaling (Moschidou et al. 2013; Li et al. 2009; Galende et al. 2010).

AECs possess a much greater ectodermal differentiation capacity, while AMSCs possess a much greater mesodermal differentiation capacity. Oct4, Nanog, and Sox2 are important transcriptional factors on stemness maintenance. But the canonical Wnt/ β -catenin signaling pathway appears to trigger human AF-MSCs osteoblastogenesis and adipogenic differentiation. The results unravel novel molecular determinants of AF-MSC commitment toward osteoblastogenesis, which may represent potential targets for improving their use in regenerative medicine. Although a plethora of molecules have been identified to have a role in modulating stem cell fate, the Wnt signaling is recognized as a key regulator of adult tissue homeostasis and remodeling through multiple so-called canonical and non-canonical pathways (D'Alimonte et al. 2013). Flt3, the receptor of Fms-related tyrosine kinase 3 ligand, is expressed in AMSCs. Fms-related tyrosine kinase 3 ligand is able to promote the proliferation of AMSCs effectively in vitro. However, the phenotype and ability of AMSCs to differentiate into mesenchymal lineages did not change (Li et al. 2014).

2.4.2 Immunomodulation

Human AECs, AMSCs, and AF-MSCs have a low immunogenic profile and possess potent immunosuppression. AM-derived stem cells have been shown to retain immunomodulatory properties and possessed strong inhibition of lymphocyte proliferation and survival when transplanted in immunocompetent animals without inducing any tumorigenic effect in vivo. It also inhibits cytokines interleukin (IL)-2 and interferon gamma IFN- γ production and suppresses the generation and maturation of monocyte-derived dendritic cells, as reported for MSC from other sources (Banas et al. 2008; Magatti et al. 2009; Xue et al. 2014). AF-MSCs are known to play a role in preventing rejection of the fetus and are thought to have low immunogenicity. As needed, AMSCs, AECs, and AF-MSCs could be stored and provided immediately for future autologous therapy. The autologous tissues made from patient-specific cells could be applied to non-rejected transplantation. AM-derived stem cells express human leukocyte antigen G (HLA-G) exposed to IFN- γ . The programmed cell death receptor 1 (PD-1, an inhibitory receptor that is normally expressed on activated T and B cells), is negative, but programmed death ligands 1 and 2 (PD-L1 and PD-L2) are typically upregulated on the AM stem cells. The two cells are also negative for the immunoglobulin-like transcript receptors 2, 3, and 4. There is some controversy about the expression of TRAIL, tumor necrosis factor alpha (TNF- α), and Fas ligand (Fas-L), all members of the TNF family for induction of apoptosis. However, in adaptive immunity, the level of IL-10 and TGF- β in the supernatant not only increased significantly in cocultures of AMSCs and peripheral blood mononuclear cells, but also inhibit proliferation. The level of IL-17 and IFN- γ also is lower in the presence of mitogens. Meanwhile, hepatic growth factor, indoleamine 2,3-dioxygenase (IDO), and cyclooxygenase 2 (COX-2) mRNA, were induced more in AMSCs. The inhibition

of cyclooxygenase pathway partially reverted the antiproliferative effect of T cells. These factors previously documented to take part in the inhibitory effects of MSCs from other sources. The AM stem cells could be an interesting source of soluble factors in clinical application, without referring to rejection reaction (Insausti et al. 2014; Rossi et al. 2012). AMSCs can significantly suppress T lymphocyte proliferation, especially CD8⁺ T cell. Otherwise, it can decrease activated Th1 and Tc1 percentages and slightly increase Th2 and Tc2 percentages. AMSCs have potential of alleviating acute graft-versus-host disease and maintaining graft versus leukemia. AMSCs and AECs that have HLA-G secretion feature may be involved in the suppression of the lytic activity of NK cells and B cells and modulate the maturation of dendritic cells (Insausti et al. 2014; Di Trapani et al. 2013; Fang et al. 2014).

Primary cultured AMSCs and AECs were treated with IL-1 β as a model for acute chorioamnionitis and with CXCL10 for chronic chorioamnionitis. IL-33 and IL1RL1 (ST2) mRNA were not detected in AECs after incubation with IL-1 β or CXCL10. IL-33 mRNA was expressed in AMSCs, and the level of expression has increased after incubation with IL-1 β . IL1RL1 (ST2) mRNA expression has decreased in AMSCs after IL-1 β treatment. However, IL-33 and IL1RL1 (ST2) mRNA expression in AMSCs did not change with CXCL10 treatment. The regulation of AMSCs and AECs is different from IL-1 β - and CXCL10-induced inflammation and immune function (Kallapur et al. 2013).

2.4.3 Epithelial–Mesenchymal Transition (EMT)

EMT is a key cause of fibrosis disease and is also the pathological process in fibrosis. EMT of normal conjunctival tissues is a major reason for pterygium generation. An important marker is α -smooth muscle actin in epithelia transition into mesenchyma, but high expression of Oct3 and E-cadherin genes are markers on inhibition of EMT. Human amnion stem cells can significantly inhibit α -SMA expression and migration of human pterygium fibroblasts by coculture of amnion stem cells and pterygium fibroblasts in vitro. These results suggested that amnion stem cells have the potential to inhibit the generation and invasiveness of pterygium (Sha et al. 2014). In the culture medium with bFGF and the inclusion of an AM in the dermal matrix, most fibroblasts were α -SMA negative. The suppression of α -SMA expression enhanced epidermal differentiation and decreased TGF- β 1 expression in the epidermis. The inhibition of TGF- β kinase completely suppressed α -SMA expression in the dermal matrix. The hyperproliferative epidermis expressed more TGF- β 1, which is responsible for myofibroblast differentiation (Yang et al. 2011). AFSCs form epithelial tubules and cyst structures in 3D collagen gel. AFSCs continue to express MSC markers during cultivation in the gel. Thus, AFSCs may undergo epithelial–mesenchymal transition (Davydova et al. 2011). The cultured human AECs undergo EMT through the autocrine production of TGF- β . Multisubcultured AECs underwent morphological changes

acquiring a mesenchymal shape. Epithelial cell markers E-cadherin and cytokeratins were lost, and typical mesenchymal markers, such as vimentin and α -SMA, appeared. The expression of SNAIL1, MMP9, PAI1, or ACTA2 genes is associated with EMT increase. The expression of the transcription activators KLF4 or MTA3 was consistent with the downregulation of CDH1. The TGF- β receptor I (ALK5) inhibitor SB-431542 or TGF- β -neutralizing antibody can prevent EMT and preserve the AECs' epithelial phenotype (Alcaraz et al. 2013).

2.4.4 Angiogenic Potential

The AM is important in clinical applications as it is proangiogenic, antifibrotic, and antiscarring and has low immunogenicity (Koob et al. 2014). It has been recently reported that human AMSCs possess great angiogenic potential in vivo, except that AECs and AF-MSCs might be induced into vascular endothelial cells in condition medium with vascular endothelial growth factor and bFGF (Alviano et al. 2007). Vascular endothelial growth factor receptors 1 and 2 were expressed in induced human AMSCs and the expression of endothelium-specific markers such as FLT-1 KDR and ICAM-1. During the ameliorating peripheral neuropathy in sciatic nerve injury, AMSC injection promoted significant recovery of motor nerve conduction velocity and voltage amplitude, also augmented blood perfusion, and increased intraneural vascularity. Whole-mount fluorescent imaging analysis demonstrated that these MSCs exhibited higher engraftment and endothelial incorporation abilities in the sciatic nerve. The higher expression of proangiogenic factors was detected. Promoting angiogenesis is a therapeutic effect and mechanism of treating peripheral neuropathy, as same as repairing other tissue injury (Li et al. 2014; Warriar et al. 2012). AM-derived stem cells may potentially assist both bone and cartilage repair, due to their angiogenic potential, they may also pave the way for novel approaches in the development of tissue-engineered vascular grafts which are useful when vascularization of ischemic tissues is required (Petsche Connell et al. 2013). AF-MSCs supported vascular tubule formation in vivo more effectively than bone marrow MSCs, further enhancing their promise as vehicles for tissue repair and regeneration. There are differences in secreted angiogenic factors for angioinhibition, inflammatory response, migration, angiogenesis-vasculogenesis, tissue repair, and blood clotting between AF-MSCs and bone marrow MSCs using proteome arrays (Roubelakis et al. 2013).

2.4.5 Secretion Function

AEC is known to produce a “cocktail” of trophic factors, such as neurotrophin-3, nerve growth factor, fibroblast growth factor-2, IL-1, IL-4, IL-6, and so on (Venkatachalam et al. 2009). AECs are found to secrete some of the factors,

brain-derived neurotrophic factor and ciliary neurotrophic factor for neuroprotective effect in rat retinal ganglion cells (Uchida et al. 2003). Amnion-derived cellular cytokine solution also accelerates the healing of skin burns. AF-MSCs express several specific neural stem/progenitor markers, such as nestin and connexin 43, and release amounts of brain-derived neurotrophic factor, as well as vascular endothelial growth factor. These factors can enhance cell recovery following neuronal damage through multiple rescue mechanisms and may provide a suitable stem cell therapeutic means for neurodegenerative disorders including Parkinson's disease (Payne et al. 2010). The use of human AF-MSCs as the feeder layer to establish human embryonic stem cell lines is promising, because of multiple biological active factor secretion of AFSCs. In addition, AMSCs and AECs quickly exert therapeutic effect on anti-inflammatory, promote angiogenesis, and reduce damage before these cells still have not found to be differentiated into specific tissue function cells in tissue or organ injury. The mechanism may be related to secretion or paracrine of AM- and AF-derived stem cells. Some of cytokines significantly enhanced in damage tissue, such as hepatocyte growth factor, bFGF, vascular endothelial growth factor, IL-4, IL-10, insulin-like growth factor-1, and granulocyte colony-stimulating factor. The action may be the same as bone marrow MSCs without direct and enough evidence on more cytokines' secretion (Carvajal et al. 2013; Yamahara et al. 2014).

2.5 The Potential Applications and Therapeutic Base

MSCs have shown therapeutic potential for repair and regeneration of tissues damaged by injury or diseases. Human adult stem cells are multipotent cells which are present in many tissues of the human body, including AM and AF, and present in bone, cartilage, muscle or fat, as well as a variety of other connective tissues by differentiating into various cells of other embryonic lineages, such as osteoblasts, chondrocytes, myoblasts, liver cells, islet cells, myocardial cells, neurocytes, and vascular endothelial cells or adipocytes. They are an important source for regenerative medicine, such as in osteogenesis imperfect, bone fracture, myocardial infarction, and liver, kidney, and spinal injury. These cells are also used to treat inflammation and immunopathy because they have anti-inflammatory and immunoregulation properties. AM- and AF-derived stem cells have low immunogenicity and have advantages on origin, ethic, amplification, and preservation in vitro, and AMSCs, AECs, and AFSCs have the extremely broad translation and application prospects in autologous and xenogenous treatments.

2.5.1 Cardiac Regeneration

After inducing cardiogenic differentiation of human AM- or AF-derived stem cells by cardiac lysis, growth differentiation factor-15, and bone morphogenetic

protein-2, fibroblast growth factor 10, 5-azacytidine, respectively, the expressions of cardiac markers inward rectifier potassium channels 2.1, cardiac troponin T and myocyte enhancer factor 2. Nanog promoter-Cre plasmid and cytomegalovirus promoter-loxP-STOP-loxP-Red-puro(r) plasmid were cotransfected into immortalized human AMSCs (Otaka et al. 2013). These cells were treated with 5-azacytidine, trichostatin A, activin A, and bone morphogenetic protein-4, or cocultured with murine fetal cardiomyocytes. Then, expression of Nanog, Oct3/4, Sox2, and Klf4 was significantly higher. They expressed Nkx2.5, GATA-4, human atrial natriuretic peptide, cardiac troponin T, myosin light chain-2 α , Mlc-2v, β -myosin heavy chain, hyperpolarization-activated cyclic nucleotide-gated channels, and inwardly rectifying potassium channels (Kir)-2.1. These induced cells could not contract (Nagura et al. 2013; Bai et al. 2012; Han et al. 2011; Shaw et al. 2011). Cocultured AF-MSCs spontaneously contracted in a synchronized manner and expressed the cardiac markers with neonatal cardiomyocytes (Guan et al. 2011). These results suggest that human AMSCs could be a useful cell source for cardiac regeneration therapy. In myocardial infarction rat, AMSCs and AECs may differentiate into myocardial cells, delay ventricular remodeling, and improve cardiac function in rats through epicardial and venous ejection fraction. Brdu-labeled positive human AM-derived stem cells were found in myocardial infarction region at 1, 4, and 6 weeks after human-derived stem cell transplantation. Cell engraftments expressed cardiac-specific protein connexin 43, α -actinin, and desmin. Ejection fraction and fractional shortening, diastolic anterior left ventricular wall, and systolic anterior left ventricular wall values of rats were all significantly higher in stem cell transplantation than in model rats (Fang et al. 2012; Wang et al. 2013a).

2.5.2 Neuronal Regeneration

Stem cell therapy is a potential treatment for spinal cord injury. Several studies have reported that AECs transplanted into the spinal cord transection rats can improve hind limb motor function and ameliorate the bladder function. Transplanted AECs survive well for a long time of 8 weeks and integrate well with the host. AECs survive in the transplanted environment, support the growth of host axons, prevent the formation of glial scar at the cut ends, and may prevent death in axotomized cells or attract the growth of new collateral sprouting (Sankar and Muthusamy 2003). Grafting AECs genetically modified to overexpress glial cell derived neurotrophic factor into spinal cord injury also rescue the axotomized rubrospinal neurons. Two weeks after spinal cord injury, human AECs were transplanted around the spinal cord lesion site of spinal cord injury-induced mechanical allodynia and thermal hyperalgesia. AECs significantly reduced mechanical allodynia, but have no effects on thermal hyperalgesia (Roh et al. 2013). This effect seems to be closely associated with the reduction of spinal cord microglial activity and NMDA receptor NR1 phosphorylation, microglial marker, and F4/80 expression of spinal cord, but not the increased expression of glial fibrillary acidic

protein or induced nitric oxide synthetase. Human AECs displayed positive immunoreactivity to MAP-2, glial fibrillary acidic protein, and Nestin could secrete the neurotrophic factors. bFGF can upregulate the TrkB receptor as a brain-derived neurotrophic factor preceding condition for activity. Neural stem cells and human umbilical cord blood mesenchymal stem cells grown either with AEC-conditioned medium or in transwells showed significantly improved survival and differentiation into dopaminergic neuron-like cells. AECs are a potential inducer to obtain DA neuron-like cells for an ethical and legal cell therapy for Parkinson's disease. Moreover, the neural differentiation and length of neurite were greater in exogenous FGF (Meng et al. 2007). This is related to secretion of brain-derived neurotrophic factor, etc. AECs may be regarded as a critical component of neural stem cells' niche. This microenvironment is the need for AEC–neural stem cells coculture and could potentially facilitate the production of neurons for future clinical applications. Transfected human bFGF to AECs was serving as neural stem cell-differentiated niche and be useful as a source of sustained trophic supported to improve neural stem cell differentiation toward neuron *in vivo*. AECs have beneficial effects by the neurotrophic factor secretion of AECs on rats with 6-OHDA-induced Parkinson's disease. AEC transplantation significantly ameliorated spatial memory deficits in double-transgenic mice of coexpressed APP^{swe}- and PS1 Δ E9-deleted genes, as well as increased acetylcholine levels and the number of hippocampal cholinergic neurites. This will be very beneficial for Alzheimer's disease therapy (Yang et al. 2010; Xue et al. 2012). Encouraging reports have revealed that human AECs can rescue injured brain tissue and improve functional recovery in experimental models of stroke or middle cerebral artery occlusion of rat (Liu et al. 2008). The potential stroke therapy may involve a reduction of local inflammation and modulation of the immune response, promotion of neural recovery, differentiation into neural tissue, re-innervation of lost connections, and secretion of necessary cytokines, growth factors, hormones, and/or neurotransmitters to restore cellular function. AECs cannot only survive in the cerebrum of rats with traumatic brain injury up to 4 weeks after transplantation, but also express the specific neuronal antigen MAP2 and improve the motor deficits of rats with traumatic brain injury. Human AECs can ameliorate behavioral dysfunction and reduce infarct size in the rat with middle cerebral artery occlusion or after stroke, due to neuronal differentiation and cytokine secretion by these cells (Liu et al. 2008). Transplanted glial cell-derived neurotrophic factor expressing AECs, as a transgene carrier in gene therapy, can protect against hippocampal neuronal death following traumatic brain injury. When it is transplanted into brain tissue as striatum of healthy or disease rats, AECs survived well for a long time, migrated for a distance, and did not induce immune rejection. The AECs labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylrhodamine perchlorate can survive in the spinal cord of monkey for up to the maximum period of observation at 60 days. There is no evidence of immunological rejection probably due to the non-antigenic nature of the human AECs (Sankar and Muthusamy 2003).

Transplantation of AMSCs also can benefit to improve neurological function restoration of rats with spinal cord injury. These human nuclei of monoclonal antibody MAB1281-positive cells survive in injury site and not express microtubule-associated protein and glial fibrillary acidic protein. Its mechanism might be related to upregulating NF-200 expression in the distal end of injured spinal cord. After spinal cord injury in rats, the combined treatment with methylprednisolone and AMSCs significantly reduces myeloperoxidase activity, the cell apoptosis, and the proinflammatory cytokines such as tumor necrosis factor- α , interleukin (IL)-1 β , IL-6, and IL-17, and interferon- γ , but increases the levels of the anti-inflammatory cytokines IL-10, transforming growth factor- β 1, and the survival rate of AMSCs in the injury site. That is to say anti-inflammatory and antiapoptotic effects are important mechanisms (Yu et al. 2012; Gao et al. 2014). AMSCs have been reported to be able to promote regeneration in central nervous tissue. AMSCs are induced to differentiate into motor neuron precursor cells. In these cells, neuron-specific enolase and synaptophysin expression levels are increased and glial fibrillary acidic protein expression is decreased. Human AMSC transplantation exhibits great potential for proliferation, is induced to differentiate into neuron-like cells and then significantly improves neurological symptoms following focal cerebral ischemia. Many of MSC-type cell studies have reported that MSCs have the ability to differentiate into neural-like cells or the neuron and neurotransmitter factors in vitro or in vivo. More and more researches have confirmed that the MSCs can be remyelinated in models of demyelination that do not involve inflammation (Hu et al. 2013; Li et al. 2012). AMSCs and AFSCs have been noted as new alternative sources that would be useful for clinical applications (Table 2.3). AM is a new composite matrix bridging both stumps of spinal cord transection in rats to promote recovery of motor function. Decellularized AM has been successfully applied as nerve conduit biomaterial to improve peripheral nerve regeneration in injury models. In the differentiation of human AM toward the Schwann cell with a sequential order of neuronal induction and growth factors, AM maintained high viability of brain derived neurotrophic factor and glial cell

Table 2.3 Potential clinical application of AFSCs

Target tissue	Disease	Application
Fetus	Fetal abnormality	Biochemical tests, prenatal diagnosis
Skin	Wound, burn	Tissue graft
Heart	Cardiac malformation	Autologous heart valve tissue engineering
β -cell	Type 1 diabetes	Preclinical animal studies
Central nervous system	Ischemic stroke	Cell transplantation, intravenous cell grafts, preclinical animal studies
	Alzheimer's disease	
	Parkinson's disease	Preclinical animal studies, cell injection
	Spinal cord injury	
Limb	Hind limb ischemia	Preclinical animal studies, cell injection, angiogenesis allograft
	Foot and ankle wounds	

derived neurotrophic factor secretion and p75 are upregulated and also exhibited a change in forming a multilayered epithelium with intense accumulations of the marker proteins (Banerjee et al. 2014; Liang et al. 2009).

After transplanting AF-MSCs into the striatum of ischemic rats, the grafted cells tended to survive and migrate toward injured brain regions. Immunohistochemical analysis showed that the cells had differentiated into neurons as well as astrocytes. This suggested that the AF-MSCs could be an alternative stem cell source on the ischemic brain tissue injury (Cipriani et al. 2007). The effectiveness of AF-MSCs has also been reported in the regeneration of the sciatic nerve. Glial cell line-derived neurotrophic factor-modified human AF-MSCs promoted nerve regeneration. More importantly, this factor expressed consecutively in the induced cells for up to four weeks. The combination of granulocyte colony-stimulating factor (50 $\mu\text{g/kg}$) administration by intraperitoneal injection and AF-MSC transplantation led to better outcomes. AF-MSCs can be recruited by expression of SDF-1 α in muscle and nerve by intravenous administration after nerve crush injury. The increased deposition of AF-MSCs paralleled the expression profiles of SDF-1 α and its receptor CXCR-4, regeneration markers in either muscle or nerve leading to improvements in neurobehavior (Yang et al. 2012).

2.5.3 Liver Disease

AM- and AF-derived stem cells were induced to liver-like cells or liver cells in vivo and vitro. The latter expressed albumin and cytokeratin 18, alpha fetal protein, oil drop, glycogen, and hepatocyte nuclear factor-3 β . In carbon tetrachloride-induced acute hepatic injury mouse model, AMSC and AF-MSC transplantation shows obvious therapeutic effect on improved liver function and pathologic histological structure in situ and in intravenous injection. Transplanted cells are planted and survived in damaged livers as in D-galactosamine-induced hepatic failure rats or in carbon tetrachloride-induced acute liver injury mice (Gong et al. 2011; Zheng et al. 2012). Serum albumin level was significantly elevated, and serum alanine aminotransferase and aspartate aminotransferase level was decreased. Transplantation of human AMSCs and AF-MSCs can improve liver function in rat with carbon tetrachloride-induced hepatic cirrhosis yet. The extent of liver cirrhosis was obviously ameliorated. It has been reported that the transplantation of AMSCs significantly decreased hepatic fibrosis by regulating TGF- β signal transduction and decreasing hepatic stellate cell activity. AMSCs can be implanted and survived for 8 weeks in liver tissue of hepatic fibrosis rat (Zhang et al. 2011). In other words, the therapeutic action of AMSCs may be included in the inhibition of epithelial–mesenchymal transition and regulation of mesenchymal–epithelial transition. Human AEC culture medium treatment suppresses decreased TGF- β 1 and collagen production in activated hepatic stellate cells, as well as inducing apoptosis and reducing proliferation. Human AEC culture medium treatment and secretion of AECs may be effective in ameliorating liver fibrosis (Hodge

et al. 2014; Manuelpillai et al. 2010). The major acute-phase mediators associated with fulminant hepatic failure, including IL-1 β , IL-6, and TNF- α , impair the regeneration of liver cells and stem cell grafts. AF-MSCs not only have the capacity to differentiate into hepatocytes, but also genetically modified to over-express interleukin-1 receptor antagonist can improve liver function and increase survival rates in injured liver rats (Zheng et al. 2012). This may provide a novel therapeutic approach to the treatment of fulminant hepatic failure. Thereby, AMSC and AF-MSC transplantation provides a new approach for the treatment of fibrotic liver diseases, and they are also shown to survive and to achieve hepatocyte differentiation without apparent immunological rejection.

2.5.4 Kidney Injury

Acute kidney injury is emerging as a public health problem in developing and developed countries. Several pharmacological approaches to improve renal function and survival after an acute kidney injury episode have been largely unsuccessful in clinical practice. Adult stem cell therapy has provided new hopes of innovative intervention to enhance the limited capability of kidney regeneration. An important origin for cell therapy is MSCs, which are an attractive therapeutic tool by virtue of their unique biological properties, tropism for damaged tissues, and proregenerative capacity of tubular epithelial cells which after acute ischemic or toxic insults undergo dysfunction and lesion. The mechanisms were explicitly underlying the renoprotective effects of stem cell therapy of acute kidney injury. MSCs interact with damaged cells via the release of soluble factors and exosomes, improving microvesicles. Several biological effects, including antiapoptotic, promitogenic, immunomodulatory, and anti-inflammatory activities, have been analyzed in renal tissue (Herrera et al. 2004; Bruno et al. 2009). AM- and AF-derived stem cells transplantation are promising therapeutic tools already validated on prevent fibrosis, renal tubular epithelial cell regeneration, decrease ameliorate glomerulosclerosis and preserve renal function in a preclinical porcine of autotransplantation, mice and rat model of kidney injury, even of acute renal failure (Baulier et al. 2014; Chang et al. 2011; Lv et al. 2014; Perin et al. 2010).

Human AMSCs are distributed abundantly in kidney tubule mesenchyme and few in renal tubular epithelial cell of cisplatin-induced acute kidney injury through mice caudal vein transplantation. Some of hyperchromatic nuclei and larger volume of regenerative renal tubular epithelial cells were found. Renal function was obviously improved. The transplantation of AMSCs could promote the recovery of acute kidney injury. AMSC transplantation shows obvious therapeutic effect on acute kidney injury and not only alleviates tubular damage, but also may participate in the repair of damaged tissue (Yu et al. 2012). Researchers injected autologous AF-MSCs in the renal artery 6 days after renal transplantation. The AF-MSC injection improved glomerular and tubular functions, leading to full renal function recovery and abrogated fibrosis development at 3 months. The strong proof of

concept generated by AF-MSCs porcine model is a first step toward evaluation of MSC-based therapies in human kidney transplantation. It is an important mechanism that AF-MSCs secrete growth factors and anti-inflammatory cytokines, anti-oxidative stress, and immunomodulatory on the efficiency and the safety (Perin et al. 2010; Gosemann et al. 2012; Feng et al. 2013; Rota et al. 2012). Although the AMSCs homing and engraftment to sites of renal damage issue have also been reported, the effect of AM- and AF-derived stem cells are engrafted injured kidney predominantly exerted antiapoptotic effect, activated Akt, and stimulated proliferation of tubular cells, possibly via local release of factors, including IL-6, vascular endothelial growth factor, and stromal cell-derived factor-1. However, their actions are not completely known in the development of interstitial fibrosis, tubular atrophy, ischemia/reperfusion renal injuries, diabetic nephropathy, and lupus nephritis.

2.5.5 Skin Wounds and Burns

Extensive burns and full-thickness skin wounds can be devastating to patients, even when treated. The autologous split-thickness skin graft involves removing a piece of skin from a secondary surgical site for the patient, stretching the skin, and reapplying the graft on the wound or burn. Although skin autograft treatment yields a reasonable clinical outcome, if the wound is extensive, then the number and size of donor sites are limited if the wound is extensive. Allograft is an additional option, but they suffer from the need of immunosuppressive drugs to prevent immune rejection of the graft. Although such polymeric scaffold, biobrane, and new dermagraft result in improved wound healing over untreated controls, they are costly to produce and result in relatively poor cosmetic outcomes (Theoret 2009; Yannas et al. 2010; Rajangam and An 2013; Papanas et al. 2012). The results showed that bioprinting AF-MSCs could be an effective treatment and conquer the problems for large-scale wounds and burns. MSC treatment with acute and chronic wounds results in accelerated wound closure, increased epithelialization, formation of granulation tissue, and angiogenesis. MSCs have recently been shown to be also effective for improving in vivo skin expansion (Jadlowiec et al. 2012; Jiang et al. 2013; Skardal et al. 2012).

AFSCs are an attractive cell source for applications in skin regeneration unlike embryonic stem cells that form teratomas. Furthermore, AFSCs remain stable and show no signs of transformation in culture. The immunomodulatory and high proliferation properties of AFSCs suggest that they can be used as an “off-the-shelf” cell therapy product for wound healing (Yoon et al. 2010). AF-MSCs release some of the paracrine factors and their ability to accelerate the wound-healing process by stimulating proliferation and migration of dermal fibroblasts. These factors include various cytokines and chemokines that are known to be important in normal wound healing, as IL-8, IL-6, TGF- β , tumor necrosis factor receptor I, vascular endothelial growth factor, and EGF (Jun et al. 2014). The proteomic analysis showed that AFSCs secreted a number of growth factors at concentrations higher

in vivo. In parallel, AF-MSC-conditioned media induced endothelial cell migration in vitro. The increased wound closure rates and angiogenesis in wound site may be due to delivery of secreted trophic factors, rather than direct cell–cell interactions. AF-MSCs are resuspended in fibrin–collagen gel and “printed” over the wound site by bioprinting technology for the treatment of full-thickness skin wounds in nu/nu mice. The wound closure and re-epithelialization were significantly greater in wounds treated by fibrin–collagen gel only. Histological examination showed more increased microvessel density and capillary diameters in the AF-MSC treated wounds (Skardal et al. 2012).

AF-MSCs in hypoxic conditioned medium could enhance the proliferation and migration of human dermal fibroblasts in vitro and wound closure in a skin injury model, as compared to AF-MSCs in normoxic conditioned medium. However, the enhancement of fibroblast migration was inhibited by SB505124 and LY294002, inhibitors of TGF- β /Smad2 and PI3 K/AKT. Therefore, this enhanced wound healing is related to the increase in hypoxia-induced paracrine factors via activation of TGF- β /Smad2 and PI3 K/AKT pathways. Expression of TGF- β 1 was more in albino rats with irradiated wounds than those injected intradermally with human AECs (Jun et al. 2014). The model groups showed severe inflammation, deficient healed dermis, and delayed re-epithelialization. SDF-1 expression was high, while CXCL-5 expression was high in AEC-transplanted rat causing accelerated wound healing. AECs showed a great effect on the quality of the dermis as well as bone marrow MSCs, while superiority of the epithelium and its appendages was achieved. Human AECs could be used safely in case of irradiated wounds (Mehanni et al. 2013).

AM is a biological dressing in the management of burns by rapid re-epithelialization and healing as it diminishes the oozing of plasma, bacterial count and fluid, protein, and heat loss. Dermal injection of freeze-dried AM extract also is a potential wound-healing substrate which can promote epidermal and dermal regeneration, while avoiding undesirable hyperproliferation of damaged tissue (Kang et al. 2013; Mohammadi et al. 2013).

2.5.6 Autoimmune Disease

MSCs have been shown to possess immunomodulatory properties, which suppress T cell proliferation, influence dendritic cell maturation and function, suppress B cell proliferation and terminal differentiation, and suppress immune modulation of other immune cells such as NK cells and macrophages. In terms of the clinical applications of MSCs, they are involved in four main areas: tissue regeneration for cartilage, bone, muscle, tendon, and neuronal cells; gene therapy vehicles; enhancement of hematopoietic stem cell engraftment; and treatment of immune diseases such as systemic lupus erythematosus, graft-versus-host disease, rheumatoid arthritis, autoimmune encephalomyelitis, acute pancreatitis, multiple sclerosis, and sepsis (Yi and Song 2012).

Rheumatoid arthritis is a chronic general autoimmune disease that is mediated by immunocompetent cell and multiple cytokines. Its main pathological feature is progressive joint damage. Human AMSCs may inhibit the development of collagen-induced arthritis by regulating the Foxp3+ Treg and Th17 cells. AMSC treatment caused lower arthritis index score, decreased volume of target joints, alleviated pathological damage of the joints, and decreased percentage of Th1, Th2, Th17, CTL1, CTL2, and NKT cells, but produced higher percentage of Foxp3+ Treg cells, compared with untreated arthritis rats and AMSC culture medium treatment (Xiao et al. 2013). The supernatant of cultured human AMSCs and AECs could inhibit lymphocyte proliferation. After mixed culturing with AMSCs, CD4⁺ T cell subsets were enhanced, but CD8⁺ T cell subsets were obviously suppressed. Th1 and Tc1 significantly decreased, but Th2 and Tc2 slightly increased in all experiments. AEC did not have significant difference compared with control. It was found that both human amniotic cells could secrete soluble cytokines to play immune suppression.

Human AMSCs (5×10^5 cells) were injected into the lateral cerebroventricle of rat with autoimmune encephalomyelitis, which was induced in guinea pigs by spinal cord homogenate, complete Freund's adjuvant, and Bordetella pertussis toxin. The behavior scores of human AMSC-treated rats were reduced gradually. After 3 weeks, AMSC-treated animals showed an improvement in inflammatory reaction in the brain and spinal cord, and the percentages of Treg, Th2, Tc1, and Tc2 lymphocyte subsets were increased obviously, whereas Th17 cells were decreased significantly. The concentrations of IFN- γ and IL-2 in the plasma were decreased, but concentrations of IL-4 and IL-10 were increased. AMSCs can improve neural function of rats with autoimmune encephalomyelitis and alleviate immunopathologic damage of neural tissues. Results suggested that the mechanism might relate to Foxp3+ Treg cell upregulation and Th17 cell downregulation (Fang et al. 2014). AEC is same as therapeutic effect on autoimmune encephalomyelitis and upregulation of Foxp3+ Treg cells and downregulation of Th17 cells (Li et al. 2013).

These immunomodulatory properties in vitro have generated enormous interest in the potential application of MSC in vivo as an immunosuppressive cellular therapy. Successful results have been obtained with the use of bone marrow MSC both for the prevention of graft-versus-host disease in solid organ transplantation and for the treatment of steroid-resistant acute graft-versus-host disease, arising after allogeneic hematopoietic cell transplantation. It is worth of further exploration that how many AMSCs and AF-MSCs are needed and how they affect the acute or chronic graft-versus-host disease of allogeneic hematopoietic cell transplantation.

2.5.7 Premature Ovarian Failure

Premature ovarian failure, a condition that causes amenorrhea and hypergonadotropic hypogonadism before the age of 40, affects 1 % of women in the general population. The occurrence of premature ovarian failure has increased in

recent years. Premature ovarian failure usually can't be reversed and though currently available treatments yet. New treatment strategies are urgently required with regenerative medicine development. Stem cell transplantation has been reported to rescue ovarian function in a preclinical mouse model of chemotherapy-induced premature ovarian failure. AMSCs and AF-MSCs were injected into a cyclophosphamide or cis-platinum-induced premature ovarian failure mouse. These cells could be detected by fluorescence microscopy up to three and eight weeks after injection. Ovarian function was improved, and full recovery is by the regulation of local cytokines and perfect microenvironment of follicular development. The level of E2 was upregulated to reach the normal level, and the level of FSH was decreased as the same as normal mice. Follicular development and potentia generandi were not different compared with normal female mice. The red fluorescence protein-transduced CD44⁺/CD105⁺ human AF-MSCs could survive and proliferate in the ovary of long-term cyclophosphamide-induced premature ovarian failure mouse. The cells could be detected by fluorescence microscopy up to three weeks after transplanted into the ovaries. The ability of human AFSCs to differentiate into germ cell and oocyte-like cells has been previously documented. The function properties and long-term survival in vivo of AMSCs and AF-MSCs make them ideal seed cells for stem cell transplantation for premature ovarian failure treatment (Liu et al. 2012; Wang et al. 2013b). The grafted GFP-labeled AFSCs and immunostaining with antihuman antigen-specific antibodies demonstrated that they survived and differentiated into granulosa cells which directed oocyte maturation. Furthermore, labeling of ovarian tissue for anti-Müllerian hormone expression, a functional marker of folliculogenesis, was strong in injured ovaries but absent in negative controls. This result highlights the possibility of using AFSCs in regenerative medicine reproductive health (Lai et al. 2013). Intravenously injected AECs reached the ovaries of chemotherapy-treated mice and restored folliculogenesis for promoting reproductive health and improving the quality of life for female cancer survivors.

2.5.8 Hematopoietic Supporting and Improvement

It was reported that human AMSCs expressed multiple hematopoietic cytokines, including leukemia inhibitory factor, stem cell factor, macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage–granulocyte colony-stimulating factor, IL-3, IL-6, and IL-11. The number of suspension mononuclear cells of umbilical cord blood was enhanced in culture medium at the days 3–21. AMSC transplantation showed that it has a function to support hematopoiesis in vitro, as well as AFSCs (Ditadi et al. 2009). The stem cells derived from amnion and AF are cocultured with mononuclear cells from cryopreserved cord blood in a medium supplemented with cytokine stem cell factor, thrombopoietin, and granulocyte colony-stimulating factor. The hematopoietic stem and progenitor cells are distinctly amplified. The comparison of MSCs from bone marrow with MSCs from cord blood and AF showed no significant difference (Klein et al. 2013). High-dose

chemotherapy often results in severe bone marrow damage. Beside drugs, stem cell transplantation has also been used as a strategy for the treatment of bone marrow damage. Human AMSC transplantation through the caudal vein, the body weight was higher than model mice after cisplatin-induced myelosuppression. The number of white blood cells, lymphocytes, mononuclear cells, red blood cells, and hemoglobin, hematocrit, and platelet of peripheral blood, and the number of bone marrow karyocytes were significantly higher than those in model mice. Bone marrow histopathological testing results showed that AMSC transplantation significantly improved femur bone marrow organizational structure compared with myelosuppression mice, especially megakaryocyte increases. The AMSCs with antihuman nuclei monoclonal antibody-fluorescein isothiocyanate were colonized in myeloid tissue (Mizokami et al. 2009; Yao et al. 2013a). It is clear that human AMSC transplantation can remarkably ameliorate hematopoietic function in myelosuppression condition.

2.5.9 Eye Injury

Limbal stem cell deficiency is a pathologic state that limbus of cornea or base material is damaged. It often leads to the corneal surface by invading conjunctival epithelium cells' ingrowth of fibrous tissue, stromal scarring, and neovascularization, which cause chronic pain and visual loss. AECs not only differentiate into corneal epithelial-like cells in vitro, but also provide a kind of niche that enhances the functional properties of human corneal endothelial cells via inhibiting P53-survivin-mitochondria axis (Yao et al. 2013b; Sha et al. 2013). Although there are differences on collagen type IV, V and laminin $\alpha 4$, BM40/SPARC, tenascin-C, amniotic and limbal epithelial basement membranes all show positive immunoreactivity for collagen type IV $\alpha 1$, $\alpha 2$, $\alpha 5$, and $\alpha 6$ chains; multiple type collagens, laminin, nidogen and fibulin; fibronectin, etc. AEC transplantation decreases corneal conjunctivization better than simple AM transplantation in limbal stem cell deficiency model through burning cornea by sodium hydroxide after cutting layers of limbus cornea (Dietrich-Ntoukas et al. 2012; Luo et al. 2013; Covre et al. 2011; Ricardo et al. 2009). The results indicate that transplantation of AECs represents an effective technique for ocular surface reconstruction in patients with severe limbal stem cell deficiency. Human AECs can be successfully reproduced in corneal stromal and formed 4–5 layer epithelial cells as the same as normal corneal epithelium. AECs also could be expected to reconstruct the corneal epithelium and by tissue engineering technology.

2.5.10 Other Tissue Injury

Human AFSCs were injected into the injured tibialis anterior muscles established by cardiotoxin and X-ray irradiation in Nod/Scid mice. The double-staining immunofluorescence showed that human-specific nuclear mitotic apparatus protein expressed

in tibialis anterior muscles and no myogenic phenotype at 2 weeks after cell transplantation. The single-cell coexpressed hepatocyte growth factor receptor or myogenic regulatory factor at 4 weeks after cell transplantation. In some myofibers, human-specific nuclear mitotic apparatus proteins and laminin or desmin were also coexpressed (Ma et al. 2012). AF stem cells can participate in the regeneration of injured mouse muscle. After cell transplantation, transplanted cell also coexpressed NuMa and c-Met, Myf-5, and laminin or desmin at 4 weeks after cell transplantation (Ma et al. 2011). AF-MSCs improve survival and enhance repair of damaged intestine in necrotizing enterocolitis via a cyclooxygenase 2-dependent mechanism (Zani et al. 2014). Research demonstrated that AF-MSCs injected into an established model of necrotizing enterocolitis could improve survival, clinical status, gut structure, and function. Understanding the mechanism of this effect may help us to develop new cellular or pharmacological therapies for infants with necrotizing enterocolitis. Furthermore, human AM- and AF-derived stem cells are employed as a tool for basic research and studied in prevision of their use for cell-based therapies, although some irregularities in their epigenetic control are not dimness.

2.5.11 Potential Antitumor Therapeutic Strategies

Cancer therapeutic strategies principally include surgery, radiotherapy, chemotherapy and biotherapy. Despite the developments of therapy, cancer mortality rates are higher worldwide. Previous study reported that bone marrow MSC transplantation resulted in antitumor activity against non-Hodgkin's lymphoma (Secchiero et al. 2010). However, some bone marrow stem cells or precursor cells have been also shown to increase growth and metastasis of colon cancer, lymphoma, and melanoma cells in vivo (Audollent et al. 2011). Therefore, it is unclear whether MSCs promote or suppress tumor growth so far. Certain studies show that bone marrow MSCs have been promoted as an attractive option to use as cellular delivery vehicles to carry antitumor agents, owing to their ability to home into tumor sites and secrete cytokines (Hamada et al. 2005). However, MSC lineage to be used in the cell therapy needs to be carefully chosen to balance efficacy and safety for a particular tumor type. MSCs from AM and AF may be one of the tumor cell growth inhibitors or a new delivery vehicle for antitumor effects (Rolfo et al. 2014; Kang et al. 2012a, b). They inhibit proliferation of cancer cell lines of haematopoietic and non-haematopoietic origin by inducing cell cycle arrest, or induce C6 glioma apoptosis in vivo through the Bcl-2/caspase pathways (Magatti et al. 2012; Jiao et al. 2012). The two source stem cells also are capable of self-renewal and can generate differentiated progenies for organ development as well. They are considered as potential source for regenerative medicine and tissue replacement after disease. They are in an intermediate stage between pluripotent embryonic stem cells and lineage-restricted adult stem cells. Their non-tumorigenicity and no or low expression level of major histocompatibility complex antigens, and contribute to low immunogenicity and anti-inflammation. In non-engineered stem cell transplantation strategies, amnion-derived stem cells effectively target the tumor and

suppressed the tumor growth by expressing cytotoxic cytokines or cancer suppressor gene. Additionally, they also have a potential as novel delivery vehicles transferring therapeutic genes to the cancer formation sites in gene-directed enzyme/prodrug combination therapy.

2.5.12 The Challenge of Clinical Translation

AM- and AF-derived stem cell-based therapeutic and seed cell-engineered strategies are showing huge potential in experimental studies. However, some problems of stem cells including safety and ethical issues have limited their clinical use. Thus, the AM and AF are considered as non-controversial sources because of the use of either heterologous embryonic stem cells or the less ethically disputed MSCs. A small amount of AF obtained by amniocentesis and amniotic membrane samples could produce enough MSCs for applications. However, some of the gene expressions of AFSCs were changed in different culture conditions. AMSCs even change into senescence as the same as other MSCs for times without number subculture. It is reported that AECs cause EMT after subculture after passage 5. How to preferably keep the biological characters and functions of AMSCs, AECs, and AFSCs in amplified culture system for more amplify and subculture in vitro? How to block or delay stem cells aging? How to effectively control EMT and mesenchymal-epithelial transition among AECs, AMSCs, or AF-MSCs in cultured conditions and induced directional differentiation? Whether are different therapeutic effects in the same tissue injury that is caused by different reasons? These are worth of further investigation. The epigenetics and regulation and control of AM- and AF-derived stem cells have not become directly involved for the biological mechanism, regeneration, restoration, and development. In tissue engineering, there are a number of problems that needed to be solved, such as interactions and histocompatibility. It is absolutely necessary that further studies on molecular mechanism of AM- and AF-derived stem cells demonstrate the prospects of potential therapeutic uses and safety for several diseases. Although we still need to do more investigation, even to break through the bottleneck, it is hoped translation that these beneficial effects of AMSCs, AECs, and AF-MSCs will gradually be developed into therapeutic outcomes for injury regeneration, autoimmune disease, and tissue engineering. Finally, we elaborate on the potential for these cells to promote regeneration of various tissue defects (Insausti et al. 2010; Murphy et al. 2010), including fetal tissue, the nervous system, heart, lungs, kidneys, bones, cartilage, and ovary.

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