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**REVIEW ARTICLE****Stem Cell Therapy for Diabetes: Are We Close Enough?***Sachin S. Kadam<sup>1\*</sup>**<sup>1</sup>Department of Biosciences and Technology, Defence Institute of Advanced Technology, Girinagar,  
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**Abstract:**

Over the last few decades, understanding of the mechanism of cellular development has increased tremendously. The knowledge of the potential of stem/pre-cursor cells in tissue engineering and cell therapy has gained the popularity. In case of diabetes, the availability of the source of stem cells and the efficacy of their isolation techniques for maximum yield of viable cells to expand is an important issue which needs attention. Attempts to make beta cells from mouse embryonic stem cells (ES) and adult stem cells have been frustrating in part because too much has been expected too soon. The problem with ES cells are that it is not known whether these cells are truly similar to normal beta cells or not and ethical issues surrounding them. ES cells is a major concern. Current claims about differentiation / transdifferentiation of adult stem cells to insulin producing cells has been demonstrated by many groups. These adult stem cells are of enormous interest because of their general accessibility and lack of ethical issues. Also, adult stem cells are non immunocompatible unless isolated from the same patient whereas ethical and scientific issues surrounding embryonic and fetal stem cells hinder their widespread implementation. Therefore, much attention is now focused on alternative sources of adult/postnatal stem cells.

**Keywords:** Diabetes, Regenerative medicine, Stem cell Therapy,

**Introduction:**

Diabetes mellitus (DM) is now taking its place as one of the main threats to human health in the 21<sup>st</sup> century [1, 2]. Over the last century, human lifestyle and behaviour changes have resulted in a dramatic increase

in the incidence of diabetes worldwide. The past two decades have seen an explosive (almost 46%) increase in the number of people diagnosed with diabetes worldwide, [3-6] with more than 40% from India only [7]. The global prevalence of diabetes is shifting significantly from the developed countries to the developing countries [2].

DM is currently a chronic disease without a cure; however, type 2 diabetes can be managed with a combination of dietary treatment, medication, exercise, and insulin supplementation. In case of Type 1 DM, a sustained C-peptide production and successful insulin independence continuously for five years after pancreatic islet transplantation has showed a ray of hope [7]. Since then, the islet transplantation is increasingly being used as a cell replacement therapy for type-1 diabetes [8]. However, the need for ongoing immunosuppressive therapy and the scarcity of donor islets have precluded the widespread adoption of islet transplantation. Although xenotransplantation (for example, porcine islets) could provide a virtually inexhaustible source of islets for transplantation [9, 10] the concern about infection by animal retroviruses and certain ethical issues limit the use of this potential source. Hence, there is a need to look for new sources of islet tissues to meet the potential demand for islet cell transplantation.

**Stem cell Therapy as a source for islet neogenesis**

A stem cell is defined by their ability to self-renew indefinitely by asymmetric cell division with a potential to differentiate into one or more specialized cell-types [11-13]. Stem cells of embryonic or adult origin have become the favourable and attractive target

of research in the biomedical sciences as they offer solutions to overcome the technical difficulties associated with conventional cell therapy by their ability to proliferate, replicate in a controlled laboratory conditions and to differentiate into a wide range of tissue types.

In developing a potential therapy for patients with diabetes, the stem cell system needs to meet several criteria. For diabetes therapy, it is not clear whether it will be desirable to produce only  $\beta$ -cells or whether other types of pancreatic islet cells (PP,  $\alpha$ ,  $\delta$  cells) and acinar cells are also essential. Recent trends indicate that isolated  $\beta$  cells cultured in the absence of the other islet cells ( $\alpha, \delta, PP$ ) are less responsive to changes in glucose concentration than intact islet clusters made up of all islet cell types. Perhaps the most important issue is the choice of the appropriate sources of stem cells.

### **Embryonic Stem Cells:**

October 2013, marks the 15<sup>th</sup> anniversary of the first reported derivation of human Embryonic Stem (ES) cells [14]. The report was met with much acclaim as it was quickly recognized that the ability of these pluripotent stem cells derived from the inner mass of the mammalian blastocyst to differentiate indefinitely into all cell types/germ layers of the human body; could provide a source of cells both *in vivo* and *in vitro* for replacing tissues lost to injury and disease - the ultimate goal of regenerative medicine.

Regardless of the species, undifferentiated ES cells express several cell surface markers; these include stage specific embryonic antigen (SSEA), SSEA1 in mouse and SSEA-3 and SSEA-4 in human, as well as tumor recognition antigens TRA-1-60 and TRA-1-81 in human [15-17]. In the uncommitted state both mouse and human ES cells express alkaline phosphatase, the POU-domain transcription factor Oct-4, and telomerase16. When removed from the specific culture conditions required to maintain an undifferentiated state, ES cell lines from both species can

spontaneously be differentiated *in vitro* to form a variety of cell types derived from each of the three germ layers. Even without the addition of any exogenous growth factors, ES cells allowed to differentiate will spontaneously form neurons [18, 19], cardiomyocytes [20, 21], muscle cells [22], hematopoietic cells [23] pancreatic precursor cells [24, 25] and many other cell types [26-29]. Although these procedures provide a starting point for producing specific cells for possible applications, whether in regenerative medicine, or as tools for drug discovery or as disease models, little is understood about the underlying mechanisms. Even if, limited success has been achieved by manipulating culture conditions to drive differentiation of human ES cells along particular lineages, there is little evidence to support the view that such conditions specifically direct differentiation rather than select for propagation or survival of cells generated by spontaneous processes. A number of chemical agents and growth factors such as retinoic acid or Bone morphogenetic proteins (BMP) are well known to have physiological roles in embryonic development and have also been used to promote differentiation of ES cells in culture [30]. However, even in these cases, there is a tendency for differentiation to follow broad lineages such as ectodermal or mesodermal; the cell types generated also tend to be heterogeneous [31].

Human embryonic stem cell (hESC)-based cell replacement therapies represents an attractive target for type 1 diabetes [32]. Recently, it has been reported that ES cells from mouse [33-40], monkey [41] and human [42-44] were able to differentiate into insulin positive cells. However, to date, studies that reported the generation of insulin-positive cells from ES cells, through cellular genetic manipulation [40,45] or by utilization of specific culture conditions [39,46] did not show a significant content of insulin or a physiological regulation of insulin secretion. Actually, insulin secretion in differentiated ES cells never exceeded 1.6% of the amount that a  $\beta$ -cell typically

secretes. This is about the same production level found in non- $\beta$  insulin producing cells, such as fetal liver cells or certain neuronal cells [47, 48].

Identification of a pancreatic progenitor cells would be an important step to isolate large numbers of cells that could be readily differentiated into islets or  $\beta$ -cells. Based on the expression of many neuronal cell markers, a neuroectodermal origin of pancreatic endocrine cells has been hypothesized for many years. It was suggested that nestin, the intermediate filament protein which is expressed in neuronal precursors might be a marker of islet progenitor cells [49]. Several groups have used protocols designed to enrich for neural precursors, characterized by expression of nestin, in attempts to coax ES cells to adopt a pancreatic islet fate [37, 50]. However, Lumelsky and coworkers [37] observed that using a differentiation protocol for neuronal progenitor cells, the differentiation of nestin-positive ES cells into  $\beta$ -like cells, had a limited effect. Only very few cells were insulin-positive and the resulting cell aggregates did not express the Pdx-1 (pancreatic development homeobox 1, a homodomain protein absolutely required for pancreatic development in both human and mice) and the majority of cells exhibited a neuronal phenotype. There is some controversy about the use of nestin as a progenitor marker and its significance in islet neogenesis. Some authors considered nestin as a neuroepithelial precursor marker (neural cell adhesion molecule), which is also essential in multipotential progenitor cells of pancreas, present both in rat and in human pancreatic islets (called nestin-positive islet-derived progenitors) [50, 51]. Others have reported that nestin only marks a population of mesenchymal or endothelial cell types, thereby excluding role of nestin in islet cell development [52, 53]. Recently, it has been demonstrated that the nestin positive cells in the pancreas of mice of different ages is immunolocalized with reference to insulin and glucagon positive cells [54].

The proliferative capacity of ES cells is attractive but

their applications are limited due to risk of teratocarcinoma formation [35, 55]. The major concern with ES cells is considerable ethical issues regarding the use of human ES cells followed by their pluripotency and plasticity, to create a mixture of many different cells failing to produce the homologous population of fully differentiated  $\beta$ -cells required for transplantation therapy [25]. Moreover, considering that ES cells are by definition immortal, their poor survival when differentiated was unexpected. Even though EC would seem to constitute an ideal source for cell replacement therapies for many human diseases, recent events have shown that some spectacular results in the field of ES cell research have to be reanalyzed very carefully [56].

The great advantage of ES cells over other stem cells is that they can generate many potentially useful cell types - but that is also their disadvantage. To use ES cells effectively in regenerative medicine, or in other applications, such as disease modeling or drug discovery, applications that are often overlooked in popular discussion, is that it is essential to understand how to control their differentiation. This is necessary if one is to expand cultures of the undifferentiated cells to a usable scale, free of potential pathogens. It is also necessary if one is to produce specified cell types, free of other unwanted cells, and if the genetic fidelity of the cells is to be preserved, a factor that was not immediately apparent when these cells were first derived. These issues present substantial challenges [31]. Nevertheless, in diabetes research, human ES cells may help to decode some crucial steps *in vitro*, since almost all the data available on pancreas development were obtained from animal models. To activate the differentiation programmes, ES cells are forced to aggregate into embryonic bodies (EBs) by culturing in suspension and in the absence of leukemia inhibitory factor (LIF). These unique properties make ES cells of great interest as a source to obtain insulin producing cells for diabetes treatment [57].

Recent success in generating insulin-secreting islet-like cells from human embryonic stem (ES) cells, in combination with the success in deriving human ES cell - like induced pluripotent stem (iPS) cells from human fibroblasts by defined factors, have raised the possibility that patient - specific insulin - secreting islet-like cells might be derived from somatic cells through cell fate reprogramming using defined factors [58]. Although the precise relationship of iPS and ES cells remains to be explored in detail, the advent of iPS cell technology circumvents the ethical, legal and logistical problems associated with the need for human embryos to derive ES cell lines, and has started a democratization process that should see a substantial expansion in the study of pluripotent cell biology. With all these results in mind, insulin producing, C-peptide and glucagon positive islet-like clusters (ILCs) from the iPS cells were derived from human skin cells by retroviral expression of Oct4, SOX2, c-MYC, and KLF4 under feeder-free conditions [59]. The major hurdles that must be overcome to enable the broad clinical translation of these advances include teratoma formation by ES and iPS cells, and the need for immunosuppressive drugs. Thus, the creation of human - animal hybrid embryos by iPS - proposed as a way to generate embryonic stem cells without relying on scarce human eggs - has met with legislative hurdles and public outcry. But a very recent report [60] suggests that the approach has another, more fundamental problem: it may simply not work - as these hybrid embryos fail to grow beyond 16 cells. Hence, it is not yet sure whether iPS cells lives up to all the great hopes given to them. The best strategy would be 'wait and watch'.

#### **Adult Stem Cells:**

Several adult tissues, including blood, epidermis, liver, enterocytes and spermatogonia are replenished throughout the life. This observation of the regenerative potential of adult tissue led to the concept of adult stem cells. Adult stem cells are rare cells with low

proliferation capacity, which according to present hypotheses reside in stem cell niches and give rise to a transient amplifying cell pool that differentiates, thereby regenerating the respective tissues [61] except germ layer and further classified as hematopoietic stem cells [HSC] and mesenchymal stem cells (MSC).

Although MSCs were originally isolated from bone marrow [62], recent years have witnessed an explosion in the number of adult stem cell populations isolated and characterized. Every tissue or organ, from adipose tissue [63, 64], placenta [65, 66], amniotic fluid [67], umbilical cord blood [68, 69] etc. exhibits stem cell population. Some studies have suggested that there may be a greater degree of plasticity, perhaps even pluripotency, associated with adult stem cells than was previously believed. The multilineage differentiation potential of MSCs derived from variety of different species has been extensively studied *in vitro* since their first discovery in 1960 [70]. An excitement ensued when, in 1998, Ferrari et al. [71] showed transplantation of genetically marked bone marrow into immunodeficient mice migrated into areas of induced muscle degeneration, undergo myogenic differentiation, and participated in the regeneration of the damaged fibers. Subsequently, numerous publications [72, 73,74] have described various events, in which adult stem cells from one organ give rise to cell type characteristics of different organ.

The capacity to differentiate into multiple mesenchymal lineages including cartilage [75, 76], bone [77-78], and adipose tissue [79, 80] is being used as a functional criterion to define MSCs. This ability has rendered MSC an ideal candidate cell source for clinical tissue regeneration strategies, including the augmentation and local repair and regeneration of specific lineage. Recent studies indicated the identification of pluripotent cells that not only differentiate into cells of mesoderm lineage, but also into endoderm and neuroectoderm lineages, including neurons [81], hepatocytes [82], and endothelia [83].

Individual colonies derived from single MSC precursors have also been reported to be heterogeneous in terms of their multilineage differentiation potential. Only one third of adherent bone marrow derived MSC clones are pluripotent [84]. Furthermore, nonimmortalized cell clones examined by Muraglia et al. [85] in 2000 demonstrated that 30% of the *in vitro* derived MSC clones exhibited a tri-lineage (osteogenic/chondrogenic/adipogenic) differentiation potential, while the remainder displayed a bi-lineage (osteogenic/chondrogenic) or uni-lineage (osteogenic). These observations are consistent with other *in vitro* studies using conditional immortalized clones [86-88]. It has also been observed that only 58.8% of the single colony derived clones had the ability to form bone within Hydroxyapatite-tricalcium phosphate ceramic scaffolds post implantation in immunodeficient mice [89]. All these results indicate heterogeneous nature of clonally derived MSCs with respect to their developmental potential.

At present, there is no specific marker or combination of markers have been identified that specifically defined MSCs [90, 91]. Phenotypically, *ex vivo* expanded MSCs express a number of non-specific markers, including CD29, CD44, CD73, CD90, CD105, CD166 [84, 92]. Despite this controversy of what defines a mesenchymal stem cell, there is general agreement that MSCs lack typical hematopoietic antigens, namely CD14, CD34 and CD45 [84]. With specific regards to pancreatic lineages there has been a report of differentiation/ transdifferentiation by Ianus and colleagues [93] who studied *in vivo* differentiation of adult bone marrow derived cells into pancreatic endocrine cells. Their results suggested that a population of cells within the bone marrow has the capacity to transdifferentiate into cells that can populate and perhaps function within, the endocrine pancreas. One study using streptozotocin (STZ) – induced pancreas damage demonstrated that pancreatic cell proliferation was induced after bone marrow transplantation [94]. The stem cells from bone mar-

row are capable of producing a whole spectrum of cell types; highlighting the opportunity to manipulate these cells for therapeutic use as they have been shown to cross the lineage boundaries [95]. It has been shown that the human bone marrow derived mesenchymal stem cells could be induced to differentiate into functional insulin producing cells using Pdx-1, delivered by recombinant adenovirus. More recent reports suggest that bone marrow stem cells reversed experimental diabetes *in vivo* by enhancing the regeneration and survival of endogenous  $\beta$ -cells rather than repopulating the islets with trans-differentiated  $\beta$ -cells [96,97]. Several *in vitro* studies have demonstrated that rodent bone marrow stem cells can adopt an insulin-expressing phenotype [98], and driving the phenotype of human bone marrow stem cells by the forced expression of  $\beta$ -cells transcription factors generated cells capable of glucose responsive insulin secretion [99,100]. Banerjee and colleagues have demonstrated reversal of experimental diabetes in STZ diabetic mouse model by multiple injections of unfractionated bone marrow leading to induction of pancreatic regeneration, which is highly promising [101], although the mechanism is not suggested. These studies point towards futuristic therapeutic approach of auto transplantation of bone marrow to cure diabetes.

Apart from bone marrow the mature liver has been shown to serve as a potential source of tissue for generating functional endocrine pancreas. This may allow the diabetic patient to be the donor of his or her own therapeutic tissue; thus alleviating both the needs for allo-transplantation and the subsequent immune suppression [102]. There have been sporadic reports that progenitor/stem cells from other tissues can be induced to differentiate into insulin expressing cells, including cells localized to intestinal epithelium, dermis, spleen, salivary gland and blood monocytes, endometrium / menstrual blood [103]. These studies have not always proved to be reproducible, and have been reviewed recently [104-106]. Thus, with the

ability to differentiate into multiple lineages (multipotent) and immunosuppressive effects, adult stem cells became an attractive alternative to human ES cells in regenerative medicine and cell-based therapy against various human diseases including type 1 diabetes [107]. In particular, adult stem cells have many attractive features as a source of functional transplantable cellular material when compared to ES cells.

#### **Fetal/extra-embryonic/post-natal stem cells:**

Fetal stem cells, comprising of the final broad stem cell class, have a comparatively recent history. They can be isolated from two distinct sources, the fetus proper and the supportive extra-embryonic structures. Stem cells derived from the extra-embryonic sources are particularly interesting due to their potential clinical utility. Over the past decades fetal stem cells have been isolated from multiple extra-embryonic tissues, reminiscent of gradual broadening of stem cell sources seen in the adult. Amniotic fluid, amnion, umbilical cord blood, Wharton's jelly and placenta have all generated putative stem cells. These tissues, collectively also known as the afterbirth (after the delivery of foetus, placenta begins a physiological separation for spontaneous expulsion afterwards along with all umbilical cord, amnion etc. therefore, called the afterbirth) are routinely discarded at parturition as a biological waste. However, this situation may change in the near future, as a growing number of reports demonstrated greater potential of these tissues as 'store house' of stem cells which makes it a valuable alternative stem cell source with fewer concerns in terms of ethical controversy and moral issues [108] of the resident stem cell populations.

The first isolated fetal stem cells were hematopoietic, derived from human umbilical cord blood. The isolated cells were capable of long-term self-renewal and differentiation to multiple hematopoietic lineages [109, 110]. Clinically, cord blood stem cells were successfully employed in a bone marrow transplant in 1988

[111]. Stem cells from extra-embryonic tissue expressed a number of mesenchymal cell surface markers, including CD90 and CD105. Subsequent work demonstrated the expression of Oct4 within a subset of most of the extra-embryonic stem cells. This is important, as Oct4 expression is associated with pluripotent cells such as embryonic germ cells and ES cells [112-114]. This observation keeps extra-embryonic stem cells poised to join embryonic and adult stem cells. Following *in vitro* expansion, the isolated cells were capable of differentiating *in vitro* into chondrocytes, adipocytes and osteocytes. In addition to tri-lineage differentiation, stem cells derived from umbilical cord [115,116], cord blood [117-119], placenta [66,120], amniotic membrane [121,122] have shown potential for differentiation into insulin producing  $\beta$ -cells and have been considered as surrogate  $\beta$ -cells source for islet transplantation. Most importantly, an emerging body of data indicates that MSCs possess immunomodulatory properties [123-126] & may play specific roles as immunomodulators in maintenance of peripheral tolerance, transplantation tolerance, autoimmunity, tumor evasion, as well as fetal-maternal tolerance. These observations have further raised clinical interest in adult and its counterpart fetal MSCs.

Recent advance in adult/fetal stem cell technologies and basic biology has accelerated therapeutic opportunities aimed at eventual clinical applications. First, the extracorporeal nature of these stem cell sources facilitates isolation, eliminating patient risk that attends other adult stem cell isolation. Most significantly, the comparatively large volume of extra-embryonic tissues and ease of physical manipulation theoretically increases the number of stem cells that can be isolated. Second, ethical questions surrounding the use of human embryonic stem cells are essentially absent from the discussion of adult stem cells. Third, because they can be isolated from the prospective patient, they would be genetically matched, thus eliminating the need for immunosuppressive therapies. Fourth, the puta-

tive ability of extra embryonic stem cells to differentiate into cells from multitude of lineage suggests their use in treating a variety of diseases. Stem cells from extra-embryonic tissues represent an emerging area of research that bears tremendous potential with broad application to many different areas including normal and pathological development, assisted reproductive technology procedures and regenerative medicine [127].

The extra-embryonic/adult stem cells have opened up the possibility of 'fixing' a particular genotype (either normal or diseased) in pluripotent stem cells. Isolation from tissues normally discarded at birth facili-

tates easy harvest and overcomes ethical concerns. The cells isolated from these tissues grow well in culture, appear capable of differentiation to multiple cell types and may be less likely to be rejected following transplantation. All these studies have prompted researchers to examine the stem cell potential of the biological waste tissues of human origin such as umbilical cord, placenta, amnion and endometrium and to test their ability to differentiate into glucose responsive insulin producing islet like clusters for possible cell replacement therapy in diabetes as well as to create an islet model for *in vitro* studies.

### References:

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic *Nature* 2000; 414(6865): 782-787.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27(5):1047-1053.
3. Amos A, McCarty D, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetic Med* 1997; 14: S1-S85.
4. King H, Aubert R, Herman W. Global burden of diabetes, 1995-2025. Prevalence, numerical estimates and projections. *Diabetes Care* 1998; 21: 1414-1431.
5. Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: can the doomsday scenario be averted? *J Intern Med* 2000; 247: 301-310.
6. Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, Rao PV, Yajnik CS, Prasanna Kumar KM, Nair JD. Diabetes Epidemiology Study Group in India: High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia* 2001; 44:1094-1101.
7. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; 54(7): 2060-2069
8. Limbert C, Path G, Jakob F, Seufert J. Beta cell replacement and regeneration: strategies of cell-based therapy for type 1 diabetes mellitus. *Diabetes Res Clin Pract* 2008; 79(3): 389-399.
9. Cozzi E, Bosio E. Islet xenotransplantation: current status of preclinical studies in the pig-to-nonhuman primate model. *Curr Opin Organ Transplant* 2008; 13(2): 155-158.
10. Dufrane D, Gianello P. Pig islet xenotransplantation into non-human primate model. *Transplantation* 2008; 86(6): 753-760.
11. Gordon MY, Blackett NM. Reconstruction of the hematopoietic system after stem cell transplantation. *Cell Transplant* 1998; 7(4): 339-344.
12. Scheffler B, Horn M, Blumcke I, Laywell ED, Coomes D, Kukekov VG, Steindler DA. Marrow-mindedness: a perspective on neurogenesis. *Trends Neurosci* 1999; 22(8): 348-357.
13. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 2000; 100: 157-168.
14. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282:1145-1147.
15. Enver T, Soneji S, Joshi C, Brown J, Iborra F, Orntoft T, Thykjaer T, Maltby E, Smith K, Dawud RA, Jones M, Martin M, Gokhale P, Draper J, Andrews PW. Cellular differentiation hierarchies in normal and culture-adapted human embryonic stem cells. *Hum Mol Genet* 2005; 14:3129-3140.

16. Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, Beighton G, Bello PA, Benvenisty N, Berry LS, Bevan S, Blum B, Brooking J, *et al.* Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat Biotechnol* 2007; 25(7): 748-750.
17. Fong CY, Peh GS, Gauthaman K, Bongso A. Separation of SSEA-4 and TRA-1-60 Labelled Undifferentiated Human Embryonic Stem Cells from A Heterogeneous Cell Population Using Magnetic-Activated Cell Sorting (MACS) and Fluorescence-Activated Cell Sorting (FACS). *Stem Cell Rev* 2009;5(1):72-80.
18. Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI. Embryonic stem cells express neuronal properties in vitro. *Dev Biol* 1995; 168(2): 342-357.
19. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell* 2002; 110(3): 385-397.
20. Maltsev VA, Wobus AM, Rohwedel J, Bader M, Hescheler J. Cardiomyocytes differentiated *in vitro* from embryonic stem cells developmentally express cardiac-specific genes and ionic currents. *Circ Res* 1994; 75(2): 233-244.
21. Wobus AM, Kaomei G, Shan J, Wellner MC, Rohwedel J, JiGuanju, Fleischmann B, Katus HA, Hescheler J, Franz WM. Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes. *J Mol Cell Cardiol* 1997; 29(6):1525-1539.
22. Rohwedel J, Maltsev V, Bober E, Arnold HH, Hescheler J, Wobus AM. Muscle cell differentiation of embryonic stem cells reflects myogenesis *in vivo*: developmentally regulated expression of myogenic determination genes and functional expression of ionic currents. *Dev Biol* 1994; 164(1): 87-101.
23. Kaufman DS, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 2001; 98(19): 10716-10721.
24. Kahan BW, Jacobson LM, Hullett DA, Ochoada JM, Oberley TD, Lang KM, Odorico JS. Pancreatic precursors and differentiated islet cell types from murine embryonic stem cells: an *in vitro* model to study islet differentiation. *Diabetes* 2003; 52(8): 2016-2024.
25. Zulewski H. Stem cell with potential to generate insulin producing cells in man. *Swiss Med wkly* 2006; 136: 647-654.
26. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The *in vitro* development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J Embryol Exp Morphol* 1985; 87: 27-45.
27. Keller GM. *In vitro* differentiation of embryonic stem cells. *Curr Opin Cell Biol.* 1995; 7(6): 862-869.
28. Baker RK, Lyons GE. Embryonic stem cells and *in vitro* muscle development. *Curr Top Dev Biol* 1996; 33: 263-279.
29. Yamane T, Hayashi S, Mizoguchi M, Yamazaki H, Kunisada T. Derivation of melanocytes from embryonic stem cells in culture. *Dev Dyn* 1999; 216(4-5): 450-458.
30. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 2000; 97: 11307-11312.
31. Gokhale PJ, Andrews PW. Human embryonic stem cells: 10 years on. *Lab Invest* 2009; 89(3):259-262.
32. Stanley EG, Elefanty AG. Building better beta cells. *Cell Stem Cell* 2008; 2(4): 300-301.
33. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat Biotechnol* 2008; 26(4): 443-452.
34. Blyszczuk P, Asbrand C, Rozzo A, Kania G, St-Onge L, Rupnik M, Wobus AM. Embryonic stem cells differentiate into insulin-producing cells without selection of nestin-expressing cells. *Int J Dev Biol* 2004; 48(10): 1095-1104.
35. Hori Y, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SK. Growth inhibitors promote differentiation of insulin producing tissue from embryonic stem cells. *Proc Natl Acad Sci USA* 2002; 99(25): 16105-16110.
36. Leon-Quinto T, Jones J, Skoudy A, Burcin M, Soria B. *In vitro* directed differentiation of mouse embryonic stem cells into insulin-producing cells. *Diabetologia* 2004; 47(8): 1442-1451.
37. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001; 292(5520): 1389-1394.



38. Miyazaki S, Yamato E, Miyazaki J. Regulated expression of pdx-1 promotes in vitro differentiation of insulin producing cells from embryonic stem cells. *Diabetes* 2004; 53(4): 1030-1037.
39. Moritoh Y, Yamato E, Yasui Y, Miyazaki S, Miyazaki J. Analysis of insulin-producing cells during in vitro differentiation from feeder-free embryonic stem cells. *Diabetes* 2003; 52(5): 1163-1168.
40. Soria B, Andreu E, Berná G, Fuentes E, Gil A, León-Quinto T, Martín F, Montanya E, Nadal A, Reig JA, Ripoll C, Roche E, Sanchez-Andrés JV, Segura J. Engineering pancreatic islets. *PflügersArchiv-European J Physiol* 2000; 440:1-18.
41. Lester LB, Kuo HC, Andrews L, Nauert B, Wolf DP. Directed differentiation of rhesus monkey ES cells into pancreatic cell phenotypes. *Reprod Biol Endocrinol* 2004; 2: 42.
42. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes* 2001; 50(8): 1691-1697.
43. Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human embryonic stem cells into insulin-producing clusters. *Stem Cells* 2004; 22(3): 265-274.
44. Best M, Carroll M, Hanley NA, Piper Hanley K. Embryonic stem cells to beta-cells by understanding pancreas development. *Mol Cell Endocrinol* 2008; 288(1-2): 86-94.
45. Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, Wobus AM. Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. *Proc Natl Acad Sci USA* 2003; 100(3): 998-1003.
46. Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. *Science* 2003; 299 (5605): 363.
47. Alarcon C, Serna J, Perez-Villamil B, de Pablo F. Synthesis and differentially regulated processing of proinsulin in developing chick pancreas, liver and neuroretina. *FEBS Lett* 1998; 436(3): 361-366.
48. Devaskar SU, Giddings SJ, Rajakumar PA, Carnaghi LR, Menon RK, Zahm DS. Insulin gene expression and insulin synthesis in mammalian neuronal cells. *J Biol Chem* 1994; 269(11): 8445-8454.
49. Hunziker E, Stein M. Nestin-expressing cells in the pancreatic islets of Langerhans. *Biochem Biophys Res Commun* 2000; 271(1): 116-119.
50. Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Muller B, Vallejo M, Thomas MK, Habener JF. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 2001 50(3): 521-533.
51. Huang H, Tang X. Phenotypic determination and characterization of nestin-positive precursors derived from human fetal pancreas. *Lab Invest* 2003; 83(4): 539-547.
52. Selander L, Edlund H. Nestin is expressed in mesenchymal and not epithelial cells of the developing mouse pancreas. *Mech Dev* 2002; 113(2): 189-192.
53. Klein T, Ling Z, Heimberg H, Madsen OD, Heller RS, Serup P. Nestin is expressed in vascular endothelial cells in the adult human pancreas. *J Histochem Cytochem* 2003; 1(6): 697-706.
54. Dorisetty RK, Kiran SG, Umrani MR, Boindala S, Bhonde RR, Venkatesan V. Immunolocalization of nestin in pancreatic tissue of mice at different ages. *World J Gastroenterol* 2008; 14(46): 7112-7116.
55. Sipione S, Eshpeter A, Lyon JG, Korbitt GS, Bleackley RC. Insulin expressing cells from differentiated embryonic stem cells are not beta cells. *Diabetologia* 2004; 47(3): 499-508.
56. Mandavilli A. Profile: Woo-Suk Hwang. *Nat Med* 2005; 11(5): 464.
57. Santana A, Ensenat - Waser R, Arribas M, Reug JA, Roche E. Insulin producing cells derived from stem cells: Recent progress and future directions. *J Cell Mol Med* 2006; 10(4): 866-883.
58. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861-872.
59. Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 2008; 283(46): 31601-31607.
60. Young C, Colin EB, Nathan RT, Stephen JW, Vladislav M, Sandy B, Irina K, Wan-Song W, Randall D, Rebecca M, Jing Su, Shi-Jiang Lu, Marc M, Young-Ho C, Scott R, Atala A, Dittman R, Lanza R. Reprogramming of Human Somatic Cells Using Human and Animal Oocytes. *Cloning and Stem Cells* 2009; 11(2): 2113-2123
61. Ebert R, Ulmer M, Zeck S, Meissner-Weigl J, Schneider D, Stopper H, Schupp N, Kassem M, Jakob F. Selenium

- supplementation restores the antioxidative capacity and prevents cell damage in bone marrow stromal cells in vitro. *Stem Cells* 2006; 24(5): 1226-1235.
62. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411): 143-147.
  63. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implication for cell-based therapies. *Tissue Eng* 2001; 7: 211-288.
  64. Chandra V G S, Phadnis S, Nair PD, Bhonde RR. Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. *Stem Cells* 2009; 27(8):1941-1953.
  65. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem cells* 2004; 22:1338-1345.
  66. Kadam S, Muthyala S, Nair P, Bhonde R. Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes. *Rev Diabet Stud* 2010;7(2):168-182
  67. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FH, Willemze R, Fibbe WE, Kanhai HH. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood* 2003; 102: 1548-1549.
  68. Bieback K, Kem S, Kulter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. *Stem cells* 2004; 200: 123-135.
  69. Kögler G, Sensken S, Airey JA, Trapp T, Müschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Müller HW, Zanjani E, Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med* 2004; 28:857-884.
  70. Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplant of bone marrow cells. *J Embryo Exp Morphol* 1966; 16: 381-390.
  71. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998; 279(5356): 1528-1530.
  72. Joshi CV, Enver T. Plasticity revisited. *Curr Opin Cell Biol* 2002; 14(6): 749-755.
  73. Goodell MA. Stem-cell "plasticity": befuddled by the muddle. *Curr Opin Hematol* 2003; 10(3): 208-213.
  74. Raff M. Adult stem cell plasticity: fact or artifact? *Annu Rev Cell Dev Biol* 2003; 19: 1-22.
  75. Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. *Cell Transplant* 1997; 6: 125-134.
  76. Johnstone B, Yoo JU. Autologous mesenchymal progenitor cells in articular cartilage repair. *Clin Orthop Relat Res* 1999; 367: S156-162.
  77. Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 1997; 64(2): 278-294.
  78. Richards M, Huibregtse BA, Caplan AI, Goulet JA, Goldstein SA. Marrow-derived progenitor cell injections enhance new bone formation during distraction. *J Orthop Res* 1999; 17(6): 900-908.
  79. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997; 276(5309): 71-74.
  80. Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 1999; 14(5): 700-709.
  81. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR. Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol* 2000; 164(2): 247-256.
  82. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002; 109(10): 1291-1302.
  83. Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in 21st century. *Trends Mol Med* 2001; 7: 259-264.
  84. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411): 143-147.

85. Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. *J Cell Sci* 2000; 113(Pt7): 1161-1166.
86. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. *J Cell Physiol* 1998; 176(1): 57-66
87. Dormady SP, Bashayan O, Dougherty R, Zhang XM, Basch RS. Immortalized multipotential mesenchymal cells and the hematopoietic microenvironment. *J Hematother Stem Cell Res* 2001; 10(1): 125-140.
88. Osyczka AM, Nöth U, O'Connor J, Caterson EJ, Yoon K, Danielson KG, Tuan RS. Multilineage differentiation of adult human bone marrow progenitor cells transduced with human papilloma virus type 16 E6/E7 genes. *Calcif Tissue Int* 2002; 71(5): 447-458.
89. Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG. Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. *J Bone Miner Res* 1997; 12(9):1335-1347.
90. Baksh D, Song L, Taun RS. Adult mesenchymal stem cells: characterisation, differentiation and application in cell and gene therapy. *J Cel Mol Med* 2004; 8(3): 301-316.
91. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007; 110(10): 3499-3506.
92. Deans RJ, Moseley AB. Mesenchymal stem cells: Biology and potential clinical uses. *Exp Hematol* 2000; 28:875-884.
93. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 2003; 111(6): 843-850.
94. Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA, Bhatia M. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 2003; 21(7): 763-770.
95. Serakinci N, Keith WN. Therapeutic potential of adult stem cells. *Eur J Cancer* 2006; 42 (9): 1243-1246.
96. Hasegawa Y, Ogihara T, Yamada T, Ishigaki Y, Imai J, Uno K, Gao J, Kaneko K, Ishihara H, Sasano H, Nakauchi H, Oka Y, Katagiri H. Bone marrow (BM) transplantation promotes beta-cell regeneration after acute injury through BM cell mobilization. *Endocrinology* 2007; 148(5), 2006-2015.
97. Urbán VS, Kiss J, Kovács J, Gócza E, Vas V, Monostori E, Uher F. Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. *Stem Cells* 2008; 26(1), 244-253.
98. Teng C, Guo Y, Zhang H, Ding M, Deng H. Identification and characterization of label-retaining cells in mouse pancreas. *Differentiation* 2007; 75(8): 702-712.
99. Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells* 2007; 25(11): 2837-2844.
100. Phadnis SM, Joglekar MV, Dalvi MP, Muthyala S, Nair PD, Ghaskadbi SM, Bhonde RR, Hardikar AA. Human bone marrow-derived mesenchymal cells differentiate and mature into endocrine pancreatic lineage in vivo. *Cytotherapy* 2011; 13(3):279-293.
101. Banerjee M, Bhonde RR. Islet generation from intra islet precursor cells of diabetic pancreas: in vitro studies depicting in vivo differentiation. *JOP* 2003; 4(4): 137-145.
102. Meivar-Levy I, Ferber S. Regenerative medicine: using liver to generate pancreas for treating diabetes. *Isr Med Assoc J* 2006; 8(6): 430-434.
103. Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, Wang H, Ge W, Bogin V, Chan KW, Thébaud B, Riordan NH. Endometrial regenerative cells: a novel stem cell population. *J Transl Med* 2007; 5: 57-67.
104. Burke ZD, Thowfeequ S, Peran M, Tosh D. Stem cells in the adult pancreas and liver. *Biochem J* 2007; 404(2): 169-178.
105. Limbert C, Path G, Jakob F, Seufert J. Beta cell replacement and regeneration: strategies of cell-based therapy for type 1 diabetes mellitus. *Diabetes Res Clin Pract* 2008; 79(3): 389-99.
106. Efrat S. Beta-cell replacement for insulin-dependent diabetes mellitus. *Adv Drug Deliv Rev* 2008; 60, 114-123.
107. Liu M, Han ZC. Mesenchymal stem cells: biology and clinical potential in type 1 diabetes therapy. *J Cell Mol Med* 2008; 12(4): 1155-1168.
108. Marcus AJ, Woodbury D. Fetal stem cells from extra-embryonic tissues: do not discard. *J Cell Mol Med* 2008; 12(3): 730-742.
109. Knudtzon S. In vitro growth of granulocytic colonies

- from circulating cells in human cord blood. *Blood* 1974; 43:357-361.
110. Ueno Y, Koizumi S, Yamagami M, Miura M, Taniguchi N. Characterization of hemopoietic stem cells (CFUc) in cord blood. *Exp Hematol* 1981; 9: 7116-7122.
  111. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, Boyse EA. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci USA* 1989; 86: 3828-3832.
  112. Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschlager M. Oct-4- expressing cells in human amniotic fluid: a new source for stem cell research? *Hum Reprod* 2003; 18: 1489-1493.
  113. Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D, Medicetty S. Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* 2003; 21:50-60.
  114. Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, Luo Y, Rao MS, Velagaleti G, Troyer D. Human umbilical cord matrix stem cells: Preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells* 2006; 24(3): 781-792.
  115. Yang CC, Shih YH, Ko MH, Hsu SY, Cheng H, Fu YS. Transplantation of human umbilical mesenchymal stem cells from Wharton's jelly after complete transection of the rat spinal cord. *PLoS ONE* 2008; 3(10): e3336.
  116. Kadam SS, Bhonde RR. Islet neogenesis from the constitutively nestin expressing human umbilical cord matrix derived mesenchymal stem cells. *Islets* 2010;2(2):112-120
  117. Laitinen A, Laine J. Isolation of mesenchymal stem cells from human cord blood. *Curr Protoc Stem Cell Biol* 2007; Chapter 2: Unit 2 A.3.
  118. Koblas T, Harman SM, Saudek F. The Application of Umbilical Cord Blood Cells in the Treatment of Diabetes Mellitus. *Rev Diabetic Stud* 2005; 2: 228-234.
  119. Denner L, Bodenburg Y, Zhao JG, Howe M, Cappo J, Tilton RG, Copland JA, Forraz N, McGuckin C, Urban R. Directed engineering of umbilical cord blood stem cells to produce C-peptide and insulin. *Cell Prolif* 2007; 40(3): 367-380.
  120. Poloni A, Rosini V, Mondini E, Maurizi G, Mancini S, Discepoli G, Biasio S, Battaglini G, Berardinelli E, Serrani F, Leoni P. Characterization and expansion of mesenchymal progenitor cells from first-trimester chorionic villi of human placenta. *Cytotherapy* 2008; 10(7): 690-697.
  121. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. *Stem Cells* 2005; 23: 1549-1559.
  122. Kadam SS, Sudhakar M, Nair PD, Bhonde RR. Reversal of experimental diabetes in mice by transplantation of neo-islets generated from human amnion-derived mesenchymal stromal cells using immunisulatory macrocapsules. *Cytotherapy* 2010 Dec; 12(8):982-991.
  123. Rasmusson I. Immune modulation by mesenchymal stem cells. *Exp Cell Res* 2006; 312: 2169-2179.
  124. Uccelli A, Moretta L, Pistoia V. Immunoregulatory function of mesenchymal stem cells. *Eur J Immunol* 2006; 36: 2566-2573.
  125. Alma JN, Willem F. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007; 110: 3499-3506.
  126. Le Blanc K, Ringden O. Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med* 2007; 262: 509-525.
  127. Hemberger M, Yang W, Natale D, Brown TL, Dunk C, Gargett CE, Tanaka S. Stem cells from fetal membranes - a workshop report. *Placenta* 2008; 29(Suppl A): S17-19.

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