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TOPIC HIGHLIGHT

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Mesenchymal stromal cells from human perinatal tissues: From biology to cell therapy

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Abstract

Cell-based regenerative medicine is of growing interest in biomedical research. The role of stem cells in this context is under intense scrutiny and may help to define principles of organ regeneration and develop innovative therapeutics for organ failure. Utilizing stem and progenitor cells for organ replacement has been conducted for many years when performing hematopoietic stem cell transplantation. Since the first successful transplantation of umbilical cord blood to treat hematological malignancies, non-hematopoietic stem and progenitor cell populations have recently been identified within umbilical cord blood and other perinatal and fetal tissues. A cell population entitled mesenchymal stromal cells (MSCs) emerged as one of the most intensely studied as it subsumes a variety of capacities: MSCs can differentiate into various subtypes of the mesodermal lineage, they secrete a large array of trophic factors suitable of recruiting endogenous repair processes and they are immunomodulatory.

Focusing on perinatal tissues to isolate MSCs, we will discuss some of the challenges associated with these cell types concentrating on concepts of isolation and expansion, the comparison with cells derived from other tissue sources, regarding phenotype and differentiation capacity and finally their therapeutic potential.

Key words: Mesenchymal stromal cells; Umbilical cord; Cord blood; Regenerative medicine; Cell therapy; Stem cells; Amnion; Chorion; Perinatal; Discarded tissue; Fetal membranes

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INTRODUCTION

Regenerative medicine is of growing interest in biomedical research. The role of stem cells to regenerate, repair and replace tissues or organs is intensely studied. In general, organ injuries or local defects induce a mobilisation of endogenous immature progenitor cells either locally or systemically. Then mediated by the milieu, regulated by factors of the extracellular matrix, cellular components or soluble mediators, the precursors differentiate along a hierarchy of committed to mature cells to functionally regenerate the cellular compartment of the organ[1]. Hematopoietic stem cell (HSC) transplantation conducted in a routine scale is mimicking these processes[2]. In HSC transplantation, umbilical cord or placental blood (CB) came into focus as

a third source after the first successful transplantation performed by Gluckman *et al*[3] in 1989. Despite this approach, cells from fetal tissues have become increasingly interesting also for tissue engineering approaches to regenerate solid organs. This is based on reports of solid organ engraftment after experimental or clinical whole CB transplantation[4].

Multipotent cells have been observed in the fetal circulation but there seems to be an inverse correlation in frequency of cells with endothelial and mesodermal differentiation potential[5]. Besides mesenchymal stromal cells (MSCs), a plethora of different stem and progenitor cell populations have been described in perinatal tissues with potential ranging from embryonal-like[6-8] to lineage-committed progenitor cells[5,9]. Whether or not these differentially named cells refer to similar cell populations obtained by different isolation or culture methods, whether these cells relate to a common ancestor[10], are in constant (epi)genetical transition enabling them to shift to different phenotypes[11], emerge upon *in vitro* culture or by dedifferentiation effects[12] remains unidentified and represents a major challenge for the future.

WHY ARE PERINATAL TISSUES INTERESTING FOR POTENTIAL CELL THERAPEUTIC USE?

Human embryonic stem cells (hESCs) are derived from the inner cell mass exhibiting a tremendous proliferative potential and in addition pluripotent differentiation potential into cells of all three germ layers. Currently however, three major factors limit their clinical application[13]. Firstly, the ethical debate: in some countries it is not allowed to generate or even work with hESC lines. Secondly, pluripotent hESCs induce teratoma formation after transplantation. Still methods are inferior to ascertain that no residual pluripotent hESCs are present in a transplant of differentiated cells. Thus the risk of teratoma formation cannot be fully excluded. Thirdly, hESCs may elicit immune reactions after transplantation. In the future, probably autologous induced pluripotent stem cells from adult somatic cells may help to overcome at least issues one and three[14,15]. By then, adult tissues will have to be regarded as an ethically sound option.

Adult stem cells have been identified in a variety of tissues; MSCs for example in every tissue tested so far[16]. Here the problem arises on how to isolate these very rare cells and from which sources. The most often analysed tissue is bone marrow (BM). But cell procurement is highly invasive and cell numbers are low and necessitate further *ex vivo* expansion. Furthermore, cell numbers, at least in BM, have been shown to decline with age[17].

Thus, postnatal gestational tissues inherit numerous advantages starting with the young chronological age that minimizes the feasibility for incorporated mutations and ending with the non-invasive procurement. hESC cells from Wharton's Jelly (WJ) and the amnion arise from the epiblast but are not ethically controversial or tumorigenic[18]. Early focus on perinatal tissues harbouring stem cells arose from HSCs identified in CB[19]. Subsequently, besides cord blood, fetal liver, lung, brain, villous placenta, fetal membranes as well as amniotic fluid were identified to host MSCs[20-22]. Apart from abortal tissues, in the majority of cases perinatal tissues are discarded at birth and thus cells are harvestable without any risk for the baby or its mother. Accordingly, there is an unlimited supply, easy access and minimal ethical/legal issues associated with perinatal tissues. Tissues may be stored for autologous use or also allogeneic settings as fetal cells have been demonstrated to be immuno-privileged[21]. Hence CB storage is one strategy increasingly followed in numerous countries, not only for allogeneic but also for potential autologous applications[23].

Antenatal stem cells may be derived from diagnostic samples during amniocentesis[24]. Due to their extensive proliferation potential, a low amount of cells may expand to similar numbers as cells obtained at high numbers from adult tissues. In addition to enhanced expansion capacities, stem cell properties might be enriched in fetal/perinatal stromal cells (Table 1). Frequencies of cells exerting telomerase activity and expressing pluripotency markers are significantly higher compared to stem cells derived from the adult[21,25,26]. Thus, perinatal tissues feature a promising source for MSCs for cell therapy due to their multipotency, immuno-privilege and not-tumorigenicity.

BIOLOGY OF MESENCHYMAL STROMAL CELLS

The acronym MSCs is used to abbreviate marrow stromal cells, mesenchymal stem cells and mesenchymal stromal cells. Classically, MSCs have been isolated from the BM. However, alternative tissues have been identified, including adipose tissue, cutaneous tissues, fetal tissues, dental pulp, hair follicle, synovium, blood, *etc*[16]. MSCs exhibit an *in vitro* expansion potential and more importantly a broad differentiation potential into not only mesodermal (including osteoblasts, adipocytes and chondrocytes) but also endodermal (hepatocyte-like cells) and ectodermal cells (neuronal, neuroglial cells) (For a comprehensive overview, we recommend the special issues 35/3 and 35/4 2008 in Transfusion Medicine and Hemotherapy).

Admittedly, the common definition of a stem cell has not been fulfilled with MSCs so far[27]: it has not been demonstrated that a single implanted MSC can regenerate and maintain a whole tissue compartment as has been shown for HSCs. In addition, MSCs grown in culture do not exert an unlimited self-renewal capacity associated by a lack of telomerase activity and telomere length shortening upon proliferation[28]. This is in contrast to hESCs which display no replicative senescence in culture. Hence, there is a trend towards redefining these cells as "mesenchymal stromal cells" (MSCs)[29].

Nevertheless, MSCs emerged as central candidates in the entire field of cellular therapies. Besides the secretion of trophic factors, MSCs exert fundamental immunomod-

Table 1 Comparison of different perinatal sources of mesenchymal stromal cells regarding mesenchymal stromal cell characteristics

Source of MSCs	Isolation	Origin	Isolation efficacy	Frequency	Expansion potential	Immune phenotype	Differentiation[a]	Immuno-suppression	Senescence/ genetic stability/ safety	Ref.
Cord blood	Ficoll gradient Lineage depletion	Fetal	At best 60%	Very rare, $1/10^8$ MNC Inverse correlation with gestational age	Dependent on seeding density	SSEA-3, SSEA-4, TRA 1-60, TRA 1-82, Nanog	Osteogenic Chondrogenic Adipogenic-significantly reduced compared to adult MSC endothelial	Inhibition of T cell proliferation	Cells senescence Stable Karyotype Non tumorigenic	[20,32] and own unpublished data [49,61,93,95,96]
Umbilical Cord (Wharton´s jelly) Including UC matrix stem cells, UC perivascular cells, UC stroma cells	Removal of vein and arteries Mechanical dissection, Collagenase + hyaluronidase digestion	Fetal	100%	10-50 × 10^3 /cm UC	High Expansion potential	20% no HLA class Ⅰ, nor class Ⅱ SSEA-4, TRA 1-60	Osteogenic Chondrogenic Adipogenic-significantly reduced compared to adult MSC Neurogenic Myogenic, cardiac endothelial	Inhibition of T cell proliferation	Late senescence Telomerase Stable karyotype Non-tumorigenic	Reviewed in [59,95,97]
Amniotic Fluid	Centrifugation	Fetal	21/23 samples	Frequency 3 CFU-f/mL AF range 1-6 0.9%-1.5%	Expansion significantly higher than for BM-MSCs doubling time 18 h	Lower expression of CD44 and CD105 compared to BM-MSCs 90% positive for Oct-4	Osteogenic Adipogenic Chondrogenic Hepatogenic Neurogenic Myogenic, cardio	Inhibition of T cell proliferation	Longer telomeres than BM-MSCs Stable karyotype Non tumorigenic	[95,98]
Placenta (Amnion, chorion and decidua basalis)	dissection digestion with collagenase and DNase, Percoll gradient	Fetal/ maternal	62.5%-100%		Expansion significantly higher than for BM-MSC	SSEA-4, TRA 1-60, TRA 1-81	Osteogenic Chondrogenic Adipogenic-significantly reduced compared to adult MSC	Inhibition of T cell proliferation	Cells senescence Rarely chromosomal changes no transformation No toxicity when injecting 1x10^7MSC/kg	[26,50,99,100]
Amnion (contains besides MSCs amniotic epithelial cells)	Mechanical peeling, mincing Trypsin treatment, digestion with collagenase or/and DNase	Fetal		4 × 10^6/100 cm^2 starting material Or 1 × 10^6 /g of tissue	First trimester proliferate better than third trimester	SSEA-3, SSEA-4, Nanog	Adipogenic Chondrogenic Osteogenic Myogenic, skeletal Myogenic, cardio Angiogenic Neurogenic Pancreatic	Inhibition of T cell proliferation	Cells senescence Stable Karyotype Non tumorigenic	Reviewed in [21] [95,101,102]
Chorion (contains besides MSCs chorionic trophoblastic cells)	Mechanical removal, dispase and collagenase digestion	Fetal			Expansion significantly higher than for BM-MSC		Adipogenic Chondrogenic Osteogenic Myogenic, skeletal Neurogenic	Inhibition of T cell proliferation	Cells senescence Stable Karyotype Non tumorigenic	Reviewed in [21]

[a]Differentiation potential assessed *in vitro* and in non-clonal cultures. MSCs: Mesenchymal stromal cells; UC: Umbilical cord; AF: Amniotic fluid-derived.

ulatory functions[30]. *In vitro* analyses provide a multiplicity of data demonstrating that MSCs, despite the constitutive expression of HLA class I and the interferon γ (IFN γ)-inducible expression of HLA class Ⅱ, can suppress allogeneic T, NK and B cell responses but also can affect dendritic cell functions and tumor cell growth[31].

MESENCHYMAL STROMAL CELLS FROM UMBILICAL CORD BLOOD

Although MSCs seem to be present in any tissue analyzed so far, the presence of circulating MSC has been controversially discussed[32]. Indeed MSCs circulate in peripheral

blood and CB but at much lower frequencies than their hematopoietic counterparts. Thus they are difficult to isolate and culture. Furthermore the frequency of MSCs within fetal blood decreases sharply with advanced gestational age[5,33]. But, interesting for future use, MSCs have also been isolated from cryopreserved term CB[34].

The first reports dealing with issues of MSCs in CB claimed that either stromal feeder cells[35,36] or osteoprogenitors can be cultured[37]. Later on, Goodwin *et al*[38] and Erices *et al*[39] demonstrated cells capable of differentiating into bone, cartilage and adipose tissue. This encouraging data were neglected by a number of authors who tried but failed to isolate MSCs from CB but succeeded with BM[40,41]. Analyzing factors which might influence the unpredictable isolation success in full-term CB ranging between 20%-40% of utilized CB units, we demonstrated that by selecting units with decreased storage time and a high volume of cell-rich CB, the isolation success can be enhanced towards 60%[20]. The infrequent isolation suggests that either MSCs circulate in extremely low frequencies in CB or that reliable culture conditions equivalent to those for BM have yet to be defined. Anyhow, currently the chance of using CB-MSCs in autologous therapeutic settings is considerably hampered[42] (Table 1).

At present no unique phenotype comparable to CD34 for HSC[43] has been identified which allows a standardised prospective isolation of MSCs with predictable differentiative potential[44]. Recent data indicate a perivascular origin of MSCs[16,45]. MSCs could be prospectively isolated using markers for pericytes, but it is doubted that all pericytes inhabit MSC characteristics[46]. With the markers to prospectively isolate pure MSCs still under debate, the expression profile of culture-expanded MSCs consists of a variety of markers typical for other cell lineages. Thus a combination of expressed and not-expressed (most importantly all hematopoietic lineage) markers is currently used to define MSCs. Classically, MSCs express CD44, CD73 (SH-3, SH-4), CD90 (Thy-1), CD105 (SH-2, Endoglin), CD106 (VCAM-1) and HLA class I but lack the expression of CD14, CD34, CD45 (Leucocyte Common Antigen) and HLA class Ⅱ[47] (Table 1).

Since currently the immunophenotype is insufficient to define MSCs, the demanded assay is to analyze their differentiative capacity at least towards the mesodermal lineage. MSCs respond to osteogenic stimuli with the upregulation of osteogenic markers, assessed either by PCR, immuno- or histochemical staining. When assessed quantitatively, CB-MSCs seem to have a stronger osteogenic potential *in vitro* compared to BM-MSCs[48,49]. The differentiation into the chondrogenic lineage can be induced by using a micromass culture and various growth factors. CB-MSCs form cartilage as well as BM-MSCs but the type of cartilage may differ dependent on the tissues source used[50].

Differing observations have been published regarding the adipogenic differentiation capacity of CB-MSCs. In some studies CB-MSCs demonstrate no to only low level adipogenic differentiation after culture in adipogenic media[48,50-52] (Table 1). However, other authors reported no limited adipogenic potential[38,39,53].

A variety of data support the idea that there is no mesodermal germ-layer restricted differentiation potential in CB-MSCs. Depending on the *in vitro* stimulus, CB-MSCs can also differentiate into neural cells, neuroglial and hepatocyte-like cells, endothelial cells, skeletal myoblasts, respiratory epithelial cells and cardiomyogenic cells[32] (Table 1). Goodwin *et al*[38] observed a variety of markers indicative for osteoblastic and neural lineages already expressed on BM-MSCs but induced in CB-MSCs upon induction, suggesting a commitment of BM-MSCs in contrast to CB-MSCs. The inducible expression as well as differing responsiveness to differentiative stimuli may therefore relate to a more primitive cell population contained in CB.

Comparative analysis of genomic and proteomic expression profiles in comparison to mature lineages as well as hESCs revealed shared patterns but also marked differences which might result from differing isolation and culture conditions and may have to be re-evaluated after adjusting to common and standardised protocols[32].

STROMAL CELLS FROM THE UMBILICAL CORD

The presence of fibroblast-like cells with myogenic properties within the umbilical cord (UC) matrix was described years ago[54]. Colony-forming units fibroblast (CFU-F) can be obtained at higher frequencies and in contrast to CB from every UC[55] (Table1). Cells derived from WJ, the gelatinous part of the UC, but also cells in the perivascular region have been associated with multilineage differentiation potential[25,56,57]. Recently, Ishige *et al*[58] compared MSCs derived from the arterial, venous or gelatinous part of the cord. They observed different frequencies, slightly different osteogenic potential but similar phenotype in these populations. In contrast to circulating MSCs, UC-derived cells express pan-cytokeratin markers. Expression however may vary when perivascular cells are compared to WJ cells[55,59].

Similar to cells derived from CB, UC-derived cells exert higher proliferative capacities compared to BM but also improved properties of *in vitro* osteogenesis and neurogenesis at the expense of adipogenesis[55]. Comparing MSCs from the same donor derived from either the cord blood or cord matrix revealed significant differences in the gene expression profiles[60]. In CB-MSCs genes related to development, osteogenesis and immune system were expressed whereas UC-MSCs express genes associated with cell adhesion, morphogenesis, secretion, angiogenesis and neurogenesis.

STROMAL CELLS FROM THE PLACENTA

The composition and origin of cells within amniotic fluid change throughout gestation[61,62]. Human amniotic fluid-derived MSCs (AF-MSCs) are abundant and can be isolated by plastic adherence in minimal medium. The proliferative capacities exceed that of BM-MSCs but markers and

Table 2 Current clinical trials listed at www.clinicaltrials.gov (research August 2010) employing mesenchymal stromal cells from umbilical cord blood, umbilical cord or placenta or case studies applying mesenchymal stromal cells from perinatal sources

UCB-MSCs	Safety and efficacy study of umbilical cord blood-derived mesenchymal stem cells to promote engraftment of unrelated hematopoietic stem cell transplantation
UCB-MSCs	Safety and efficacy study of umbilical cord/placenta-derived mesenchymal stem cells to treat myelodysplastic syndromes
UCB-MSCs	Stem cell therapy for type 1 diabetes mellitus
UCB-MSCs	Study to compare the efficacy and safety of cartistem® and microfracture in patients with knee articular cartilage injury or defect
UC-MSCs	A research study looking at specific tissue of the umbilical cord
UC-MSCs	Allogeneic mesenchymal stem cell for Graft-versus-host disease treatment
UC-MSCs/Placenta-MSCs	Safety and efficacy study of umbilical cord/placenta-derived mesenchymal stem cells to treat myelodysplastic syndromes
Placenta-MSCs	Safety of intramuscular injection of Allogeneic PLX-PAD cells for the treatment of critical limb ischemia
UCB-MSCs	Successful stem cell therapy using umbilical cord blood-derived multipotent stem cells for Buerger's disease and ischemic limb disease animal model[103]
UCB-MSCs	A 37-year-old spinal cord-injured female patient, transplanted of multipotent stem cells from human UC blood, with improved sensory perception and mobility, both functionally and morphologically: a case study[104]

properties are shared with MSCs from adult tissues. One exception is observed regarding the expression of HLA class I and II which seems to be reduced, suggesting even more pronounced immunological inertness compared to adult MSCs.

The human placenta, besides supporting fetal development, may also represent a reservoir of stem/progenitor cells. Four regions can be distinguished harbouring different cell types: the amniotic epithelial (AEC), amniotic mesenchymal (AMSC), chorionic mesenchymal (CMSC) and finally the chorionic trophoblastic cells[21]. As these cell preparations might be contaminated with maternal cells, the fetal origin has to be demonstrated with methods sensitive enough to detect less than 1% of maternal cells (Table 1).

The two fetal membranes, amnion and chorion, emerge from the basal surface of the placenta to encase the fetus within the amniotic fluid. Amnion is composed of a mono-epithelial layer and a fibroblast layer beneath the basal membrane. A layer of collagen fibres separates the amniotic and chorionic mesoderm. After delivery, to separate stem cells from the fetal membranes, both membranes are peeled apart and enzymatically digested. AMSCs are gained after removing the epithelial layer by trypsin followed by collagenase treatment. For chorion, the maternal parts are mechanically removed and then the trophoblast layers digested using dispase and the chorion stromal cells released by collagenase. Primary yields are high with approximately 6×10^6 AECs and 2×10^6 AMSCs per gram amnion[63].

Like BM-MSCs, AMSCs and CMSCs display a fibroblastoid phenotype upon adherence to plastic, can form typical colonies, display differentiation potential into mesodermal lineages and express the range of markers used to characterise MSCs. Furthermore these cells express some pluripotency markers, SSEA-4, TRA-1-61, and TRA-1-80[26] (Table 1). Regarding the differentiation potential, slight differences have been described. AMSCs seem to be more directed to an adipogenic potential whereas CMSCs more to chrondo-, osteo-, myo- and neurogenic lineages[24]. Portmann-Lanz *et al*[64] demonstrated no obvious effects by the gestational age but the excess of adipo- and neurogenic potential was detected to be higher in ASCs compared to CSCs. ASCs seem to inherit also some vasculogenic and hepatic potential[21,65].

CELL THERAPY WITH FETAL MESENCHYMAL STROMAL CELLS

MSCs are promising candidates for use in regenerative medicine. Most clinical studies have been conducted using BM-MSCs (www.clinicaltrials.gov). Current studies listed there and case reports published focusing on perinatal MSC sources are summarized in Table 2.

Studies in general focus on either local or systemic administration of MSCs. Preclinical models indicated that the site-directed administration appears to result in engraftment and integration of the MSCs mediated by extent of tissue injury. But owing to problems in quantifying engraftment, published results vary enormously for MSC biodistribution and assessing the therapeutic outcome[66].

The systemic administration, in contrast, seems to result in general in even less persistence of tissue-localized MSCs. After infusion, MSCs remain in the circulation for no more than 1 h[67]. Thereafter MSCs are detectable primarily in the lungs and then secondarily in the injured organs, albeit at low frequencies. Specific homing to and survival in the BM has been shown not only for BM-MSCs but also for CB-MSCs after transplantation into immunodeficient nude mice without conditioning pretreatment[68].

Basically, clinical expectations are associated with three functional aspects of MSC: (1) Tissue repair by either reparative cells directly or by secretion of paracrine effectors; (2) Stromal capacities to support engraftment, of especially HSCs; and (3) Immune modulation.

Tissue repair by either reparative cells directly or by secretion of paracrine effectors

Figure 1 depicts a range of those tissues which have been the object of pre-clinical investigation to induce tissue repair or tissue regeneration with perinatal MSCs. Interestingly, in preclinical models, tissue targets of all three germ

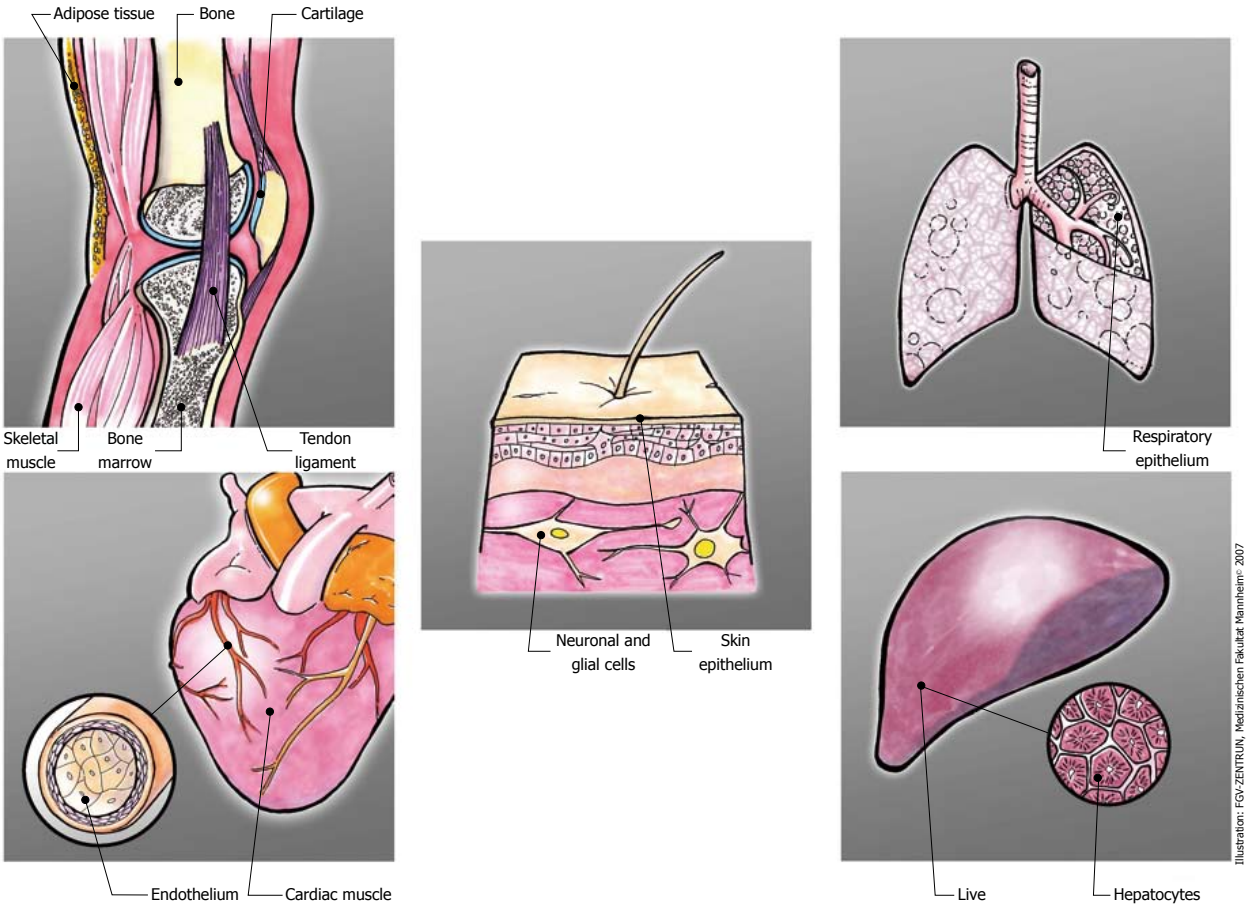

Figure 1 Therapeutic potential of perinatal mesenchymal stromal cells assessed in preclinical models.

Table 3 Differentiation potential of perinatal mesenchymal stromal cells

Mesoderm		Endoderm		Ectoderm	
Bone	[74,75,105-109]	Skin, skin epithelium	[110]	Lung, respiratory epithelium	[111,112]
Cartilage	[76,113,114]	Nerve, neuronal, glial	[64,115-124]	Liver, hepatocytes	[125-128]
Adipose tissue	[48,49,51,129]			Islets, beta cells	[130,131]
Skeletal muscle	[54,132-136]				
Cardiac muscle	[77,137-142]				
Vessels, Endothelium	[116,129,143-145]				
Stromal support	[79,81,82,146,147]				
Immune regulation	[83,148-151]				

Some refrences-if highly interesting or comparing mesenchymal stromal cells (MSCs) from different tissues- refer to *in vitro* data. This list can by no means be regarded as comprehensive. It shall just give an overview on the broad (pre)clinical potential of MSCs.

layers have been investigated and proved some success of perinatal stem cell transplantation (Table 3). Whether this success resulted from direct engraftment and cellular differentiation is doubted more and more because overall engraftment levels are extremely low and lack long-term persistence. Rather, in a variety of settings, the secretion of regenerative or immune-suppressive factors ameliorated clinical signs.

Preclinical models are difficult to design and interpret because a multitude of parameters like animal and disease model, mode of application, cellular source (species and tissue source), cell number, *ex vivo* culture and differentiation, post transplantation analysis have been demonstrated to affect the experimental outcome. This is exemplified in the following: Zhao *et al*[69] and Satore *et al*[70] transplanted AF-MSCs to treat acute ischemic myocardium in a porcine or rat model. In the study of Zhao *et al*[69], the transplanted cells survived for at least 2 mo and exhibited myocardial commitment with finally myocardiocyte *in situ* differentiation. However, Sartore *et al*[70] could not detect significant myocardial engraftment. These contrary results may be due to the mixed cell population used because Zhao *et al*[69] excluded AEC cells before transplantation. Furthermore, *ex vivo* culture conditioning and species sources differed. Sartore *et al*[70] *ex vivo* cultivated the cells before autotransplantation into porcine ischemic hearts. Zhao *et al*[69] co-cultivated human MSCs with neonatal rat heart explants before xenotransplating in rat ischemic hearts. Also, cell

passaging and cell dose may affect the capacity of engraftment. This example highlights that preclinical data have to be extremely carefully compared and evaluated to extrapolate therapeutic efficacy.

The concept of using fetal stromal cells for fetal tissue engineering is the one most intensely studied to treat congenital abnormalities[71]. Obtaining fetal biopsies for autologous purposes is amendable but associated with a not negligible risk and thus currently considered for life-threatening diseases. By means of tissue engineering, a few studies indicate that a variety of defects may be treatable. In one study, diaphragm reconstruction was achieved using hAMSCs[72]. The Hoerstrup group is focussing on fetal MSCs in cardiovascular engineering, e.g. to generate living heart valves[73]. Apart from treating congenital abnormalities, the tendency of improved osteogenic differentiation potential, prompted investigators to study bone and cartilage formation with fetal stromal cells[22,74-76]. All data indicate that fetal tissue derived MSCs might be candidates for hard tissue engineering.

Further publications suggest that the search for the optimal tissue to derive MSCs might be advisable. For example Iop *et al*[77] indicate dissimilar cardiovascular properties of fetal compared to adult MSCs. This is also suggested by a variety of authors with regard to neuroregenerative protocols[64].

Stromal capacities to support engraftment, especially of hematopoietic stem cells

In CB transplantation, the cell dose as major determinant of rate and incidence of hematopoietic recovery is limited to the volume which can be collected from one placenta. Strategies to improve engraftment are under investigation and include *ex vivo* expansion of HSCs and co-infusion of MSCs. *Ex vivo* expansion of CB-HSCs has been achieved on monolayers of MSCs derived from various tissues. In fact CB-MSCs constitutively secrete a variety of growth factors affecting HSCs[78]. Thus, stromal layers derived from CB-MSCs as well as from BM-MSCs were capable of maintaining and amplifying colony-forming cells over a prolonged period of time[79,80]. Furthermore the co-transplantation of CB-MSCs can lead to an enhanced and accelerated engraftment of CB-HSCs within the murine NOD/SCID (nonobese diabetic/severe combined immune deficiency) transplantation model[81,82].

Immune modulation

Several studies based on initial reports by Le Blanc *et al*[31] report that MSCs not only evade immune recognition but furthermore play a role in modulating immune and inflammatory responses. They are currently clinically exploited as a tool for managing tolerance in clinical transplantation, including graft versus host disease. MSCs interact with a variety of immune cells to affect both the innate and adaptive immune system modulating various effector functions to induce immunosuppression and an anti-inflammatory milieu in various injury models. Immunological responses elicited by fetal and adult MSCs seem to be comparable and fetal MSCs are also anti-proliferative to T cells[83]. Comparing MSCs from BM, adipose tissue, CB and WJ indicated similar immunomodulatory capacities of all cell types, demonstrating that this is a broad stromal capacity not restricted to a developmental or tissue origin[84]. Analyzing specific aspects of immune regulations in more detail, however, indicate that cells from different tissues or developmental age may behave slightly differently. Unrestricted somatic stem cells from CB can induce a balance between T cell effector responses and dendritic cell maturation depending on the cytokine milieu. Here especially, interferon-γ and tumor necrosis factor-alpha play a role[85,86].

MSCs are considered to be non-immunogenic. The fetal-maternal interface seems to be immunologically special to enable maternal acceptance of the fetal allograft. Thus fetal or perinatal MSCs might be specifically interesting for allogeneic settings[21]. This idea is challenged by findings by Cho *et al*[87]. They demonstrated in a swine model that the first injection of WJ derived MSCs was non-immunogenic. However, repetitive administrations as well as injection into inflamed skin or interferon activation mediated alloreactive immune responses.

CLINICAL MANUFACTURING OF FETAL STROMAL CELLS

The employment of adult stem cell types in clinical studies, in general, necessitates manufacturing, processing and testing of cellular products according to the current national regulations, including current good tissue practice (GTP) and good manufacturing practice (GMP)[17].

As indicated, fetal MSCs have proven valuable in fetal tissue engineering. Having diagnosed a structural birth defect and performed diagnostic amniocentesis to procure AF-MSCs, the months until birth allow for cell isolation, expansion, cryopreservation, thawing, secondary expansion and tissue engineering of the graft. A protocol validating a three stage procedure for manufacturing AF-MSCs has been introduced by Steigman *et al*[88] and Brooke *et al*[89] provide a protocol for placental MSCs.

The authors also discuss the aspect of using fetal bovine serum (FBS). FBS contains xenogeneic proteins which are internalized by MSCs. Consequently, a host of potential problems can arise such as viral and prion transmission or immunological reactions. These risks have initiated the search for alternative substitutes: recently serum, plasma or platelet derived supplements have been introduced enabling the FBS-free propagation of MSCs[90-92]. Obviously the same holds true for CB-MSCs: very recently human platelet lysate has been established as supplement to expand CB-MSCs for clinical applications, paving its way to the clinic[93,94].

CONCLUSION

The potential application of autologous stem cells collected at birth requires immense technical and financial

resources for storing the frozen cell samples throughout the period of life. Although such a procedure seems possible from a technical point of view, it is highly debatable whether the eventual use and possible benefits justify these efforts.

For allogeneic use, the presence of stem cell populations collectable at birth provides a readily accessible and currently probably under-utilized stem cell source with little ethical conflict and numerous advantages. For allogeneic applications however, efficient and reproducible methods to isolate, expand and differentiate and quality control these perinatal progenitor cells are required. If one can extrapolate from the lessons learned in HSC transplantation, stem cell populations harvestable at the time of birth promise to develop as adequate alternatives to other adult tissues.

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