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Review Article

Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art

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Abstract: Mesenchymal stem cells (MSCs) are multipotent adult stem cells present in all tissues, as part of the perivascular population. As multipotent cells, MSCs can differentiate into different tissues originating from mesoderm ranging from bone and cartilage, to cardiac muscle. MSCs are an excellent candidate for cell therapy because they are easily accessible, their isolation is straightforward, they can be bio-preserved with minimal loss of potency, and they have shown no adverse reactions to allogeneic versus autologous MSCs transplants. Therefore, MSCs are being explored to regenerate damaged tissue and treat inflammation, resulting from cardiovascular disease and myocardial infarction (MI), brain and spinal cord injury, stroke, diabetes, cartilage and bone injury, Crohn's disease and graft versus host disease (GvHD). Most of the application and clinical trials involve MSCs from bone marrow (BMMSCs). Transplantation of MSCs from bone marrow is considered safe and has been widely tested in clinical trials of cardiovascular, neurological, and immunological disease with encouraging results. There are examples of MSCs utilization in the repair of kidney, muscle and lung. The cells were also found to promote angiogenesis, and were used in chronic skin wound treatment. Recent studies involve also mesenchymal stem cell transplant from umbilical cord (UCMSCt). One of these demonstrate that UCMSCt may improve symptoms and biochemical values in patients with severe refractory systemic lupus erythematosus (SLE), and therefore this source of MSCs need deeper studies and require more attention. However, also if there are 79 registered clinical trial sites for evaluating MSC therapy throughout the world, it is still a long way to go before using these cells as a routinely applied therapy in clinics.

Keywords: Umbilical cord blood, mesenchymal stem cells, regenerative medicine, cell therapy, umbilical cord blood banking

1. Mesenchymal Stem Cells (MSCs)

Introduction to MSCs

Mesenchymal stem cells (MSCs), also known as marrow stromal cells [1] or mesenchymal progenitor cells [2], are definied as self-renewal, multipotent progenitor cells with the capacity to differentiate into several distinct mesenchymal lineages [3]. To date, MSCs of multiple adult vertebrate species have been demonstrated to differentiate into connective skeletal tissue, bone, cartilage, marrow stroma and adipocytes [4,5]. In addition, controversial data suggest that MSCs may give rise to sarcomeric muscle (skeletal and cardiac) [6-8], endothelial cells [9] and even cells of non-mesodermal origin, such as hepatocytes [10], neural cells [11] and epithelial cells [12,13]. Hence, the descriptive terms pluripotent or multipotent are reciprocally used to describe the capacity of MSCs to differentiate into a wide arrange of mammalian tissues [14].

MSCs were initially identified in bone marrow (BM) and later in muscle, adipose and connective tissue of human adults [15-18]. However, because the frequency and differentiating capacity of MSCs decrease with age [19], alternative sources of MSCs have been sought. MSCs have been identified in human amniotic fluid, placenta, umbilical cord blood (UCB) and veins [20-22] as well as in several fetal tissues including bone marrow, liver, blood, lung and spleen

[23-25]. MSCs isolated from the synovium as an adherent cell population were capable of differentiation into chondrocytes, osteocytes and adipocytes [26]. They also showed that these cells were capable of contributing to skeletal muscle regeneration in a nude mouse model and restored expression of dystrophin in the sarcolemma in dystrophic muscle of immunosuppressed *mdx* mice [27].

Stem cells from adipose tissue, variously referred to as processed lipoaspirate (PLA) cells [28] and adipose-derived adult stem (ADAS) cells [29], have been shown to have similar differentiation potential. De Ugarte *et al.* [30] suggest that there is little difference between cells from marrow and fat in terms of yield, growth kinetics, cell senescence, multi-lineage differentiation capacity, and gene transduction efficiency. The utility of these cells in therapeutic applications may then depend on the availability of tissue specimens and the ease of *in vitro* expansion. Kaviani *et al.* [31] first described the presence of a sub-population of amniotic fluid cells with mesenchymal features, able to proliferate *in vitro* more rapidly than comparable fetal and adult cells. In 't Anker *et al.* [20] demonstrated that the amniotic fluid can be an abundant source of fetal cells that exhibit a phenotype and a multilineage differentiation potential similar to that of bone marrow-derived MSCs; these cells were named amniotic fluid mesenchymal stem cells (AFMSC). These characteristics, together with the absence of ethical issues concerning their employment, suggest that stem cells present in amniotic fluid might be promising candidates for tissue engineering and stem cell therapy of several human disorders.

Also if some confusion exists regarding the muscle-derived stem cells (MDSCs) and their potential application in regenerative medicine and gene therapy, more recent evidence supports the existence of a population of multipotential MDSCs able to differentiate into other mesodermal cell types. Muscle-derived cells have been shown to differentiate into mesenchymal tissues, functionally regenerating bone and muscle, as well as play a role in cartilage healing [32-35].

The considerable therapeutic potential of MSCs has generated markedly increasing interest in a wide variety of biomedical disciplines. In different papers investigators report studies of MSCs using different methods of isolation and expansion, and different approaches to characterizing the cells. Therefore, Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSCs. First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSCs must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* [36,37].

MSCs and bone marrow

BM is a complex tissue containing hematopoietic progenitor cells and a connective-tissue network of stromal cells. Blood and the system that forms it, known as the hematopoietic system, consist of many cell types with specialized functions. Red blood cells (erythrocytes) carry oxygen to the tissues. Platelets (derived from megakaryocytes) help prevent bleeding. Granulocytes (neutrophils, basophils and eosinophils) and macrophages (collectively known as myeloid cells) fight infections from bacteria, fungi, and other parasites such as nematodes (ubiquitous small worms). Some of these cells are also involved in tissue and bone remodeling and removal of dead cells. B lymphocytes produce antibodies, while T lymphocytes can directly kill or isolate by inflammation cells recognized as foreign to the body, including many virus-infected cells and cancer cells. Many blood cells are short-lived and need to be replenished continuously; the average human requires approximately one hundred billion new hematopoietic cells each day. The continued production of these cells depends directly on the presence of Hematopoietic Stem Cells (HSCs), the ultimate, and only, source of all these cells [38].

Marrow stroma includes a subpopulation of undifferentiated cells that are capable of becoming one of a number of phenotypes, including bone and cartilage, tendon, muscle, fat, and marrow stromal connective tissue which supports hematopoietic cell differentiation [39,40]. MSCs represent a very small fraction, 0.001-0.01% of the total population of nucleated cells in marrow [5]. Many studies have defined conditions for isolation, expansion, and *in vitro* and *in vivo* differentiation of the stromal cells. These cells are referred to as MSCs, since they are

known to have capacity of proliferation and differentiation into the mesenchymal lineage. Their mesenchymal differentiation potential is retained even after repeated subcultivation *in vitro* [41,42].

Though BM has been represented as the main available source of MSCs [5,43], the use of BM-derived cells is not always acceptable owing to the high degree of viral exposure and the significant decrease in the cell number and the proliferative/differentiation capacity along with age. In addition, it requires a painful invasive procedure to obtain a BM sample. Therefore, the identification of alternative sources of MSCs may provide significant clinical benefits with respect to ease of accessibility and reduced morbidity [44-47].

MSCs and umbilical cord blood

The umbilical cord (UC) contains two arteries and one vein, which are surrounded by mucoid connective tissue, and this is called the Wharton's jelly. The cord is covered by an epithelium derived from the enveloping amnion. The network of glycoprotein microfibrils and collagen fibrils in the Wharton's jelly has been previously studied [48]. The UCB has been used as an alternative source since 1988 [49]. The blood remaining in the umbilical vein following birth contains a rich source of hematopoietic stem and progenitor cells, has been used successfully as an alternative allogeneic donor source to treat a variety of pediatric genetic, hematologic, immunologic, and oncologic disorders [50-53]. Fresh cord blood is also a promising source of non-hematopoietic stem cells. Among others, it contains endothelial cells, MSCs and unrestricted somatic stem cells (USSC) [54-57].

Primitive stromal cells can be isolated from umbilical cord Wharton's jelly and can be differentiated into different cells, like osteoblasts, chondrocytes, adipocyte, cardiomyocyte and neurocyte [58,59]. Most of the time UCB is still regarded as medical waste in the delivery rooms, whereas, in contrast to BM aspiration, it is obtained by a simple, safe and painless procedure when the baby is delivered.

There are many advantages of UCB as a source of Human Stem Cells (HSCs) as compared to BM and Peripheral Blood (PB). First, the collection of cord blood units is easy and non-invasive for the donor and therefore the number of potential donors is higher than for bone marrow. Umbilical cord can be easily obtained without causing pain, and the procedure avoids ethical and technical issues [58]. Then, cord blood units are stored in advance and are therefore rapidly available when needed while bone marrow has to be collected from the donor just before transplantation and there is always a risk of last minute consent refusal. Moreover, MSCs from UCB are more primitive than MSCs isolated from some other tissue sources [60-63]. Despite, reports to date have focused on obtaining those cells after culture expansion from a segment of the umbilical cord [64-66]. Culture expansion has a disadvantage, the cells cannot be frozen on the same day as UCB cells arrive in the laboratory, and there is the increased risk of contamination with any culture manipulation. Moreover, MSCs from UCB have lower success rate of isolating if compared with MSCs from BM (63% vs 100%) [67].

Finally, the human leukocyte antigen type (HLA) does not need to be a perfect match in case of allogeneic cord blood cell transplantation because these cells are less likely to induce immunological reactions than bone marrow cells. UCB cells are good substitutes for BM-derived hematopoietic progenitors due to the immaturity of newborn cells [68]. The immaturity of cells is associated with lower immunogenicity, therefore, UCB reduces graft-versus-host reactivity when compared with adult-derived marrow grafts. Furthermore, UCB raises no ethical issues for basic studies and clinical applications.

2. Technical characteristics of human cord mesenchimal stem cells (hUCMSCs)

Isolation and characterization of MSCs from UCB: state of the art

Although the isolation of hematopoietic stem cells from UCB has been well established, the isolation and characterization of MSCs from UCB still need to be evaluated and are controversial. Erices *et al.* [21] reported that UCB-derived mononuclear cells gave rise to two adherent cell types, with only one of them expressing MSC-related surface antigens. Mareschi *et al.* [69] reported that under given conditions, it was possible to isolate MSCs from BM, but not from UCB; Goodwin *et al.* [70] have reported the multi-lineage differentiation ability of UCB-

isolated cells. Neither of these reports provided sufficient evidence to fulfill the qualifying criteria for MSCs because relatively heterogeneous cells were reported by both groups. Wexler *et al.* [71] have recently reported that UCB is not a rich source of human MSCs, while Musina *et al.* [72] found that a specific feature of human umbilical cord blood mesenchymal stem cells (hUCBMSCs) is their low count per volume of the initial material and very low proliferative activity. On the other hand, Romanov *et al.* [73] suggested that umbilical cord contains a high number of MSC-like elements forming colonies of fibroblastoid cells that may be successfully expanded in culture. These MSC-like cells contain no endothelium- or leukocyte specific antigens but express alpha-smooth muscle actin (α-SMA) and several mesenchymal cell markers. Therefore, umbilical cord/placenta stroma could be regarded as an alternative source of MSCs for experimental and clinical needs.

Consistent findings within the literature include the extent of patient variability between each donor blood sample and the contamination of a large number of cells such as fibroblastic cells, dendritic cells, adherent monocytes, macrophages, and osteoclastic cells arising within the cultures [69,70,74].

Methods of isolation and hUCMSCs culture

There are four methods for isolation of MSCs from UC: density gradient centrifugation, flow cytometer isolation, attachment screening and two step, enzymatic digestion [75]. In 2003, Romanov *et al.* using enzymatic digestion and centrifugation methods isolated well developed colonies of fibroblast like cells and further characterization revealed that these cells expressed MSC markers [73]. Lu *et al.* [66] attempted to isolate MSC according to the protocol described in the report and obtained MSCs from three of ten UCs. Wang *et al.* [76] centrifuged the mesenchymal tissue scraped from the Wharton's jelly, treated it with collagenase and 2.5% trypsin and the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Finally, they obtained 25 $\times 10^3$ cells per centimetre of umbilical cord. In 2006, Weiss *et al.* [65] reported a more efficient method of starting the cultures via enzymatic degradation of the extracellular matrix to release the cells from the WJ.

hUCMSCs were isolated from 78% of the cords; including from one of the two cords refrigerate for 24 hours prior. Using this improved procedure, UCMSCs were isolated from every cord and up to 1.5 $\times 10^6$ cells per cm of UC.

In Fu's [77] study, approximately 1×10^6 hUCMSCs were collected from 20 cm of UC and the number of hUCMSCs doubled (2×10^6) in 10% FBS DMEM in 3 days. They found that hUCMSCs in Wharton's jelly of the UC can be easily obtained and processed compared with embryonic and bone marrow stem cells. Lu *et al.* [66] established a simple, two step enzymatic digestion, to isolate and culture hUCMSCs from each of 36 UCs, which may be the most efficient way to isolate MSCs from UC.

A new and simple method of obtain and cryopreserving hUCMSCs extracted from a small piece of UC was described by Friedman *et al.* [78]. This method is followed by mincing the tissue and cryopreserving it in autologous cord plasma to prevent exposure to allogeneic or animal serum, thus showing that UCMSCs are a reliable, easily accessible, noncontroversial source of MSCs.

Immunophenotype and comparison of hUCMSCs and hBMMSCs

MSCs express numerous receptors important for cell adhesion with hematopoietic cells. Much valuable information can also be gained from a systematic analysis of cell surface molecules on MSCs. Majumdar *et al.* [79] determined that MSCs express a large spectrum of cell adhesion molecules of potential importance in cell binding and homing interactions. MSCs exhibit high expression of integrins that could also play a role in homing to sites of injury and binding to specific matrix molecules in the manner suggested by Bogenrieder and Herlyn [80].

The morphology of hUCMSCs in the culture show a typical MSCs immunophenotypic markers and fibroblastoid morphology. The absence of endothelial CD31 and leukocyte surface markers support classifying hUCMSCs as mesenchymal progenitors. Very important, hUCMSCs are negative for CD14, CD28, CD31, CD33, CD34, CD45, CD56, CD133, HLA-DR, and for graft versus host disease CD80, CD86, CD40, which shows that they are appropriate for transplantation.

Table 1. Surface markers expressed by hUCMSCs and BMMSCs [186]

Surface markers	hUC	BM
CD13	++++	++++
CD14	-	-
CD29	++++	++++
CD31	-	-
CD34	-	-
CD38	-	-
CD44	++++	++++
CD45	-	-
CD73	++++	++++
CD90	++++	++++
CD105	++++	++++
CD106	+	+++
CD146	++++	+++
HLA-ABC	+++	++++
HLA-DR	-	-

Recent studies show that hUCMSCs share most of their immunophenotype with Bone Marrow Mesenchymal Stem Cells (BMMSCs), including a cluster of differentiating markers, neural markers and extracellular adhesion molecules. Surface markers of hUCMSCs and BMMSCs are shown in **Table 1**. They share the expression of CD13, CD29, CD44, CD73, CD90, CD105, CD146. Moreover, they share the cell cycle status, the adipogenic and osteogenic differentiation capacity and finally the cytokines as well as haematopoietic supportive function.

Despite this, recent studies show that there are still several differences between them [81]. Firstly, the fibroblast colony-forming units (CFUF) frequency was significantly higher in UC derived nucleated cells than in BM derived nucleated cells. Since CFUF represents the mesenchymal progenitor cell, this suggested a higher frequency of MSCs in the nucleated cells of UC than in those of BM. Secondly, the proliferation analysis revealed that hUCMSCs have a faster population doubling time, that not change after 30 passages. In contrast, BMMSC showed significantly slower population doubling time which became even longer after Passage 6. hUCMSCs had a higher proliferative capacity of in comparison with BM-MSCs indicating that UCMSCs may be a novel alternative source of human MSCs for clinical application.

In addition, hUCMSCs showed lower expression of CD106 and HLA-DR in comparison with hBMMSC. The different expression of CD106 in hUCMSCs and BMMSCs may represent a specific indicator for identifying peripheral MSCs from BMMSCs because low expression of CD106 has also been identified in adipocyte derived MSCs. Furthermore, Lu *et al.* [66] noted low expression of HLA-ABC on hUCMSCs and the absence of HLA-DR expression. Because HLA-ABC could be a hurdle for allogeneic cell therapies, the lower expression of HLA-ABC may favour the use of hUCMSCs for allogeneic cell therapy.

Collection strategies

Collection strategy is the first step for collecting good-quality UCB units. There are many techniques of UCB sample collection. The key difference regards whether samples are taken while the placenta is still in the uterus (*in utero*), or after the delivery of the placenta (*ex utero*). The *in utero* collections are usually performed by obstetricians and midwives in the delivery room, while *ex utero* UCB collection is performed after delivery of the placenta, in an adjacent room, by trained personnel of UCB bank.

Many authors have investigated the impact of *in utero* and *ex utero* collection strategy on the quality of UCB units. They compared both strategies in vaginal deliveries and concluded that *in utero* collection yielded a significantly higher volume, total mononuclear cells (tMNCs) number, CD34 cells and total CFU [82,83]. In one of this study, Surbek *et al.* [82] has confirmed that larger UCB volume and the tMNCs were

obtained in the samples collected before placental delivery for Caesarean deliveries also. Wong *et al.* [84] reported collection of UCB from the same cord before and after the placenta was delivered, and they observed that the concentration of nuclear cells was higher when the UCB was *in utero* than after it was delivered. Solves *et al.* [83] showed that the proportion of excluded UCB units (discard rate) after collection was significantly higher for *ex utero* group. The main reasons for discarding these units were low volume and total cell count. All these authors concluded that it is beneficial to collect UCB while the placenta is still *in utero*. Nevertheless, Lasky *et al.* [85] in a large retrospective multicentre study did not find differences in volume, total cell count and discard rate between *in utero* and *ex utero* strategies. Several other methods of UCB collection have been previously described [86,87].

However, Bertolini *et al.* [88] showed that UCB collection using an open system was associated with significant risk of bacterial and maternal cell contamination compared to closed collection system. According to these data, open systems have been replaced by closed systems. Other means to increase UCB volume include multiple punctures or placenta manipulation, but these should increase the risk of bacterial or maternal cell contamination [89]. All of mentioned studies conclude that the *in utero* collection strategy, performed with single puncture of umbilical vein and into closed system is the best approach for collecting good-quality UCB units.

In order to improve *in utero* technique of UCB collection, more recently Skoric *et al.* [90] designed closed blood-collection set for active Syringe/Flush/Syringe method. The samples were collected before placental expulsion by trained personnel. They compared that method with the usual *in utero* method performed by obstetric staff. In all cases, the umbilical cord was clamped within 30 s after delivery and single umbilical vein puncture was used. Results from this study showed that the median volume, total number of nuclear and mononuclear cells were significantly higher and proportion of excluded UCB units was significantly lower in group A (active Syringe/Flush/Syringe method) compared with group B (by gravity method). The reasons for discarding these units were low volume and total cell count. Anywhere they did not have bacterial contaminations in any case.

Cryopreservation of UCB

The cryopreservation is the accepted method for cell preservation before or after cell expansion for clinical use, and it is important to determine the optimized cryopreservation conditions of cell derived UCB. Many studies have reported the cryopreservation strategies to prevent cell damage during the freezing and thawing processes [91-94]. To reduce the damage caused by the formation of ice crystals, a cryo-medium containing cryoprotectants (CPAs) was used. The standard CPAs is dimethyl sulfoxide (DMSO), which is usually used at a concentration of 10% and combined with normal saline and serum albumin. DMSO is an intracellular cryoprotectant as it can move across the cell membrane by displacing the water within the cell, thus preventing the formation of ice crystals in the cell and protecting the cell from rupture [91,95]. However, DMSO has toxic effects on cells depending on the temperature and the exposure time for both pre-freeze and post-thaw periods [91,95,96]. Therefore, many studies to avoid the toxic effects on the cells have been performed new protocol to reduce concentration of DMSO (from 3.5% to 7%) for bone marrow cells and peripheral blood stem cells , as well as for HSC from UCB [97-100].

Several studies compared the effect of cooling rate and cryoprotectant concentration on UCB recovery. Donaldson *et al.* [101] cryopreserved UCB samples with different concentrations of DMSO and hydroxyethylstarch in combination with a variety of cooling rates. They concluded that good recovery of UCB can be achieved with 5-10% DMSO at a controlled-rate freezing of 1° C/min. Recently, Hunt *et al.* [102] reported a statistically significant difference in recovery between cooling at 1°C and 5°C /min in favour of -1°C/min. In 2007 Skoric *et al.* [90] confirmed an evident effect of cooling rate on the recovery of UCB cells. This study highlights the comparative cryo-investigation of BM cells have shown that the post-thaw recovery and clonogeneic ability of immature pluripotent and mature committed progenitors were the highest when controlled-rate freezing and 5% DMSO was used [103]. Ethylene glycol (EG) in a high concentration (about 50%) is frequently used as a permeating CPA for vitrification of mammalian embryos and oocytes because it has a lower toxic-

ity compared to other cryoprotectants, such as DMSO [104,105]. However, there are no studies on whether a low concentration of EG (10%) could be used for cryopreservation of cord blood cells.

The purpose of a recent study [106] is to optimize the cryopreservation conditions of HSC from UCB. Improved cryopreservation conditions of HSC were established with a new cryo-medium including EG, which is the least toxic and most stable cryoprotectants, compared to the control cryo-medium. The results presented in this study provided optimal cryopreservation conditions for HSC from UCB using EG combined with the controlled-rate freezing method and thus contributes to improvements in cryopreservation of cells from UCB for research purposes and could be applied to UCB banking in the near future.

3. Umbilical cord blood banking

Public and private UCB banks

Since the first successful umbilical cord blood transplants in children with Fanconi anemia, the collection and therapeutic use of these cells has grown quickly [107]. In order to have cord blood cells available for transplantation a number of banks were created worldwide. These banks are run by either hospitals or non-profit organizations that collect the samples from donors and provide them when the cells are needed for transplantation [108]. The New York Blood Center's Placental Blood Program, supported by NIH, is the largest U.S. public umbilical cord blood bank and now has 13,000 donations available for transplantation into small patients who need HSCs [http://stemcells.nih.gov/info/scireport/chapter5.asp].

It is agreed by most ethical review boards that blood donated to a public bank cannot be permanently linked to the donor. The larger obstacle facing public banks is that the high costs required to maintain them has prevented more than a handful from opening. Because public banks do not charge storage fees, many medical centers do not have the funds required to establish and maintain them [109]. Then, a number of private for-profit companies have been established encouraging parents to bank their children's cord blood for their own autologous use or for directed donor allogeneic use for a family member should the need arise. Parents have been encouraged to bank their infants' cord blood as a form of "biological insurance" [110].

There are different opinions regarding cord blood use. A prospective by Sullivan [111], states that the information on cord blood banking that is provided for parents needs to be scientifically accurate, and he cites 16 publications that give negative opinions about the value of private cord blood banking. He states that the probability that a privately stored CB unit will be used for an autologous transplant is extremely low. Nietfield *et al.* [112] performed the first calculation of lifetime probability that a person has to undergo a hematopoietic stem cell transplant (HSCT). The result of almost 1:200 Is much higher than commonly appreciated, and therefore the opinions regarding CB use need to be considered with attention and need an update. The 2007 annual report of the European Group for Blood and Bone Marrow Transplantation (EBMT) describes the current status of HSCT activity in Europe. It highlights the increasing role of allogeneic HSCT and gives the first quantitative information on novel cellular therapies. In 2007, there were 25 563 first HSCTs, 10 072 allogeneic (39%), 15 491 autologous (61%) and 3606 additional transplants reported from 613 centers in 42 countries.

Regulation and accreditation

Unlike other blood banks, that have had specific guidelines and regulations in place for many years, cord blood banks have only recently become regulated. This is because cord blood storage is a fairly new area, therefore the usual authoritative bodies have not been as quick to established the appropriate regulations on the industry.

In the United States, the Food and Drug Administration (FDA) finally set out in 2005 a full body of regulations outlining the particular procedural methods that banks and laboratories must follow. These regulations cover the appropriate collection, processing, packaging, labelling and distribution of cord blood cells. The FDA regulates cord blood under the category of "Human Cells, Tissues, and Cellular and Tissue Based-Products." The Code of Federal Regulations under which the FDA regulates public and private

cord blood banks is Title 21 Section 1271 [113]. In addition, private cord blood banks can apply for voluntary accreditation with either the American Association of Blood Banks (AABB) or the Foundation for the Accreditation of Cellular Therapy (FACT) [www.aabb.org].

The FDA governs the collection, processing, storage, labeling, packaging, and distribution of cord blood stem cells. There are two different standards which can apply: cGTP (current Good Tissue Practices) and cGMP (current Good Manufacturing Practices). cGTP standards apply to the collection, processing and storage of human cells, tissues, and cellular/tissue-based products (HCT/Ps) and are regulated by the Center for Biologics Evaluation and Research. All US cord blood banks must be compliant with cGTP standards. cGMP standards apply to the manufacture of a product that is considered a drug [114].

In Europe, The European Union Group on Ethics (EGE) has issued Opinion No.19 titled Ethical Aspects of Umbilical Cord Blood Banking. The EGE concluded that "the legitimacy of commercial cord blood banks for autologous use should be questioned as they sell a service, which has presently, no real use regarding therapeutic options [115].

So, the article 6 of directive 2004/23/ec of the European parliament and of the council of 31 March 2004 (on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells) declare: "Member States shall ensure that all tissue establishments where activities of testing, processing, preservation, storage or distribution of human tissues and cells intended for human applications are undertaken have been accredited, designated, authorised or licensed by a competent authority for the purpose of those activities" [www.irishstatutebook.ie/2007/en/si/0598.html]. In UK, Since 5 July 2008, Cord blood banks, both public and private, must be licensed by the Human Tissue Authority in order to release transplants to hospitals in the National Health Service.

The Human Tissue Authority (HTA) is an independent watchdog that supports public confidence by making sure human tissue is used safely and ethically, and with proper consent [www.hta.gov.uk]. They license and inspect organisations that store and use human tissue for purposes including teaching about the human body, carrying out post-mortem examinations, using human tissue to treat patients, carrying out research on human tissue and displaying human bodies or tissue in public.

4. Mesenchymal stem cell stherapy

Tissue engineering and regenerative medicine are the terms that are nowadays used to describe the approach to generate complex tissues and organs from simpler pieces. The main goal is to create new therapies for patients with severe injuries or chronic diseases in which the body's own responses do not suffice to restore functional tissue. Both are multidisciplinary, young and emerging fields in biotechnology and medicine, which are expected to change patient treatment profoundly, generating and regenerating tissues and organs instead of just repairing them. Whereas tissue engineering is a more technical concept of tissue and organ reconstruction by the use of cells, scaffolds, and biomolecules, the term regenerative medicine is more focused on the support of self healing capabilities and the use of stem cells. Stem cell therapy utilizing MSCs are the focus of a multitude of clinical studies currently underway.

Widely described, MSCs are an excellent candidate for cell therapy because (a) are easily accessible; (b) the isolation is straightforward and cells can expand to clinical scales in a relatively short period of time [116,117]; (c) can be biopreserved with minimal loss of potency [118,119]; and (d) human trials of MSCs thus far have shown no adverse reactions to allogeneic versus autologous MSC transplants. This last, was proved that culture-expanded MSCs did not have MHC class II cell surface markers, but rather only MHC class I and no co-stimulator molecules [120]. Thus, human MSCs could not be antigen-presenting cells and would be invisible to the host's immune system [121,122]. These observations were used to suggest that MSCs could be used as allogeneic cells therapeutically.

Transplantation of BMMSCs is carried out in two rather different settings, autologous and allogeneic. Autologous transplantations employ a patient's own bone marrow tissue and thus present no tissue incompatibility between the do-

nor and the host. Allogeneic transplantations occur between two individuals who are not genetically identical (with the rare exceptions of transplantations between identical twins, often referred to as syngeneic transplantations). Nonidentical individuals differ in their human leukocyte antigens (HLAs), proteins that are expressed by their white blood cells. The immune system uses these HLAs to distinguish between "self" and "nonself.". For successful transplantation, allogeneic grafts must match most, if not all, of the six to ten major HLA antigens between host and donor. Even if they do, however, enough differences remain in mostly uncharacterized minor antigens to enable immune cells from the donor and the host to recognize the other as "nonself".

Other proprieties of MSCs including their wide-ranging differentiation potential, their possibility of engraftment [123], their immunosuppressive effects [124] and their expansion through culture have led to increasing clinical interest in the use of MSCs in numerous pathologic situations.

The reason for which the MSCs can repair damaged tissue may be due to different mechanisms, for example, differentiation towards tissue-specific pathways, repair of the microenvironment with paracrine/juxtacrine effects of growth factors and cytokines produced by the cells [125] or extracellular matrix reorganization [126].

Bone marrow derived- mesenchymal stem cells (BMMSCs)

Transplantation of MSCs from BM is considered safe and has been widely tested in clinical trials of cardiovascular [127,128], neurological [129, 130], and immunological disease [131,132] with encouraging results. More recently, groups around the world have investigated MSCs transplantation for the treatment of myriad diseases based on a newfound appreciation for MSCs' pleiotropic functions that enhance endogenous repair and attenuate immunological dysfunction. Currently, there are 79 registered clinical trial sites for evaluating MSC therapy throughout the world (http://clinicaltrials.gov/).

Today there is a strong international interest in MSCs as a potential therapy, under this the United States has 28 registered trial sites, while the rest of the world accounts for more than half of the total number (19 in Europe, 16 in China, 5 in the Middle East, 4 in India, 3 in Canada, 2 each in Africa and Japan, and 1 in Australia). Within the past year, several of the pivotal lead trials either have undergone early termination or have failed to meet primary endpoints, but on the other hand, the list of reports indicating that MSCs contribute to tissue repair *in vivo* enlarges. There are examples of MSCs utilization in the repair of kidney [133], muscle [27] and lung [134]. The cells were also found to promote angiogenesis [135], and were used in chronic skin wound treatment [136].

Here we report the most important application with MSC from bone marrow.

Vessel and heart

Myocardial tissue had been considered incapable of regeneration. However, in 2001, Orlic *et al.* [137] showed that injections of bone marrow cells into infarcted mice hearts resulted in improved cardiac function. Two years later, Beltrami *et al.* [138] demonstrated that cardiac progenitor cells are able to differentiate to cardiomyocytes, endothelial, and smooth muscle cells. This group also demonstrated that injection of these cells into damaged hearts improved cardiac function through regeneration of the myocardium itself. When MSCs are exposed to the DNA demethylating agent (5-azacytidine), they express specific cardiac genes, adopt myotube morphology, produce intercalated disks, and have other functions associated with myocytes [7,139,140]. Once injected in an infarcted heart, they contributes to the processes of myocardial remodeling, reduction of infarct size, scar formation, vascular repair, angiogenesis, recruitment of other regenerative factors, and ultimately homing of stem cells, thus possibly facilitating myocyte regeneration. According to this experimental work, some clinical trials have been performed, mainly to treat heart damage in patients.

Some studies used freshly isolated autologous bone marrow-derived mononuclear cells, delivered to the myocardium anytime from less than 3-day post-infarct to late stages of congestive heart failure, and showed improvements in common indicators of cardiac function [141-144]. A recently completed phase I trial, using a single infusion of allogeneic BMMSCs in pa-

tients within 10 days of acute Myocardial Infarction (MI) corroborates these findings [145]. In the randomized trial, patients receiving MSC experienced a 4-fold decrease in arrhythmias and premature ventricular contractions (PVCs), and showed improved overall health compared to patients receiving placebo. After 1-year patients revealed a significant increase in left ventricular ejection fraction (LVEF). Importantly, there were no significant adverse events in this trials and so it is validated the safety of allogeneic MSCs. Despite this, these results should be considered with cautious optimism because the viability of MSCs post-treatment and the role of MSCs in the recovery of cardiac function remain to be elucidated.

Two group performed independently intracoronary short injection of autologous cultured bone marrow MSCs cells after acute myocardial infarction and for chronic ischemic cardiomyopathy [13,146,147]. In Chen study 65 of the 68 patients tolerated the injections of cells and three of them showed a transient episode of pulmonary edema, which was controlled by intravenous injection of diuretics. In these two randomized trials, the authors found improved cardiac function in patients receiving the cells. Another group in Greece [147] performed a similar study in 11 patients and found the procedure to be safe and possibly contributing to regional regeneration of myocardium. Numerous clinical trials are going to begin or will begin next years (see http://www.clinicaltrial.gov), and results may be available in the next 3 years.

Orthopedic applications and treatment of cartilage lesions

Because the MSCs were able to differentiate into osteoblasts, many researcher embarked on clinical efforts to cure gene defects by allogeneic transplantations, using normal cells that did not exhibit the genetic defect [148,149]. One current stem cell-based orthopedic therapy includes bone marrow-derived MSC transplantation for osteogenesis imperfecta (OI), a genetic disorder in which osteoblasts synthesize defective collagen type I, which leads to a variety of skeletal pathologies.

Researchers conducted innovative studies that leveraged the therapeutic potential of allogeneic MSC transplants to treat six children with a OI [8]. The therapeutic outcome was successful (1.5%-2% of engraftment), showing donor-derived MSCs located in the bone marrow of the recipient. Bone marrow MSCs were able to give rise to properly functioning osteoblasts, resulting in the increase in bone mineral content, as well as the improvement in growth velocity and the reduction of bone fracture frequencies . Encouraged by the results, the authors performed next trials [150]. Bone marrow was obtained from allogenic, HLA-compatible, sibling donors and was given twice to each patient. Among the five children enrolled in this study, three appeared chimeric and showed donor osteoblast engraftment. As a result, those children gained significant increase in total body length measured 6 months after transplantation, in comparison to 1.25 cm for control patients. Moreover, the bone mineral content improved by 45% to 77% of the baseline values. The number of fractures, visualised by radiography, declined from an average of 10 during 6 months before treatment, to 2. Unfortunately, the follow-up study demonstrated that the growth ratio either decreased or remained unchanged. In contrast, bone mineralization continued to increase.

Another great challenge for tissue engineering using MSCs is the treatment of cartilage lesions in orthopedic medicine there are also many examples of applications involving local delivery of marrow stem cells. These include spine fusion [151], the repair of segmental bone defects [152] and craniotomy defects [153]. Similar approaches have also been described for the repair of focal defects in articular cartilage and tendon [154].

Skin defects

BMMSCs have shown potential in improving the healing of skin defects in animal models [155-157] and in humans [158]. Autologous and allogeneic MSCs seem to be equally effective for wound repair [159]. *In vivo*, cultivated bone marrow and adipose-tissue MSCs have been effective in repairing the cornea after alkali burn in a rat model [13]. This repair could be due to transdifferentiation of MSCs into cornea cells [160,161] or paracrine effects and decreased inflammatory / immune reaction [161]. However, the experience with use of MSCs for wound healing is encouraging, although many questions remain about optimal culture conditions, dosage, route of application, combination

with scaffolds and type of MSCs.

Kidney disorders

Many kidney disorders involve both ischemic/inflammatory and immunologic injury. Therefore cell-based therapies such as those using MSCs which function through multiple mechanisms and have the potential to target the inflammatory and immunologic pathways have been considered a clinically relevant solution in contrast to pharmacologic agents that target only a single event or pathway in the pathophysiology of a given disease.

Several preclinical studies demonstrated that ex vivo expanded MSCs can ameliorate renal injury and accelerate repair. Effects have been demonstrated in models of acute ischemia and reperfusion, acute tubular epithelial injury and experimental glomerulonephritis [162,163]. MSCs can home to sites of injury, where they modulate the repair process. They may improve functional and structural recovery of both glomerular and tubular compartments. Morigi *et al.* [163] esplored human BMMSCs treatment could prevent acute kidney injury (AKI) induced by cisplatin and prolong survival in an immunodeficient mouse model. Results showed that human BMMSC infusion decreased proximal tubular epithelial cell injury and ameliorated the deficit in renal function, resulting in reduced recipient mortality. These findings indicate that human MSCs of bone marrow origin hold potential to prolong survival in AKI and should be considered for testing in a clinical trial.

Neuronal disorders

When used *in vivo* for neurodegenerative disorders, MSCs do not normally seem to pass through the liquoral barrier. However, MSCs can survive, migrate and differentiate into neural-glial cells after *in utero* intraventricular injection inside fetal rat brains [164]. Neurological recovery has been shown in animal models of Parkinson's disease, hypoxic-ischemic neural damage and retinal injury following *in vivo* transplantation of these cells inside the lesion [165].

A few researchers investigated cell-based treatment modalities for Huntington's disease (HD). Rossignol *et al.* [166] investigated the potential treatment of BMSCs in the 3-nitropropionic acid rat model of HD. They demonstrated that intrastriatal injection of BMMSCs improved motor dysfunction modestly, and those transplanted cells were still viable and metabolically active 71 days post-transplantation. They suggested that the observed recovery of function was attributed to the release of trophic factors from the MSCs because very few MSCs showed evidence of trans-differentiation.

Recently, Venkataramana *et al* [167] reported a first open label clinical pilot study with autologous bone-marrow-derived stem cells transplanted into the striatum of patients with advanced Parkinson's disease (PD). This disorder is a progressive neurodegenerative disease for which stem cell research has created hope in the last few years. Seven PD patients aged 22 to 62 years were enrolled to participate in the prospective, uncontrolled, pilot study of single-dose, unilateral transplantation of autologous BMMSCs. The BMMSCs were transplanted into the sublateral ventricular zone by stereotaxic surgery. Patients were followed up for a period that ranged from 10 to 36 months. These results indicate that this protocol seems to be safe, and no serious adverse events occurred after stem-cell transplantation in PD patients. The number of patients recruited and the uncontrolled nature of the trial did not permit demonstration of effectiveness of the treatment involved. However, the results encourage future trials with more patients to demonstrate efficacy [167,168]. Although this was trial carefully designed with a favourable outcome in respect to adverse effects, many questions remain and preclinical studies need to demonstrate whether such cells either can differentiate into exogenous functional neurons or provide trophic support for endogenous cells.

Human umbilical cord blood derived mesenchymal stem cells (hUCBMSCs)

Spinal cord and brain injury applications

hUCMSCs have an higher capability of differentiating into nerve like cells and a hold great promise as tools for understanding development and as therapeutic agents for brain injury and spinal cord injury. Transplantation of hUCMSCs into the injured spinal cord may have the following functions: compensation for demyelination; removal of inhibition; promotion of axonal regeneration; direction of axons to appropriate targets and replacement of lost cells. Weiss *et al.*

[65] has treated rat models affected by Parkinson's disease with hUCMSCs. The results demonstrated that the hUCMSCs produce significant amounts of glial cell line-derived neurotrophic factor (GDNF), one of the most potent trophic factors for dopaminergic neurons and fibroblast growth factor, the animals with transplanted cells showed a significant recovery in behaviour. Although the above data from hUCMSCs indicate that these cells may be therapeutically useful in treating CNS disorders, transplantation of hUCMSCs for treatment of spinal cord injury is just the beginning.

In ischemia studies, most data show that cell therapy is performed using hUCB. The first evidence of a therapeutic effect of hUCB came from a study where rat was used to induce focal ischemia. Intravenous administration of hUCB reduced behavioral deficits after stroke in rats [176]. In an other recently study the scientist examined the effects of hUCBMSCs in canine thromboembolic brain model [177]. Cerebral ischemia was induced through occlusion of the middle cerebral artery by injecting thrombus emboli into 10 beagles. hUCBMSCs were transplanted through the basilar artery 1 day after ischemic induction using an endovascular interventional approach. Infarct volume was reduced after intra arterial delivery of hUCBMSCs in canine cerebral ischemia whereas infarct volume was increased in the control groups. hUCBMSCs expressed neuroprotective factors, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), at 4 weeks after the transplantation. Jeong *et al.* [178] reported that transplantation of hUCBMSCs into contralateral regions of injured rat brain at 7 d after injury resulted in significant behavioral improvement. These results suggested that transplantation of hUCBMSCs showed their efficacy by reducing the infarction lesion volume and through earlier recovery from the neurological deficit. Therefore intra-arterial transplantation of hUCBMSCs could be useful in clinical treatment of cerebral ischemia.

UCB stem cells have shown promise in the treatment of Cerebral palsy (CP) in both animal models and early human trials. CP is a devastating brain disorder that affects many children worldwide, with 10 000 infants diagnosed annually, and stem cells ultimately have the capacity to generate new cells to replace those lost through injury or disease.

Recently, considerable excitement has been generated by anecdotal reports of improvement after umbilical CB stem cell infusions in children treated in a clinical study at Duke University. Although not a randomized trial, this treatment has been used to treat more than 50 children with cerebral palsy. Preliminary observations have been encouraging (see <http://www.msnbc.msn.com/id/> 23572206/), and many additional patients are being enrolled. It should be noted that not all children have benefited to the same extent, and it appears that the younger the patient the more significant the benefits that have been observed. However, the optimal therapeutic regime and the mechanism (s) behind any beneficial effects have yet to be determined.

Lung disease

Progression of acute respiratory distress syndrome is demonstrated by loss of lung tissue as a result of inflammation and fibrosis. Recently, the exogenous administration of BMMSCs significantly attenuated the bleomycin-induced lung injury by downmodulating the inflammatory responses and ameliorating their fibrotic effects [134]. However, it is not known if hUCBMSCs can differentiate into lung specific cell types *in vivo*, and whether these cells are suitable exogenous stem cell sources in lung injuries of experimental or clinical settings. A new study [179] examined whether intratracheal or intraperitoneal transplantation of hUCBMSCs can attenuate hyperoxia-induced lung injury in immunocompetent newborn rats. Wild-type rats were randomly exposed to 95% oxygen or air from birth. hUCBMSCs was administered either intratracheally or intraperitoneally at postnatal day (P) 5 and after 10 days the harvested lungs were examined. The transplantation of hUCBMSCs to the wild-type newborn rat pups significantly attenuated the hyperoxia-induced lung injury in the surviving animals. This protective effect was associated and probably mediated by the down-modulation of the pulmonary inflammatory and the ensuing fibrotic responses. These findings suggest that the administration of hUCBMSCs might be a possible candidate for the new therapeutic modality for the hyperoxia-induced neonatal lung diseases, such as clinical Bronchopulmonary dysplasia (BPD).

Kidney injury

The application of hUCBMSCs in treating acute renal failure (ARF) has not been reported in a lot of studies. However a recently issue [180] showed the transplantation of hUCMSCs via the left carotid artery into ARF rats. Serum creatinine and urea nitrogen decreased compared to control groups. In addition, the transplanted hUCBMSCs could reside in local injury sites, leading to the relief of hyperemia and inflammation, but no obvious trans-differentiation into renal-like cells. The results lay the foundation for further study on the potential application of hUCMSC in human disease.

For acute Kidney Injury (AKI) a recently study showed the potentiality of human hUCBMSCs to cure mice with this disease. Infusion of hUCBMSCs in immunodeficient mice with cisplatin-induced AKI ameliorated both renal function and tubular cell injury, and prolonged survival. Transplanted hUCBMSCs are able to reduce the apoptosis and increased the cell proliferation by the capacity of the stem cell to produce growth factors. Altogether these results highlight the potential of human hUCBMSCs as future cell therapy for testing in human AKI [181].

Juvenile diabetes

Approximately 15 000 youth in the US are newly diagnosed with Types 1 Diabetes (T1D) annually (www.diabetes.niddk.nih.gov/dm pubsstatistics/#youngpeople) and 5-10% of all adults living with diabetes display the T1D phenotype (<http://diabetes.niddk.nih.gov). At present, autologous hUCBMSCs are being evaluated in a clinical trial to treat T1D in children [182]. To date, 23 children have been treated, and the first child treated under the study protocol showed significant improvement in glucose versus control and was able to produce insulin much longer than children with a similar prognosis [183]. Most of the treated children have reported enhanced blood glucose control and management. In addition, it appeared that there was retention of endogenous insulin production as assessed by stimulated C-peptide secretion.

Autoimmune Applications

Such diseases, which can affect either specific organs or the entire system, include multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (SLE). Chang *et al.* [184] investigated whether hUCBMSCs transplantation is useful in alleviating lupus nephritis in a murine model. It was found that hUCBMSCs transplantation significantly delayed the development of proteinuria, decreased anti-dsDNA, alleviated renal injury, and prolonged the life span. Together, these findings indicated that human MSCs were effective in decreasing renal inflammation and alleviating experimental lupus nephritis by inhibiting lymphocytes, inducing the cytokines and inhibition of pro-inflammatory cytokines production rather than direct engraftment and differentiating into renal tissue. Therapeutic effects demonstrated in this preclinical study support further exploration of the possibility to use hUCBMSCs from mismatched donors in lupus nephritis treatment.

In recent times, a new study showed a single-arm trial that involve 16 SLE patients whose disease was refractory to standard treatment for who had life-threatening visceral involvement. Sun and colleagues [185] explored if hUCBMSCs transplant may improve symptoms and biochemical values in this patients with SLE. All 16 patients received umbilical mesenchymal stem cell transplants. 10 patients completed at least 6 months of follow-up and 2 patients were followed for more than 2 years. There was no treatment-related mortality or other adverse event during or after hUCMSCt.

In conclusion, this study shows very clearly that UC-MSCT exerts a profound therapeutic effect in severe and refractory SLE patients. All the patients achieved at least 3 months of clinical and serologic improvement, and for 2 patients this was achieved without any immunosuppressive drugs. This study shows for the first time that allogenic hCBMSCs transplanatation is safe and effective, at least short term, in treating patients with severe SLE. Further clinical trials with more patients included and longer periods of follow-up compared to standard treatment will be needed to determine the efficacy and safety of this novel approach to the treatment of lupus.

5. Conclusion

MSCs are multipotent, non-haematopoietic progenitor cells that are being explored as a promising new treatment for tissue regeneration. It seems well-founded that MSCs constitute a su-

perb potential tool in regenerative medicine approaches. They possess an extensive proliferative potential and are able to differentiate into various cell lineages. Due to these important features, the use of MSCs in clinical trials increases. It has been documented that these cells engraft successfully in patients and cause beneficial effects.

Developing new therapies that affect multiple disease pathways is of growing importance for patients care. MSCs transplantation represents an exciting approach that could potentially treat complex diseases by providing combinatorial therapy. Furthermore, the continued use of MSCs therapy can be recast to better our understanding of the natural role of these cells during health and disease *in vivo*.

After learning more about their properties, it will be possible to start new, more advanced and better treatment strategies for various diseases, even those, which seem to be incurable at present. Moreover, knowing that each patient is genetically different and may give different response to a treatment, and carry variable predisposition to different diseases, specifically targeted strategies using autologous MSCs, may be designed. However, it is still a long way to go before using these cells as a routinely applied therapy in clinics.

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HIGHER TELOMERASE ACTIVITIES IN MESENCHYMAL STEM CELLS FROM UMBILICAL CORD BLOOD THAN FROM BONE MARROW AFTER CULTURE EXPANSION

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Introduction Mesenchymal stem cells (MSCs) have unique capabilities of self-renewal and multilineage differentiation, therefore, they can be used to repair or regenerate damaged cells and tissues, particularly bone tissues. The enzyme telomerase serves to maintain telomere length and is thought to play an important role in the maintenance of self-renewal abilities of stem cells. The aim of this study is to investigate the telomerase activities of MSCs from different sources, including human bone marrow (BM) and umbilical cord blood (UCB) after culture expansion.

Materials and Methods MSCs were harvested from UCB and BM by negative immuno-selection and limiting dilution and surface immunophenotyping of clonally-expanded MSCs was carried out to confirm their characteristics as previously reported[1-3]. Multi-lineage differentiation potentials into osteoblasts, chondrocytes, and adipocytes were also confirmed. To measure the telomerase activity, total RNA was extracted form the MSCs using RNEasy (Qiagen) per the manufacturer's instructions, and cDNA was then amplified by RT-PCR. Telomerase repeat amplification protocol (TRAP)[4] was utilized for telomerase activity assay. For telomere restriction fragment (TRF) assay, the lengths of telomere of UCB-MSCs and BM-MSCs were also measured by Southern blot analysis and the telomeric DNA amount was calculated by integrating the volume of each smear using computer software (ImageQuant, Amersham Biosciences). For cell cycle analysis, MSCs were cultured and subjected to DNA staining by propidium iodime (PI) for 30 minutes. The percentage of cells for each different cycle phase was determined by flow cytometric analysis. Osteogenic differentiation abilities in UCB-MSCs and BM-MSCs after culture-expansion were also tested by induction with osteogenic medium containing with 0.1 uM dexamethasone, 10 mM β-glycerol phosphate, and 0.2 mM ascorbic acid under serum-free condition.

Results Growth kinetic analysis showed that doubling time of UCB-MSCs and BM-MSCs at 30th population doublings (30th PD) was 46 and 52 hours respectively (data not shown). Telomerase activity analysis by TRAP assay revealed that both UCB-MSCs and BM-MSCs exhibited telomerase activities after culture expansion to the 30th PD, and telomerase activity in UCB-MSCs is higher than in BM-MSCs (Figure 1A). Southern blot analysis also showed longer telomere length in UCB-MSCs at 30th PD (Figure 1B and Table 1). However, after further sub-cultivation, telomerase activities of both UCB-MSCs and BM-MSCs declined. Extensively sub-cultivated UCB-MSCs (65th PD) and BM-MSC (43th PD) demonstrated no telomerase activities (Fig. 1A).

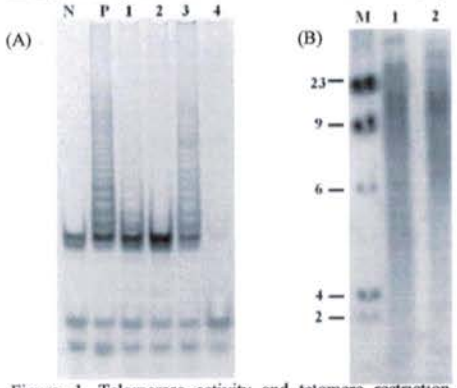

Figure. 1. Telomerase activity and telomere restriction tragment of MSCs derived from BM and UCB during expansion. (A) Telomerase activity of MSCs: N, negative control; P, positive control; lane1, BM-MSCs at 30th PD; lane2, BM-MSCs at 43th PD; lane3, UCB-MSCs at 30th PD; lane4, UCB-MSCs at 65th PD. Cell lysates of laryngeal carcinoma were used as a positive control. (B) Southern-blot analysis of telomere length: M, marker; lane1, BM-MSCs at 30th PD; lane2, UCB-MSCs at 30th PD.

Table. 1. Telomere length of BM-MSCs and UCB-MSCs quantified by ImageQuant.

	Telomere length (kb)
BM-MSCs at 30th PD	5.68
UCB-MSCs at 30th PD	5.90
BM-MSCs at 43th PD	5.12
UCB-MSCs at 65th PD	5.53

Cell cycle analysis showed that more UCB-MSCs remain in quiescent state (G0/G1) than BM-MSCs(97.03% vs. 92.85%) (Figure 2).

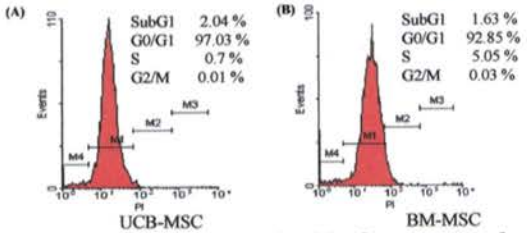

Figure. 2. Cell cycle was analyzed by flow cytometry after DNA staining with PI. (A) UCB-MSCs (B) BM-MSCs

Osteogenic differentiation assay demonstrated that both UCB-MSCs maintained osteogenic differentiation ability at 30th PD (data not shown). It is found that UCB-MSCs maintain telomerase activity after osteogenic induction. However, BM-MSCs lose telomerase activity express after 10 days of osteogenic induction (Figure 3).

Figure. 3. Telomerase activity of UCB-MSCs and BM-MSCs after osteogenic induction: N, negative control; P, positive control; lane1, BM-MSCs after 10 days of osteogenic induction; lane2, UCB-MSCs after 10 days of osteogenic induction

Discussion It is demonstrated in this study that MSCs from both UCB and BM possess telomerase activities after culture-expansion for 30 population doublings, and UCB-MSCs demonstrated greater telomerase activities and longer telomere length than BM-MSCs, as shown by TRAP assay and TRF measurement. Besides, greater telomerase activities in UCB-MSCs are associated with the shorter doubling time. Interestingly, it is found that a greater proportion of UCB-MSCs are in the quiescent (G0/G1) state than BM-MSCs. This may also be associated with greater telomerase activities of UCB-MSCs, which make the time required for cell division in each cell cycle shorter. Taken together, UCB-MSCs possess greater telomerase potential after culture-expansion after 30 population doublings while maintaining their osteogenic differentiation abilities. UCB-MSCs may therefore be excellent candidates for cell therapy and tissue engineering applications of bone reconstruction.

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