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EXPERT OPINION

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Bone and cartilage regeneration with the use of umbilical cord mesenchymal stem cells

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Introduction: The production of functional alternatives to bone autografts and the development new treatment strategies for cartilage defects are great challenges that could be addressed by the field of tissue engineering. Umbilical cord mesenchymal stem cells (MSCs) can be used to produce cost-effective, atraumatic and possibly autologous bone and cartilage grafts.
Areas covered: MSCs can be isolated from umbilical cord Wharton's jelly, perivascular tissue and blood using various techniques. Those cells have been characterized and phenotypic similarities with bone marrow-derived MSCs (BM-MSCs) and embryonic stem cells have been found. Findings on their differentiation into the osteogenic and chondrogenic lineage differ between studies and are not as consistent as for BM-MSCs.
Expert opinion: MSCs from umbilical cords have to be more extensively studied and the mechanisms underlying their differentiation have to be clarified. To date, they seem to be an attractive alternative to BM-MSCs. However, further research with suitable scaffolds and growth factors as well as with novel scaffold fabrication and culture technology should be conducted before they are introduced to clinical practice and replace BM-MSCs.

Keywords: bone, cartilage, mesenchymal, stem cells, tissue engineering, umbilical cord, Wharton's jelly

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1. Introduction

The increasing demand for bone grafts over the past decades necessitates the development of cheap, atraumatic and safe bone grafting strategies [1]. Moreover, the high prevalence of osteoarthritis and rheumatic joint disorders dictate the need for a novel, effective treatment of cartilage defects [2]. Solutions to the aforementioned problems could be given by a multidisciplinary field such as tissue engineering, which attempts to combine cells with scaffolds and growth factors in order to create artificial tissues.

Stem cells have been extensively used for tissue engineering purposes, with bone-marrow derived mesenchymal stem cells (BM-MSCs) being nowadays the gold standard of bone and cartilage tissue engineering research [3]. However, the use of BM-MSCs has several limitations such as donor-site morbidity, low yield of stem cells from a single aspiration, difficult culture expansion, the possibility of donor-site infection and a differentiation potential inversely proportional to their age [4-7]. Those limitations could be possibly overcome with the use of another type of mesenchymal stem cell (MSC) from human umbilical cord. Isolated from either umbilical cord tissue or umbilical cord blood (UCB), those MSCs are multi-potent cells with a proven high proliferation capacity which can be atraumatically obtained from discarded umbilical cords or UCB banks [8,9].

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Article highlights.

- Umbilical cord-derived mesenchymal stem cells (MSCs) are promising candidates for bone and cartilage tissue engineering.
- MSCs can be isolated from various umbilical cord parts such as Wharton's jelly, perivascular tissue and umbilical cord blood.
- Umbilical cord-derived MSCs share similar phenotypic characteristics with bone marrow-derived MSCs.
- Conflicting results exist on the suitability of umbilical cord-derived stem cells for bone regeneration purposes.
- Cartilage regeneration is feasible with umbilical cord-derived MSCs, although the creation of fibrocartilage seems more profound.

This box summarizes key points contained in the article.

MSCs can be isolated from various umbilical cord compartments as the epithelium, Wharton's jelly (WJ), perivascular tissue (PVT), vascular endothelium and the endovascular compartment that is UCB [8,10-12]. The most commonly used are MSCs derived from WJ, PVT and UCB. The purpose of this article is to review the current data on the isolation and phenotypic characterization of the most commonly used cord-derived MSCs, as well as their ability to differentiate toward the osteogenic and chondrogenic lineage.

2. Methods

An extensive literature search was performed in an attempt to review the current data on MSCs derived from various parts of the umbilical cord and their ability to create bone and cartilage. For the purposes of our review two search engines were used: SCOPUS (1960 – November 2014) and MEDLINE (1950 – November 2014). Various keywords such as 'mesenchymal', 'stem cells', 'MSC' 'umbilical', 'cord', 'blood', 'UCB', 'osteogenesis', 'osteogenic', 'bone', 'chondrogenesis', 'chondrogenic', 'cartilage', 'tissue', 'isolation', 'phenotype', 'characterization', 'engineering', 'cord blood', 'WJ', 'perivascular', 'CD146 ($^+$)','pericytes' were used with all possible combinations. In order to select only relevant articles, all abstracts were first checked and irrelevant studies were excluded from the present review. Namely, we have focused on literature regarding human MSCs from WJ, PVT and UCB. Thus, studies utilizing nonhuman MSCs or umbilical cord epithelial cells and umbilical vessel endothelial cells were considered to be out of the spectrum of our review.

3. Isolation of MSCs from various umbilical cord departments

3.1 Isolation of WJ-MSCs

Isolation of WJ-MSCs has been achieved by enzymatic [13,14] and nonenzymatic [15] means [16]. During enzymatic procedures, cords are dissected, vessels detached, WJ removed and diced/minced into pieces. Those pieces are then left to degrade enzymatically with the use of collagenase and/or hyaluronidase, resulting in a suspension of cells which are then cultured as MSCs from other sources (**Figure 1Aii**). In order to avoid cell strain, isolation can also be achieved with non-enzymatic methods, placing minced or whole [17] WJ on Petri dishes and allowing MSCs to migrate and adhere to the plates (**Figure 1Ai**). Indeed, Iftimia-Mander *et al.* have shown that the enzymatic method can achieve a higher cell yield, but its non-enzymatic alternative achieves less variability in the number of cells isolated from different cords. Moreover, the same group proved that cryopreservation lowers the cell yield only when enzymatic methods are employed [18]. Those results indicate that the selection of method requires balancing between the risk of variability and the risk of low cell yield.

3.2 Isolation of PVT-MSCs

For the isolation of PVT-MSCs, cords are dissected and the epithelial layer is removed, exposing the WJ that contains the blood vessels. Then, vessels are gently detached one by one from the gel. The two ends of each vessel are sutured together and the resulting loop is placed into a PBS-proteolytic enzyme solution for a maximum of 24 h. Subsequently, vessels are removed from the suspension and the solution is centrifuged to receive a cell pellet of PVT-MSCs [16,19], which are then plated (**Figure 1C**).

3.3 Isolation of UCB-MSCs

Ficoll gradient centrifugation is used for the isolation of UCB mononuclears from whole UCB samples diluted 1:1 up to 1:5 with PBS. The mononuclear layer is subsequently obtained and cells are pelleted, washed repeatedly with PBS and left to adhere overnight in flasks with culture medium. Finally, the supernatant with non-adherent cells is removed and the adherent ones are trypsinized or scraped and plated in flasks to proliferate (**Figure 1B**) [20-23].

However, cell yield from UCB is extremely low [24] and the isolation of MSCs is not guaranteed as with WJ specimens. Indeed, it has been shown that the number of cells obtained from a UCB sample is dependent on a variety of factors, such as the amount of cord blood obtained, the duration of pregnancy and the delay of umbilical cord clamping [25,26]. In a study where 10 umbilical cords and their respective blood samples were analyzed for the presence of MSCs, only 1 out of 10 samples was found to contain MSCs compared to 100% of umbilical cord WJ samples [24]. Moreover, it has been proposed that in terms of proliferation, time to reach confluency and cell viability, WJ-MSCs are also superior to UCB-MSCs [27,28]. In an attempt to give a solution to the low cell yield problem, Zhang *et al.* managed to isolate cells from 90% of UCB samples by simply aspirating > 90 ml of blood and isolating cells within 2 h from delivery [29].

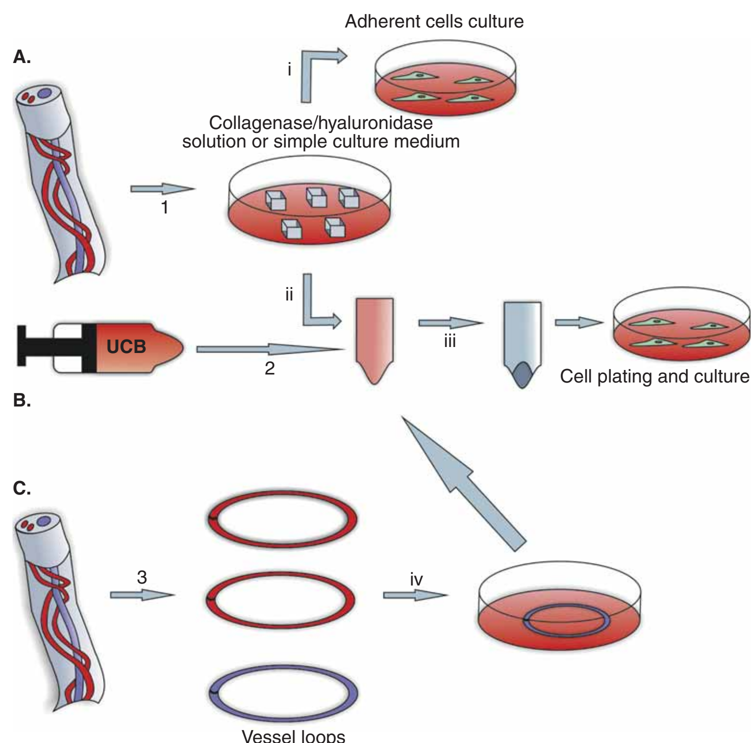

Figure 1. Illustrations showing the methods used for the isolation of umbilical cord-derived mesenchymal stem cells **(A)** Isolation of Wharton's jelly mesenchymal stem cells. Umbilical cords are dissected, blood vessels are removed and the remaining jelly is diced or minced (1) and left to degrade either nonenzymatically (i) or inside a collagenase/hyaluronidase solution (ii). After the enzymatic degradation the cell suspension is obtained cells are pelleted (iii) and washed and subsequently plated. **(B)** Isolation of UCB mesenchymal stem cells. After Ficoll density-gradient centrifugation (2) mononuclear layer is aspirated, cells are pelleted washed and finally plated. **(C)** Perivascular mesenchymal stem cell isolation. Cords are dissected and vessels are removed and tied end-to-end to create loops (3). Loops are then treated enzymatically in plates (iv) as done for Wharton's jelly.
UCB: Umbilical cord blood.

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4. Phenotypic characterization of umbilical cord MSCs

Although cell markers such as CD29, CD44 and STRO-1 have been identified to be present on MSC populations [30], according to the International Society for Cellular Therapy [31], they should at least fulfill the following criteria:

- When plated in plastic non-coated flasks they should adhere to them.
- No > 2% of them should express hematopoietic cell markers.
- At least 95% of the population must express CD73, CD90 and CD105.
- They must at least be able to *in vitro* commit to the osteogenic, the chondrogenic and the adipogenic lineage.

Several studies have proved that stem cells isolated from different umbilical cord parts bear a phenotype similar to other MSCs [20,32,33]. First, they have immunosuppressive properties, as proved by the expression of molecules such as human leukocyte antigen G [34], indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 [35]. Their immune avoidance properties are also enhanced by the low levels of MHC class I molecules and the absence of MHC class II and costimulatory antigen (CD80, CD86) expression [8]. In addition, they express embryonic markers such as Oct-4, Nanog, Sox-2 and ITSN1 [34-36]. Moreover, PVT cells characteristically express the endothelial cell surface molecule CD146 [12]. The previously mentioned characteristics not only confirm the mesenchymal origin of those stem cells but also render them attractive in terms of survival within the hostile inflammatory environment of a fracture or a cartilage defect.

Finally, although cells of mesenchymal origin were found in UCB in early 2000s [37,38], scientists questioned their existence for years [39,40]. However, we are now certain that UCB contains MSCs [20] with the same phenotypic characteristics as WJ-MSCs, which are preserved even after cryopreservation and thawing of cord blood for banking purposes [21].

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5. Bone tissue regeneration

Umbilical cord MSCs are multipotent cells, which should at least be able to differentiate into the osteogenic, chondrogenic and adipogenic lineages. The possibility to create bone with the use of umbilical stem cells, would offer a very attractive alternative to allografts. This is extremely important because although allografts are currently used for a variety of orthopedic procedures, they are expensive and bear risks of viral infection and immunorejection associated with poor biological and mechanical properties.

The osteogenic capacity of WJ-MSCs has been evaluated in several studies with conflicting results (**Table 1**). Indeed, Ciavarella *et al.* proved that WJ-MSCs maintain their cell surface markers after several passages, are able to express genes related to osteogenesis and share common differentiation mechanisms with BM-MSCs [41]. In addition Hou *et al.* showed that one of those common features is the involvement of the BMP-2 pathway in osteogenesis and concluded that UC-MSCs are also suitable for use in tissue engineering [42]. Another *in vitro* study by Schneider *et al.* compared the osteogenic capacity of BM-MSCs and UC-MSCs both in two-dimensional (2D) cultures and three-dimensional (3D) collagen I/III scaffolds. They found that although WJ-MSCs produced higher amounts of extracellular matrix (ECM) proteins, BM-MSCs expressed higher levels of alkaline phosphatase (ALP) and osteopontin. However, constructs of both cell types were mineralized and the authors conclude that due to their increased biosynthetic activity UC-MSCs deserve to be used in bone tissue engineering [43]. Favorable osteogenic results have been also demonstrated by Penolazzi *et al.*, who proved excellent cell viability, expression of osteogenic markers and secretion of cytokines with chemotactic properties when they encapsulated WJ-MSCs in alginate hydrogels [44]. A study published 2 years later by the same group has demonstrated the successful induction of osteogenesis in WJ-MSCs seeded in porcine urinary bladder matrix [45]. Finally, their ability to produce osteoblasts has been also highlighted in serum free cultures [46], with *in situ* mineralized collagen scaffolds [47], with scaffolds of umbilical cord-derived fibrin [48] and with polycaprolactone (PCL)/collagen/hydroxyapatite scaffolds [49].

On the other hand, other authors have questioned the suitability of WJ-MSCs for bone tissue engineering. In fact, a genetic network analysis has led to the conclusion that although WJ-MSCs are similar to embryonic stem cells (ESCs) in terms of proliferation and more naive than BM-MSCs, they have low osteogenic capacity. Thus, they concluded that BM-MSCs are more suitable as a cell source for bone tissue engineering purposes [50]. The study of Sudo *et al.* proved that WJ-MSCs are not cable of differentiating into osteoprogenitor cells [51] and Capelli *et al.* showed that even though they expressed the osteoprotegerin gene, minimal calcium deposition and ALP activity was present compared to BM-MSCs [52]. Moreover, in their attempt to create osteochondral constructs with the use of non-woven poly-L-lactic acid scaffolds and WJ-MSCs, Wang *et al.* reported less ECM deposition in the bone side of their graft, as indicated by lowest glycosaminoglycan (GAG) deposition and hydroxyproline contents [53]. Finally, Kuo *et al.* also identified differentiation discrepancies between BM-MSCs and WJ-MSCs. By means of proteomic differential displays, they attributed those differences to the increased expression of the chaperone heat shock protein (HSP)90β and managed to enhance the osteogenic differentiation of WJ-MSCs with the use of an HSP90β knockdown model [54].

The major problem with all the previously mentioned studies is that they usually try to differentiate cells isolated from all around the area of the umbilical cord without attempting to separate WJ from PVT-MSC populations. This may seem of minor importance because it has long been thought that PVT-MSCs share the same properties with WJ-MSCs [55]. However, CD146 ($^+$) cells exist only around umbilical blood vessels and have at least the same or even higher osteogenic differentiation capacity than BM-MSCs. This conclusion is supported by evidence suggesting common osteogenic pathways with BM-MSCs (e.g., WNT signaling), as well as higher ALP activity and better mineralization of PVT-MSC constructs [12]. It has also interestingly been shown that a subpopulation of PVT-MSCs can spontaneously start differentiating toward the osteogenic lineage, even from their first passage and without needing to be exposed to osteogenic medium [19]. The only results against the increased osteogenic potential of PVT-MSCs are those of Girdlestone *et al.* who found no differences between the differentiation potential of WJ-MSCs and PVT-MSCs. In fact, they reported that UCB-MSCs and BM-MSCs have a higher ability to produce bone, when compared to their umbilical tissue counterparts [56].

Osteogenesis with the use of UCB-MSCs seems to be more efficient (**Table 1**). First, it has been reported that they have an osteogenic potential even equal to that of BM-MSCs [57,58] as proved by measurements of ALP activity, activation of osteogenic genes, Alizarin Red [59] and von Kossa stains, as well as by the expression of ECM proteins [58]. Furthermore, a study compared cord blood mononuclears with ESCs in regard to their ability to produce ectopic bone following intramuscular implantation of differentiated stem cell-collagen constructs. They found that UCB mononuclears were superior to ESCs, from the first post-implantation month, in terms of mineralization [60]. Moreover, it has been shown that osteogenesis in UCB-MSCs is feasible and is maximized when MSCGM (a commercial growth medium containing DMEM and FCS) conditioned with 10% SingleQuot and dexamethasone in a concentration of 10^{-7} M is utilized for the differentiation [61]. Finally, the study of Bosch *et al.* comes to add to the previously mentioned favorable results and at the same time to highlight the inferior osteogenic potential of WJ-MSCs. Indeed, authors reported that UCB-MSCs have an equal osteogenic potential to BM-MSCs, whereas WJ-MSCs are not

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Table 1. Major studies on the osteogenic differentiation of umbilical cord-derived mesenchymal stem cells.

Study (year)	Method	Results	Conclusion	Ref.
Hou *et al.* (2009)	Studied the osteogenic differentiation of WJMSCs using BMMSCs as controls. They assessed osteogenesis and expression of proteins of BMP-2 pathway after 14 days of treatment with BMP-2	Expression of osteogenic genes, positive mineralization stains, expression of BMP-2 pathway proteins	Osteogenesis in WJMSCs is BMP-2 mediated as in BMMSCs	[42]
Baba *et al.* (2013)	Cultured WJMSCs with UCB auto-serum in UC fibrin matrix, differentiated them with classic osteogenic medium and implanted them subcutaneously in mice for 6 weeks	Areas of calcification in the constructs, surface capillaries, expression of osteogenic markers	Moderate osteogenic results, bone can be created with the use of UC stem cells and UC fibrin	[48]
Baksh *et al.* (2007)	Compared PVTMSCs with BMMSCs in terms of proliferation, transfection efficiency, osteogenesis and WNT pathway activation. Vitamin D3 was used for osteogenesis	Expression of CD146 on PVTMSCs, WNT-related gene expression, differentiation into mesenchymal lineages, higher ALP activity and better mineralization than BMMSCs, comparable chondrogenesis	PVTMSCs have an MSC-like phenotype and can be used instead of BMMSCs for cell-based therapies	[12]
Gauthaman *et al.* (2011)	Compared the proliferation, differentiation, mineralization, attachment of WJMSCs when seeded into 4 different scaffolds: PCL, PCL/Coll, PCL/HA, PCL/Coll/HA	All scaffolds were mineralized at 21 days but WJMSCs seeded on PCL/Coll/HA scaffolds displayed better results	WJMSCs on PCL/Coll/HA scaffolds can efficiently differentiate into the osteogenic lineage	[49]
Karadas *et al.* (2014)	Seeded WJMSCs and menstrual blood stem cells on pre-mineralized collagen scaffolds and studied their osteogenic potential	Foam porosity was 70% and pore size 50 – 200 μm. Mineralized collagen was superior to either culture plates or pure collagen scaffolds in terms of MSC ALP activity. WJ were slightly superior in osteogenesis than menstrual blood MSCs	Collagen foams with CaP are superior to culture plate surfaces for the osteogenic differentiation of both types of cells. Human MSCs can be used for *in vivo* bone regeneration	[47]
Schneider *et al.* (2010)	Compared bone marrow with umbilical MSCs on 2D cultures and 3D Coll I/III scaffolds	ALP and OPN were expressed more in BMMSCs and Coll I, IV and laminin were expressed more by umbilical MSCs. Production of MMPs lead to cell migration, colonization of Coll matrix, as well as construct contraction and strengthening	Umbilical MSCs were better in ECM synthesis in 3D constructs, whereas BMMSCs exhibited more pronounced osteogenesis in 2D cultures. Umbilical MSCs have the potential to be used for bone tissue engineering purposes	[43]
Penolazzi *et al.* (2010)	Developed a microencapsulation method for WJMSCs in alginate with a vibrating nozzle	Cell alginate constructs had a smooth shape and did not alter cell viability and protein secretion profiles compared to free cells. Moreover cells were able to undergo osteogenic differentiation despite encapsulation	WJMSCs can be encapsulated for tissue engineering purposes without affecting their viability and protein secretion	[44]
Penolazzi *et al.* (2012)	WJMSCs were seeded on porcine urinary bladder matrix scaffolds	Cells adhered to scaffolds. Scaffolds did not affect cell viability and osteogenic gene expression was noted following differentiation	WJMSC can undergo osteogenesis when seeded on porcine urinary bladder matrix scaffolds	[45]
Hsieh *et al.* (2010)	Genetic network analysis of BMMSCs and WJMSCs. Gives gene expression profiles of the two cell types	WJMSCs proliferated faster and expressed angiogenic genes. BMMSCs express genes related to the commitment to the osteogenic lineage	BMMSCs are more suitable for osteogenesis. WJMSCs have embryonic properties	[50]

Unless stated otherwise, osteogenesis was induced with classic osteogenic medium containing dexamethasone, β-glycerophosphate and ascorbic acid, for 21 days.

BMMSC: Bone marrow-derived mesenchymal stem cells; Coll: Collagen; HA: Hydroxyapatite; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells; PCL: Polycaprolactone; PVTMSC: Perivascular tissue-derived mesenchymal stem cells; UC: Umbilical cord; UCB: Umbilical cord blood; UCBMSC: Umbilical cord blood-dervied mesenchymal stem cells; WJMSC: Wharton's jelly-derived mesenchymal stem cell.

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Table 1. Major studies on the osteogenic differentiation of umbilical cord-derived mesenchymal stem cells (continued).

Study (year)	Method	Results	Conclusion	Ref.
Ciavarella *et al.* (2009)	Assessed the expression of Runx2, SOX9 PPARγ and TAZ during WJMSC differentiation	Osteogenic genes are expressed during WJMSC osteogenesis. TAZ expression is also important for WJMSCs osteogenesis	WJMSCs have a similar osteogenic potential to BMMSCs and thus could possibly serve as an alternative cell source for bone tissue engineering	[41]
Wang *et al.* (2011)	Attempted to create osteochondral grafts by suturing together a chondrogenic and an osteogenic layer of cell seeded poly-L-lactic acid scaffolds and placing an MSC layer in between (sandwich approach). Differentiation process lasted 6 weeks and suturing took place after the first 3 weeks	Osteogenesis and chondrogenesis started before suturing and continued after it. Good chondrogenic results were noted but apart from Runx2 which was activated earlier, other osteogenic markers were not present till week 6. The transition interface between bone and cartilage was better in constructs created with the sandwich approach	Suturing technique is an efficient way of creating osteochondral grafts and UCMSCs can be used for this purpose	[53]
Girdlestone *et al.* (2009)	PVTMSCs and WJMSCs were cultured in low serum medium and their multipotent differentiation potential was assessed	MSCs cultures in low serum conditions were slightly different in shape but had the same proliferation capacity and immunosuppressive properties but poor osteogenic potential	UCMSCs can be used for further tissue engineering research	[56]
Rebellato *et al.* (2008)	Adipose-derived, MSCs, BMMSCs and UCBMSCs were compared regarding their isolation and differentiation efficiency	Isolation of MSCs from cord blood was successful only in 30% of samples. Osteogenic and chondrogenic potential of all cell types was comparable. Cord blood MSCs had a lower adipogenic potential	Further research is needed on MSC biology to justify their use in clinical practice	[57]
Wagner *et al.* (2005)	Gene expression of adipose-derived MSCs, BMMSCs and cord blood MSCs was analyzed with the use of microarrays and compared to fibroblasts	25 genes were upregulated in all cell types and could possibly be used as a molecular MSC signature	Their findings could be possibly used as a genetic MSC definition	[58]
Khorshied *et al.* (2012)	Cultured UCBMSCs and HSCs and attempted to differentiate both into osteoprogenitor cells	Not only MSCs but also HSCs were differentiated into osteoblasts when treated with common osteogenic medium	HSCs can also be used for tissue engineering purposes	[59]
Handschel *et al.* (2010)	Seeded UCBMSCs and ESCs in insoluble collagenous bone matrix scaffolds and implanted intramuscularly in immunocompromised rats. CT scans were performed at the end of each month for 3 months and specimens were histologically examined	Ten times higher mineralization was seen in UCBMSC than in ESC constructs	UCBMSCs are better than ESCs in inducing *in vivo* ectopic bone formation when combined with insoluble collagenous bone matrix scaffolds	[60]
Hildebrandt *et al.* (2009)	Investigated the effect of three differentiation media and dexamethasone in 10^{-7} and 10^{-8} M concentrations on UCBMSC osteogenic differentiation	Concentrations of 10^{-8} M of dexamethasone in contrast to the 10^{-7} M equivalent promoted differentiation only when combined with BMP-2. MSCGM medium with 10% SingleQuot was better at supporting differentiation	UCBMSCs differentiate more effectively into the osteogenic lineage when cultured with MSCGM with 10% SingleQuot and 10^{-7} M dexamethasone	[61]
Bosch *et al.* (2013)	Gene expression of UCBMSCs was compared to that of BMMSCs by means of microarray and qRT-PCR analysis	BMMSC expression of osteogenic genes was higher and UCBMSCs did not express BMP-4 and BSP genes. Moreover, UCBMSC displayed more primitive gene expression profiles	UCBMSCs do not have the potential for skeletal lineage differentiation and that should be taken into account when used for tissue engineering purposes	[63]

Unless stated otherwise, osteogenesis was induced with classic osteogenic medium containing dexamethasone, β-glycerophosphate and ascorbic acid, for 21 days.

BMMSC: Bone marrow-derived mesenchymal stem cells; Coll: Collagen; HA: Hydroxyapatite; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells; PCL: Polycaprolactone; PVTMSC: Perivascular tissue-derived mesenchymal stem cells; UC: Umbilical cord; UCB: Umbilical cord blood; UCBMSC: Umbilical cord blood-dervied mesenchymal stem cells; WJMSC: Wharton's jelly-derived mesenchymal stem cell.

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suitable for bone regeneration purposes [62]. Nonetheless, the same group has published results which state that BM-MSCs have a higher expression of osteogenic genes and are thus more suitable than UCB-MSCs for use in bone tissue engineering [63].

6. Cartilage tissue regeneration

Human hyaline cartilage is the avascular tissue that covers articular joint surfaces and consists of chondrocytes inside an ECM of proteoglycans and type II collagen. Articular cartilage has a low regeneration capacity following degeneration or trauma, and cartilage defects would not heal when left untreated. Treatment of those defects is extremely important, because progression will probably lead to osteoarthritis. Current treatment options include autologous chondrocyte implantation (ACI), osteochondral autograft and allograft transplantation. Moreover, microfractures can be caused arthroscopically to the subchondral bone, triggering the production of fibrous repair tissue to cover the defect. This technique can be also combined with the application of collagen membranes to cover the underlying bone [64]. However, the possibility of using stem cells for the treatment of such lesions is very promising, as it may potentiate the production of real hyaline cartilage without the donor site morbidity of ACI or the infection and immunorejection risks of allograft transplantation [65].

The use of UC-MSCs for cartilage regeneration is an active and promising research field with generally better results than the field of bone regeneration (**Table 2**). The capacity of WJ-MSCs to differentiate into chondroprogenitors has been examined both *in vitro* and *in vivo*. Most of the studies conclude that WJ-MSCs are able to differentiate into the chondrogenic lineage [66,67] and preserve their immunosuppressive phenotype even after differentiation [35,68]. Moreover, evidence suggests that they have superior chondrogenic properties when compared to BM-MSCs. Namely, when both were seeded on PGA scaffolds, total collagen production was higher for UC-MSCs despite the more pronounced BM-MSC collagen gene expression [69]. When also tested on PCL/collagen scaffolds and treated with insulin-transferrin-selenium and basic fibroblast growth factor, they differentiated more efficiently than BM-MSCs [70]. In addition, on collagen type I hydrogels they expressed large amounts of type II collagen and cartilage oligomeric matrix protein and upregulated the expression of SOX-9 [67]. Nevertheless, this was not the case when compared to adipose-derived mesenchymal stem cells (AMSCs) in the presence of BMP-6, where the production of GAGs and expression of chondrogenic genes was more profound in AMSC samples. It should be stressed though that BMP-6 is known to be a potent chondrogenic growth factor for AMSCs, which could probably not mediate WJ-MSC chondrogenesis [71].

Despite the previously mentioned results, it has to be stressed that WJ-MSCs seem to produce fibrous cartilage instead of hyaline cartilage. This has been demonstrated with WJ-MSCs seeded on PGA scaffolds [69,72] and in a comparison with temporomandibular chondrocytes, where WJ-MSCs produced fair amounts of type I and only minute amounts of type II collagen [73].

The use of UCB-MSCs for the production of cartilage has also been evaluated with more robust results regarding the production of hyaline cartilage. They phenotypically resemble BM-MSCs and are able to form lacunae-like structures and produce type II collagen [74], which is further increased when stimulated with BMP-6 [75]. Moreover, when seeded on 4% hyaluronic acid hydrogels and implanted into femoral defects they managed to cover them with tissue containing type II collagen, which was macroscopically similar to native cartilage [76]. They were also found equivalent to amniotic fluid and neonatal BM-MSCs in terms of cartilage ECM production on PGA scaffolds [77]. Their ability to produce hyaline-like cartilage was also demonstrated in co-cultures with chondrocytes and the use of IGF-1 [78] and in co-cultures with ESCs and the use of BMP-4 [79].

Only one study, to the best of our knowledge, has reported the production of fibrocartilage from UCB-MSCs. In this *in vivo* study, authors assessed the suitability of fibroblasts, chondrocytes, BM-MSCs and human UCB-MSCs on PLA scaffolds, to repair damaged cartilage of New Zealand rabbit models. Investigators checked the repair progress at 6 and 12 weeks after implantation and found that UCB-MSC constructs had almost completely covered the defect at the end of the experiment. However, at 6 weeks, no type II collagen had been produced and at 12 weeks only 40% of specimens had detectable amounts of type II collagen whereas the rest consisted of fibrous cartilage [80].

7. Expert opinion

Tissue engineering is a rapidly advancing field and papers published on the production of artificial bone and cartilage with the use of stem cells increase year after year. However, less progress has been made in the field of umbilical cord-derived stem cells. Their high proliferation capacity and immunomodulatory properties, together with the possibility of autologous use, make them promising candidates for future clinical applications on bone and cartilage tissue engineering. The main problem with the use of UC-MSCs is the debate on their ability to repair bone and cartilage. Many authors have proved that they are suitable for bone and cartilage regeneration [41-49,66,67,69,70], whereas others state that they are unable to either differentiate into osteoprogenitors and chondroprogenitors or produce satisfactory ECM [50-54,69,72,73].

Those important discrepancies should be addressed before progressing to clinical studies and are probably caused by specific flaws in research methodology. Namely, many studies do not discriminate between UC-MSCs and PVT-MSCs. Moreover, it has been reported that osteogenic and chondrogenic gene activation in umbilical cells starts later in the

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Table 2. Major studies on the chondrogenic differentiation of umbilical cord-derived mesenchymal stem cells.

Study (year)	Method	Results	Conclusion	Ref.
Liu *et al.* (2009)	Attempted to differentiate WJMSCs into chondro, adipo, and Schwann cell progenitors. One week after chondrogenic induction, cells were transferred to a rotatory bioreactor for 14 days	Cells expressed chondrogenic genes such as SOX9 and Col2A1. Type II collagen and GAGs were produced. After bioreactor cultures cartilage ECM production was profound and structures resembling chondrocytes in lacunae were noticed	WJMSCs could be used for cartilage tissue engineering following extensive *in vivo* studies	[65]
Chen *et al.* (2013)	WJMSCs were seeded in type I collagen hydrogels and chondrogenic/osteogenic differentiation was attempted	Significant amounts of type II collagen and cartilage oligomeric matrix protein were produced. Chondrogenic gene expression steadily increased reaching a zenith in day 21	WJMSCs in collagen hydrogels could be possibly used for cartilage tissue engineering	[66]
Wang *et al.* (2009)	A 6-week study where WJMSCs were compared with BMMSCs as per their chondrogenic differentiation when seeded on PGA scaffolds. Chondrogenesis was checked every 3 weeks (3 time points)	WJMSCs displayed higher proliferation and seeding efficiency and produced more collagen although their collagen gene expression was lower. However they mainly created type I collagen compared to their counterparts that produced more type II collagen	WJMSCs are suitable for fibrocartilage engineering. In-depth study of chondrogenic signals in them could possibly lead to efficient hyaline cartilage engineering	[68]
Fong *et al.* (2012)	Compared WJMSC and BMMSC chondrogenesis on PCL/collagen nanoscaffolds with the use of three differentiation media	Two-stage differentiation (complex medium – chondrogenic medium) culture environment enhanced chondrogenic differentiation of WJMSCs	WJMSCs can be used for chondrogenesis when seeded with PCL/collagen scaffolds and cultured with the two-stage process	[69]
Hildner *et al.* (2010)	Compared chondrogenesis of WJMSCs with that of AMSCs when culture in 3D micromass pellets and differentiated for 35 days	WJMSCs proliferated faster whereas GAG production was much higher in AMSCs. However BMP-6 was known to favor AMSC chondrogenesis	Both cell types can be differentiated into chondroprogenitor cells but AMSCs were more effective in ECM production	[70]
Wang *et al.* (2009)	Attempted to produce fibrocartilage with human WJMSCs on PGA scaffolds and to test the influence of three different scaffold seeding densities on 4-week differentiation cycles	Type I and type II collagen was produced and authors recommend an initial seeding density of 25×10^6 cells/ml. Compression testing yielded best results with higher cell densities	Human WJMSCs are suitable for generation of fibrocartilage and that could optimally be achieved in a specific initial cell seeding density	[71]
Bailey *et al.* (2007)	Compared WJMSCs with temporomandibular chondrocytes in PGA scaffolds and 1 month static cultures	WJMSC constructs produced higher amounts of collagen I, more GAGs and had a 55% higher cellularity than chondrocyte constructs	Human WJMSCs may have the potential to be used in cartilage engineering although they mainly produce type I collagen	[72]
de Mara *et al.* (2013)	Stimulated UCBMSCs with BMP-2 and BMP-6 and assessed the production of cartilage ECM by means of collagen type II and aggrecan deposition	BMP-2 induced chondrogenesis more sufficiently. Type II collagen was expressed 21 days after differentiation, whereas type I was expressed from day 14	BMP-2 is better in inducing chondrogenesis in UCBMSCs which could possibly serve as a cell source for cartilage tissue engineering	[74]
Chung *et al.* (2014)	Compared chondrogenesis with UCBMSCs on 4 different hydrogels. Cell-hydrogel constructs were implanted in rat cartilage defects for 16 weeks	Constructs with 4% hyaluronic acid hydrogels created microscopically and macroscopically better cartilage as proved by the levels and organization of collagen type II bundles inside the implanted constructs	Human UCBMSCs combined with hyaluronic hydrogels are an attractive option for cartilage regeneration	[75]
Zheng *et al.* (2013)	Human UCBMSCs were cultured together with rabbit chondrocytes in 2:1 and 3:1 ratios and with or without the presence of IGF-1 and type II collagen and aggrecan production were measured at 14 days	At 14 days of culture, cells cultured at a 3:1 ratio with IGF-1 producing more collagen and aggrecan	Co-culturing human UCBMSCs with chondrocytes and IGF-1 yields sufficient cartilage ECM production	[77]

Unless stated otherwise, chondrogenesis was induced with classic chondrogenic medium containing insulin-transferrin-selenium, non-essential amino acids, dexamethasone, L-proline, TGF-β, sodium pyruvate and ascorbic acid, for 21 days.

AMSC: Adipose-derived mesenchymal stem cells; BMMSC: Bone marrow-derived mesenchymal stem cells; ECM: Extracellular matrix; GAG: Glycosaminoglycan; MSC: Mesenchymal stem cells; PCL: Polycaprolactone; PGA: Poly-glycolic acid; UCBMSC: Umbilical cord blood mesenchymal stem cells; WJMSC: Wharton's jelly mesenchymal stem cell.

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differentiation process compared to BM-MSCs [50,53]. Most of the studies however, tend to end differentiation at day 21 influenced by traditional BM-MSC cultures. Thus, studies with prolonged differentiation periods have to be conducted to clarify whether different results correlate to different differentiation times.

It has also been shown that UC-MSCs differ from adult stem cells. They express embryonic genes and proliferate faster. Therefore, future research should be focused on the in-depth understanding of those mechanisms and the adoption of new differentiation strategies based on those findings. Such research examples have already been published, as was the finding that UC-MSCs prefer embryonic to adult stem cell culture conditions [36].

Furthermore, research on tissue regeneration with umbilical MSCs is still mainly focusing on 2D cultures with classic differentiation protocols, and studies on the response of those cells when seeded on different scaffolds are still limited. Moreover, different growth factors (e.g., statins [81]), which have yielded fair osteogenic results when used on other stem cell types should also be tested on umbilical stem cells. Bearing in mind the discrepancies in the molecular differentiation pathways, factors inducing osteogenesis in BM-MSCs could be proved useless when tried on UC-MSCs and vice versa.

UCB-MSCs seem to be better candidates than umbilical tissue derived MSCs for tissue engineering purposes. Until now, most studies utilized UCB for the isolation of hematopoietic stem cells. Only after the middle of 2000s researchers focused on MSCs isolated from UCB and have proved that they are able to effectively produce both cartilage and bone. Those cells have the advantage that can be abundantly found in UCB banks. The increasing trend to cryopreserve UCB for future use could probably potentiate the use of UCB mononuclears for future 'off-the-shelf' autologous customized biological therapies.

Finally, technological advances in scaffold fabrication techniques and culture methods could be applied to produce bio-inspired UC-MSC-laden constructs. 3D bioprinting and other rapid prototyping methods could be utilized to create tissue-like structures with predefined cell positioning and graft shapes based on CAD models of real bone or cartilage defects. In addition, 3D culture systems – bioreactors – could be used to facilitate scale up of the differentiation process under strictly defined culture conditions.

Overall, the field of bone and cartilage regeneration with the use of umbilical cord stem cells is still naive. However, the future of personalized medicine and orthopedic tissue engineering could possibly lie in the umbilical cord. The impact of research related to it and the sophisticated combination of its derivatives with novel scaffolds and effective growth factors, will probably lead to the creation of cheap, atraumatic, personalized and autologous bone and cartilage grafts.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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