INTRODUCTION: Bone marrow (BM) represents the most common source of adult mesenchymal stem cells (MSCs) for cell-based therapies, including bone repair. However, due to the limited number of MSCs available in BM for autogenous use, the concern over immune rejection from allogeneic donors, and the possibility of donor site morbidity, there is a need to identify alternative MSC sources. A potential alternative source of MSC-like cells is the connective tissue (Wharton’s jelly) of human umbilical cord (UC), specifically human umbilical cord perivascular cells (HUCPVCs) [1]. The present work is focused on the comparison of the proliferative and multilineage differentiation capacity of HUCPVCs with adult human BM-derived MSCs to demonstrate the potential of HUCPVCs as a candidate source of stem cells for cell-based therapeutic strategies.

MATERIALS & METHODS: HUCPVCs and MSCs isolation: HUCPVCs were isolated from UCs by consenting full-term caesarian sections according to the methods described by [1]. HUCPVCs (n=3) were generously provided by Dr. J.E. Davies (University of Toronto, Canada). BM MSCs were harvested from the hips of consenting patients (n=3). For both cell types, adherent cells were maintained in monolayer culture in DMEM supplemented with 10% FBS. Passage 2-3 cells were used for subsequent studies. Phenotypic Analysis: Standard flow cytometric analysis was used to determine CD49e, CD146, CD117, CD90, CD31, CD117, CD45 and Stro-1 expression on HUCPVCs and MSCs. Cell Proliferation Assays: HUCPVCs and MSCs were plated at 3x10^4 cells/cm^2 in 12-well plates. On days 3, 7, 14 and 20, MTS assay was performed according to manufacturer’s protocol (Promega Corp.). Multilineage Differentiation Assays: Osteogenesis (OS): Cells were grown in osteogenic growth media (10 nM dexamethasone (DEX), 5 mM β-glycerophosphate, 50 μg/ml ascorbic acid (AA) and 10 nM Vitamin D3). On day 21, cultures were stained for alkaline phosphatase (ALP) activity and mineralization was assessed by von Kossa staining. RT-PCR was performed to detect the expression of osteoblast specific genes. Chondrogenesis: Cells were grown in high density pellets (2.5x10^5 cells) for 21 days in serum-free media (DMEM, ITS-premix, 50 μg/ml AA, 40 μg/ml L-proline, 100 μg/ml sodium pyruvate, 0.1 μM DEX), with and without 10 ng/ml TGF-β3. On day 21, pellets were prepared for histology for detection of sulfated glycosaminoglycan (sGAG) (Alcian Blue staining) and collagen (Picrosirius Red staining). sGAG content was quantified using the Blyscan sGAG assay. RT-PCR was performed to confirm the expression of Sox9, aggrecan and Col II. Adipogenesis: Adipogenesis was assayed by culturing cells in the presence of 1 μM DEX, 1 μg/ml insulin and 0.5 mM IBMX. On day 21, cultures were stained with Oil Red O stain and quantified by isopropanol elution and spectrophotometry.

RESULTS & DISCUSSION: Phenotypic Profile of HUCPVCs: Flow cytometric analyses revealed that HUCPVCs represent a non-hematopoietic cell population (CD45 expression), comparable to BM MSCs (Fig. 1A). However, HUCPVCs expressed elevated levels of CD146 and CD117 (Fig. 1B). Proliferative Potential: MTS assay showed similar proliferation profile for the two cell types (Fig. 1C). By day 20, HUCPVCs and BM MSCs had undergone 2.7±0.17 and 2.8±0.21 population doublings, respectively. However, beyond day 20, MSCs experienced contact-inhibited growth, while HUCPVCs continued to grow by multilayering (results not shown). Multilineage Differentiation Potential: HUCPVCs expressed elevated ALP activity by day 21 relative to MSCs (data not shown). Furthermore, von Kossa staining revealed more mineralization in OS cultures initiated with HUCPVCs than MSCs (Fig. 2A-B) and mineralization was detected earlier in HUCPVC OS cultures than MSC OS cultures (day 10 vs. day 14). HUCPVCs cultured as high density cell pellets cultures under chondrogenic conditions (+TGF-β3) generated larger pellets, compared to MSCs (data not shown).

While both HUCPVC and MSC pellet cultures exhibited similar sGAG staining intensities, as determined by Alcian Blue staining (Fig. 2C-D) and sGAG content (Fig. 2G), the amount of collagen in the extracellular matrix, as assessed by Picrosirius Red staining, was significantly higher in HUCPVC pellets than in MSC pellets (Fig. 2E-F). RT-PCR analysis revealed elevated levels of Col II gene expression in HUCPVC pellets vs. MSC pellets (data not shown).

Oil Red O staining demonstrated significantly more lipid-positive cells in HUCPVC cultures than MSC cultures grown in adipogenic conditions (Fig. 3A,B), confirmed by the statistically higher quantity of Oil Red O stain extracted from HUCPVCs (Fig. 3C).

CONCLUSIONS: This work demonstrates that HUCPVCs represent a non-hematopoietic cell population with the capacity to proliferate and differentiate down the osteogenic, chondrogenic and adipogenic lineages. Our direct comparison of HUCPVCs to MSCs further substantiates HUCPVCs as a candidate source of stem cells for cell-based therapeutic strategies.