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Exosome Origins: Why the Cell Source Matters

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ABSTRACT

As the field of exosome therapeutics expands, several companies are providing products sourced from different cells or tissues, often claiming equivalent or superior safety and efficacy profiles with little data to support these claims. A cursory review of scientific literature quickly reveals that not all exosomes are created equal. Exosomes are secreted by most, if not all cells. The composition of exosome content, however, is variable and dependent upon the originating cell type and its environment. Therefore, understanding the primary critical attributes of the cell source will provide the end-user important information to evaluate the quality, safety, and potential efficacy of the exosome product. Currently, bone marrow-derived mesenchymal stem cells (BM-MSCs) and placenta tissue-derived cells are primary cell sources for commercial products. The purpose of this paper is to elucidate important considerations of exosome origin before use.

Keywords

Stem cells, Tissues, Diseases, Exosomes.

Characterization of Exosome Origin Cell Source

Bone marrow-derived MSCs were first shown to possess multipotential differentiation capabilities in the mid-1980s. Since that time, they have been characterized in over 25,000 peer-reviewed studies. Their capacity to address acute and chronic injury and disease in both preclinical models and clinical trials has been well demonstrated. Furthermore, significant advances in understanding the therapeutic mechanisms of action of these cells have been made. In a PubMed search of "bone marrow" AND "mesenchymal stem cells" OR "mesenchymal stromal cells," over 63,000 results were listed.

In comparison, MSCs derived from birth tissues (Placenta/amnion and Wharton's jelly/cord blood) have more recently been characterized and studied (since the mid-2000s). As a result, the depth of characterization and comparison of birth tissue derived cells to the bone marrow derived cells is lacking. A PubMed search using the phrases "placental mesenchymal stem cells" OR "placental mesenchymal stromal cells" yielded 1,199 results, or approximately 30-fold less than for BM-MSCs (Figure 1). Similarly, a PubMed search using the phrases "Extracellular vesicles" OR "exosomes" AND "bone marrow derived mesenchymal stem cells versus extracellular vesicles" OR "exosomes" AND "placenta derived mesenchymal stem cells" yielded 313 results to only 14 results (Figure 1).

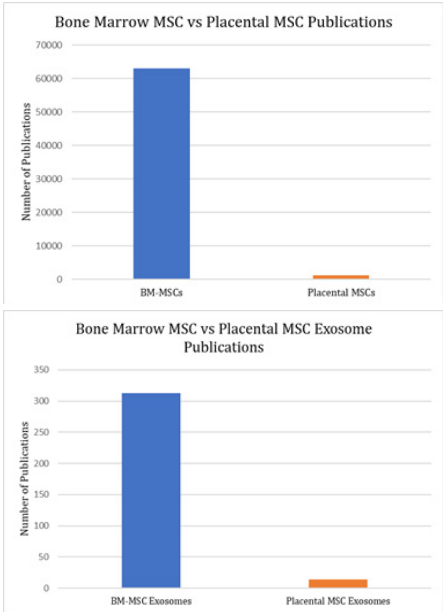


Figure 1: Publications characterizing bone marrow and placenta MSCs (L) and their Exosomes (R).

Safety Profiles of Bone Marrow and Placental MSCs

The most important considerations for exosome therapeutic use are safety, quality, and their ability to provide a positive clinical outcome. Several comparative studies of MSCs from bone marrow and placental tissue have been performed and indicate there are some crucial differences. There are two key areas that present differences that could have safety and efficacy implications. Two publications comparing MSCs from placenta and bone marrow found differences in the immune-modulation actions between the two cell types and as well as their degree of immune-privilege [1,2]. In both articles the BM-MSCs appear to have favorable characteristics relative to the placental cells.

In another study comparing the two different cell types, it was determined that placental-derived MSCs (PD-MSCs) express proteins associated with the induced pluripotent and embryonic stem cell phenotypes such as SSEA-4 and TRA1 (i.e., cells have a profile that may be similar to these more pluripotent cell types) [3,4]. Interestingly, these same proteins, which are not expressed in BM-MSCs, are also biomarkers for breast and prostate cancer cells [5,6]. It is possible, therefore, placenta cell-derived exosomes may have a profile of factors that are more comparable to cancer-derived exosomes which have been indicated to have potential safety issues. In particular, higher levels of proteins such as PDL1 and HLA-G, both important for establishing immune-tolerance of fetal cells with the maternal placenta, persist within placental MSCs [1,5,7]. Theoretically, uptake of HLA-G and PDL1 at high levels delivered by exosomes into dormant cancer cells could enable them to escape immuno-surveillance. BM-MSCs express much lower levels of both PDL1 and HLA-G and therefore have a much lower risk profile.

Are the Placental MSCs Really Younger than the Bone Marrow MSCs?

A popular marketing and sales argument for the use of placental cell-derived exosomes is that they originate from more primitive (younger) cells and therefore must have a profile that is more regenerative than adult (>18 years old donor) bone marrow derived cells. This argument initially makes intuitive sense, given our culture's deep-seated notion that younger is always better. However, this cultural concept is likely irrelevant to characterizing exosomes from placental tissue. Fazekasova et al. 2010, showed that when they used the same isolation methodology for MSC isolation from the same regions of different placental tissues, the derived cell populations were of both infant and maternal origin [1]. They noted, "It was not possible to reproduce the same isolation results between different whole placental cell isolates, even though the same methods were used." Therefore, the exosomes derived from placental populations are likely to be a combination of both mother and fetal cells, and in the worst-case scenario, completely from the mother. Similarly, other groups characterizing MSCs of placental origin also found chimeric culture compositions consisting of both maternal and fetal cells [8,9]. Sardesai and colleagues found that under common cell culture media and environmental conditions that the maternal cell growth was favored over the fetal origin cells [9]. The implication of an age-related advantage for placental derived MSCs is therefore unwarranted because the mother's cells were likely older than the bone marrow donor cells. It was also noted that over passages in culture, the proportions of maternal to neonatal cells in mixed isolates were variable, again suggesting exosome content from Placental-derived MSC populations may be variable in composition and quality [1,8,9]. A combination of cells from two different donors will create variability in exosome composition, purity, and quality between production lots that results in higher variability of the finished product and possibly clinical outcomes. For companies using placenta-derived cell lines to generate exosome products, rigorous quality control testing of cell purity and identity should be performed during the production run to control this source of variability.

A critical question for exosome users to ask of placental cell exosome providers is for proof that the exosome source is fully neonatal in origin and not maternal. In contrast, utilization of well-characterized bone marrow derived cell source, ensures that full identification, characterization and profiling of the cells has been undertaken. Thus, BM-MSC exosome users may have confidence in the donor cell source quality.

Another critical safety parameter for exosome-based products, as with all cell or tissue culture-based therapeutics, is the presence of ancillary reagents or materials. For example, fetal bovine serum (FBS) or newborn calf serum are commonly used to support cell survival and growth in culture. The first use of bovine serum to support cell cultures is attributed to Thomas Puck in the late 1950s, and has since become standard cell culture methodology [10]. With the advent of tissue and cell-based grafts, therapeutic removal of animal proteins, in general, is indicated to reduce the safety risk of adventitious agent introduction [11]. Due to its xenogeneic nature, however, for tissue and cell-based graft and therapeutic products, FBS must be removed to such a degree that the bovine proteins do not invoke an immune response in the recipient of the product. Indeed, Scala and colleagues determined in a survey of 23,077 individuals that 3.6% of these individuals were allergic to bovine serum albumin, the major protein component of bovine serum [12]. Failure to satisfactorily reduce this residual ancillary animal protein content in the final product, therefore, could result in mild to severe reactions that result in failure or worst case, additional exacerbation of the disease state for which the product was being used to alleviate.

Recently, an independent laboratory evaluated samples of 3 commercially available exosome products for bovine protein content. Assessment of bovine protein presence in the products was made using antibody-protein arrays designed to detect bovine cytokines and growth factors (RayBiotech, Norcross, GA). Thirty different bovine proteins known to be present in serum were quantified. Five of the 30 were found in none of the samples. Nine were determined to use antibodies that were not species-specific (i.e., the antibodies bound conserved protein epitopes

and cross-reacted with both bovine and human proteins). Of the 16 remaining bovine proteins quantified, 0 of 16 were detected in exosomes generated by cells maintained in animal protein-free defined medium Product 1; whereas 15 of 16 bovine proteins were present in Product 2 samples and 13 of 16 were detected in Product 3 indicating both products had pg/ml quantities of bovine proteins remaining in the released products (Table 1).

Bovine Protein ID	Product 1 [pg/mL]	Product 2 [pg/mL]	Product 3 [pg/mL]
aFGF	0.0	279.7	736.5
ANG-1	0.0	301.1	601.2
CD40L	0.0	17.3	230.2
Decorin	0.0	237.0	293.2
IFNg	0.0	0.4	0.0
IL-1 F5	0.0	0.3	8.2
IL-10	0.0	1,368.3	0.0
IL-13	0.0	26.7	8.8
IL-17A	0.0	16.6	31.4
IL-1b	0.0	0.2	0.4
IP-10	0.0	0.0	2.2
LIF	0.0	122.0	181.8
MCP-1	0.0	5.0	352.5
MIG	0.0	5.5	27.6
TNFa	0.0	26.9	0.0
VEGF	0.0	39.4	8.6

Table 1: Quantification of Bovine Cytokine Proteins in Commercially Available Exosome Products.

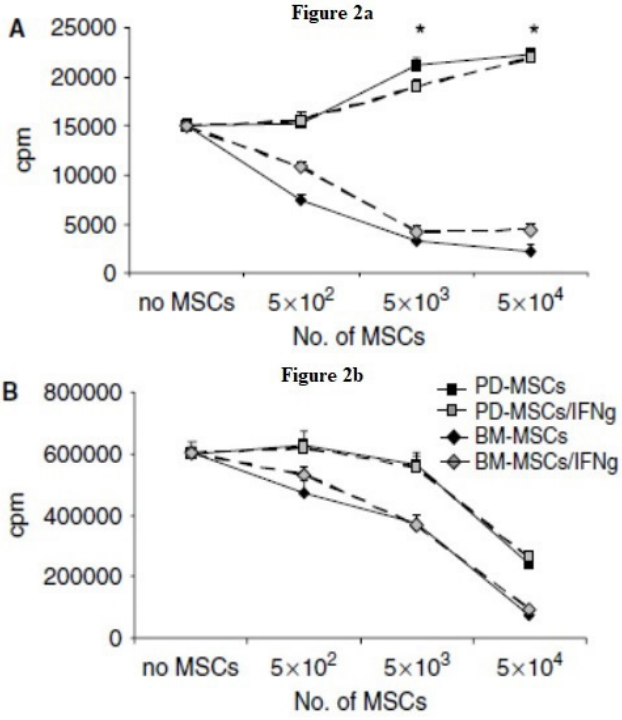
Since serum albumin (the most concentrated serum protein) was not measured in this study, the total amount of residual bovine protein that could induce an allergic reaction is not known.

Clinicians using products containing detectable levels of animal proteins should be aware of the potential risks associated with their use and evaluate the prudence of their transplantation accordingly.

Exosome Products Reflect Differences Seen Between the Different Origin Cells

The functionality of source cells is reflected within the signaling molecules of their growth factors and exosomes. Heo et. al. demonstrated in a side by side comparison of BM-MSCs and Placental-MSCs abilities to regulate immune function, that BM-MSCs expressed a greater capacity to inhibit T-cell proliferation in a mixed lymphocyte reaction assay and also expressed higher levels of Interleukin-10 compared to placental derived cells. The BM-MSCs also expressed higher levels of TGF-β1 and Interkeukin-6 compared to placental cells collectively supporting a more favorable immunomodulatory profile.

Fazekasova et. al. also demonstrated a more favorable immunomodulatory profile of BM-MSCs compared to Placental-MSCs [1]. In this paper, the immunomodulatory functions of different isolates of Placental-MSCs were compared to those of BM-MSCs. "CD4+ T cells were stimulated with PHA or anti-CD3/CD28 beads in the presence of Placental- or BM- MSCs (grown for 48 hours, with or without interferon gamma IFNγ). BM-MSCs inhibited both PHA (Figure 2A) and anti-CD3/CD28 bead (Figure 2B) stimulation of CD4+ T cells in a dose-dependent manner. Some inhibition with the highest number of Placental-MSCs was observed when T cells were stimulated with anti- CD3/CD28 beads. The treatment of MSCs with (IFNγ) for 48h did not change the immunoregulatory capacity of MSCs" [1]. These results suggest that BM-MSCs are superior in their immunomodulatory capacity compared to Placental-MSCs."



"Placental-MSCs seem to be more immunogenic and less immunomodulatory than Bone Marrow MSC preparations and are altogether less appropriate for use in a clinical setting." From: Fazekasova H, Lechler R, Langford K, et al. J Tissue Eng Regen Med. 2011 Oct;5(9):684-94.

The capacity of exosome origin cells to induce differentiation and other physiological changes is likely due to cell signaling from exosomes as well. Indeed, MSCs from different tissues have been shown to have different potential to differentiate into different cell types. It may be expected then the exosomes secreted from these different origin cells project these differences when communicating with host progenitor cells. Heo et. al. demonstrated a reduced capacity of placental cells to display trilineage differentiation in vitro compared to bone marrow MSCs [2]. DLX5 an important signaling molecule for osteogenesis was shown to be expressed only in BM-MSCs, and these cells were shown to possess a higher capacity to turn into bone forming osteoblasts. Barlow and colleagues consistently found human placental MSCs less able to demonstrate adipogenic differentiation in vitro as compared to BM-MSCs [13]. It is important to understand these differences to determine which exosome population is appropriate to use.

Conclusion

Published peer reviewed studies comparing bone marrow derived MSCs versus peri-natal (Placenta/amnion and Wharton's jelly/cord blood) derived MSCs clearly indicate that BM-MSCs have a much higher safety profile and yield higher quality exosomes that deliver a more favorable profile of signaling factors. Bone marrow derived MSCs are a much more characterized cell source. Over 63,000 peer reviewed papers have been published on bone marrow derived MSCs. This compares to 1,200 on perinatal derived MSCs. Given the reported differences in safety, quality, and efficacy of exosome products, it is important for physicians to investigate the exosome product being considered beyond the sales and marketing brochures to ensure they provide their patients with the best chance for a safe and successful clinical outcome.

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