iba Solutions For Life Sciences

Cell Selection & Expansion

Application Note

ISOLATION OF EXOSOMES FROM MESENCHYMAL STEM CELLS

High quality exosome isolation with Fab-TACS® technology

Introduction

Exosomes are small extracellular vesicles that range from 30 to 150 nanometer in size. They originate from endosomes of various cell types and mediate cell-to-cell communication. Different studies show that they participate in a variety of physiological and/or pathological processes. Exosomes are packaged with a conserved set of proteins, including tetraspanins (CD9, CD63 and CD81). However, they also contain specific proteins that represent their parental cell source and reflect their microenvironmental niche. This leads to considerable differences in exosome cargo[1–3]. Exosomes derived from mesenchymal stem cells (MSCs) have shown promising therapeutic effects in pre-clinical and clinical settings[4].

MSCs have the potential to differentiate into various cells types. They are used in regenerative medicine because of their immunomodulatory and anti-inflammatory properties and their ability to treat tissue injury. The therapeutic effect of MSCs is thought to be mediated through the secretion of a variety of factors, including exosomes. Via those vesicles, they can affect the physiology of both adjacent and distant responder cells. In fact, exosome-based therapies may hold some advantages over cell-based therapies as they avoid many risks associated with cell transplantations. However, this approach requires high quality exosome preparations. In this application it will come apparent that our CD9 and CD81 specific Fab-TACS® isolation technology is applicable for purifying exosomes secreted from umbilical cord-derived MSCs.

Mesenchymal stem cell isolation and culture

Umbilical cords (UCs) were collected in phosphate-buffered saline (PBS) supplemented with antibiotics. The whole cord segment was washed two times in sterile PBS. It was pinned at opposite ends, and an incision was made along the middle of umbilical cord lining. The cord lining was peeled back using a scalpel and underlining connecting tissue was cut away.

The Wharton's jelly is located in the space between the vein and arteries (see Fig. 1). 1-2 grams of tissue

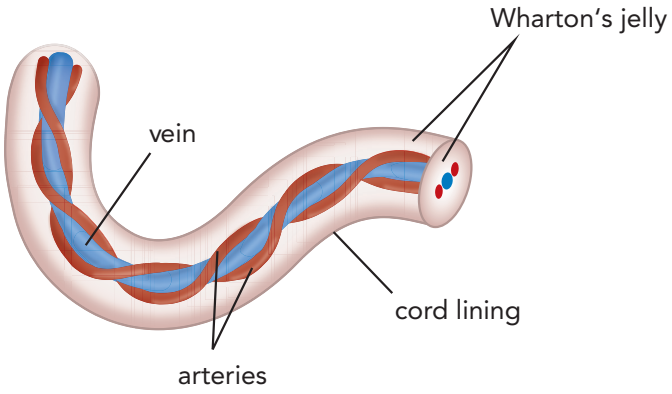

Fig. 1: Location of the Wharton's jelly

were collected. After isolation, tissue was washed once with 20 ml of sterile PBS at 2000 rpm for 5 mins. PBS was removed carefully and 10 ml collagenase-hyaluronidase solution added. The tissue was digested with 0.075% collagenase I for 30 min at 37°C. The cell suspension was centrifuged at 310 x g and washed once with 20 ml PBS. Cells were resuspended in MSC Media (DMEM supplemented with 10% human platelet lysate (hPL) and 1% penicillin-streptomycin (P/S)) and seeded in 6-well plates[5].

Short Protocol for MSC Cultivation and Exosome collection

MSCs were incubated at 37°C in a humidified atmosphere with 5% CO_2. Adherent cells were allowed to expand until they reached about 80% confluence. Morphology of isolated and expanded MSC cells was assessed during the cell culture period using light microscopy (Fig. 2) and FACS analysis.

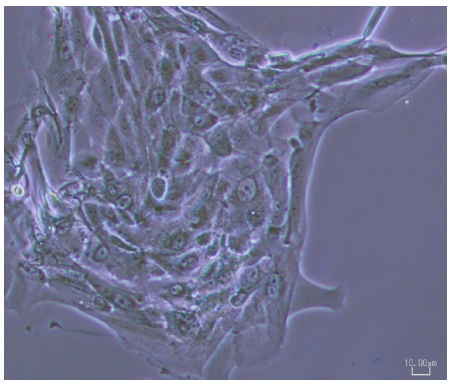

Fig. 2: Mesenchymal stem cell morphology

For exosome production and harvest, cells were cultivated in starvation medium up to 72 h (starvation condition, < 2% exosome-free hPL). After the starvation period cell supernatant was collected and exosomes isolated using a Fab-TACS® Exosome Isolation Kit.

Exosome isolation of mesenchymal stem cell supernatants

Enrichment of CD9 and CD81 positive exosomes from MSC cell culture supernatant was performed using the respective Fab-TACS® Exosome Isolation Kits according to the included manuals. Briefly, the MSC cell culture supernatant was centrifuged at 3000 x g for 10 min. The supernatant was filtrated through a 0.2 µm polyether-sulfone filter. Elution Buffer and PBS were filtered through a cellulose-acetate filter (0.2 µm). After draining the storage buffer, columns were loaded with Fab-Streps. Filtrated culture media were transferred to the column in 1 ml steps. After washing, filtrated Biotin Elution Buffer was applied to the matrix to collect exosomes. Exosome size was analyzed with the NanoSight LM10 instrument (Malvern Instruments) and data were processed using NTA software 2.3. Protein content was determined by Western Blot using monoclonal detection antibodies for Alix and CD63.

Results and discussion

The size of isolated particles is a good indicator for the actual exosome content. Using our CD81 and CD9 Fab-TACS® exosome isolation technology, we purified particles of which 97- 99% fell within the range of 30 – 150 nm (Fig. 3A, B). This indicates significant exosome

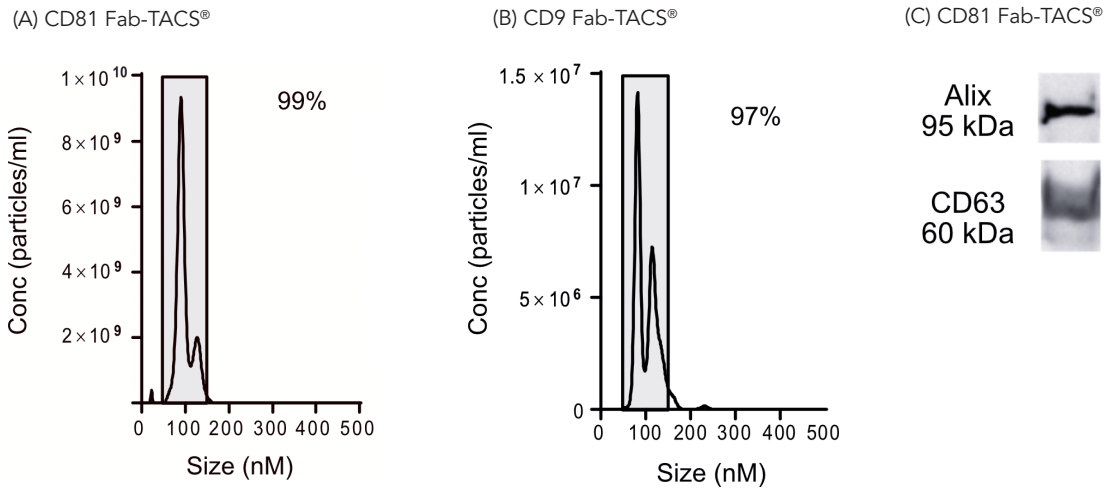

Fig. 3: (A-C) Exosomes of mesenchymal stem cell supernatants were isolated using CD81 (A, C) or CD9 (B) Fab-TACS® Exosome Isolation Kits. Particle size was analyzed with the NanoSight LM10 instrument (Malvern Instruments) and data were processed using NTA software 2.3. The gray windows show the target size for exosomes (A,B). Protein content of exosomal proteins Alix and CD63 was determined by Western Blot (C).

enrichment. However, this small particle size between 30 nm and 150 nm is not exclusively indicative for exosomes as same-sized non-exosome contaminants may contribute to this pool. To confirm that the particles isolated from MSC supernatants with our Fab-TACS® system are indeed exosomes, we tested for the presence of marker proteins CD63 and Alix. Both proteins were highly expressed within the isolated particles (Fig. 3C), proving their exosomal phenotype.

Cell culture supernatant compositions may vary depending on mesenchymal stem cell donor. A stable quality for exosome isolations is required for the application in pre-clinical and clinical settings. Therefore, we investigated the reproducibility of the Fab-TACS® Exosome Isolation Kit. Three independent purifications of different MSC donors yielded particles of which > 90% were exosome-sized. In addition, they were very comparable in their average diameter that ranged from 86 nm to 90 nm (Fig. 4).

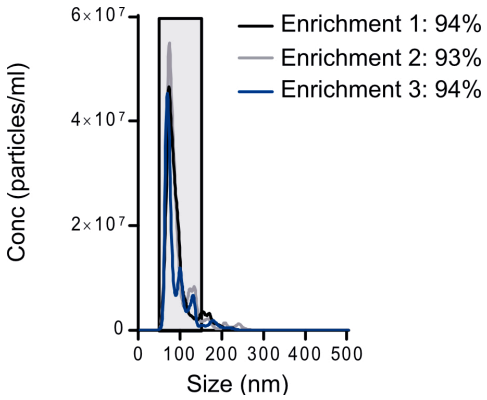

Fig. 4: Exosomes of mesenchymal stem cell supernatants were isolated using the CD9 Fab-TACS® Exosome Isolation Kit analyzed with the NanoSight LM10 instrument (Malvern Instruments). Data were processed using NTA software 2.3. The gray window shows the target size for exosomes.

Conclusion

Our CD81 and CD9 Fab-TACS® exosome isolation technology consistently delivers pure and specific exosome populations from MSC supernatants that are suitable for various downstream applications.

References

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