Exosomes Secreted from Bone Marrow-Derived Mesenchymal Stem Cells Protect the Intestines from Experimental Necrotizing Enterocolitis

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insulin syringes with 29 gauge needles (Becton Dickinson, Franklin Lakes, NJ) immediately prior to IP injection.

2.4 Isolation and characterization of exosomes BM-MSCs were cultured until ~80% confluent, and were then cultured in serum-free media (SFM) for 48h, at which time exosomes were isolated from the BM-MSC-conditioned media (CM) by one of two methods. The first method, used for *in vivo* studies, was performed according to the manufacturer's instructions for the P100 PureExo Exosome Isolation kit (101bio, Palo Alto, CA). Exosomes were isolated from one T-75 flask containing ~80% confluent BM-MSCs, resuspended in 150 µl PBS, and used for three separate IP injections (50 µl / pup). These isolated exosomes were stored at 4°C and used within 72 hours or were frozen at -80°C. Exosomes isolated using this method were characterized by nanoparticle tracking analysis (NTA) using the NanoSight NS300 (Malvern Instruments Inc., Westborough, MA). Quantification indicated that this isolation method yielded ~$2.5x10^9$ BM-MSC-derived exosomes / 50 µl volume. The second method, used for *in vitro* wound healing studies, used serial centrifugation to obtain both BM-MSC-derived exosomes as well as exosome-depleted BM-MSC-CM for use as a control [31], [32]. One T-75 flask containing ~80% confluent BM-MSCs was used to prepare BM-MSC-CM which was collected and centrifuged at 300xg for 10 min to pellet cells which were discarded. The supernatant was collected and centrifuged at 2,000xg for 20 min, and the resulting supernatant was centrifuged at 10,000xg for 30 min to pellet contaminating cellular debris, which was discarded. The remaining supernatant was then ultra-centrifuged at 100,000xg for 18h to obtain both isolated exosomes (pellet) as well as exosome-depleted BM-MSC-CM (supernatant) [32]. The pellet was resuspended in 150 µl PBS, and both isolated exosomes and exosome-depleted BM-MSC-CM were stored at 4°C and used within 3 days or frozen at -80°C. These preparations were used for three separate treatments of IEC-6 cells, at a volume of 50 µl each, as described in section 2.9. Exosomes isolated using both methods showed strong Western blot immunostaining to the well-established exosome markers CD-9 and flotillin-1.