Scalable production of exosomes and their potential use as a therapeutic for tendinopathy

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Abstract

While the recognized regenerative properties of stem cells show promise in bioengineering tendon constructs for repairs, there is limited evidence that direct injection of stem cells for tendon healing has beneficial effects. It has been suggested that much of the observed benefit of these stem cell injections arises from stem cell-secreted factors carried in discreet microvesicles called exosomes. Here, we present our findings on the physical and functional characteristics of exosomes from adipose tissue-derived MSCs relevant to wound healing and tissue regeneration. Scalable production of exosomes was accomplished using a hollow-fiber bioreactor. Having successfully developed scalable exosome production, isolation procedures, and in vitro assays to functionally characterize these particles which are secreted by the cultured MSCs, our goal is to leverage the regenerative and healing properties of adult stem cells by developing exosomes as a non-surgical and non-cellular treatment for tendon repair.

Human stem cell derived exosomes

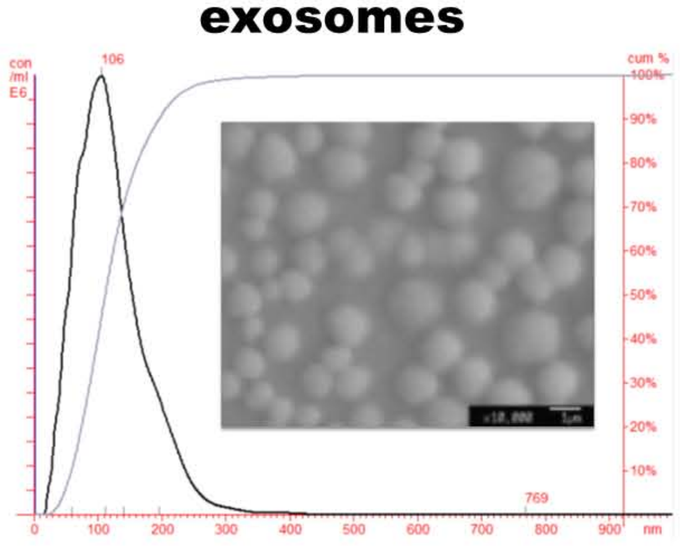


Figure 1. shows the size distribution of a sample with mean of 122 nm ± 58 nm and a mode of 106 nm. Exosomes visualized by electron microscopy to determine their relative size and shape (**Figure 1 inset**). Revealed they had a larger average particle size by SEM than the preparation shown in the histogram.

Bioreactor

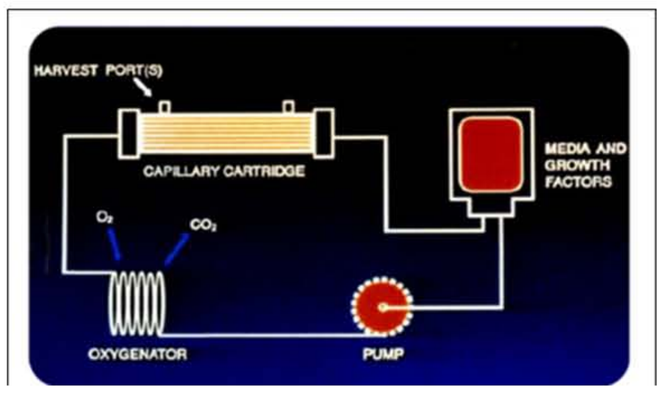


Figure 2. Schematic of hollow fiber bioreactor. Medium flows from the reservoir bottle by means of a pulsatile perfusion pump through the oxygenation circuit where it is oxygenated and saturated with CO^2. Cells growing in this environment are bathed in fresh medium as it is perfused though the hollow fibers. Metabolic waste is dialyzed away from the cells and into the circulating medium. MSCs (300 x 10^6 cells) were seeded into the bioreactor and maintained for 8 weeks. Exosomes were isolated at 2-week intervals.

Exosome Yields

	Media Volume (mL)	Total Exosome Protein (mg)	Total Number of Exosomes (10^9)
Bioreactor #1	40	1.87	22
Bioreactor #2	40	2.77	320
Bioreactor #3	40	1.95	14
Bioreactor #4	40	1.2	17
130 Flasks (T225)	4000	0.9	16

Table 1. summarizes exosomes yields from 4 sequential bioreactor isolations and 130 (1.3 x 10^9 cells) T225 tissue culture flasks over an 8-week time course.

	Growth Medium (L)	Collection medium (L)
Bioreactor	7	2
T225 Flasks	24	4

Table 2. summarizes the volumes of growth and collection medium used for exosomes isolated 4 times from the bioreactor and from 130 T225 tissue culture flasks.

Functional Activity

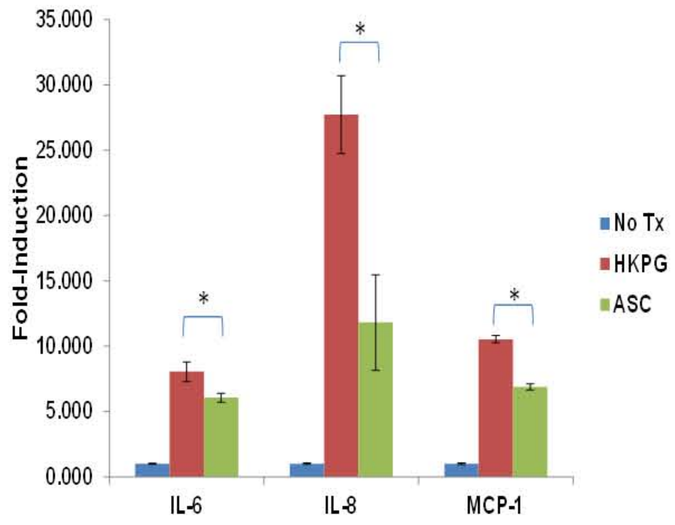
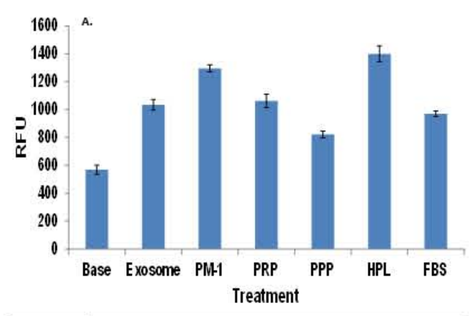


Figure 3. ASC exosomes show anti-inflammatory activity in primary human fibroblasts (PHFs). As an assay to establish the biological activity of the exosomes, we examined ASC-derived exosome preparations for their potential to inhibit IL6 and IL8 expression. Previous reports indicate the induction of inflammatory cascades in keratinocytes and fibroblasts in response to P. gingivalis lysates, including IL6 and IL8. To evaluate the anti-inflammatory impact of exosome treatment, cells were concomitantly exposed to lyophilized heat killed P. gingivalis (HKPG, 107/ml) and exosomes. To measure inflammation, RT-qPCR for the inflammatory cytokines IL6, IL8 and MCP-1 was performed. PHKs and PHFs were seeded in 6-well plates and incubated overnight in the absence or presence of exosomes (6.7 µg/ml). RT-qPCR indicates dramatically increased expression of the cytokines in response to HKPG, with a significant degree of rescue exerted by ASC-derived exosomes.

Exosome effects on cultured tenocytes



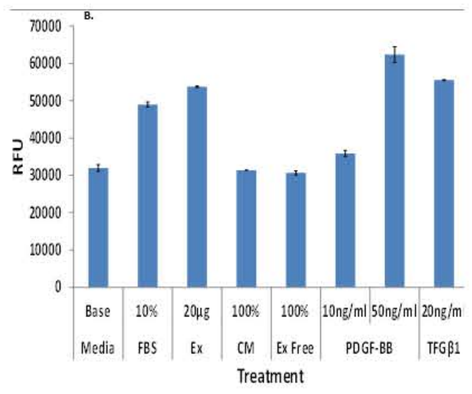


Figure 4. A) Tenocyte and B) TDSC proliferation

Exosomes (25 µg) were added to low density tenocytes (3,000 cells/well) in 96-well culture plates using serum free medium and incubated for 3 days. To compare the proliferative effects of exosomes, tenocytes were treated with other inducers including 10% PRP, PPP, FBS or Human Platelet Lysate (HPL), or ZenBio's serum containing medium PM-1. After 3 days, the cells were treated with Cell Titer Blue reagent (Promega) for 2 hours to assess tenocyte proliferation. **Figure 4A** shows that treatment with exosomes significantly increases tenocyte proliferation to the level induced by PRP and FBS containing base medium. Exosomes (20 µg) induced TDSC proliferation whereas the unconcentrated placental stem cell conditioned medium (CM) and the exosome depleted conditioned medium (Ex free) were ineffective (**Figure 4B**). Additionally, growth factors, PDGF-BB or TGFβ1 were as effective as exosomes on TDSC proliferation. To determine if exosomes induced collagen I synthesis, serum-free conditioned medium from vehicle or exosome treated tenocytes was used in a procollagen I C-peptide ELISA (TaKaRa). Cells were treated for three days with or without 25 µg exosomes, the conditioned medium removed and clarified by centrifugation and diluted into the ELISA assay. **Figure 5** shows that tenocytes treated with exosomes significantly increased collagen I production.

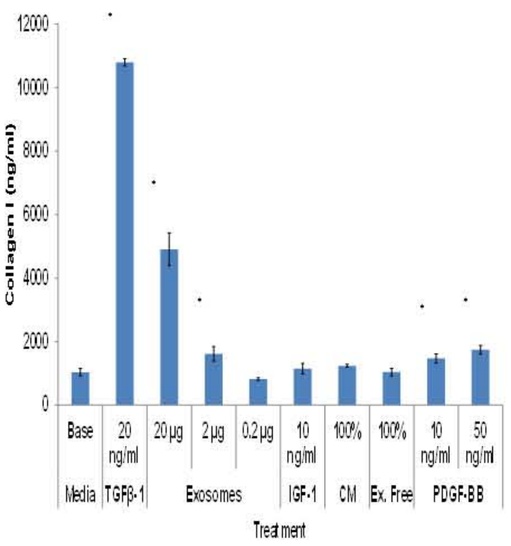


Figure 5. Collagen production

Summary

- Exosomes can be isolated multiple times over the life of the bioreactor, without passaging of the cells, using much less culture medium, and in higher concentrations, compared to isolations from cells cultured in T225 flasks.
- Exosomes can inhibit the production of inflammatory cytokines that act locally, IL6 and IL8, and MCP-1, which recruits monocytes to the site of inflammation.
- Our initial studies show that adipose-derived stem cell exosomes are capable of inducing tenocyte activities related to healing.
- We observed a significant exosome-induced increase in collagen I production in cultured tenocytes and DSCs. This could lead to improved healing strength or could cause excess scar formation depending on the balance of ECM production versus remodeling.

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