2022 Best Research Poster Award

Barwon Health

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Bovine extracellular vesicles contaminate human extracellular vesicles produced in cell culture conditioned medium when 'exosome-depleted serum' is utilised

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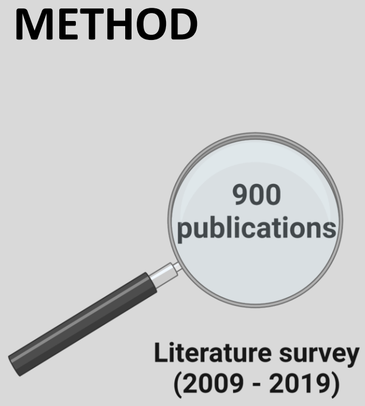
INTRODUCTION

Extracellular vesicles (EVs) are important intercellular communication messengers [1]. Despite the initial warning of the potential of bovine EV carried over by the 'Exosome depleted serum' (EDS) to interfere with the human or rodent EV studies in 2014, there has been an increase in use of the EDS in cell culture-based EV studies (46% of publications on human or rodent EVs between 2015 and 2019). The exogenous bovine RNAs and proteins carried over by EDS could affect gene expression in human/rodent cells [2], leading to false implication in disease pathogenesis and futile pursuit of cancer biomarkers and/or therapeutic targets.

OBJECTIVES

Hence, we decided to study to what extent the gold standard ultracentrifugation is able to 'deplete' the bovine EVs. We also examined whether the ultracentrifugation procedure will alter the bovine EV profile in EDS and the contamination of EDS-derived EVs in human EVs.

METHOD



EDS production

- 18-hour ultracentrifugation of 20% fetal bovine serum

EV count

- Nanoparticle tracking analysis

Bovine EV contamination

- Human HT-29 cell culture
- Bovine CD9 ELISA

Identity of bovine miRNAs to counterparts in humans and rodents

- Bioinformatics

RESULTS

1. Increasing EDS use in literature

Approximately 43% of the 900 publications employed EDS in the cell culture-based production of EVs.

2. Abundant vesicles are remained in EDS

EDS produced by the gold standard ultracentrifugation was assessed with particle and vesicle counts by nanoparticle tracking analysis (NTA). Although the 18-hour ultracentrifugation could reduce approximately 94% and 97% of total nanosized particles and vesicles in original fetal bovine serum (FBS) respectively, there were ~5.33 $\times 10^7 \pm 7.14 \times 10^6$ bovine EVs/mL detected in EDS (Fig. 1).

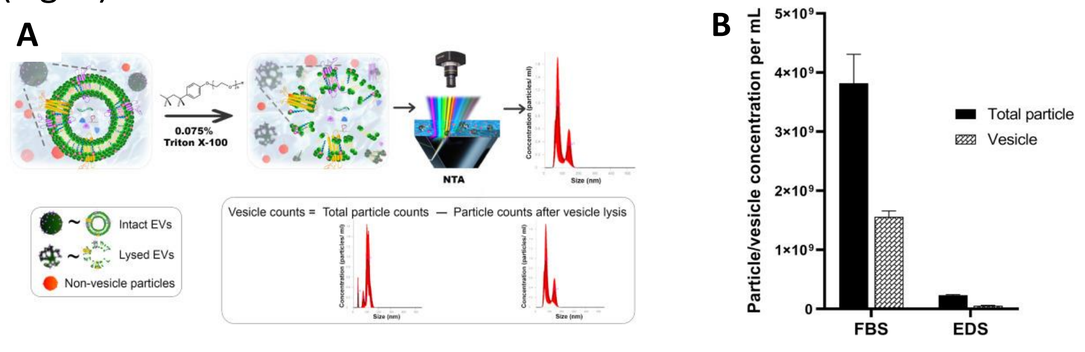


Figure 1. Total particle and vesicle counts in FBS and EDS. (**A**) Schematic illustration NTA measurement particles and vesicle concentration using 0.075% Triton X-100. (**B**) The relevant counts were determined using NTA as indicated in **A**. Data shown are means ± S.D., n=3.

Moreover, the 18-h ultracentrifugation changed the EV profiles in resultant EDS with enriched small EV proportion as compared to the EV profiles in original FBS (Table 1).

RESULTS

Table 1. Percentage of small EVs and large EVs in FBS and EDS with relevant ratios

Sample	Percentage of small EVs in total vesicle	Percentage of large EVs in total vesicle	Ratio of small EVs to large EVs
FBS	47.5 ± 6.9	6.8 ± 1.6	7.0 ± 1.2
EDS	92.4 ± 10.8	4.8 ± 1.1	19.2 ± 2.3

Data shown are means ± S.D., n =2–3

3. Persistence of bovine-derived EVs in human EVs produced in cell culture

Next, we investigated the contamination of EDS-derived EVs in the production of human EVs by the HT-29 cells.

The HT-29 cell culture conditioned medium containing 0.5% EDS was found to contain higher concentration of total vesicles than that in the cell culture with DMEM-only (Fig. 2B). As shown in Fig. 2C, no signal above the limit of detection for bovine CD9 was observed for the cell culture conditioned medium containing DMEM-only, however we could consistently detect a signal that is at least 300% above the detection limit of the assay for samples containing 0.5% EDS.

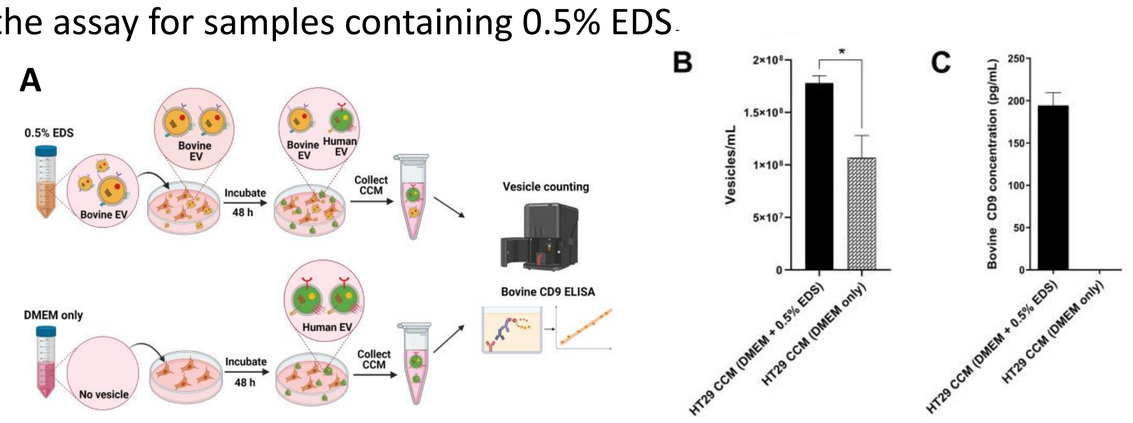


Figure 2. Characterization of the contamination of human EVs in HT-29 cell culture conditioned medium by bovine EV from 0.5% EDS. (**A**) Schematics of analysis of HT-29 cell culture conditioned medium using either DMEM or DMEM with 0.5% EDS. (**B**) Total vesicle counts of HT-29 cell culture conditioned medium prepared using two different media as indicated. (**C**) The concentration of bovine CD9 marker in HT-29 cell culture conditioned medium prepared using two different media by ELISA. Data shown are means ± S.D., n = 3. *$p < 0.05$.

4. Identity of bovine serum miRNAs to their counterparts in humans and rodents

The comparisons of miRNA sequences reveal 11 miRNAs with 100% sequence identity between bovine and the corresponding human/rodent miRNAs (Table 2).

Table 2. Identity of bovine serum miRNAs

Bovine miRNA	Identity to human	Identity to mouse	Identity to rat
Let7*	100%	100%	100%
bta-miR-26a-5p	100%	100%	100%
bta-miR-122	100%	100%	NA
bta-miR-127-3p	100%	100%	100%
bta-miR-148a	100%	100%	100%
bta-miR-181a-5p	100%	100%	100%
bta-miR-320a	100%	100%	100%
bta-miR-379-5p	100%	100%	100%
bta-miR-423-5p	100%	100%	100%
bta-miR-432-5p	100%	100%	100%
bta-miR-1246	100%	100%	100%

DISCUSSION AND CONCLUSION

Our results indicate that the widely used ultracentrifugation procedure is not only unable to deplete bovine EV but also alters the ratio of small EVs vs large EVs in the resultant EDS. Importantly, even with the lowest concentration of 0.5% EDS, bovine EVs persist in the human EVs produced in cell culture. A fresh reminder for the recognition and mitigation of the pervasive and consequential interference from bovine EVs in cell culture-based studies will promote the validity as well as the reproducibility of future EV research, especially PCR-based studies.

REFERENCES

[1] M. Mathieu, L. Martin-Jalular, G. Lavieu, C. Théry. Nat Cell Biol., 21 (1) (2019), pp. 9-17. [2] H. Aswad, A. Jalabert, S. Rome. BMC Biotechnol, 16 (2016), p. 32.