Development of a method to isolate extracellular vesicles from cell culture

Jana, Caflisch, BMA 21-24

Biomedizinische Analytik HF

Department of Visceral Surgery and Medicine University of Bern

1. Abstract

Extracellular vesicles (EVs) are small vesicles released by cells that transport various substances and serve as biomarkers. In this thesis, a method was developed to isolate EVs from hepatocyte cell cultures using an ultracentrifugation technique and subsequently extract RNA from them.

2. Introduction

EVs are cell derived particles crucial for intercellular communication, One way this communication occurs is through the transport of RNA by EVs. They show promise as biomarkers, but their small size (50-200nm) challenges isolation[1].The Department of Visceral Surgery and Medicine aims to develop a method for isolating hepatic EVs to enable early detection of liver diseases through RNA sequencing. This could potentially replace invasive biopsies with blood tests, allowing for earlier diagnosis and therapy monitoring.

3. Aims and leading questions

Aims

1. Development of a method to isolate EVs from the supernatant of cell cultures.
2. Extract RNA from the isolated EVs.

Leading questions

1. Can particles the size of EVs, which originate from the cells, be detected in the samples using nano tracking analysis after application of the developed isolation method?
2. Is it possible to extract RNA from the isolated EVs in sufficient concentration and purity to meet the nanodrop reference ranges?

4. Method and materials

EVs were isolated from 24 T75 Cell culture flasks containing HepG2 cells. After all cells were confluent, the normal medium was replaced with foetal bovine serum (FBS)-free medium to avoid contamination with EVs from the serum. The flasks were incubated for 72 hours. The samples were ultracentrifuged twice at 120’000g to concentrate the EVs. To verify that the isolated EVs originated from cells the FBS-free medium, alone was used as a negative control.



Figure 1 photo of the cultured HepG2 cells(Caflisch, 2024)

5. Results

Results NanoSight300

Subsequently, the obtained EVs were measured with the NanoSight300 (NS300). The NS300 measures the particle concentration with a laser and calculates the particle size based on their Brownian motion. Positive results required >1.00E+07 particles/ml[2] and sizes between 50-2000nm which is the size range of EVs[1].

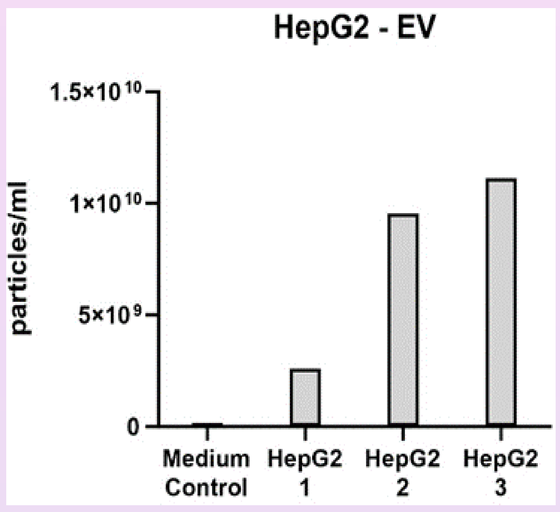


Figure 2 presentation in charts of measured particles/ml from the different experiments (Caflisch, 2024)

Table 1 Results NS300 (Caflisch, 2024)

Experiment	Median Size	Concentration particles/ml
HepG2 1	151.7 nm	2.58E+09
HepG2 2	124.3 nm	9.56E+09
HepG2 3	163.4 nm	1.11E+10
Control HepG2	115.2 nm	1.01E+07

RNA Extraction

RNA was extracted using the Qiagen exoRNeasy kit and quantified on the Nanodrop from Thermo Fischer. The Reference range for the Nanodrop was a concentration minimum of 40 ng/µl RNA and a purity ratio of A260/A280: 1.8-2.2 [3].

Table 2 Results Nanodrop (Caflisch, 2024)

Sample Name	Nucleic Acid(ng/µl)	A260/A280	Reference range met?
Reference Range	> 40	1.8-2.2	
Experiment HepG2 one	85.4	1.97	yes
Experiment HepG2 two	6.9	1.32	no
Experiment HepG2 three	64.0	1.85	yes

6. Discussion

Three experiment to isolate and EVs and extract their RNA using HepG2 cells were performed. The first experiment yielded 2.58E+09 particles/ml, and successfully extracted RNA.

The second experiment yielded a lower particle concentration (1.91E+09 particles/ml), raising suspicions of measurement error due to high particle density. Further dilution (1:400) revealed a higher concentration of 9.56E+09 particles/ml, suggesting that the first experiment's results had been underestimated. An error during RNA extraction in the second experiment necessitated a third trial.

The third and final experiment, required an even greater dilution of 1:800, which supported the hypothesis. The RNA extraction was successful in this trial. The particle concentration of the HepG2 medium negative control was a thousand times lower than the experimental samples, indicating negligible influence on the results.

Since 80% of cell free RNA which is not protected by a EVs degenerates within 20min and the EVs samples were stored for min 12h before RNA extraction, it is proven that the RNA originates from EVs[4]. The experiments demonstrated successful EV isolation from HepG2 cells, with improved accuracy through appropriate sample dilution.

Repetitions of this experiment are still necessary to prove that it is reproducible.

References

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Figure 1 Caflisch, J. (2024). foto HepG2 cells. Medi

Figure 2 Caflisch, J. (2024). (Nanosight 300) visceral surgery and medicine.

Tables

Table 1 Caflisch, J. (2024). (Nanosight 300) visceral surgery and medicine.

Table 2 Caflisch, J. (2024). (Nano drop) visceral surgery and medicine: Chart nano drop.