SEC Multi-Faceted Analysis of Exosome (EV) Combination of UV, Florescence, and Light Scattering Detectors

Introduction

Exosome, an extracellular vesicle (EV), is responsible for transmitting various information and substances in living organisms. EVs are receiving great interests in the fields of new modality[1], because of their potential uses as highly sensitive diagnostic markers and therapeutic applications. Their transmission and target specific characteristics make them potentially significant in the treatment of various diseases. EVs have unique common physical and physiological properties including their sizes and surface antigens. However, their complex cell generation processes create diversities in their properties and encapsulated substances. Thus, they are often treated as mixtures of nano vehicles with certain variations. Therefore, the manufacturing of EV as a biopharmaceutical via bioprocessing requires development of production and evaluation methods that ensures consistent reproduction of EVs with the same characteristics throughout basic research, proof of concept, all clinical trial stages and actual production for clinical use. In addition, it is also important to control impurities generated during the production procedures. Those impurities include cell-originated components with which also have diversities.

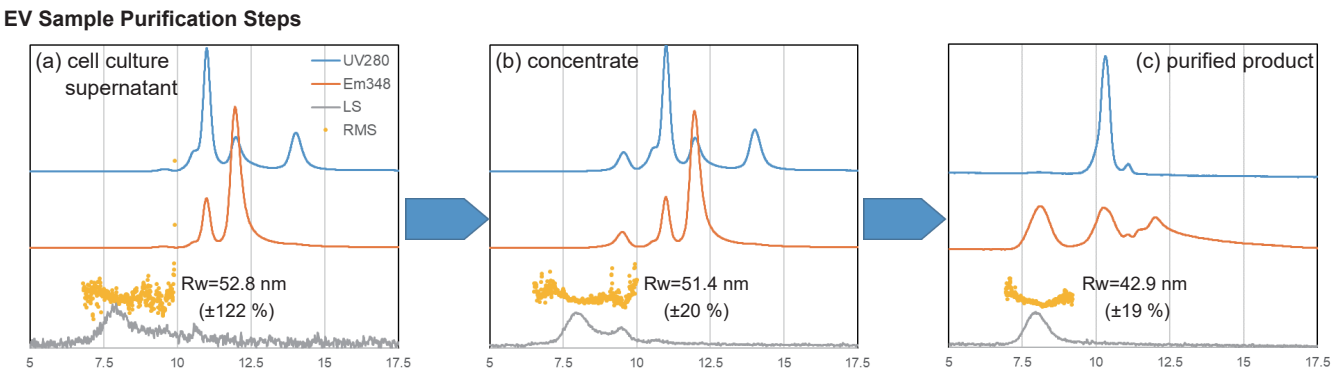
Based on the concept of Quality by Design (QbD), quality control of EV as a "product" may take a form of following combination: multiple layers of design space specified by appropriate parameters at each manufacturing process and quality evaluation by multi-faceted analysis at key process points. Among cell-originated components, the size of EVs are relatively large with diameters around 100 nm[1]. For the separation and analysis of EVs, their sizes become one key factor and this is where size exclusion chromatography (SEC) contributes. However, the amount of final EV produced is very small and the majority of its component is lipid membrane. This makes it difficult to be detected by a UV detector, which is the most frequently used detector in the bioanalysis area. Moreover, the cultured sample contains a large amount of biological impurities, which has high UV absorbance. And thus, this makes the monitoring and identification of EVs difficult. As mentioned, EVs are relatively large among the cell-originated components, but the cultured samples are also known to contain impurities of other nano-scale size components such as dead-cell genome-originated chromatin aggregates[2]. Thus, separation and monitoring using only size exclusion method will not be sufficient in the process development and quality control.

Shodex offers hydrophilic aqueous polymer-base SEC columns that are suitable for analyzing various biological components. Shodex SEC columns with various pore sizes cover a wide molecular weight range, even the large nanoscale objects including EVs. Especially, SB-806 HQ with its sufficient pore size holds and separates EV-class nanoscale objects (50-200 nm). Combined with a variety of detectors, this is a useful comprehensive analysis tool for a complex bio-nano compounds preparation process. This technical article introduces an example of multi-faceted analysis and monitoring of an EV sample purification process using a SEC column, Shodex OHpak SB-806 HQ, with 3 different detectors.

Methods

The samples analyzed were Sample (a): cell culture supernatant, Sample (b): concentrate, and Sample (c): purified product. Sample (a) was prepared from "Human Mesenchymal Stem Cells from Adipose Tissue (MSC-AT)". MSC-AT was planar cultured, then transferred to a recovery medium. Supernatant was collected after 2 days. The supernatant was filtered to remove cells and used as an injection sample. Sample (a) was then ultra-filtrated (Vivaspin 20 MWCO 100 kDa, Sartorius), and the resulting concentrated fluid was used as Sample (b). Sample (b) was further purified using a commercial EV purification kit (MagCapture Exosome Isolation kit PS, FUJIFILM Wako Pure Chemical Corporation) and used as Sample (c). The kit uses an affinity method which targets EV-specific surface lipids.

The SEC column used was Shodex OHpak SB-806 HQ (8.0 mm I.D. x 300 mm). A combination of three detectors, UV, fluorescence (FL), and Multi Angle Light Scattering (MALS) were used. Each detector was used to monitor different target components based on their response capabilities. The UV detector (280 nm) was used to monitor components of culture origin and general culture-derived impurities. The FL detector (Ex 280 nm/Em 348 nm) was used to monitor proteins (tryptophan residue as an index). The MALS detector was used to monitor larger nano particles in the range of EV size. Also, LS scattering angles provided estimated RMS radius[3].



*Y-axis was normalized for comparison. The RMS radius (Rw) is the average near the peak top.

Column	: Shodex OHpak SB-806 HQ (8.0 mm I.D. x 300 mm)
Fractionation range	: 100,000 - 20,000,000 (pullulan std)
Eluent	: PBS(-)
Flow rate	: 1 mL/min
Column temp.	: 25 ºC
Injection volume	: (a) 50 µL, (b) 50 µL, (c) 15 µL
Detector	: UV Shimadzu SPD-M30 A (280 nm) FL Shimadzu RF-20Axs (Ex 280 nm/Em 348 nm) LS MALS DAWN8+ Wyatt (90° rms radius)

Sample:
(a) Cell culture supernatant
Prepared from Human Mesenchymal Stem Cells from Adipose Tissue
(b) Concentrate
Concentrated Sample (a) using a ultra-filtration
About 40 times concentrated.
(c) Purified product
Purified Sample (b) using a purification kit
About 10 times concentrated.

Cooperation of sample preparation by Showa Denko Materials Co., Ltd.

Fig.1 Multi-detector (UV, FL, MALS) analysis of EV samples at each purification step

Results and Discussion

Figure 1 shows the results of multi-detector analysis. Based on the obtained LS strength and estimated RMS radius (mean: 42.9 nm ± 19 %) of Sample (c), the fraction that most likely include target EV eluted around 8 minutes. On the other hand, UV and FL chromatograms of Samples (a) and (b) did not show any remarkable peaks in the same fraction. This result indicates that UV and FL detectors are not suitable for identifying the fraction which contains the target EV. This is because EV's components are a large lipid membrane portion and a very small cargo portion in which is consisted of proteins and nucleic acids, and thus as overall, EVs have weak UV and FL absorbance. Thus, although UV and FL detectors are important tools for monitoring purification process and presence of impurities, they can lead to a wrong conclusion for the determination of the target fraction to be monitored and collected. This is especially significant at the early stage of the purification process if only focusing on strong UV and FL peaks. On the other hand, an LS has a high detection sensitivity towards nanoparticles including EVs. LS detected components in Sample (a) at the target fraction which were not detected by UV nor FL. As the sample goes through concentration and purification processes using an ultra-filtration and a purification kit, its response to the LS and the RMS precision increased. The peaks in Samples (b) and (c) indicated that the target components have a certain size distribution (RMS plots in Fig.1(b)(c)). Therefore, the method with MALS coupled with the appropriate SEC column can be a strong tool for monitoring EV purification processes especially in where UV and FL have low detection capabilities. The LS pattern obtained (about 9 minutes) showed that EVs as well as concentrated impurities of similar sizes were found in Sample (b). The purification kit effectively reduced those impurities (Fig.1.(c)) and also the analysis result indicated that they had different surface physical characteristics from the target EV. As mentioned earlier, UV and FL are not suitable for monitoring EVs in the early stages of purification. However, they provide important impurity profiling information throughout the purification stages. For example, the fraction with high UV sensitivity but with low FL indicates that the major components present are cell originated nucleic acids and their complexes plus other organic molecules with less proteins (about 11 and 14 minutes). Another example is that in Sample (c), processed with a commercial affinity purification kit, many peaks with different sensitivities were detected by both UV and FL. This indicates the presence of impurities even after the purification step. The analysis results suggest that there are several parameters which can lead to further improvement of product purity and purification efficiency. This includes optimization of extraneous filtration pore size, handling of residual nucleic acid, and an additional purification step using preparative SEC and/or other separation modes.

Conclusions

The role of analysis is not limited to quality control and profiling of final products in the practical production process development of EV which is thought to reflect the QbD concepts strongly. The success of practical development is supported and accelerated in two ways. In the actual manufacturing process, the effects of each unit process operation with different purposes and functions should be comprehensively and easily monitored. While during purification process development, it is favorable to have an appropriate analysis method which leads to process improvement proposals. This improvement is not only in the production process development, but also leads to further advancement of basic research. This helps to establish more efficient sample preparation method, and also contributes to improved reproducibility of every experiment in the field of basic EV evaluation. Overall, said improvements support further advancement of basic research quality in the EV field, and consequent success of its applied research. Therefore, a multi-faceted analysis using combinations of an appropriate SEC column and different detectors is a method that can be considered to contribute promoting the practical EV use in research and development and in production.

References

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