Tangential flow filtration systems based on polymer hollow fibres for pharmaceutical and personal care applications

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Introduction

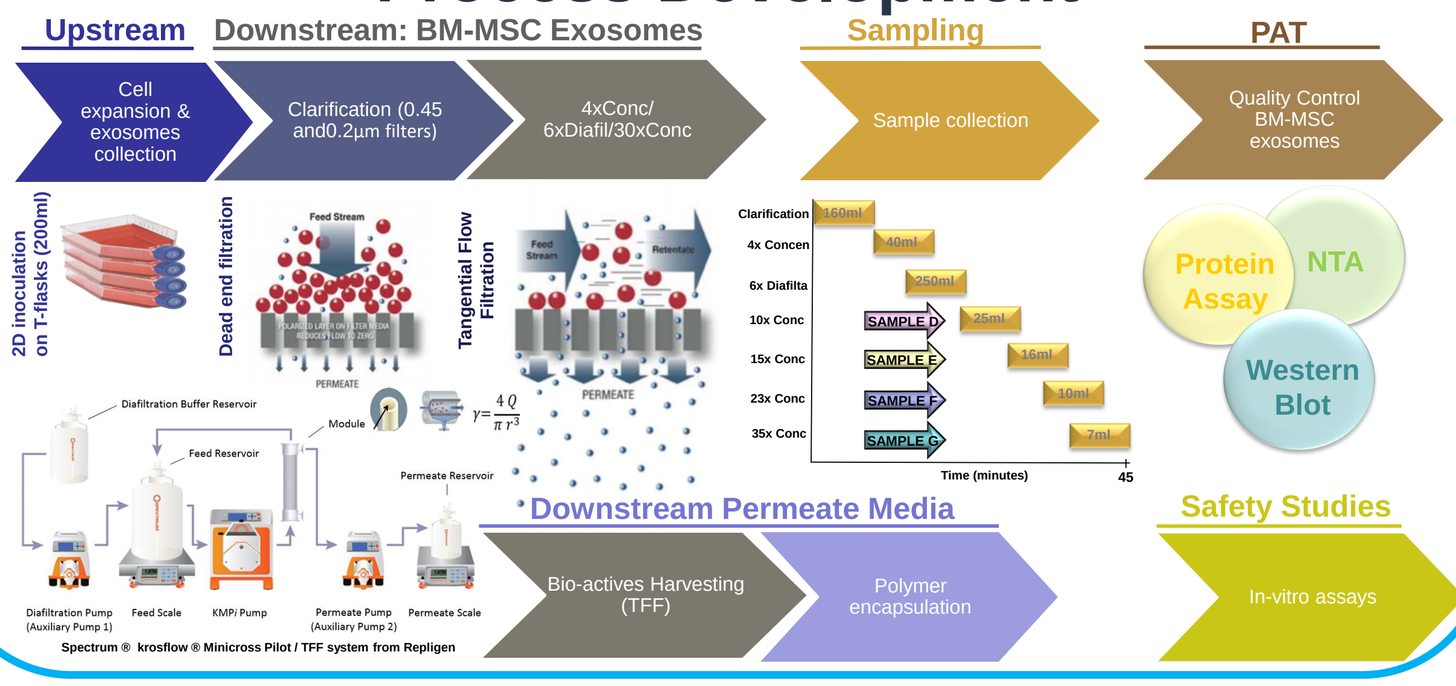
Esco Aster, CDMO organisation focused on **cGMP** compliant up- and downstream manufacturing process development, has incorporated **TFF system** as a first downstream clarification/purification step in different pipelines such as **MSC cells, extracellular vesicles, viruses and polymer-based nanoparticles**.

In this work we evaluate the performance of TFF systems that utilise polymer hollow fibres **(HF)** in the isolation and purification of large volumes of exosomes from bone marrow mesenchymal stem cells **(BM-MSC)** by:

- Controlling shear rates below **3000 s-1**.
- Investigating the optimal concentration factor that allows the highest **yield recovery** of exosomes while preserving their biological activity.
- Additionally we propose a cGMP compliance strategy that enables the production of exosomes in concentrations required to achieve biological outcomes **in clinical studies**. .

The **bioactive ingredients** present in the waste media **(permeate media)** are of great interest in other applications. We also propose a strategy to harvest these key components in the media with the use of TFF. The stability of the bioactive substances can be preserved by **encapsulation** with **biocompatible polymers**, which can be then applied in **skincare cosmetics**.

Process Development



Experimental Results: Exosomes Purification

1 Cell Monitoring and Sample Preparation

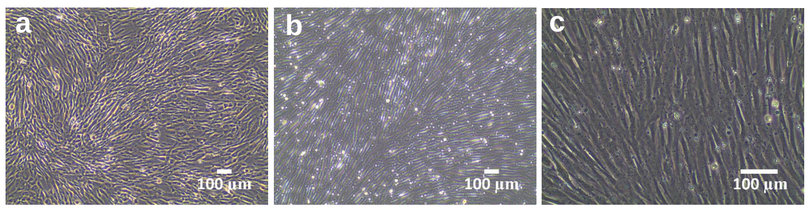


Figure 1. Microscope traces on BM-MSC expansion and conditioning (a) Cell expansion after 3 days of seeding from 2D flask (b, c) Cell expansion in conditioned media at day 6, images were recorded under different magnification. On day 6 the cell culture conditioned media was collected for further downstream processing.

2 Qualitative Analysis: Western Blot

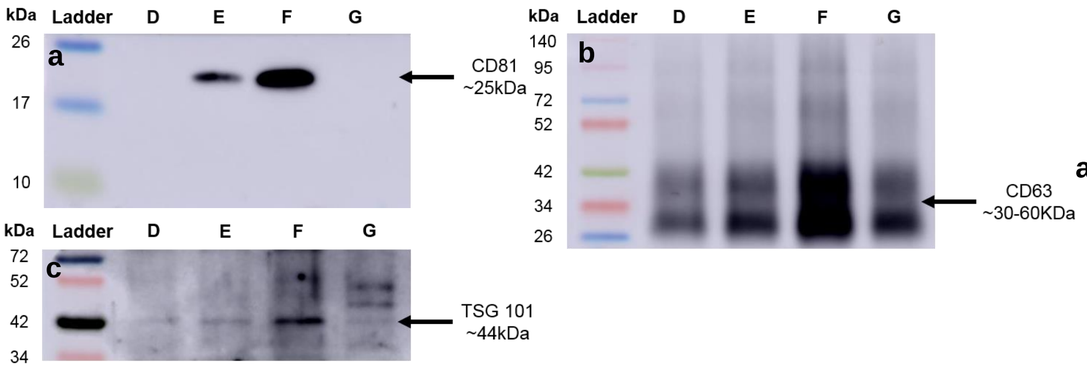
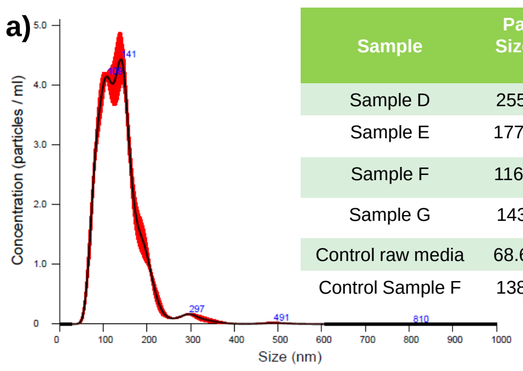


Figure 2. Western blot gel on samples D, E, F, G. Samples were collected at different time points as described in the process development section. Samples were blot using three different exosomes markers (a) CD81 (b) CD63 (c) TSG101.

3 Quantitative Analysis: NTA analysis and Bradford Protein assay

a)



Sample	Particle Size (nm)	Particle Concentration (particle/ml)
Sample D	255.5±27	1.5x 10^{10}
Sample E	177.1±8.3	6x 10^{9}
Sample F	116.6±2.8	2.2x 10^{10}
Sample G	143.6±11	4x 10^{9}
Control raw media	68.6±32.5	3.7x 10^{9}
Control Sample F	138.8±12	5x 10^{9}

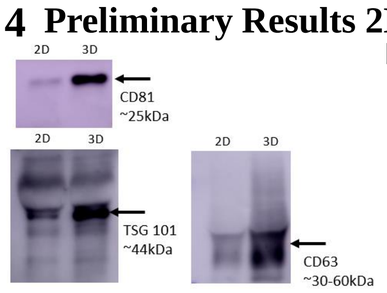
b)

Sample	Concentration (µg/ml)
Sample D	27.1
Sample E	23.1
Sample F	41.1
Sample G	28.6
Control raw media	30.6
Control Sample E	23.1
Control Sample F	26.1

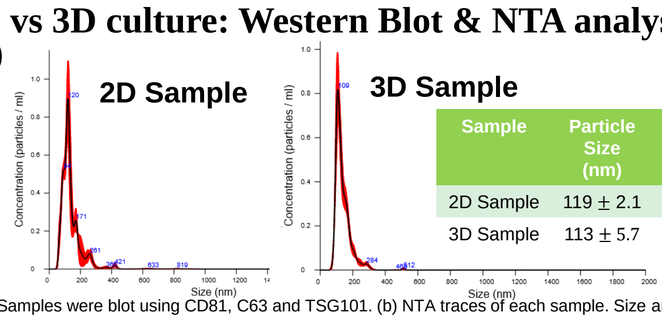
Figure 3. Quantitative analysis (a) NTA trace of sample F represents the particle size (nm) as a function of the particle concentration (particle/ml). Results for samples D, E, F and G as well as the control sample (raw Rooster Bio-media and sample F) have been summarized in the table. (b) Quantitative analysis of protein concentration in each sample. Samples were analyzed by using Bradford protein assay.

4 Preliminary Results 2D vs 3D culture: Western Blot & NTA analysis

a)



b)



Sample	Particle Size (nm)	Particle Concentration (particle/ml)
2D Sample	119 ± 2.1	1.28x 10^{10}
3D Sample	113 ± 5.7	1.36x 10^{12}

Figure 4. (a) Western blot gel on samples 2D and 3D. Samples were blot using CD81, C63 and TSG101. (b) NTA traces of each sample. Size and samples concentrations are summarized in the table.

Experimental Results: Permeate Media for Personal Care

1 Stability studies on polymer nanoparticle components

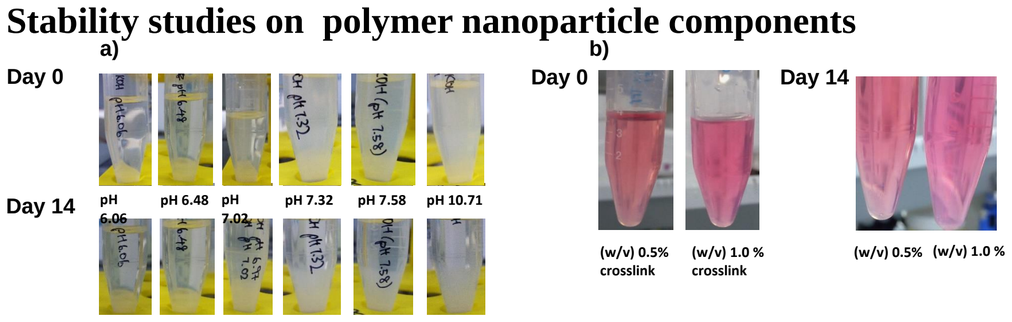


Figure 5. Stability studies (a) Monomer solubility at different pH and stability studies as a function of time (b) The images show the compatibility of different concentrations of crosslinker; 0.5% and 1.0% crosslinker, with the waste media and stability studies as a function of time

2 Quantitative analysis of blank polymer nanoparticles: NTA analysis

Conc of polymer (%w/v)	Conc of crosslinking (%w/v)	Particle size (nm)	Span (nm)	Particle/mL
0.05	0.100	166.3	10.0	1.72 x 10^{9}
0.05	0.200	207.3	12.1	7.26 x 10^{8}

Conclusion

1) BM-MSC exosomes

TFF systems based on polymer HF allow purification and volume reductions of exosomes from 200ml to few ml. However concentration factors higher than 20 times showed a significant decreased on the vesicles recovery.

To overcome this potential limitation, we propose the production of exosomes using three-dimensional cell culture approach. Our preliminary results comparing 2D vs 3D exosomes production followed by a TFF purification of small sample volumes (10ml to 1ml) seems a promising cGMP strategy for the production of high concentration of exosomes: 1x 10^{12} particles/ml.

2) Permeate media and polymer compatibility

We have selected a monomer and crosslinker:

- Able to form monodispersed polymer particles below 200nm in aqueous solutions.
- Compatible and stable with the permeate media. Our stability studies indicate the solubility of the monomer at pH below 6.5 as well as different concentrations of crosslinker to be compatibility with the media.

Strategy cGMP compliance

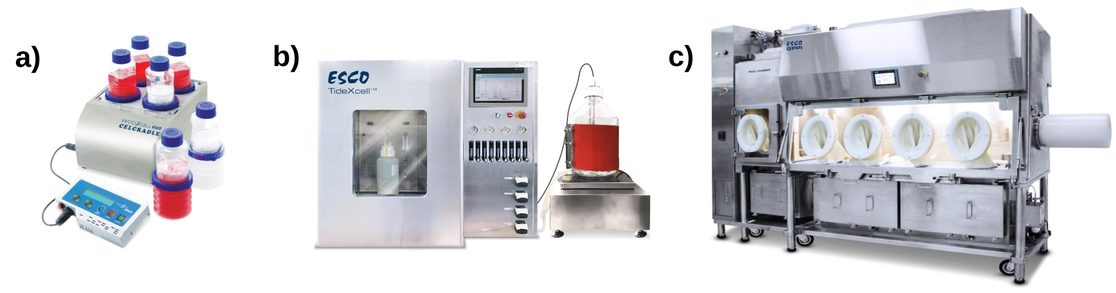


Figure 6. a) Celcradle™ , (b) TideXcell™ bioreactors and commercial TFF system (scheme in the process development section) can be integrated with (c) cell processing isolators.

References

[1] *Molecular Therapy* **Vol. 26** No 12 December 2018.

[2] Cells 2018, 7, 273; doi:10.3390/cells7120273

[3] Juergen Moll and Sebastian Carotta (eds.), Target Identification and Validation in Drug Discovery: Methods and Protocols, Methods in Molecular Biology, vol. 1953, https://doi.org/10.1007/978-1-4939-9145-7_18, © Springer Science+Business Media, LLC, part of Springer Nature 2019 .

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