Monolith-like Particle (MLP) for Next-Generation Virus Purification -Full/empty AAV Capsids Separation and Vaccine Production-

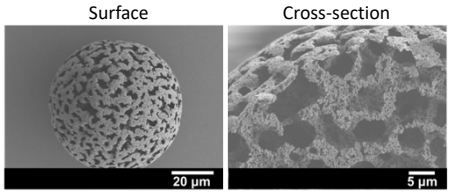
Eri Iwamoto[1], Kenji Kadoi[1], Junya Toba[1], Malcolm G. Pluskal[2], Tsuyoshi Nakama[1] and Kazuhiro Takizawa[1]
([1]JNC Corporation, Yokohama R&D Center, Kanagawa, Japan and [2]JNC America, Cellufine Application Lab, Leominster, MA)

Abstract

JNC has developed a novel cellulose-based monolith-like particle (MLP) with large through-pores. The MLP is an innovative bead architecture with high industrial applicability, optimized for the efficient separation of large targets such as viral particles. Structural analysis revealed that the MLP possesses a unique architecture with large through-pores, enabling easy access for large targets to reach the intraparticle chromatographic surface. This poster will provide detailed insights into the development of the MLP and explore their potential applications in biomolecule and virus purification processes.

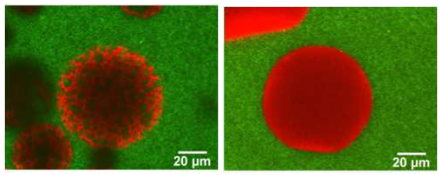
Introduction of Cellufine MLP

Fig. 1. SEM Morphology of Cellufine MLP.

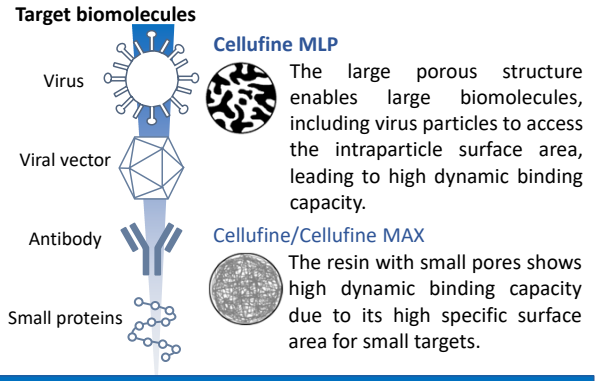


The through-pores of MLP were estimated to have a mode pore radius of 1.5 μm by using mercury porosimetry [1]. The average particle diameter of MLP is about 90 μm.

Fig. 2. CLSM observation of Cellufine MLP

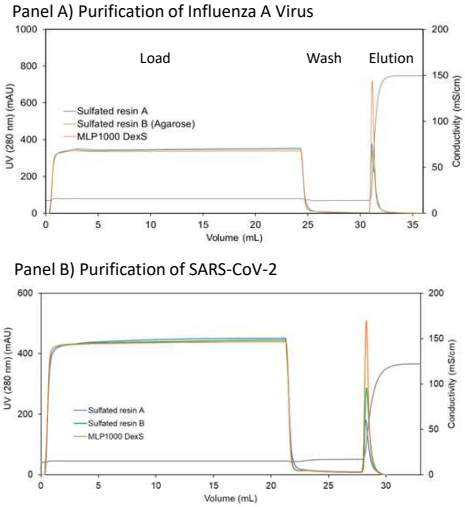


Green fluorescent nanoparticles (100 nm) can diffuse into the intraparticle area of Cellufine MLP.



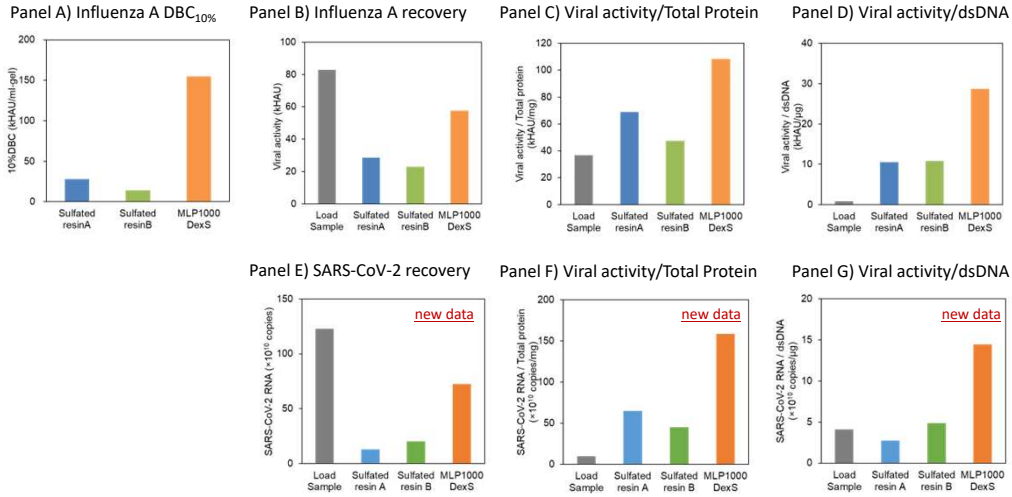
Cellufine MLP1000 DexS – *Pseudo Affinity Bind and Elute Purification of Influenza A virus and SARS-CoV-2*

Fig. 3. Bind and Elution Chromatogram of virus purification.



Column Vol : ID 5 mm × 1.5 cm L (CV=0.29 mL)
Flow rate : 0.5 mL/min
Load Sample : Panel A ; Influenza A virus (H1N1) from MDCK cells
: Panel B ; SARS-CoV-2 XBB

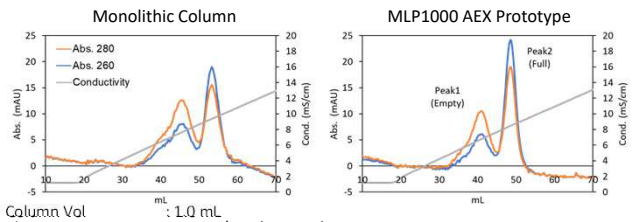
Fig. 4. Results of purification process - Virus recovery and contaminants removal



- ✓ **MLP1000 DexS showed high dynamic binding capacity ($DBC_{10\%}$) for influenza A virus and SARS-CoV-2.**
- ✓ **The purification efficiency leading to removal of contaminants by the MLP1000 DexS in bind and elute mode is higher than that of conventional sulfated resins.**

Cellufine MLP1000 AEX resin for AAV empty/full particle – *Comparison with benchmarks*

Figure 5. Comparison of chromatograms between MLP1000 AEX prototype and benchmarks



Column Vol : 1.0 mL
Flow rate : 1.0 mL/min (RT 1min)
Load sample : AFF purified AAV2: 1.06 x 10^{12} vg
Equilibration buffer : 50 mM Tris-HCl, pH 9.0 + 2 mM MgCl2
Elution buffer (B) : 50 mM Tris-HCl + 150 mM NaCl + 2 mM MgCl2, pH9.0

Table 1. Comparison of quantitative results

		260/280			
	*Full (%)	Peak1	Peak2 (peak top)	Peak2 (Peak area)	Resolution
Before purified	15.9	-	-	-	-
Commercial AEX	-	0.60	1.20	-	-
Monolithic Column	42.8	0.64	1.22	1.19	0.83
MLP1000 AEX Prototype	70.6	0.58	1.27	1.24	1.09

*Determined by Mass Photometry

- ✓ **The MLP1000 AEX showed superior separation of empty and full AAV2 particles compared to benchmarks.**

This research was supported by AMED under Grant Number JP18ae0201001

Conclusion

- ✓ **A novel cross-linked Cellufine MLP beads with large pore structure allowing large biomolecules to access the intraparticle area have been developed.**
- ✓ **MLP1000 DexS exhibits a high dynamic binding capacity and high purification efficiency for whole virus particles.**
- ✓ **MLP based AEX resin demonstrated high capacity and superior separation compared to benchmark.**

References

[1] K. Kadoi, E. Iwamoto, T. Nakama, Fabrication and characterization of a cellulose monolith-like particle for virus purification, *Biochem Eng J.* 192 (2023) 108849.
[2] K. Kadoi, J. Toba, A. Uehara, N. Isoda, Y. Sakoda & E. Iwamoto, Enhanced sulfate pseudo-affinity chromatography using monolith-like particle architecture for purifying SARS-CoV-2, *Vaccine.* 53 (2025) 126951.

Learn about Cellufine™ technologies at https://www.jnc-corp.co.jp/fine/en/cellufine/

Please visit our website