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Application of Cellufine MLP for Viral Vector Purification : Case Studies of AAV and Lentiviral Vectors

Junya Toba [1], Chigusa Mori [1], Malcolm G. Pluskal [2], and Eri Iwamoto [1]
([1] JNC Corporation, Yokohama R&D Center, and [2] JNC America, Cellufine Application Lab, Leominster, MA)

Abstract

JNC has developed a novel cellulose-based Monolith Technology Leveraged 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 viral vector purification processes.

Introduction of Cellufine MLP

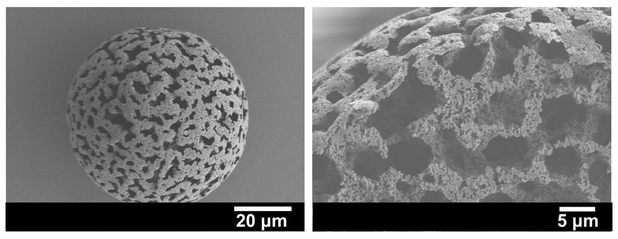


Fig. 1. SEM Morphology of Cellufine MLP

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

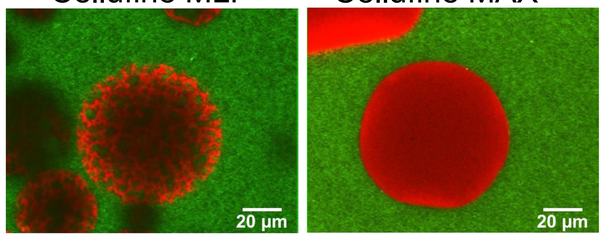
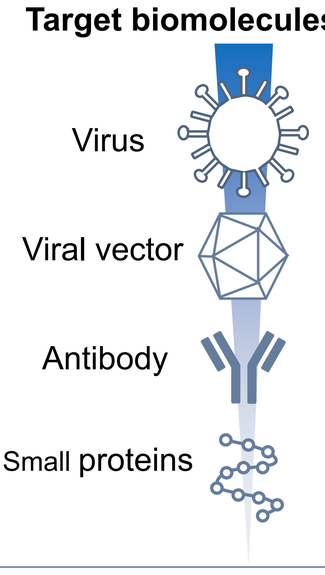


Fig. 2. CLSM observation of Cellufine MLP

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

Target biomolecules



Cellufine MLP

The large porous structure enables large biomolecules, including virus particles to access the intraparticle surface area, leading to high dynamic binding capacity.

Cellufine/Cellufine MAX

The resin with small pores shows high dynamic binding capacity due to its high specific surface area for small targets.

Cellufine MLP AEX for adeno-associated virus (AAV) Empty/Full Separation

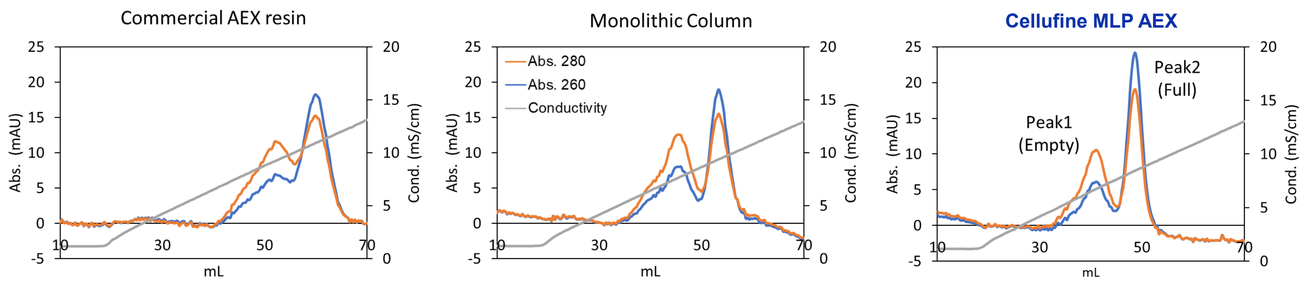


Fig. 3. Comparison of chromatograms from empty/full separation of AAV2

Column	: Super Edge (ID 6.7mm x 30 mm) 1.06 mL
Flow rate	: 1.0 mL/min (RT 1min)
Load	: Affinity 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. Quantitative comparison of AAV2 empty/full separation on different media

		260/280			
	Full*1 (%)	Peak1	Peak2 (peak top)	Peak2 (Peak area)	Resolution
Loading	15.9	-	-	-	-
Commercial AEX	-	0.60	1.20	-	-
Monolithic Column	42.8	0.64	1.22	1.19	0.83
Cellufine MLP AEX	**70.6**	0.58	**1.27**	**1.24**	**1.09**

*1 Determined by Mass Photometry

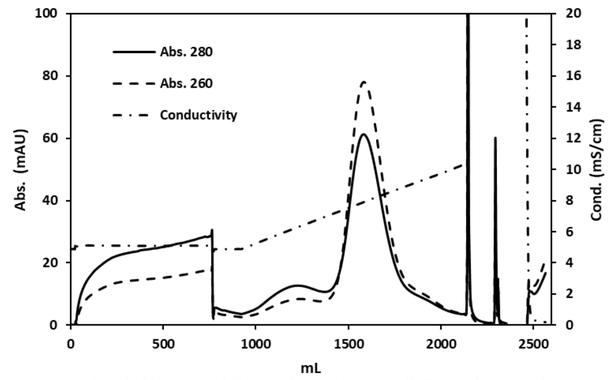


Fig. 4. Chromatogram of AAV2 purification in a 30 mL column scale-up process

Column	: HiScale 26 (ID 2.6 cm x 5.6 cm) 30 mL
Flow Rate	: 7.5 mL/min (RT 4 min, 84.8 cm/h)
Load	: Affnity purified AAV2 (2.08 x 10^{14} vg)
Equilibration buffer	: 50 mM Tris-HCl + 2 mM MgCl2, pH9.0
Elution buffer (B)	: 50 mM Tris-HCl + 90 mM NaCl + 2 mM MgCl2, pH9.0
Elution buffer (EL2)	: 50 mM Tris-HCl + 1.0 M NaCl + 2 mM MgCl2, pH9.0

※All kinds of buffer contain 0.1 % Poloxamer-188 (Pluronic F68)

Table 2. Fraction analysis from AAV2 scale-up purification

	Full particle ratio*2 (%)	Recovery*3 (%)
Loading	19.7	-
Elution	**90.2**	**91.1**

*2 Determined by AUC (analytical ultracentrifugation)
*3 qPCR, correction value based on mass balance

✓ We achieved a full particle ratio of nearly 90% in the scale-up process using Cellufne MLP AEX.

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Cellufine MLP DexS – Pseudo Affinity Bind and Elute Purification of Lentiviral vector

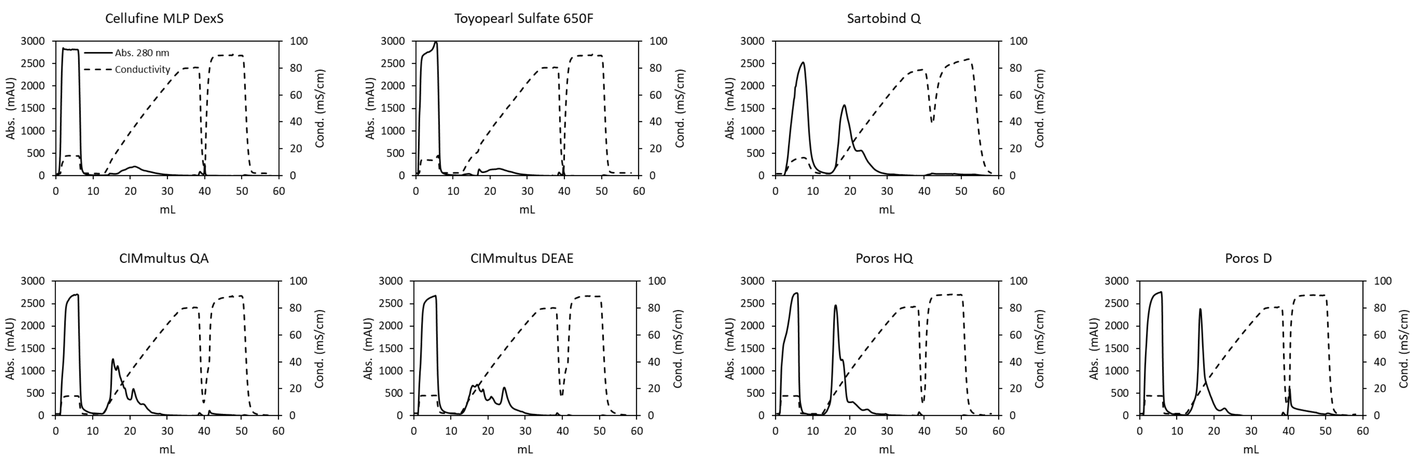

Fig. 5. Chromatograms of lentivirus purification using different chromatography resins

Table 3. Recovery and impurity removal by various chromatography resins

Resin	qPCR Recovery (%)	Protein removal (%)	DNA removal (%)
Cellufine MLP DexS	**85.2**	**93.6**	**73.3**
Toyopearl Sulfate	49.2	94.1	80.3
Sartobind Q	47.2	85.0	64.4
CIMmultus QA	22.4	70.1	58.5
CIMmultus DEAE	19.1	72.6	50.7
Poros HQ	70.7	75.3	56.1
Poros D	7.54	47.6	60.1

Column	: Super Edge (ID 6.7mm x 30 mm) 1.06 mL
Flow rate	: 1.06 mL/min (RT 1min)
Load	: Lentivirus supernatant from HEK293T cell culture
Equilibration buffer	: 20 mM Tris buffer, pH7.5
Elution buffer	: 20 mM Tris buffer, pH7.5 + 1 M NaCl

✓ Cellufne MLP DexS showed high recovery for lentiviral vector and efficient removal of impurities compared to commercially available sulfated resin and AEX resins.

Conclusions

- ✓ We have developed Cellufine MLP beads, a novel cross-linked cellulose resin featuring large pore structure allowing large biomolecules to access the intraparticle area.
- ✓ Cellufine MLP AEX could separates AAV2 empty and full capsids efficiently, achieving > 90% full particle ratio.
- ✓ Cellufine MLP DexS provides superior lentiviral vector recovery and impurity removal compared to existing sulfated resin and AEX resin.

Reference
[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.

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