Development of a Cellulose Monolith-like Particle for Sulfate Pseudo-affinity Chromatography Targeting Virus Purification

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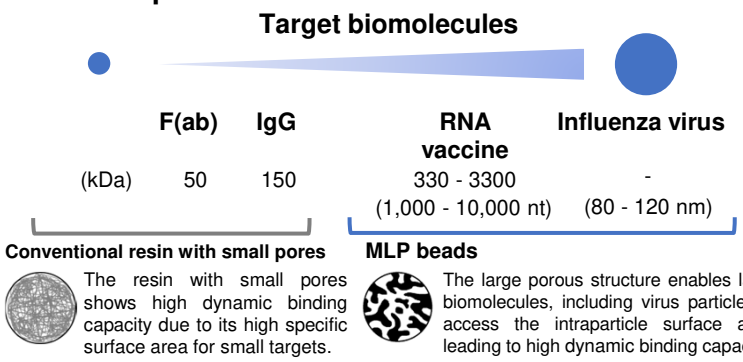
Introduction

Monolith, three-dimensional (3D) porous network-based media, have been shown to be a promising new material for chromatography of large molecules. This poster introduces a novel cellulose monolith-like particle (MLP) with large through pores for whole virus purification. Cellulose resin MLP1000 is a highly crosslinked particle that incorporates a large continuous pore structure optimized for purification of large biomolecules. MLP1000 DexS, which has been modified with a dextran sulfate ligand, exhibits an enhanced viral dynamic binding capacity resulting in high purification efficiency of whole virus particles for vaccine development.

Concept



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

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

Structure of MLP1000 beads

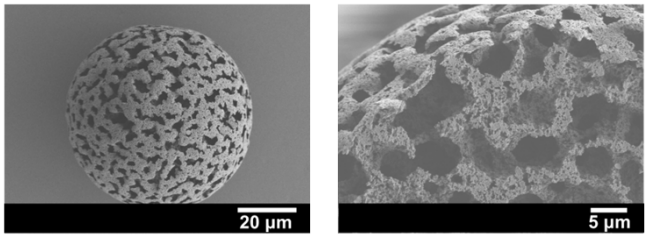


Fig. 1. SEM Morphology of cellulose resin MLP1000 beads

- 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.

ISEC with PEG/PEO and silica nanoparticles

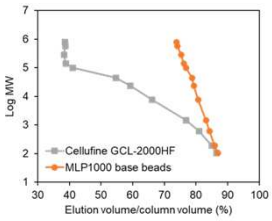
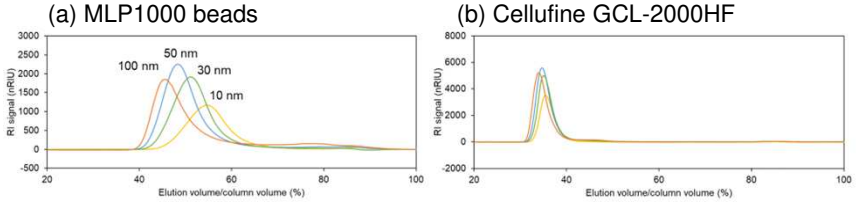


Fig. 2. ISEC with PEG and PEO



Column:
ID 7.8 mm × 30 cm L
Flow velocity:
0.4 mL/min
Mobile phase:
Pure water

Fig. 3. ISEC with silica nanoparticles

- MLP1000 exhibits high porosity, allowing 100 nm silica nanoparticles to access its intraparticle area.

CLSM observation

(a) MLP1000 beads (b) Cellufine™ MAX Phenyl

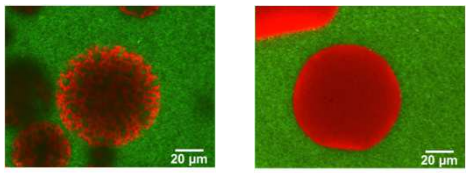
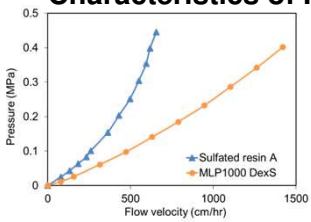


Fig. 4. Internal structure of MLP1000 beads

- Green fluorescent nanoparticles (100 nm) can diffuse into the intraparticle area of MLP1000.

Characteristics of MLP beads

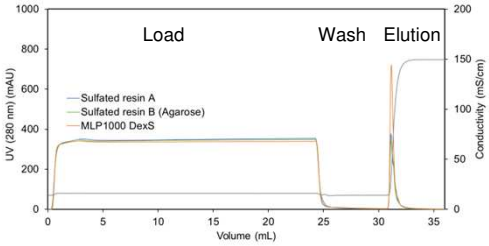


Column:
ID 2.2 cm × 20 cm L
Mobile phase:
Pure water

Fig. 5. Flow Characteristics of MLP1000 DexS

- MLP1000 exhibits high mechanical resistance due to cross-linking reaction.

Virus purification



Column: ID 5 mm × 1.5 cm L (CV=0.29 mL)
Flow velocity: 0.5 mL/min
Load Sample (24 mL): Influenza A virus (H1N1) from MDCK cells.
Wash (6 mL):10 mM Phosphate Buffer + 0.12 M NaCl
Elution (2.4 mL):10 mM Phosphate Buffer+ 2.0 M NaCl

Fig. 6. Pseudo-affinity chromatography for influenza A virus purification

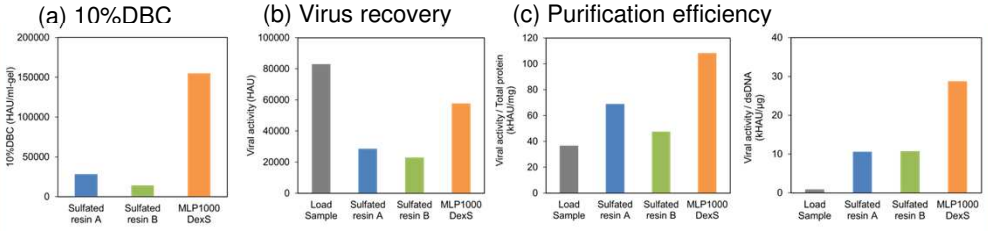


Fig. 7. Results of purification process

- MLP1000 DexS shows high dynamic binding capacity for influenza A virus.
- The purification efficiency of the MLP1000 DexS is higher than that of conventional sulfated resins.

Conclusions

- A novel cellulose resin MLP beads that allow 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.

Please visit our website

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.