Microscale Chromatography Toolkits for Rapid Screening and Purification of Therapeutic Proteins

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American Chemical Society National Conference; Division of Biochemical Technology; Downstream Processing Session, April 2nd, 2019 6pm-8pm

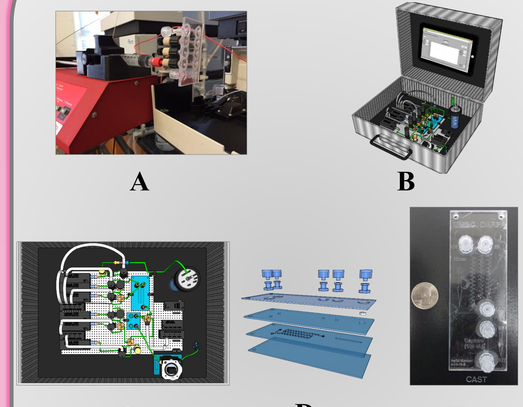
Introduction

We report versatile, customizable, robust, low-cost, and easily manufacturable chromatography micro-columns (μCols) made using thermoplastic solvent bonding and used for rapid screening of therapeutic quality protein purification. We compared granulocyte-colony stimulating factor (GCSF) protein purification, expressed using a cell-free CHO *in-vitro* translation (IVT) system, between a conventional 1mL immobilized metal affinity chromatography (IMAC) column and the fabricated μCols ranging from 25 μL to 200 μL. Experimental data revealed comparable purity with a 10-fold reduction in the amount of buffer, resin, and purification time for the μCols, with an 80% reduction of cost.

Objective

Provide an alternative and innovative solution for quick prototyping of μCols for process development and optimization for affinity-based purification.

Applications



A **B** **C** **D** **E**

Figure 1. A) μCols connected to a conventional HPLC machine. B) Fully customizable μCols are also integratable in next generation portable HPLC machines, highlighting their use for point-of-care therapeutic protein purification. C) Top view of μCols integration into portable HPLC machine. D) Schematic of μCol chip with the integration of a microfluidic mixer. E) Integrated chip with micro-fluidic mixer and μCol.

Methods

Design

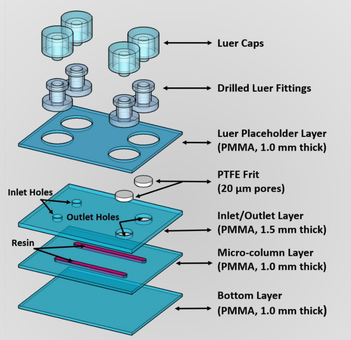


Figure 2. μCols extruded schematic showing four distinct laser-cut PMMA layers, along with luers, and PTFE frit required for a functional μCol.

Manufacturing

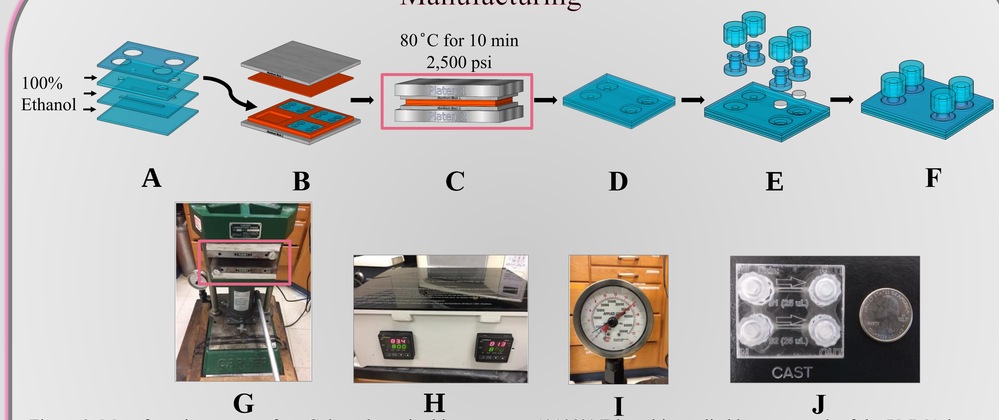


Figure 3. Manufacturing process for μCols and required instruments. A)100% Ethanol is applied between each of the PMMA layers. B)Assembled PMMA layers are then placed in pre-cut silicon rubber layers. Aluminum blocks are placed below and above silicone rubber. C) The assembly is then put between the two heated platens in the carver press at 80˚C , for 10 minutes, with a 2,500 psi applied pressure. D) Bonded PMMA layers. E) 20 μm PTFE frit is placed in the outlet hole and luers are carefully glued using clinical grade cyanoacrylate glue. F) Fully assembled μCols. G) Carver press. H)) Digital Temperature Control Box I) Pressure gage for Carver Press. J) Fully assembled μCols. [1][2]

Resin Loading

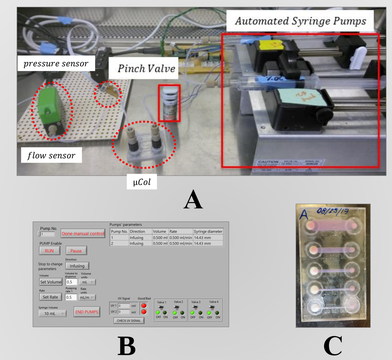


Figure 4. Resin solution is prepared using 1 ml of resin and 20 ml of 20% ethanol. Resin solution is loaded into a 10 ml syringe. A second 10 ml syringe is loaded with 20% ethanol. A) μCol is connected to sensors and loading syringes. B) Resin is loaded using LabVIEW software at 0.5 ml/min. Air bubbles are flushed out using 20% ethanol at 0.5 ml/min. C)Loaded μCols ranging from 200 ul to 25 ul.

Results

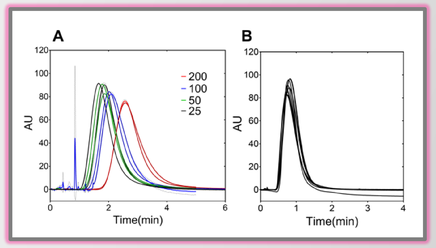


Figure 5. Acetone injections for column validations. A) Acetone injections performed on each of four different volume (25–200 μl) columns where the flow rate was 0.2 ml/min. B) Acetone injections performed on five different 100 μl column where the flow rate was 0.5 ml/min. These experiments demonstrate the manufacturing consistency across tested columns. [1]

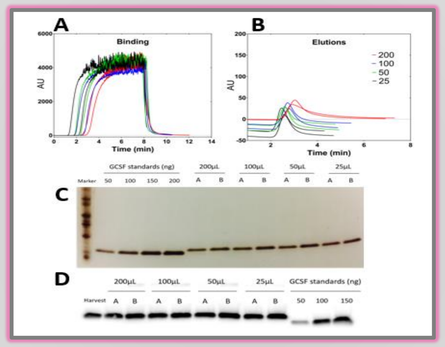


Figure 6. Binding and elution profiles for G-CSF purification on multivolume arrayed μCol device. A) UV effluent profile for binding step where 0.3 ml harvest is used. B) UV effluent profile for elution step. C) Silver-stained SDS-PAGE gels of collected fractions. D) Western blots of harvest and collected fractions. [1]

Table 1. Comparison of μCols and conventional 1 ml IMAC columns. [1]

Column Conditions	μCol (volumes 25-200 μl)	Thermo Column
Binding Capacity	~1 mg	~10 mg
Volume (ml)	0.025-0.1	1
Wash Buffer (15 CV wash; ml)	0.38-1.5	15
Wash Buffer 2 (10 CV wash; ml)	0.25-1	10
Eluted volume (ml)	0.25-1	2.5
Total Purification Time	10-20 min	2 hr
Purity of Eluted Protein	93.4 ± 1.4	≥ 90
Theoretical Plates (for flow rates between 0.1-0.5 ml/min)	31.5 ± 12.6	~ 50
Asymmetry Factor (for flow rates between 0.1-0.5 ml/min)	1.5 ± 0.1	0.88
Manufacturer	CAST, UMBC	Pierce-Thermo Fisher Scientific
Cost of each device	$5-15	$30-50

Conclusions

The reported μCols are easily customizable, robust, low-cost, easily manufacturable, and offer comparable protein separation to conventional columns. Experimental data revealed comparable purity with a 10-fold reduction in the amount of buffer, resin, and purification time for the μCols, with an 80% reduction of cost. They are compatible with most HPLC systems, as well as future generations of miniaturized HPLC systems. Besides protein capture with affinity resins, these devices can be adapted for other biomolecular separation systems such as ion-exchange, size-exclusion and buffer-exchange chromatography by choosing the appropriate resin, column design, and column volume.

Acknowledgments

We would like to acknowledge DARPA, Biologically-derived Medicines on Demand (Bio-Mod) Project Grant (N66001-13-C-4023) and UMBC Technology Catalyst fund (113-021-10622-TCF17ABH) for the financial support without which this work would not have been possible. We would also like to thank Dr. Xudong Ge, Rajani Adiga, Mike Tolosa, and Vandan Thakkar for their assistance and technical support through the course of project.

References

[1] Andar, Abhay U., et al. "Low-Cost Customizable Microscale Toolkit for Rapid Screening and Purification of Therapeutic Proteins." *Biotechnology and Bioengineering*, John Wiley & Sons, Ltd, 31 Dec. 2018,

[2] Al-Adhami, M., Andar, A., Tan, E., Rao, G. & Kostov, Y. A solvent-based method to fabricate PMMA microfluidic devices. Chips tips RSC Nov, Published online (2017).