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Forum

Additive Manufacturing Tools to Improve the Performance of Chromatographic Approaches

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Chromatography is widely applied industrially. However, some limitations are associated with its common supports, and the impossibility to fully control their structural features is particularly restrictive. Additive manufacturing (AM) is emerging as a fast, highly precise, and reproducible technology for producing chromatographic supports that can improve its performance.

Current Trends on the Chromatography Field

Chromatography plays a crucial role in several industries, and the chromatographic market is expected to grow by 6.4% over the next 5 years, reaching US$1670 million in 2024 (https://www.decisiondatabases.com/ip/38033-chromatography-resin-market-analysis-report).

The most common chromatographic supports are based on microparticulated materials that have a randomly compacted configuration. Monolithic supports are an alternative, but the internal structure of these supports cannot be fully controlled either. Each column has a slightly different internal morphology and structure, which makes its chromatographic performance impossible to predict [1]. This limitation on strictly controlling the morphology and porosity of traditionally manufactured materials can result in low reproducibility, so each column usually needs to be prepared and validated individually (Box 1). Additionally, since the performance of chromatographic separations and the column efficiency depend on several factors, including the flow of the mobile phase within the column, axial dispersion, and peak widening (resulting in low degrees of purity), recent studies showed that ordered media provides significantly improved chromatographic performance [2].

AM technology can better control the geometry of fabricated pieces, and it is being widely explored for different purposes. For chromatographic support production, it is just starting to be applied: the first uses were reported a few years ago for analytical chemistry [3]. Despite this promise of total customized production, AM technologies are still far from becoming the gold standard for producing chromatographic supports, mainly due to constraints regarding the low resolution, which limits its application in this field [4].

This forum article discusses the potential application of AM developments in the chromatography, pointing out its advantages, limitations, and new trends.

AM Technologies to Produce Chromatographic Structures

AM is starting to be used as a highly accurate and reproducible technology for chromatographic support production [5,6]. The pieces produced using AM are representations of computer-aided design (CAD) models and can be reproduced repeatedly and easily. This strategy allows fine control of the size, shape, position, alignment, and configuration of the (external and internal) structure to create complex constructions that are impossible to produce by conventional methods [7]. Computational fluid dynamics (CFD) can predict several parameters of these structures (e.g., flow dispersion, hydrodynamic constants, molecules diffusion, capacity, and others) with good accuracy.

In chromatography, AM allows the production of a more-defined and uniform convective flow path in opposition to the flow in randomly interconnected pores of a conventional chromatographic support. It should be possible to increase the theoretical plate number and the peak capacity as well as decrease the analytical time needed for the separation of small molecules and proteins [3]. Since the printed pieces are real representations of a design file, it is possible to follow step-by-step instructions to produce a piece with desirable, user-defined characteristics to minimise fouling and clogging. Even if the printed pieces do not totally avoid these limitations, the user will be aware of the feasibility of the chromatographic structure and the developer or producer will be able to predict how many runs/cycles can be performed with the chromatographic support, avoiding the loss of time and money trying geometries that will be not suitable for the desirable application. This could then lead to an increase in the economic viability of the chromatographic process applied in the recovery, purification, and quantification of molecules of interest.

Besides these features, 3D-printed columns are an attractive option for purifying more complex structures, such as viruses, as shown by Moleirinho and colleagues. They demonstrated that 3D-printed chromatographic supports allowed the purification of oncolytic adenoviruses, maintaining their size and shape (which is a common limitation in using conventional purification structures) [8].

So far, several AM techniques, as well as different types of materials, have been applied to produce chromatographic supports. Among the most commonly used AM processes, fused deposition modelling, stereolithography, and selective laser melting have already been applied to producing structures with different geometries and morphologies. Electrospinning

Box 1. Limitations of the Most Common Chromatographic Supports

Chromatographic supports (Figure I) in the form of dispersed particles are associated with several drawbacks:

- the packing of the column cannot be controlled, which means that whenever the matrix is changed in the column, it may be necessary to readjust the conditions used in the chromatographic experiments;
- high flows are limited to a few types of supports, where the majority cannot support high pressures, resulting in an increased time for each chromatographic run;
- low surface contact, when compared with other technologies on the market.

Membranes are generally used for filtration processes and are simple to use. Additionally, they present increased productivity compared with chromatographic beads. However, there are also some limitations:

- low yields and purity degrees;
- limitation on temperature control during assays;
- fouling and clogging can easily happen.

Monoliths exhibit high porosity and a structure of interconnected pores/channels. However, some limitations have been identified:

- fouling and clogging can happen, due to the high pressure that can be achieved inside the monoliths;
- impossibility of controlling the temperature at which the chromatographic tests are carried out (they have no recirculating fluid system to maintain the temperature across the chromatographic run);
- limited reproducibility due to the difficulty in fully controlling geometry/morphology of the supports, as they are produced by polymerization techniques.

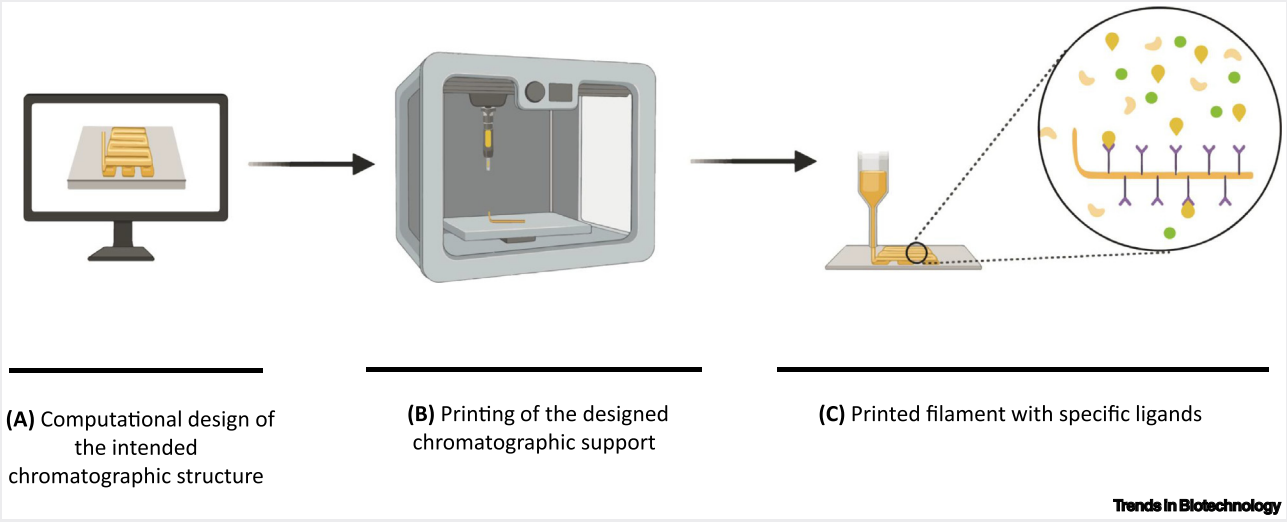

Figure I. Trends in the Production of Chromatographic Supports.

has also been applied, mainly in membrane production, but there is some controversy in describing it as an AM technique. This doubt is mainly due to the difficulty of controlling the deposition of the layers, which is now starting to be possible [1].

The materials used in the production of chromatographic supports can highly influence their properties, robustness, and even the separation performance. The material choice is directly related to the intended printing technology because each piece of AM equipment is limited to printing certain materials. Among the materials already used to produce 3D-printed chromatographic supports are polycaprolactone, poly(methacrylate), and others. Table 1 summarizes the most recent studies on chromatographic support production, listing the printing techniques as well as the main results obtained.

Layer-by-layer methodology can either be used to directly produce chromatographic matrices (as presented in Table 1) or be applied to produce a mould that will originate other chromatographic supports. One US Patent describes a chromatographic structure, whose mould was printed by AM with a very specific geometry (a Schoen gyroid structure). Although only the mould of the structure was produced through 3D printing, the material proved to be quite efficient in separating different biomolecules, compared with a conventional agarose chromatographic column [5].

Table 1. Summary of Chromatographic Support Production Using AM Methodologies[a]

	Additive manufacturing approach	Description	Handicaps	Material	Application	Main results	Refs
Extrusion	FDM	Uses a layer-by-layer deposition technique based on molten polymers extruded through a nozzle with a small orifice, which merges with the material on the previous layer. This technology is cheap and usually user friendly.	- Limitations in geometric complexity when compared for example with SLA or SLS -Low-resolution surface and rough surface finish when compared with other techniques (SLA, DLP, or Polyjet).	Different types of polypropylene	Separation of uracil+phenol+ethylbenzene	The feasibility of performing liquid-chromatographic separations in 3D-printed channels was demonstrated to separate small molecules	[13]
Light assisted	SLA, DLP and plastic jet printing (Polyjet)	Printing methods using light to cure liquid resin layer-by-layer. It allows printing models with good detail (far more than a standard filament printer can produce).	-Smaller build plates than filament (FDM) printers -Usually, a slow process since the produced pieces need to cure	PEG+PEGDA/AETAC	BSA Adsorption of BSA and CPC	Adsorption of 73.7 ± 5.9 mg/mL and 38.0 ± 2.2 mg/mL of BSA and CPC	[6]
				Cellulose	Purification of viral particles	Oncolytic adenoviruses were successfully purified with a recovery yield of 69 ± 6%, while maintaining their size and shape. Additionally, lentiviral vectors had a recovered yield of 57%.	[8]
	SLM	Use high power-density laser to melt and fuse metallic powders. The pieces are built by selectively melting and fusing powders within and between layers. It enables the production of pieces impossible to be produced through other processes.	-Slow production process and extremely expensive when compared with other subtractive processes -Produces a lot of waste -Usually needs post-processing processes	Titanium (Ti-6Al-4V)	Effect of column geometry on the separation of several proteins and enzymes	3D serpentine column provided a 58% reduction in the analysis time and 74% increase in the peak capacity for the isocratic separations of the small molecules and proteins, when compared with a 3D spiral column.	[14]
				Titanium	Protein separation	The monoliths were successfully used to separate a mixture of four intact. Further chromatographic characterisation showed a permeability (Kf) of ~4 × 10^{-15} m^2 and a total porosity of 60%.	[15]

[a]Abbreviations: AETAC, 2-(acryloyloxy)ethyl)trimethylammonium chloride; BSA, bovine serum albumin; CPC, c-phycocyanin; DLP, digital light projection; FDM, fused deposition modelling; PEG, polyethylene glycol; PEGDA, polyethylene glycol diacrylate; SLA, stereolithography; SLM, selective laser melting; SLS, selective laser sintering.

Despite this promising potential and results, AM manufacturing does not *per se* have the ability to structurally adapt the printed filaments/beads to a target sample. Some of the most important parameters in this context are the pore dimension, the volume of the piece produced, the production time, and the ability to print mono- or multimaterial pieces. Some existing companies, such as Nanoscribe, have printers that can create porosities close to what is required for chromatographic applications. However, only some details on the printed structure could achieve nanometer resolution, with micrometre or millimetre scales being more common [9]. Additionally, the maximum printed piece can only reach 100 × 100 × 8 mm^3 and the process takes a long time (~20 h). This printing technology cannot satisfy industrial demands, where the bed volume could reach several litres. Therefore, the maximum printable piece volume possible so

far would not be enough to achieve the desired surface area, and the printing process is too time consuming, which would create problems when scaling up this technology to meet the needs of industry.

In this regard, it is crucial to invest in the development of new printing systems or work on combining existing printing methods. For example, combining electrospinning technology with other AM techniques could help to decrease the dimensions of the pores. Indeed, this combination was already used in the field of tissue engineering [10], where it achieved decreased pore dimension and increased contact surface area for enhanced cell adhesion. Although a different application, what is intended here – increasing the surface area and decreasing the pore volume – is similar and should be achieved by this proposed technology. Additionally, this technology will become more valuable if it can fully control the fibre design instead of only the fibre alignment [11]. This adaptation or combination of printing mechanisms still needs some improvement, especially when industrial applications are envisioned. However, it will be a critical step in the functional and effective applications of AM in the chromatographic world.

Another key factor for using AM in the large-scale production of chromatographic supports could be the possibility to directly immobilise specific ligands onto the support surface during the printing process. This would be an important advance, mainly because the target molecules could be specifically captured, representing a huge gain in the final product recovery and purity. Indeed, the purity of the samples is a key point in the pharmaceutical sector, mainly because impurities present in the final formulations could lead to serious problems in patients' health [12].

Concluding Remarks and Future Perspectives

AM technology is a promising future trend in the chromatography field mainly in predicting the structural configuration expected from a specific geometry of chromatographic support, and in achieving a total reproducibility of the produced structures. Additionally, this production approach will make it possible to develop supports with an increased superficial area of contact and large pores, not only allowing a higher number of binding sites to the desired molecule but also promoting a better flow of molecules within the support. In this regard, innovation in the technologies used to produce supports is critical. AM has the capacity to respond to this challenge and will lead to the production of organized chromatographic supports, with fully controlled geometry that can adapt to the target compound. Considering the translational application and transfer potential of this technology, the successful application of AM to chromatographic support production may result in progress in various industrial sectors, but with especially great impact on the biomedical field in diagnosis, prognosis, and disease therapy.

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Declaration of Interests

The authors declare no conflict of interest.

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References

1. Kalsoom, U. *et al.* (2018) Current and future impact of 3D printing on the separation sciences. *Trends Anal. Chem.* 105, 492–502
2. Schure, M.R. *et al.* (2004) Simulation of ordered packed beds in chromatography. *J. Chromatogr. A* 1031, 79–86
3. Fee, C. *et al.* (2014) 3D printed porous media columns with fine control of column packing morphology. *J. Chromatogr. A* 1333, 18–24
4. Capel, A.J. *et al.* (2018) 3D printing for chemical, pharmaceutical and biological applications. *Nat. Rev. Chem.* 2, 422–436
5. Fee, C.J. *et al.* Separation medium, US 2018 / 0369785 A1.
6. Simon, U. and Dimartino, S. (2019) Direct 3D printing of monolithic ion exchange adsorbers. *J. Chromatogr. A* 1587, 119–128
7. Hearn, M.T.J. (2017) Trends in additive manufacturing of chromatographic and membrane materials. *Curr. Opinion Chem. Eng.* 18, 90–98
8. Moleirinho, M.G. *et al.* (2021) 3D-printed ordered bed structures for chromatographic purification of enveloped and non-enveloped viral particles. *Sep. Purif. Technol.* 254, 117681
9. Fendler, C. *et al.* (2019) Microscaffolds by direct laser writing for neurite guidance leading to tailor-made neuronal networks. *Adv. Biosyst.* 3, 1800329
10. Vyas, C. *et al.* (2020) Three-dimensional printing and electrospinning dual-scale polycaprolactone scaffolds with low-density and oriented fibers to promote cell alignment. *3D Prin. Addit. Manufact.* 7, 105–113
11. Yuan, H. *et al.* (2017) Improving fiber alignment during electrospinning. In *Electrospun Nanofibers*, pp. 125–147, Woodhead Publishing
12. Valente, J.F.A. *et al.* (2018) The biological performance of purified supercoiled p53 plasmid DNA in different cancer cell lines. *Proc. Biochem.* 75, 240–249
13. Abdulhussain, N. *et al.* (2020) Fabrication of polymer monoliths within the confines of non-transparent 3D-printed polymer housings. *J. Chromatogr. A* 1623, 461159
14. Gupta, V. *et al.* (2018) Investigating the effect of column geometry on separation efficiency using 3D printed liquid chromatographic columns containing polymer monolithic phases. *Anal. Chem.* 90, 1186–1194
15. Passamonti, M. *et al.* (2019) Confinement of monolithic stationary phases in targeted regions of 3D-printed titanium devices using thermal polymerization. *Anal. Chem.* 92, 2589–2596