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Sartobind® Rapid A: Comparable High Product Quality as Protein A Resins at High Productivity

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Abstract

The mAb capture step represents the current bottleneck in downstream processing. Protein A resins are diffusion-limited chromatography materials that require low flow rates to achieve a binding capacity above 30 g/L, which results in low productivity. Here, we present a novel chromatography membrane that combines high binding capacities with high flow rates for increased productivity while achieving comparable product quality as state-of-the-art protein A resins.

This novel technology increases productivity 10× compared to resins, which remains consistent during scale-up, and it purifies monoclonal antibodies with 10× less chromatography material used per batch, allowing for full utilization of the membrane within one batch. Plus, as a disposable consumable, it provides the opportunity to remove column handling in bioprocesses and resin reuse over multiple batches.

Introduction

Processes to purify recombinant monoclonal antibodies (mAb) for therapeutic treatment are well established in the market. However, manufacturer's are continuously improving their processes to lower patient risks and treatment costs. Over the past decade, efforts to improve efficiency have focused on upstream processes, resulting in a shift of the bottleneck towards downstream processes [1, 2]. Downstream purification typically relies on three chromatographic steps. Of these, the mAb capture step still depends on protein A resin in a packed bed column format, leading to obvious shortcomings. For example, packed bed columns have high diffusional resistance and long process times. They must also be reused over multiple batches to make them economical, which in turn requires extensive cleaning and validation efforts, as well as tedious column packing [3].

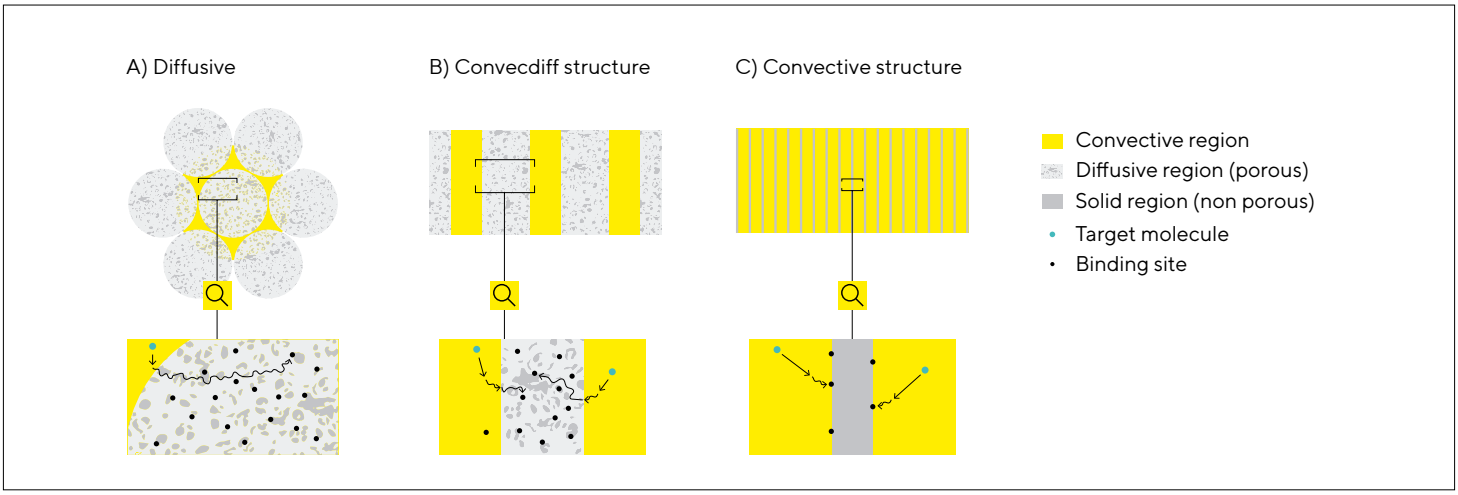
So far, available chromatographic matrices fall into two categories based on their dominant mass transport capabilities. The first category, porous chromatographic resins (i.e., diffusive materials, see figure 1A), has a mass transport dependent on diffusion into the porous structure. Inside of resins, the effective pore diffusion is slow and the distances to be covered are comparatively large (~30 – 50 µm). This leads to process operation at low flow rates and high residence times, resulting in low productivities (10 – 30 g/L×h) [4, 5].

The second category contains purely convective materials such as membranes, fiber beds, or monoliths (see figure 1C). In these materials, the binding sites reside at the surface of the convective pores, so the predominant transport mechanism is based on convection. This structure supports good accessibility of the ligand, but a trade-off between binding capacity and pressure drop occurs because both values are linked to the pore size but in an inverse manner. Pore sizes that ensure acceptable binding capacities for a protein-A-functionalized material (> 30 g/L) are in the sub-micron range (e.g., 0.3 µm). This forces the user to either accept high pressure drop, increased fouling propensity, or low binding capacity [5].

Researchers are making considerable efforts to identify alternative chromatographic materials that overcome these challenges while providing comparable product quality and support therapeutics that are affordable to more people [6]. A new generation of chromatographic materials is emerging that combines the structural and performance aspects of resins with the benefits of purely convective materials. This new convecdiff membrane contains a high binding gel phase with a short diffusional path length (2-3 µm) and large convective pores for fast transport to the gel phase (see figure 1B). This combination offers robust and scalable high binding capacities at short residence times. In addition, the large convective pore sizes ensure low fouling propensity, easy cleanability, and high permeability, allowing for bed heights of about 4 mm with low pressure drops [5].

In this application note, we compare the critical quality attributes (CQAs) of mAb purified using the convecdiff Sartobind® Rapid A membrane with the CQAs of mAb purified using state-of-the-art resin, proving that both materials show the same good product quality.

Figure 1: *(A) Diffusive, (B) Convecdiff and (C) Convective Chromatography Material Characteristics.*



Materials and Methods

Buffers, Reagents and Monoclonal Antibodies

Chemicals used for buffer preparation were purchased from Carl Roth (Karlsruhe, Germany), with buffer constitutions listed in Table 1. Buffers and recipes used in this study are subject of internal platform approach.

Table 1: *Buffers Utilized for the Chromatographic Experiments.*

Buffer	Phase	Ingredients	pH
PBS	(Re-)Equilibration, Wash, HPLC Mobile Phase	1 × PBS[5]	7.4 ± 0.2
Elution-buffer	Elution	0.1 M acetic acid, 150 mM NaCl	2.9 ± 0.1
Reg-buffer	Regeneration | Cleaning	0.2 M NaOH	> 12.5

The recombinant human monoclonal antibody was expressed in Chinese hamster ovary (CHO) cells using standard cell culture techniques (stirred bioreactor). The cultivations were done in Sartorius 5 L Biostat® reactors in batch mode for 14 days. Cell clarification was performed in a two-step depth filtration using Sartorius Sartoclear® DL20 and DL60 with subsequent sterile filtration using Sartorius Sartopore® 2 XLG. Table 2 summarizes the characteristics of the antibody used in this work.

Table 2: *Monoclonal Antibody Properties.*

Molecule	Class	pI	MW [kDa]
mAb1	Antibody IgG1	8.36	145.41

Protein A Chromatography Devices

Protein A chromatographic devices used were novel Sartorius Sartobind® Rapid A membrane devices with membrane volumes (MV) of 1.2 mL as well as a HiTrap® MabSelect SuRe™ column from Cytiva (Uppsala, Sweden) with a column volume (CV) of 1 mL (comparison resin adsorber).

Protein Concentration and Monomer Determination by Size Exclusion HPLC

Protein concentrations and monomer | aggregate levels of HCCF and purified samples were determined by analytical high-performance size exclusion chromatography (SEC-HPLC) using a TSKgel® G3000SWXL-column (30 mm ID × 7.8 cm) from Tosoh (Griesheim, Germany) with an UltiMate™ 3000 HPLC System from Thermo Fisher Scientific (Dreieich, Germany). The HPLC system was operated at 1 mL/min with PBS as mobile phase applying 10 µL of sample. The elution profile was monitored at λ = 280 nm using the system's spectrophotometer. Elution peak area was converted to protein concentration using a standard curve generated with purified material. Aggregate levels were determined as a ratio of peak areas of the early-eluting aggregate peak(s), late-eluting fragment peak(s), and the monomer peak.

Dynamic Binding Capacity Measurements

DBC is defined as maximum amount of target protein that can be loaded onto a stationary phase without causing unnecessary loss, measured under realistic experimental conditions. Dynamic binding capacity (g of mAb per L of membrane | resin) was determined for chromatographic devices (see previous chapter: Protein A Chromatography Devices) using an ÄKTA™ Avant 150.

$DBC_{10\%}$, is defined as the amount of protein loaded at 10% breakthrough (g of mAb per L of membrane | resin) and was determined as follows. Purified protein load was adjusted to pH 7.0 ± 0.2. The device was equilibrated and then loaded with protein (feed concentration: C_{Feed} ~1.0 g/L) until the stationary phase was saturated. The exact protein concentration of the feed was determined by offline A280 measurement using the Unchained Labs Little Lunatic (Pleasanton, USA).

$$(1)\ DBC_{10\%} = \frac{(V_{10\%} - V_0) \times C_0}{V_{column\ |\ membrane}}$$

Where $V_{10\%}$ is the volume at which 10% breakthrough was observed, V_0= system void volume (L), C_0 is the mAb concentration (g/L) and $V_{column\ |\ membrane}$ is the volume of the resin respectively of the membrane in the chromatographic devices. The breakthrough was determined at a residence time of 12 seconds for Sartobind® Rapid A and 4 minutes for HiTrap® MabSelect SuRe™.

Determination of Productivity

The productivity of the utilized chromatography devices, as well as of the purification of the different mAbs, was calculated according to:

$$(2)\ PR = \frac{m_{mAb}}{V_{column\,|\,membrane} \times t_c}$$

where PR [g/L_{MV} × h] is the productivity, m_{mAb} [g] is the average eluted mass of monoclonal antibody, $V_{column\,|\,membrane}$ [L] is the volume of the resin | membrane in the chromatographic devices and t_c [h] is the average cycle time over the whole process.

Protein A Capture Chromatography From Harvested Cell Culture Fluid

Capture of monoclonal antibodies from harvested cell culture fluid (HCCF) was conducted with Sartobind® Rapid A membrane with a membrane volume of 1.2 mL and Cytiva HiTrap® MabSelect SuRe™ (see previous chapter: Protein A Chromatography Devices) as comparison with regard to different critical process parameters (CPP) for one mAb using an ÄKTA™ Avant 150 chromatography system from Cytiva (Uppsala, Sweden). Chromatography was performed with buffers and chromatography recipes mentioned in Table 1 and Table 3.

Table 3: *Chromatography Recipes of mAb Capture With Protein a Membrane Adsorber Versus Resin*

	Sartobind® Rapid A		Protein A Resin	
Phase	V [MV]	Flowrate [MV/min]	V [CV]	Flowrate [CV/min]
Equilibration	5	10	5	0.5
Load [g/L]	34.4	5	24.3	0.3
Wash	12	10	6	0.5
Elution	12[1]	5	12[1]	0.5
Regeneration	9 – 10[2]	5	2	0.2
Re-Equilibration	15 – 16[3]	10	6	0.5
Avg. Cycle Time [min]	–	9.6	–	100.4

[1] Fractionation of elution peak from 100 – 100 mAU at λ = 280 nm
[2] Hold until pH ≥ 12.3, then 4 MV
[3] Hold until pH ≤ 7.5, then cycle ends

The load was calculated as 80% of the $DBC_{10\%}$ measured at a residence time of 12 seconds for the membrane or respectively as 80% of the $DBC_{10\%}$ at 4 minutes residence time for the resin depending on the HCCF. The load density was chosen conservatively to achieve the desired number of cycles without product loss. Elution pools were collected from 100 – 100 mAU (using ÄKTA™ spectrophotometer with a 2 mm path length at λ = 280 nm). Step yield was determined using mass of product in the load and pool (both determined by SE-HPLC).

Host Cell Protein, hcDNA and Leached Protein A Measurements

Host cell protein (HCP) concentrations were measured using the CHO HCP ELISA Kit3G F550-1 Kit from Cygnus Technologies (Southport, USA). Host cell DNA concentrations have been measured using the Quant-iT PicoGreen dsDNA Assay Kit from Thermo Fisher Scientific (Dreieich, Germany). The log-reduction-value (LRV) of both impurities has been determined by means of the decadic logarithm of the quotient of impurity concentration in the feed and the impurity concentration in the elution fraction. Leached protein A has been measured using the Protein A ELISA Kit (9000-1) from Repligen (Waltham, USA). The values listed refer to ng protein A per mg mAb. All assays have been performed according to the manufacturer's instructions and analyzed in an Infinite M Nano+ plate reader from Tecan (Maennedorf, Switzerland). For each HCCF used in this study, every 10th elution fraction was collected and analyzed regarding different CQAs (critical quality attributes) and CPPs.

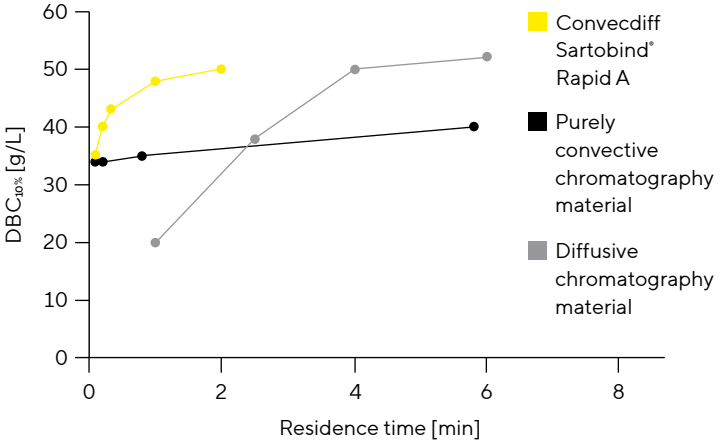
Results

Comparability of Product Quality Attributes and Critical Process Parameters After Purification Using the Convecdiff Membrane and a Standard Resin

First, the dynamic binding capacity of Sartobind® Rapid A was experimentally determined at varying residence times (by varying the flow rate) from 12 seconds to 2 minutes (see Figure 2). This was compared with $DBC_{10\%}$ data for a purely convective material and a diffusive chromatography material [5, 7, 8]. As shown in Figure 2, similar to diffusion-limited resins, the convecdiff Sartobind® Rapid A membrane also showed an increase in $DBC_{10\%}$ when residence time increased. A dynamic binding capacity of 50.1 g/L at 2 minutes residence time and 35.2 g/L at 0.1 minutes residence time respectively is reached by this prototype. At about 1–2 minutes residence time, the plateau of max. $DBC_{10\%}$ is achieved for the convecdiff membrane, while resins reach the plateau around 4–6 min of residence time. The purely convecdiff material showed a very limited dependence of residence time on $DBC_{10\%}$.

As expected, the purely convective chromatography material, like a fiber material without diffusive regions, is not limited by diffusion and therefore showed a very limited dependence of $DBC_{10\%}$ on residence time. The convecdiff and diffusive materials have diffusive regions and therefore exhibit a residence-time-dependent binding capacity. This is caused by a mass transport based on diffusion, which is a slow process and is dominant especially inside of the material; the process increases by lowering the residence time or increasing the diffusive distance (particle size) [6].

Figure 2: *$DBC_{10\%}$ As a Function of Residence Time for Commercially Available Materials and the Convecdiff Sartobind® Rapid A.*



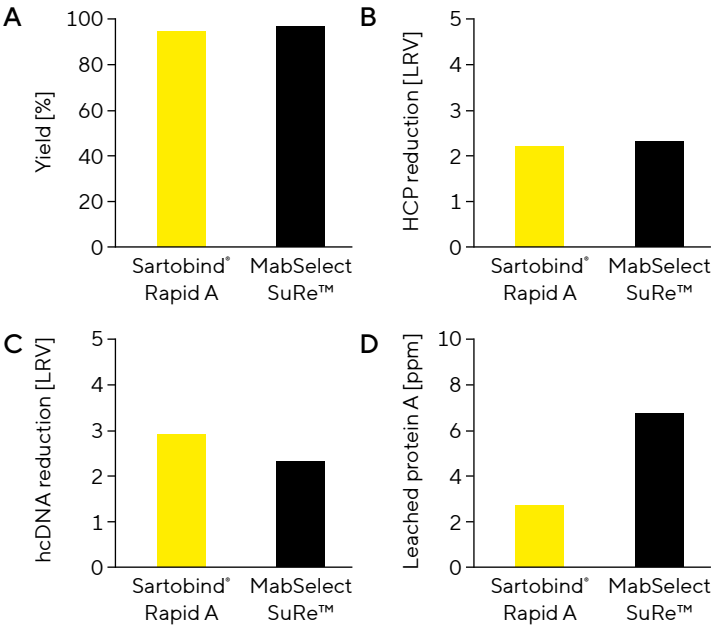
Note. Data adapted from [5, 7, 8]

We further compared CPPs and CQAs of the same antibody, after purification with Sartobind® Rapid A and MabSelect SuRe™ using standard protocols. For the resin, the recommended protocol from the manufacturer was chosen (Table 3).

Table 4: *CPPs and CQAs of mAb 1 Purified With Convecdiff Membrane and Standard Resin.*

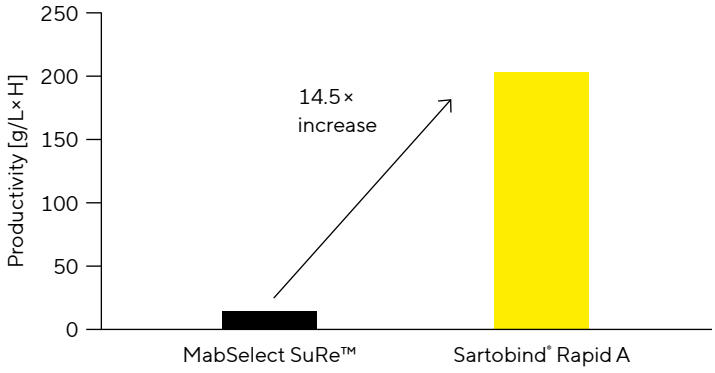
	Sartobind® Rapid A	MabSelect SuRe
$DBC_{10\%}$ [g/L]	42.9 ± 0.8	30.4 ± 0.5
Residence time[min]	0.2	4.0
Yield [%]	94.7 ± 0.2	96.4 ± 0.4
HCP reduction [LRV]	2.2 ± 0.2	2.3 ± 0.1
hcDNA reduction [LRV]	2.9 ± 0.2	2.3 ± 0.1
Protein A leached [ppm]	2.7 ± 0.7	6.7 ± 0.3
Avg. productivity [g/L × h]	203.6	14.1

Figure 3: *Comparison of Critical Quality Attributes After Purification With Sartobind® Rapid A and MabSelect SuRe™: A) Yield, B) HCP Removal, C) hcDNA Removal and D) Ligand Leaching*



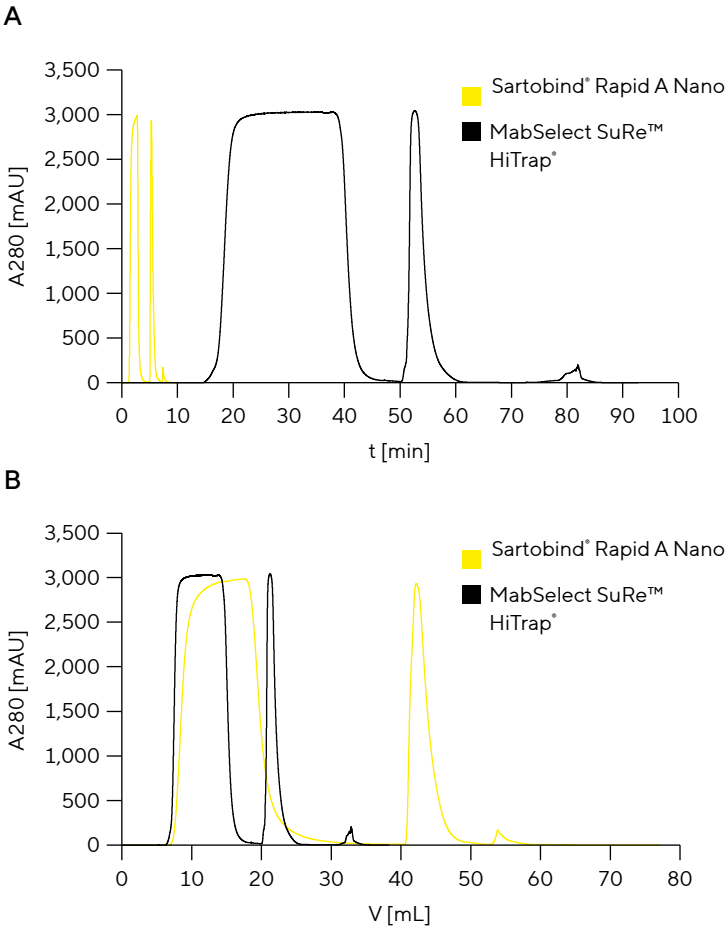
The membrane showed an average $DBC_{10\%}$ of 42.9 g/L at 12 seconds residence time compared to the resin with a $DBC_{10\%}$ of 30.4 g/L at 4 minutes residence time. Based on this first evaluation of the $DBC_{10\%}$ for each chromatographic matrix, the resin | membrane was loaded with 80% of $DBC_{10\%}$ to simulate common practice in the industry. This resulted in a comparable yield for both materials in the range of 94.5 – 96.8% in the elution fraction (see figure 3A). In addition, HCP removal Sartobind® Rapid A for both materials were found to be within a comparable range (see Table 4, figure 3B-D). Slightly higher LRV for hcDNA removal were detected for the membrane (see figure 3C) and less leached protein A ligand was detected from Sartobind® Rapid A compared to MabSelect SuRe™ (see figure 3D).

Figure 4: *Productivity Comparison of HiTrap® MabSelect SuRe™ to Sartobind® Rapid A*



However, a significant difference in the average productivity can be seen in both materials. Here, the membrane shows 14-fold higher productivity compared to the resin (see figure 4). As described above, this high productivity is mainly achieved because the membrane showed a binding capacity in the range of the resin. However, this is achieved with a residence time that is 20 times shorter (higher flow rate respectively), resulting in very short cycle times (see figure 5A). Figure 5B shows the buffer consumption of both materials used to perform one purification cycle, demonstrating that the membrane required 1.74 L/g_{mAb} while the resin required 1.21 L/g_{mAb}. The 30% higher buffer consumption of the membrane compared to the resin is a result of the membrane volume (1.2 mL MV) to void volume (4 mL) ratio in the device.

Figure 5: *Comparison of (A) Cycle Time and (B) Buffer Volumes of One Single Cycle of mAb Capture With MabSelect SuRe™ Versus Sartobind® Rapid A.*



Discussion

In this study, we demonstrate comparability of a novel convecdiff membrane to resin beads as stationary phase in chromatography. The convecdiff Sartobind® Rapid A showed very similar CPP and CQA results. The investigated parameters differ slightly in terms of impurity reduction and ligand leaching. It should be emphasized that for the chosen HCCF, for which Sartobind® Rapid A performed either equally good or better compared to the resin. The binding capacity of Sartobind® Rapid A was significantly higher at a lower residence time compared to the resin. As a result, the convecdiff membrane led to a 14-fold increase in productivity compared to the standard resin. This is an increasingly important aspect in the purification of biopharmaceuticals, enabling substantial cost, time and space savings. It also provides a steppingstone to explore process intensification of future processes [9, 10, 11].

In summary, this novel convecdiff membrane technology will solve the bottleneck of the mAb capture step in downstream processes of monoclonal antibodies. Provided in ready-to-use devices, it will reduce hands-on time to prepare packed bed columns. The short cycle times enable lifetime utilization of the membrane within one batch.

Conclusion

Despite their high costs, state-of-the-art protein A materials are the industry workhorse for mAb purification, as they offer the best compromise between high regulatory acceptance and specificity. Certainly, in terms of costs and process intensification, they leave considerable room for improvement. Some downstream purification teams are making efforts to improve productivity and reduce the cost of resins by adopting a multi-column approach. However, this makes the mAb capture step very complex and prone to risks. Both types of technologies require relatively large volumes of resins to purify a batch. Plus, the columns are reused over multiple batches to lower cost, which adds high risks of a bioburden event to the process and the product.

The introduction of this new membrane technology can eliminate two main pain points of the industry: packed bed chromatography, which can fail, and column reuse to make them economically viable. In addition, this new ready-to-use and disposable alternative will provide cost benefits in certain processes, such as clinical-scale processes and low-demand molecules, where resins are typically underutilized. Human resources can focus on higher-value tasks because they won't be occupied with column packing and cleaning validation. Further, from a regulatory perspective, this technology will mitigate a number of common problems, such as bed failure events, bioburden issues, and cross-contamination of batches.

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