INTERVIEW with Pete Gagnon, Chief Scientific Officer of BIA Separations, a company that produces purification tools to advance the field of gene therapy.

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Taming the Last Frontier: Harmonizing the Upstream-Downstream Interface

Pete is the Chief Scientific Officer of BIA Separations, a company that produces purification tools to advance the field of gene therapy. Prior to joining BIA, Pete was VP of Process Sciences for Avid BioServices, and before that, going back to 1987, President of Validated Biosystems, an international consulting firm specializing in downstream processing. He has worked with most of the major international biopharma companies, dozens of start-ups and intermediate size companies, and nearly all of the downstream processing suppliers in the field to develop solutions to a wide range of bioprocessing challenges. He is best known for his work in the field of antibody purification but has also worked extensively with other proteins, viruses, DNA plasmids, and RNA. He has been awarded more than 50 patents worldwide

and written more than 100 publications addressing various aspects of bioprocessing. He is a frequent advisor and contributor to major conferences and serves on the editorial boards of BioProcess International and Genetic Engineering News.

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Q How do you see the current status of bioprocessing?

Pete: Good, and improving. The industry has genuinely achieved miracles. We see the proofs every day in the availability of medicines that were unthinkable a generation ago. At the same time, we have left things undone that have serious potential to slow our forward momentum. In fact, they have slowed our progress in getting to where we are, and significantly narrowed the scope of our success. It's time to fix them.

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Q For example?

Pete: As an industry, we have not given adequate attention to harmonizing the interface between upstream and downstream processing. At a superficial level, it's simple. Remove the cell debris and other physical foulants that would render purification tools ineffective, and then proceed with downstream. The piece we have missed is that cell culture harvests also contain chemical foulants; soluble materials that remain after harvest clarification. In the few systems where they have been studied extensively, these chemical foulants have proven to depress the performance of every purification method known. They impose an artificial ceiling on process efficiency and on product quality.

Q How much of ceiling do they impose?

Pete: Just to give you an idea, they reduce processing capacity up to 50% per cycle, they reduce product recovery up to 25%, they increase aggregate content up to 10-fold, they inflate host cell protein contamination by 100-fold, and they inflate DNA contamination by more than 1000-fold. A recent publication showed that if you remove the chemical foulants in advance, you achieve much higher purity IgG with one chromatography step than you can achieve with the 3-chromatography step processes currently used across the industry, and with higher recovery, and more reproducibly. You can imagine the potential impact on process economics.

Q That is a big difference! What are these foulants, and why haven't they been recognized?

Pete: The most serious troublemakers are characterized by the presence of residual chromatin debris. During cell culture, dying cells degrade to the point of becoming undetectable within 24 hours, but some of their contents survive for months after harvest. Those survivors include chromatin; chromosomal remnants in the form of degraded nucleosomal arrays and fragments. They act as nucleation centers for accretion of other contaminants, leading to formation of chemically "sticky" aggregates. Some refer to them as complexes. They bind to all chromatography and filtration media, modify their surfaces, and interfere with their function. They sometimes bind to drug products as well. On top of that, extracellular chromatin is antigenic.

There are several reasons they have not been recognized. One is that they fly under the radar. Their primary constituents are invisible to the assays that are routinely performed to characterize cell culture harvests. Another is that the field is so accustomed to suboptimal purification performance that most people don't realize how much better it can be. With affinity chromatography, for example, people tend to be so satisfied with 95% purity in one step that they don't worry about why they aren't getting the 99.9% they should be getting. Another reason is that when new drug products fulfill regulatory requirements using the historical approach, there is no sense of urgency to upgrade the system. That includes no urgency to understand the obstacles that prevent more effective processing.

Q Are there cultural barriers, for example in how upstream and downstream are typically organized within companies?

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Pete: Not like in the past. There was a long period of time when the relationship between upstream and downstream was viewed, or at least portrayed, as adversarial. I don't think it was actually ever that way but it was certainly true that productivity and success were defined in different ways for the respective groups. I remember a conference about 15 years ago where a presenter was featuring a cell culture process that delivered IgG at 35 g/L. That was a big deal then and it was naturally emphasized as an important advance, but without consideration for its overall process impact. I asked the presenter privately later, what percentage of the IgG was aggregated. 80%. That 80% would need to be eliminated during purification, but imagine a downstream manager trying to explain to a CEO why they recovered less 20%.

The situation is much better now. Cell lines and culture conditions are commonly developed with an awareness that they contribute directly and indirectly to overall productivity and product quality, but there is still room for improvement, and downstream shares as much obligation as upstream. Downstream has a responsibility to identify the contaminants that cause downstream problems so that they can be addressed effectively, either during upstream production or at the upstream-downstream interface. When upstream begins to see itself as part of downstream and vice versa, that's when you can start to make big improvements.

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Q Why haven't these improvements already happened?

Pete: As I mentioned earlier, the chemical foulants that depress downstream process efficiency are not detected by the assays routinely used to characterize cell cultures or monitor purification processes. And it's not just a matter of overlooking them. Good assays for the constituents of chromatin—chiefly DNA and histone proteins—are laborious, time-consuming, and expensive. They involve skills, extraction procedures, and equipment outside the usual scope of cell culture and downstream processing. Getting to the bedrock of how these contaminants affect overall processing efficiency will require that analytical groups re-tool to make such assays a part of routine cell culture and downstream process monitoring, at least during process development. That represents a very substantial commitment. There is now enough published information available to justify that commitment. The primary causal agents and analytical methods have been defined. The specific impacts on purification methods and product quality have been defined. Forward-looking organizations can get on with the task of developing better solutions.

Q Is this issue pertinent to BioSimilar drugs?

Pete: Very much so. In spite of the patents having expired on the gene sequences that produce these drugs, there are often later patents protecting various aspects of the cell culture or purification methods used to manufacture them. If a significant aspect of a purification process is changed, for example by removing chromatin-associated contaminants before chromatography, then some of those pitfalls can be avoided, maybe all of them. If you also demonstrate a superior contaminant-impurity profile, that further documents the distinction between your process and the originator process. Given that regulators may be evaluating several candidate biosimilars for the same originator drug, a better contaminant profile might favor approval, for example contributing to reduced formation of therapy neutralizing antibodies. This is a major issue for biosimilar candidates.

Q Where do vendors fit into the equation?

Pete: Suppliers are an integral part of the industry's global supply network. That does not mean they have primary responsibility to

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solve this particular issue, but they are certainly in an ideal position to develop innovative materials and processes that address it. End-users have also evolved their practices to support vendor efforts. As recently as a decade ago, it was rare that biopharma companies would share samples outside their own doors. Now they've recognized that for vendors to develop effective solutions, they need access to materials that embody the challenges. Ultimately, we are all in the same boat. It serves us to work together.

Q How does harmonizing upstream and downstream fit with current industry trends toward automation and continuous processing? Or does it fit?

> "Smarter chemistry arguably fulfills the intent of continuous processing as well or better than continuous processing itself. Combined, the benefits will be exponential."

Pete: It definitely fits and it definitely represents an imperative for the industry, but it represents the other side of the coin. Heads: automation and continuous processing. Tails: smarter chemistry. Even leaving aside the issue of product quality, if you are going to invest billions in automated continuous facilities, would you prefer to invest in a process with three chromatography steps, or a process with only one? Smarter chemistry arguably fulfills the intent of continuous processing as well or better than continuous processing itself. Combined, the benefits will be exponential.

Q Is anyone pursing both sides of the coin at present?

Pete: This industry isn't known for discussing its technology strategies in public, but I am aware of companies that are pursuing this goal. When one of them succeeds, publicly, the rest will need to follow to remain competitive. This will happen first with new drug products. If the economic benefits justify the costs, older processes might eventually be retrofitted.

Q Does the need for smarter chemistry apply to all biological products?

Pete: It does. The issues with chromatin in particular are best documented with proteins, especially IgG, but it affects all biologics produced by cell culture. That includes all recombinant proteins, virus and virus-like particles, exosomes, and DNA plasmids. The more surprising point is that it also affects synthetic products like RNA. Many of the process components and intermediates in RNA synthesis come from biological sources contaminated with chromatin. That foreign chromatin has extraordinary affinity for RNA. It forms stable associations that carry it through purification steps that don't specifically target it. I have wondered many times the extent to which chromatin contamination might have contributed to failure of first-generation gene therapy products. There is no way to know with certainty, but at least now we have the analytical and process technology to make sure it doesn't de-rail present efforts.

Q How do think regulators will respond?

Pete: Regulators are the real drivers for innovation in this industry so I think they will be receptive. They were the ones that came up with initiatives to improve Process Analytical Technology, Quality by Design, and now Continuous Processing. Will they embrace Smarter Chemistry? In other words, will they embrace products with 100 times lower host protein contamination, more than 1000 times lower DNA contamination, and which compound the productivity impact of continuous processing? I think it's safe to assume they will listen attentively to a new drug sponsor who significantly advances their own initiatives.

Q Where do we go from here?

Pete: International Biopharma is a big machine with lots of moving parts. It takes time for any new perspective to find its place. We saw that with protein A affinity chromatography, which is now used universally for purification of therapeutic IgG. It was well received from the beginning but certainly not an overnight success. I expect we will see a similar trajectory for harmonizing upstream with downstream and achieving the full benefits of affirmative chromatin management.

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