Pharmaceutical BIOPROCESSING

Battlefield medicine: disrupting (bio) pharmaceutical production

Conventional pharmaceutical manufacturing, whose outdated processes are fraught with significant operational and logistical issues, fails to address the 'on demand' needs of today's military and civilian patient populations. Recent advances within the DARPA Battlefield Medicine program suggest that innovative and flexible platforms for producing pharmaceuticals and biologics can be developed that minimize waste, improve capacity to handle wide-ranging operational conditions, and manufacture multiple types of therapeutics – all within short time frames. A distributed 'on demand' therapeutics manufacturing system obviates the need for individual drug stockpiling, cold storage requirements and complex logistics, while enabling cost-effective production of small quantities of medications, such as orphan drugs, and permits the flexibility and responsiveness required in manufacturing to adequately meet the general supply chain needs.

Keywords: battlefield medicine • biosimilar • CHO • DARPA • pharmaceutical manufacturing • quality by design

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The need for disruption

In this age of major advancements in on-demand manufacturing (3D printing) and personalized medicine (genetics-based diagnostic testing), pharmaceutical manufacturing has remained largely unchanged for the past 50 years. In the meantime, drug production continues to be a highly inefficient process with even generic drug prices increasing on a more frequent basis [1–4], and drug shortages occurring at any moment due to manufacturing and quality issues, delays and discontinuations [5–7]. In the military, current procurement of organic small-molecule pharmaceutical drugs and large-molecule protein therapeutics usually takes weeks or months to prepare and airlift to battlefield front lines. As a result, medicine often does not reach the patients who most urgently need them. The same applies to civilians living in remote and underserved areas of the world or those affected by a natural disaster who cannot access life-saving drugs. This inefficiency also extends to potential pandemic outbreaks – when a new threat emerges but is not fully realized, all of the preparedness efforts, including materials and labor, are largely wasted. Therefore, there is a specific need to fundamentally disrupt how organic and biologic drugs are manufactured and delivered, beginning with the development of underlying science and technologies that will enable rapid, distributed and flexible manufacturing of drugs.

It is worth noting that in many developed countries, drug shortages are plaguing the healthcare system. These shortages are leading to patient harm, increasing the costs of care and driving large increases in drug price [8,9]. Shortages of what used to be inexpensive and commonly available drugs that are used routinely in healthcare settings are becoming the new norm. In the USA, there were 185 new drugs that went on shortage in 2014, and by the end of December 2014 over 300 drugs were on the active shortage list [5]. The majority of drugs on the list tend to be generic

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Key terms:

Quality by design: Understanding the manufacturing process and identifying the key steps for obtaining and assuring a predefined product quality.

Chinese hamster ovary: Preferred host expression cell lines for the production of therapeutic proteins, in particular, for recombinant protein expression.

***Pichia pastoris*:** Methylotrophic yeast used as cellular host for expression of recombinant proteins.

Post-translational modification: Proteins that are translated from mRNA undergo chemical modifications, including glycosylation, that can play a critical role in biological processes, such as translocation across organelle membranes.

Biosimilar: A biological product that is demonstrated to be highly similar to an FDA-licensed biological product (reference product) regarding safety, purity and potency.

Intein: Proposed application for purification process for any target protein and involves protein splicing which naturally automates the very specific biochemical reactions of cleavage and peptide bond formation.

injectables, and while there are multiple etiologies for shortages, antiquated manufacturing practices that are prone to quality issues and irresponsive to changes in demand are thought to be one of the major drivers.

The Defense Advanced Research Projects Agency's (DARPA) Battlefield Medicine program is developing innovative, distributed pharmaceuticals and biologics manufacturing platforms for multiple drugs. The program is composed of two integrated research thrusts to enhance the medical capabilities of far-forward providers: Pharmacy on Demand (PoD) and Biologically derived Medicines on Demand (Bio-MOD). DARPA's aggressive timeline and metrics for the program include production of multiple US FDA-approved pharmaceuticals and therapeutics within the same miniaturized manufacturing platform at appropriate purity and potency levels and very short end-to-end manufacturing times (<24 h). During the course of development for the PoD and Bio-MOD manufacturing platforms, novel concepts have been created and utilized, including the use of continuous flow to enable new reaction schemes and conditions for multiple active pharmaceutical ingredients (APIs) [10,11].

Manufacturing of small-molecule organic APIs

Batch processing versus continuous processing

Traditional batch processing used in state of the art API manufacturing is materials, labor, time and cost intensive. It leads to inefficiencies in reaction pathways and conditions as larger quantities are needed and scaled up (Figure 1). Reiterative optimizations of these pathways and conditions are amazingly wasteful, especially as batch sizes increase, and it is difficult to control reaction temperatures within a batch reactor, sometimes resulting in nonoptimal heat transfers and product impurities. Specifically, batch processing has the following limitations:

- Requires large investments in both capital and space.
- Flaws are not immediately recognized, and there is a high risk of material waste, as the entire batch must be discarded if the product fails testing for essential quality attributes.
- Limited by difficulty of cooling large vats (temperature gradients in batch reactors can be large).
- Chemical (synthesis) and biopharmaceutical (drug product formulation) operations are conducted in separate facilities.

This last limitation has significant negative effects on quality control as compliance with Good Manufacturing Practices (GMP) becomes more burdensome with multiple facilities. In recent years, the FDA has encouraged new quality by design (QbD) approaches which require significant improvement over current manufacturing practices by ensuring consistent product quality throughout the manufacturing process and identifying when contamination or other production failures may occur [12–14]. Additionally, by reducing the likelihood of production failures, QbD principles also have the potential to reduce product development and manufacturing costs.

In contrast to batch processing, the PoD platform uses continuous-flow synthesis that allows for multiple-step reactions to be conducted by adding new reactants to the flow at specific points and reactions that produce large amounts of heat to be run safely. Additionally, development of chemical reactions that can take place as reactants flow through chemically compatible materials and device components enables miniaturization and minimizes waste. Furthermore, continuous flow enables integration of reaction, crystallization, purification and formulation with advanced control and real-time monitoring capabilities, thus enabling miniaturization of the entire end-to-end manufacturing platform (Figure 2). These attributes result in the following benefits:

- Reduces capital investment costs and facility footprint
- Lowers operating costs (reduced labor)
- Lower total product costs
- Shorter throughput times

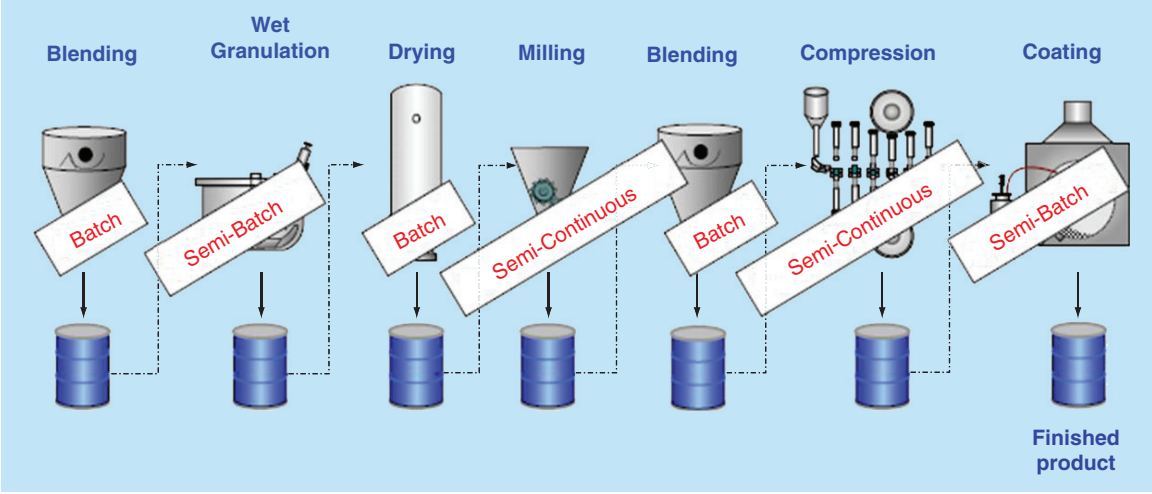

Figure 1. Example of traditional tablet manufacturing process. Product is collected after each unit operation and finished product is tested at off-line laboratories, resulting in actual processing times of days to weeks [14].

- Lower working capital requirements
- Reduced inventory of raw materials
- Enhanced ability to fine tune production rates to meet demand and minimize waste
- Improved quality assurance testing in real time

Leveraging recent advances in continuous flow synthetic and manufacturing techniques [15–34], DARPA's technical approach to the PoD system has focused on developing continuous manufacturing capabilities to enable a modular platform containing a 'reaction toolbox' whereby each reaction step is decoupled from others and then selected only for a given API synthesis. This is then followed by assembly of individual reaction steps into fully integrated, multistep synthesis of each API.

Pharmacy on Demand accomplishments

Having begun just over 3 years ago, the PoD thrust has already developed a fully operational miniaturized API synthesis platform. The PoD platform is composed of two merged modular components each measuring 6 feet × 2 feet × 1.5 feet for upstream (API synthesis) and downstream (API crystallization, filtration and formulation) processes. The platform integrated both upstream (chemistry) and downstream (crystallization, filtration and formulation) processes to demonstrate production of more than 1,000 doses a day for each API. The PoD platform has demonstrated the first-ever end-to-end manufacturing of four APIs, including Diphenhydramine (Benadryl), Lidocaine, Diazepam (Valium) and Fluoxetine (Prozac), all with purities that meet the standards of the United States Pharmacopeia (Figure 3). The fully automated and integrated processes produced the following outputs:

- Diphenhydramine = 439 g/day (17,560 25 mg doses)
- Lidocaine = 225 g/day (2,250 100 mg doses)
- Diazepam = 195 g/day (19,500 10 mg doses)
- Fluoxetine = 22 g/day (1,100 20 mg doses)

The PoD platform significantly improves upon multiple operational parameters over traditional batch processing, primarily through reduced residence times, inventory volumes and end-to-end manufacturing times (Table 1). Overall, end-to-end manufacturing of the formulated APIs using continuous flow synthesis took significantly less time (10–15 h vs 300 h or more for one batch, depending on the particular API). During the synthesis of the APIs, the average total residence volumes of reagents and solvents were no more than 50 milliliters and that of the API purification no more than 2 liters compared with a minimum of thousands of liters required for batch operations. In addition, the PoD team created water-based solution and suspension-based formulations for the APIs. This greatly reduced the number of excipients, simplified the formulation process and produced savings on material costs. The PoD team also devised various materials and engineering solutions to counter chemical compatibility issues in the spiral reactors, pumps and tubing to enable the use of high pressures (average 250 psi, maximum 300 psi), high temperatures (maximum 200°C) and aggressive/corrosive chemicals via continuous flow. Finally, the modular components of the PoD system (including the reactors, pumps and separators) enable multiple APIs to be manufactured in the same PoD system and multiple reaction and extraction steps to be conducted for a given API. Estimated total cost savings of using

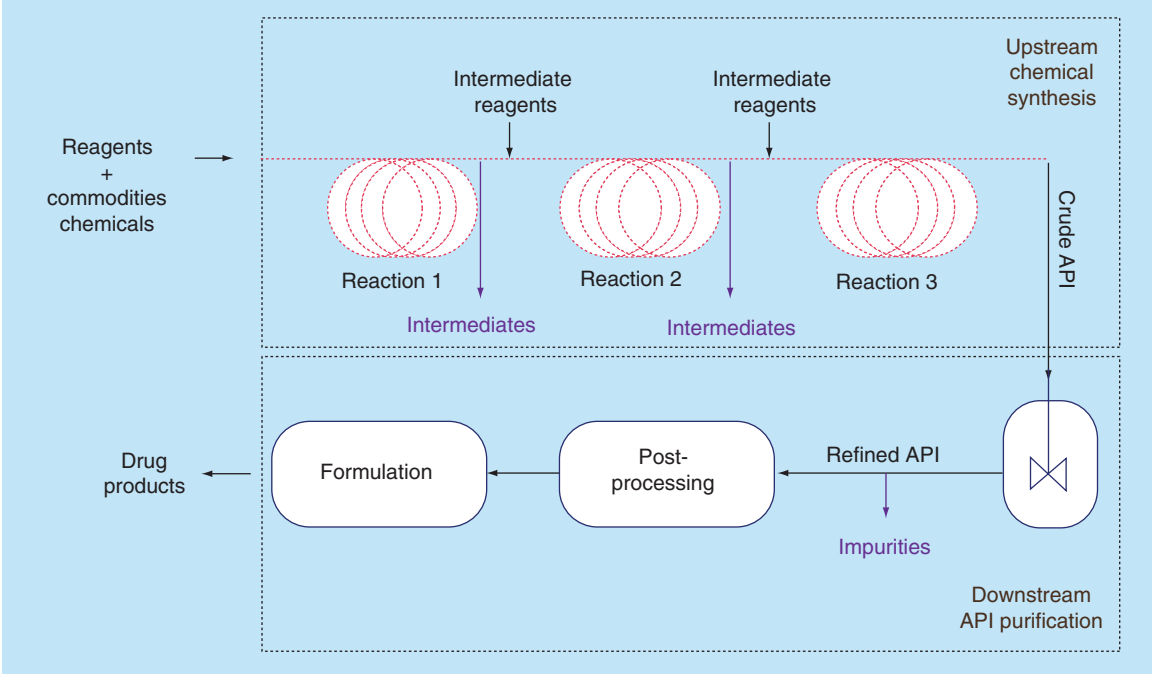

Figure 2. Pharmacy on Demand platform showing both upstream and downstream processes.
API: Active pharmaceutical ingredients.

continuous manufacturing range from 15 to 50% with a huge decrease in the plant footprint. Issues associated with scale up are eliminated as multiple units can be used rather than making a plant bigger.

Major challenges

In the course of developing the PoD platform, significant challenges emerged, including the ability to address a wide range of chemical compatibility, temperature and pressure conditions using current commercially available components. For example, pumping aggressive chemicals at elevated pressures (250 pounds per square inch) and flow rates (up to 5 ml/min) required the custom development of specialized components, including microreactors, pumps, tubing and in-line separators. These are not insurmountable challenges. On the contrary, they might present an entirely new field and industry for materials and devices.

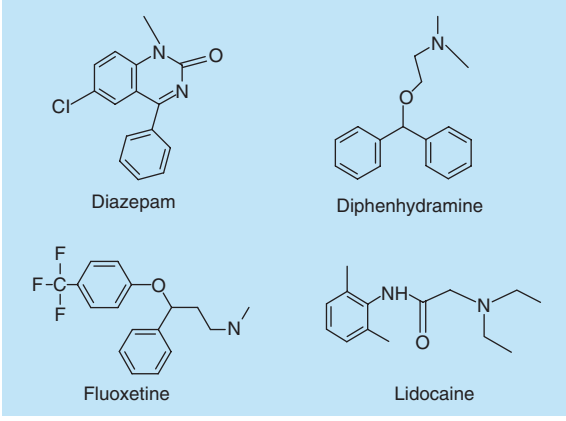

Figure 3. Four active pharmaceutical ingredients manufactured end-to-end by the Defense Advanced Research Projects Agency Pharmacy on Demand platform.

The next major challenge also represents a significant opportunity. It was discovered in the early development of the PoD program that synthetic routes and reaction schemes, which work well under batch operations, do not necessarily scale well under continuous conditions. Therefore, new synthetic methods, reaction schemes and conditions were successfully created under continuous flow. Due to the ability of microreactors under continuous conditions to more efficiently transfer heat, more precisely control temperatures and to handle volatile organic compounds, increased flexibility in the types of chemistries to be developed and implemented expands the 'reaction toolbox' and invites innovative approaches not imagined previously.

Manufacturing of biologics

Protein-based therapeutics are currently produced in large manufacturing plants using cell cultures that are grown over multiple days or weeks in 10–10,000-liter bioreactors under batch conditions [35]. The bioreactors are then harvested, and the expressed proteins are recovered, purified, stabilized and stored as an intermediate bulk drug substance. This intermediate bulk drug substance is then formulated, filled into delivery devices and released by a quality assurance entity before ship-

Table 1. Comparison of operational parameters for Batch Processing and the Pharmacy on Demand platform.

Estimates of operational parameters	Conventional batch processing	Pharmacy on Demand system
Residence times	Hours to days	Few minutes
Inventory volumes	1000 liters	Few liters
End-to-end manufacturing times	>300 h for 1 batch	10–15 h

ment to a distribution warehouse for future delivery to the point-of-care. This entire process for a biologic can take weeks or months for an FDA-approved therapeutic. For novel drugs, this process can take several years for a single therapeutic, and includes proper characterization and validation measures needed to meet regulatory approval. Similar to the manufacturing of small-molecule organic drugs, the biologics manufacturing process is materials, labor, time and cost intensive. Unlike small-molecule organic drug manufacturing, the processing of large-molecule protein therapeutics is arguably much more complex and unique as additional purification and post-translational modification steps are less straightforward in producing biologics that have appropriate purity, efficacy and potency. Additionally, process analytical technologies (PATs) and process control for QbD production and product qualification for release are essential in measuring critical quality attributes of a biologic. Add to this the evolving regulatory activities focused on biosimilars production and approval in the USA [36,37], and the biologics manufacturing landscape becomes even more complex.

Biologically derived medicines on demand (Bio-MOD)

To produce multiple biologics in response to specific demands, the Bio-MOD thrust requires new and flexible approaches for manipulating and engineering protein synthesis systems, including microbial, mammalian and cell-free translation systems. The goal of Bio-MOD is to produce multiple and wide-ranging FDA-approved protein-based therapeutics with high purity, efficacy and potency, at the point of care in time frames of less than 24 h.

Started 2 years ago, Bio-MOD is taking two different approaches. One is a cell-free protein synthetic approach using Chinese hamster ovary (CHO) cell lysates. This *In vitro* translation (IVT) approach focuses on developing cell lysate mixtures containing only the components required for protein production, such as the ribosomes and other cellular organelles, and includes the addition of DNA that codes for expression of a specific protein therapeutic. IVT methods [38–44] are advantageous for a few primary reasons, including:

- Shorter time for protein synthesis, including cell extract preparation (1–2 days compared with 1–2 weeks for *in vivo* approaches) due to reduced number of steps (e.g., no genetic engineering, no cell culture growth, less extensive purification schemes).
- No concern of toxicity of target proteins to cell host.
- More flexibility to synthesize virus-like particles, which are an important components for vaccines.

The other Bio-MOD approach for protein synthesis focuses on using a eukaryotic yeast organism (*Pichia pastoris*) to develop biologics. The use of a yeast cell-based platform like *P. pastoris* provides benefits [45–48] that are critical to rapid biomanufacturing capabilities, including:

- Unlike IVT methods, cells are at a reduced risk of DNA degradation by endogenous nucleases.
- Unlike mammalian cells, yeast can be freeze dried for storage which reduces the need for a cold chain.
- Unlike bacteria, *P. pastoris* reduces the purification complexity because they can more easily secrete proteins, and virus removal and inactivation steps are not required.

The downstream processing for Bio-MOD will be non trivial. The Bio-MOD protein production platforms will include developing innovative targeted capture processes combined with traditional but miniaturized versions of ultrafiltration/diafiltration and chromatographic processes. Integration of these purification modules into a miniaturized manufacturing system and development of a semi-universal purification scheme to address multiple target proteins will be another unique challenge.

Bio-MOD accomplishments

Preliminary results thus far have shown that miniaturized bioreactors can be developed that can control temperature and agitation while monitoring dissolved oxygen, oxidation-reduction potential and pH of cell lysates. These bioreactor prototypes have also demonstrated the ability to produce therapeutic doses of

erythropoietin (EPO) and streptokinase (SK) within 4 h after addition of complementary DNA into the bioreactor prototype containing the lysate mixture. The goal is to combine these bioreactors with an intein-based protein purification system into a lap-top sized device that could be carried in a backpack and contains all the critical elements necessary to express, purify and analyze any target protein.

Microfluidics-based bioreactors (with dimensions of 7.5 cm length, 5 cm width and 1 cm height) have demonstrated stability of 2-week fermentations in the *P. pastoris* of IFNα2b and human growth hormone (HGH), with crude protein being produced within a few hours. These microfluidic chips have unique channels and chambers to enable cells from the growth chambers, perfused material and waste to be independently collected and allows for diffusion of molecules between gas and liquid phases within the chip. Since this yeast is genetically stable, it is conceivable that the genetic circuitry can be developed for tunable production of two biologics from the same organism in a sequential manner. Preliminary results have demonstrated the ability to sequentially induce the production of IFNα2b and HGH by changing the medium, and thus, the chemical environment. These components will be integrated into a milliliter-scale table-top system for (semi)continuous operation, consisting of a parallel set of microbioreactors, filtration of cell debris from secreted protein product, novel affinity-based purification, polishing and finishing, as well as integrated on-line PAT and process control for QbD production and product qualification for release.

Major challenges

There are various technical challenges that need to be addressed, including purification of the target protein whether it is produced from a cell lysate or secreted from a cell. In particular, major impurities, including host cell proteins, DNA, aggregates and viruses, will need to be separated and removed at acceptable levels to attain therapeutic grade purity. During current batch processing of biologics, the downstream process must be validated for its ability to remove or inactivate these impurities and contaminants, but it is not practical to perform these validation studies on the entire manufacturing process due to the large size scale involved. Therefore, a downscale of the purification process (e.g., laboratory-scale mimic of the process) is often used. However, the challenge here is to ensure that the scaled down process is an accurate indication of what is occurring in the full-scale process. The current Bio-MOD platforms have both size and speed on their side, so it is feasible to think that a reliable method of purifying the target proteins as they are synthesized can be developed so that the purity of the entire small volume of target product is known at any given time during the downstream process. Furthermore, IVT and cell-based protein production platforms will also encounter different challenges for purification. For example, in the IVT system DNA contaminants potentially will be more of a focus, whereas for cell-based systems, host cell proteins potentially will be larger issues.

Another technical challenge is post-translational modification (PTM) of the target proteins. Specifically, achieving human-like N-glycosylation so that structural and functional activity is preserved will be a unique challenge for both mammalian IVT-produced and yeast-secreted proteins [46,49–51]. To address this issue, part of the solution will involve engineering of the cell extracts or cells, as well as developing additional glycosylation enzymes and sugar substrates in order to successfully produce the final glycosylation pattern. To detect the glycoprofiling, the Bio-MOD platforms will include development of a suite of on-line PAT tools, such as novel optical, microfluidic and nano-sensor technologies. In addition to analyzing PTMs, these tools will also be able to analyze target product identity, purity, quality, biological activity and aggregation.

One of the largest regulatory challenges is the manner in which the FDA will approve biosimilars. While the FDA has provided recent overview and guidance on how they will approach development of biosimilars [52], this will be an evolving discussion that will drive what the Bio-MOD platform will look like beyond the DARPA developmental phase.

Conclusion

Having just begun over a few years ago, the DARPA Battlefield Medicine program has already developed innovative drug manufacturing platforms that offer a more decentralized and mobile approach over the current state of the art. The PoD platform has demonstrated the first-ever end-to-end manufacturing of four chemically different medications using a single miniaturized platform. The Bio-MOD platforms have already demonstrated end-to-end manufacturing of biologics in a timeframe much shorter than the weeks and months it currently takes to produce these therapeutics.

Future perspective

The DARPA Battlefield Medicine program is ongoing, with progress made on a daily basis. Therefore, it is difficult to formulate conclusions with systems that are still under development. However, it is appropriate to comment on DARPA's remaining plans within battlefield medicine and speculate how it will impact the future of drug manufacturing.

For example, future plans for the PoD effort will focus on further optimizing the system to accelerate manufacturing times and enable additional flexibility in operations by overcoming a few remaining but significant challenges. For example, the downstream process, like the upstream process, will operate under continuous conditions. By switching to continuous conditions, automation of downstream processing will be simpler, quality control monitoring can be more easily conducted throughout the entire manufacturing process and further miniaturization of the PoD platform is possible [53]. For example, continuous crystallization processes that include contact secondary nucleation and which decouple nucleation and growth will aid in improved control of the rate of nucleation and crystal size [54,55], and improve the purification process. Additionally, if these processes under continuous conditions can enable crystallization to occur at room temperature as opposed to cooler temperatures, then the cold-chain is removed from the manufacturing process which further simplifies the logistics chain.

The Bio-MOD platforms have made significant progress to date, have opportunities for continued improvement, and face some significant challenges as presented above. However, the DARPA Battlefield Medicine program has accomplished what it originally set out to do. Namely, taking the technical risks off the table so the technology can continue to develop and mature, and seeking to demonstrate end-to-end manufacturing of at least six biologics. One can now imagine a very real scenario in which a target protein is produced and purified within hours, with real-time analytics validating that the biologic has the acceptable safety, purity and potency attributes. The ability of the PoD and Bio-MOD platforms to produce single-dosage levels of therapeutics at minimal expense will enable personalized medicine 'on demand', address gaps in the orphan drug market, reduce drug manufacturing shortages and expand access to medications for patients.

The concept of continuous manufacturing has started to resonate within the pharmaceutical industry [56], but has yet to be fully embraced. With new incentives, such as expiring patents on blockbuster drugs and an emerging generics market, this industry is now just realizing the massive potential value of developing cost-effective and more efficient pharmaceuticals processing technologies that have, until now, been largely non existent. On the regulatory front, the FDA has been encouraging of the pharmaceutical industry to adopt novel approaches to manufacturing, and with recent advances in addressing biosimilars [52], there is now growing optimism that current drug manufacturing operations can be disrupted in order to meet medical needs today and into the future.

Executive summary

The need for disruption

- Conventional batch processing is wasteful (labor and material costs), inefficient, and has not changed much in the last 50 years, while drug costs have skyrocketed and drug shortages are more common.
- The DARPA Battlefield Medicine program aims to disrupt how pharmaceuticals and biologics are currently produced by developing innovative technologies and flexible, distributed manufacturing platforms to produce multiple drugs 'on demand.'

Pharmacy on Demand accomplishments

- Leveraging continuous flow synthesis and manufacturing techniques will enable new types of chemistries, minimize waste and allow for platform miniaturization.
- The refrigerator-sized DARPA Pharmacy on Demand platform has demonstrated end-to-end manufacturing (upstream synthesis and downstream processing) of four active pharmaceutical ingredients at single-dosage levels within a few hours, providing feasibility for a distributed 'on demand' system and a significant improvement over conventional batch processing conditions.

Bio-MOD accomplishments

- The DARPA Biologically derived Medicines on Demand platforms have already demonstrated production of crude biologics within 24 h in both *in vitro* translation and yeast-based systems.

Future perspective

- With both the pharmaceutical industry and US FDA more accepting of technical and regulatory advances in drug manufacturing, revolutionary approaches are now possible.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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