Editorial

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Plug and Play? Interconnected multifunctional chips for enhancing efficiency of biopharmaceutical R&D

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FUTURE SCIENCE

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The end of 2012 marked the end of the 'patent cliff', an 18 month period during which many major drug companies lost exclusivity on blockbuster drugs [1]. In anticipation of this precipice, they have turned to new models for future growth – partnering with (or buying) biotechnology companies, focusing on drugs with unmet medical needs, entering generic markets, redefining relationships with innovative research organizations, looking to potentially lucrative foreign markets, and restructuring burdensome research divisions. Along with these strategy shifts, the market potential and development cost of all drugs will need radical transformation, particularly as personalized medicine becomes reality. A prime target for increased efficiency is the discovery/development process. Currently, 66% of all drugs fail within clinical Phase II processes alone. A reduction of this attrition rate to merely 50% will decrease per drug costs from US$1.8 billion to $1.3 billion, a saving of approximately 25% [2]. In parallel, the US FDA will now evaluate new molecular entities based not only on efficacy and toxicity, but also on improvement over existing treatment, with insurance companies following suit on reimbursements [1]. Clearly, driven by these economic and regulatory pressures, we find great opportunity to redefine bioprocess R&D – its efficiency needs vast improvement within a climate of reduced expenditures. If one thinks of the entire drug discovery, development, production, testing and validation pipeline as an expanded bioprocess, one might consider next generation microfabricated devices as potential high content/high throughput vehicles that can change the paradigm – dramatically reduce cost and enhance success.

We provide two exemplars for decreased cost and improved efficiency for the development of biopharmaceuticals. Namely, transformative advancements are envisioned in both preclinical toxicity and efficacy screening, as well as in the more traditional bioprocess development. In the preclinical phase, extensive absorption, distribution, metabolism and elimination and toxicology testing of therapeutics in model organisms that are needed before a drug can proceed to human trials should be reexamined. On the horizon are new Tox 21 efforts to transform toxicology from *in vivo* animal testing to *in vitro* methods using cell lines [3]. Additionally, animal-on-a-chip or even human-on-a-chip methodologies were first envisioned nearly two decades ago [4], but are now rapidly gaining momentum [5,6]; attracting many of the brightest young scientists and engineers to their development. The manufacturing bioprocess

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pipeline too, can be redefined. Like target screening and toxicity studies, bioprocess development comprises many steps, including upstream strain development, defining cell growth and production characteristics, elucidating separation and purification methodologies, followed by biochemical and formulation studies.

Common to both the research and manufacturing pipelines are the placement of proteins, cells and tissues within spatially defined locales under an overarching requirement that process liquids with defined concentrations and residence times be controlled [7,8]. Well-controlled conditions are necessary to improve process understanding, reproducibility and validation. The current paradigm in bioproduction generally involves refinement first using 'black-box' plastic disposable devices then laboratory-scale bench-top reactors that provide control of pH, dissolved oxygen and turbidity. Large volumes of expensive media restrict experimentation to improve process control, and microwell plates are subject to well variability, edge effects and evaporation. Additionally, culture flasks and dishes both lack instrumentation, reducing the amount of information needed on process variables. Microfabricated devices have the potential to overcome all of these issues in laboratory-level process development. The advantages of microfabricated devices include the smaller sample volumes, shorter analysis time, higher sensitivity, multiplexing, as well as precise spatiotemporal control. Additionally, there are process control advantages as new noninvasive optical sensors [9] can be integrated with various computational models for accurate scale-down and optimization [10].

The flexibility of microfabricated devices allow the upstream cultivation of *Escherichia coli*, yeasts and mammalian cells, which comprise the vast majority of host systems used for target therapeutic expression [11]. Terrell *et al.* developed an 'in-film' chip-based bioprocessing system consisting of both a production address wherein NS0 cells produced antibody and a capture address that enabled its quantification [12]. The production address is functionalized by electrodeposition of a reversible alginate gel potentially allowing a rapid multiplexing screening procedure for strain and process parameter optimization. Finally, in concert with platform advances, synthetic biology methodologies can be exploited to yield complementary hosts that operate autonomously, minimizing complex fluid handling [13].

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The downstream purification process should also perform the separate functionalities of sample extraction, clean-up, proteolytic digestion, separation and analysis [14]. For downstream purification processes, multifunctional microfluidic devices have coupled separation with MS analysis and electrospray ionization or matrix-assisted laser desorption ionization interfaces. These integrated units enable high-throughput, low sample consumption, automated separation and analysis of protein purification.

An often overlooked advantage to microfabricated devices is their disposability. Current practices have led to an increase in single-use disposable technologies even at the industrial scale, with the elimination of cleaning time, reduced incidence of contamination, cost savings, and increased flexibility and convenience [15]. Rao *et al.* have helped create the possibility of a disposable microfluidic device by developing disposable patch optical sensors that can be used to monitor the main process parameters. Optical sensors are manufactured in single sheets have been incorporated into a variety of formats [16].

For drug discovery and absorption, distribution, metabolism and elimination-toxicology testing, cell culture analogs when used with physiologically based pharmacokinetic models can be viewed as surrogates for human organs. Xu *et al.* have developed a device to reproduce anticancer drug toxicity studies by creating a physical replica of the human system on a disposable microfabricated device [6]. There has been significant work in developing devices for mimicking the environment of the liver, kidneys, lungs and other organs [17]. Fortunately, the NIH, the American Institute for Medical and Biological Engineering, the Defense Advanced Research Projects Agency and the FDA have already begun the process of incorporating validation and qualification concepts into the grassroots designs of the animal-on-a-chip systems [101]. A partnership with the FDA, Maryland's Center of Excellence in Regulatory Science and Innovation, is promoting these activities [102].

Potentially transformative innovations are emerging in this animal-on-a-chip technology as well. Screening methodologies that support high content analyzes are needed. Unlike cell cul-

ture protein production and purification processes that depend on monoculture and sample purity, animal-on-a-chip devices must enable the assembly of complex sets of cells within specific registries so that associated fluids can be perfused or contacted at physiologically relevant time scales. Geometries and conditions that closely match the *in vivo* system are needed [17]. That is, on a cell number or tissue weight basis, what is the fluid residence time in a kidney? How long does it take for bacteria or their signals to transverse the GI tract? Methodologies of assembly must also be matched by developments that enable analytical interrogation (optical, chemical, mechanical, and so forth). For example, it would be advantageous to connect bidirectionally, the communication systems within microfabricated biodevices (devices 'talk' via electrons and photons; biology 'talks' via small molecules and ions) [18]. Then, advanced signal processing methodologies that have transformed the study and practice of neurobiology could be overlaid onto complex biological systems, such as the immune system and its interaction with the microbiome, yielding vastly improved insight on molecular and cellular interactions [19]. Microfabricated chips or microfluidic devices that are 'biofabricated', meaning composed of biological components using biologically motivated assembly processes, could transform our understanding of drug actions, drug/tissue interactions and even tissue/organ interactions. When all constituencies (e.g., drug and insurance companies, FDA, NIH and The National Institute of Standards and Technology) are involved in these technological advances, they will become more readily accepted – leading to vast improvements in efficiency and cost.

Rao *et al.*, used the term "process scouting devices" (PSD) to refer to 'black box' microsystems that when designed, fabricated and employed can be brought to bear on the particular problem at hand [20]. Perhaps an expanded use of the term is warranted that incorporates sensor innovations, advanced cell/tissue assembly and robust analytical methodologies so that the PSDs of the future are 'smart'. There has been great progress in each aspect and function of the overall drug pipeline described above. Process interconnections are envisioned so that next generation PSDs could be physically pieced together to accomplish a specific task. That is, an integrated all-in-one device linking upstream, downstream and testing processing is perhaps too expensive, impractical and logistically infeasible – yet individual component chips might be linked in a combinatorial manner to study and optimize subtasks. Perhaps systems engineering approaches will emerge so that device connectivity is simplified and mathematical models can be tangibly linked. Then, drug interactions, toxicity screens and efficacy studies could be performed hand-in-hand with bioprocess development, all enabled by informed scouting devices and econometric analyses. In all, such endeavors might lead to radically improved R&D efficiency on an industrial scale.

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