RoosterBio®
Radically Simplifying Use of MSCs

# Adherent Cell Manufacturing's Call to Action:

## Manufacturing Platform & Media Strategy Matters for Mesenchymal Stromal/Stem Cell Production Economics

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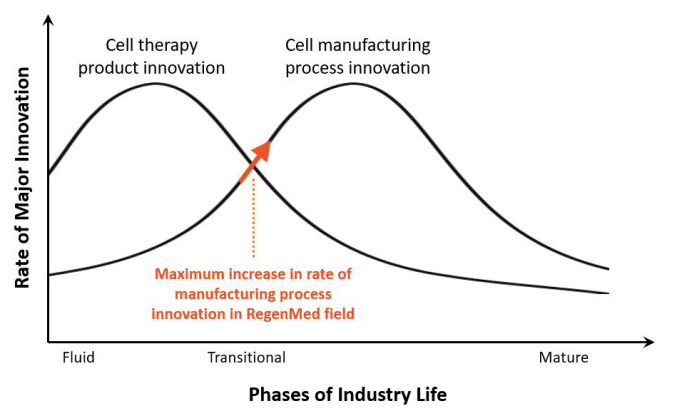
**Note to readers:** This White Paper is an updated and revised version of an article published in BioProcess International on 14-December-2018 "The Need for Adherent Cell Manufacturing: Production Platform and Media Strategies Drive Cell Production Economics" by Jon Rowley and S. Anne Montgomery.

# Introduction

Today's marketed biopharmaceuticals span diverse modalities, disease indications, and addressable patient populations. Each of these products needed to scale up from laboratory to commercial production at a key point in their development. Their milestone achievements from research to development, and then manufacturing, drew our attention to the need for clearly defined process economics and critical velocity toward commercial success of biopharmaceutical products.[1-6] However, unlike most biologics manufacture which rely on suspension-adapted mammalian or microbial cells to secrete the product, cell therapy products are formidably challenged with the direct cultivation of the product itself – the cells.[7-9] Thus, it becomes critical to recapitulate conditions that would not only maintain biological integrity of these cells, but also utilize processes that would allow us to deploy them as safe and effective therapies.[10-13]

The often-prohibitive barrier to entry for manufacture scale-up incentivizes myriad collaborative arrangements or acquisitions between Biotech and Big Pharma.[14,15] On the other hand, there remains a thirst for innovative cell production approaches for pursuit of pipeline milestones with far more autonomy and agility.[16] These new approaches could pivot cell product development into commercially relevant scales, while enabling cost structures to support flourishing business models for the smaller cell therapy enterprises. *In short, we need a democratization[17] of adherent cell therapy manufacturing capability.*

**Figure 1.** The Product Cycle Model of Innovation




▲**Fig. 1** (*above*) schematically depicts the Product Cycle Model of Innovation in relation to cell therapy and cell manufacturing. Here, a wave of cell therapy product innovation accelerates, crests, and then wanes — prior to a less-heralded but equally vital wave of process innovation that drives down costs and democratizes the industry.

Stem cells and other primary cells, most of them grown in adherent culture, have a rigorous history as basic scientific research tools for learning about mechanistic biology.[18-21] Their non-transformed phenotype allows one to observe phenomena that are unknown in the classic immortal cell lines from tumors. In fact, primary cells have now advanced from a simple, empirical research tool into their own category of platform "technology," a fertile field for novel and useful innovations.[22-29] Accordingly, human mesenchymal stromal/stem cells (MSCs) are a perennially favored cell platform for regenerative medicine. Nevertheless, MSC propagation on an adherent surface requires adaptations to be made that are distinct from biopharma's suspension-based culture processes. This calls for process innovation across the cell manufacturing and production pipeline, to achieve product development milestones at greater speeds.

Regenerative medicines, whether for cell therapies or biofabricated tissues, now face the same challenging transition once known to the historical pioneers of monoclonal antibodies (mAbs), gene therapies, and CAR-T therapies, alike. MSC bioprocess developers today require an industrialized supply chain for cellular starting materials in appropriate product formats, pursuant to a seamless route between product and process development and clinical manufacturing.

Over the past 2-3 decades, the biopharmaceutical industry gained a greater understanding of aspects around primary cell manufacturing, scale-up, quality parameters, in-process controls, and the regulatory sciences for this broad class of biologics products.[30,31] That established a template for accelerated cell technology adoption into regenerative medicine products. As we envision this, therapeutic adherent cells like MSCs now proceed along the same kind of innovation path as the components of electronic devices — i.e., transistors and microchips.[32] Like transistors, adherent cells are material "hardware" components needed by regenerative medicine product system developers, who in turn create emerging therapies in simple-to-use (or "plug and play") formats.

The analogy of a semiconductor chip "fab" provided inspiration for next-gen companies such as RoosterBio.[33] RoosterBio's goal is to simplify and streamline regenerative medicine product development, enabling access to cellular materials in volumes consistent with development, that also maintain quality and function when produced with

scalable bioprocesses. It aims to deliver in formats that standardize simple and cost-effective clinical products, with reduced time needed for a therapeutic innovator for product development, technology transfer, and clinical manufacturing. To attain this benefit, the cellular technology supply chain must be re-imagined. It must develop with quality specifications for repeat uses via product developers and manufacturers.[34-36]

In 2014, RoosterBio began by offering ready-to-use MSC bioprocess systems in the form of cell banks and highly optimized and enriched culture media. They were designed to be "off-the-shelf" products, with standardized quality and protocols, so that anyone with basic cell culture skills could advance their practical MSC manufacturing expertise simply by employing the commercially available cell banks and paired media system and following established protocols.[37] A biotech innovator can then quickly generate significant volumes of MSCs to kick-start product development and enter clinical translation rapidly, cutting years from typical development timelines. Newly formed product teams within industry — having no prior MSC experience — can now access sophisticated MSC componentry to develop new product concepts for rapid biological prototyping. If successful, such products will progress along a scalable, fully "*built-in*" raw material manufacturing process.

In this white paper, we'll explore **(1)** the promise of MSCs as a robust vehicle for widespread technology development across regenerative medicine applications; **(2)** the pathway cleared for product developers to pursue rational and scientifically validated scaleup practices; **(3)** the roadmap we envisaged, together with NIST and other collaborators to develop our industry; **(4)** the related economic progression and how it will contribute to consumer-benefitting economies of scale; **(5)** the new strategy we can leverage in the world of cell therapies, previously pioneered by analogous protein biologics manufacturers; and **(6)** the overall boost that these efforts will contribute to the whole of biomanufacturing.

## *MSCs as Foundational Technologies*

Human MSCs are widely sought after for the development of regenerative medicine products, with indications ranging from immune modulation to cardiology, oncology, and engineered tissues and organs. These cells have been used to treat more than 60,000 patients globally to date (celltrials.org, LINK), through registered clinical trials. With a physiologic role to home towards damaged tissue and secrete regenerative factors, MSCs translate into high-value, *designed* components for tomorrow's cell and gene therapies, engineered tissues, and combination medical devices.[26] They also comprise the raw material for novel processes and methods. While MSCs have had a good safety profile, the current focus of attention is on maintaining or improving their functionality through the trial-and-error of manufacturing sciences. For cell therapy efficacy endpoints to breach the ever-elusive "$p < 0.05$," the MSC's bioactivity per cell must be as robust and uniform as possible. Due to the diverse range of possible in vivo biological outcomes, MSCs hence need to be tuned toward the desired indications from the very start of bioproduction.

Just as marketed CAR-T therapies require specialized *ex vivo* stimulation protocols to elicit the desired signal transduction, MSC manufacturing bioprocesses will likewise need to be crafted around optimized conditions and/or a cocktail of pre-priming factors to target specific disease indications. Emerging gene-engineering and editing technologies now further enable precise MSC modifications to increase cell potency *in vivo* as "drugs."[38-40] Similar modifications can likewise be employed to better adapt these into a manufacturing environment.

Extracellular vesicles (EVs) and/or exosomes are gaining in popularity as a cell-free and perhaps safer approach to develop novel therapeutics for various indications. Conditioned media from MSCs are a common source for EVs. Nevertheless, challenges faced across this field lie not only in "downstream" isolation and characterization strategies to purify the target lipoparticle population, but also in scalable manufacturing "upstream." Large scale production of high quality and potent MSC EVs will require significant cell numbers, depending on dose requirements. Unfortunately, planar based flask cultures are often ill-suited to meet target production at necessary scale. New bioreactor processes that can facilitate superior cell densities per liter of media are thus needed.

The evolving field of tissue engineering has benefitted from the explosion of three-dimensional (3D) printing technology and biofabrication tools over the past two decades. While the fabrication tools and techniques have become more sophisticated to enable precision and intricacy of 3D tissue constructs, manufacturing science of cells and tissues at scale remains a constraint in this field. Development of a tissue construct in support of a Phase I study will require an abundant, consistent, and reliable supply of multiple cell types (including MSCs) as input materials for co-culture. In the related opportunity area of cultured "clean" meat, a nuanced appreciation of how cells from different non-

human livestock, fowl, or fish species can be industrially expanded will be likewise necessary.

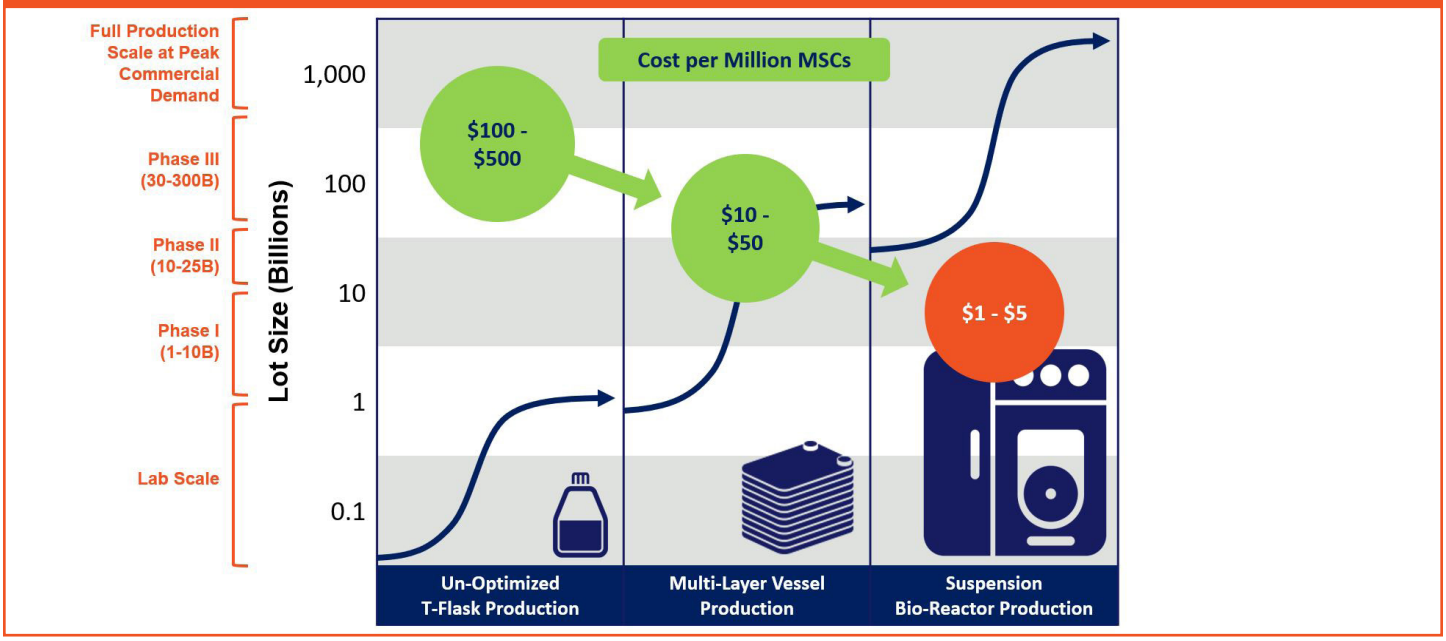
Insufficient cell production for a future trial comprises an additional bottleneck in preclinical research and product development. This considerably slows innovation in the multiple arenas served by MSCs.[41] Fortunately, bioprocess innovations in adherent cell manufacturing are now poised to end that supply constraint. With the first news of commercial breakthrough by MSC pioneers, dozens of product companies today soon will expand to hundreds.[42] All will need a steady supply of high quality cellular starting materials supported by scalable production technologies. The availability to such off-the-shelf solutions will provide companies with ready access to tens of billions of cells for development, and trillions of cells to support commercial scale manufacturing.

## *Advancing Manufacturing Sciences*

Similar to all technological industries, products based on cell technologies have followed the product cycle of innovation (*see **Figure 1***)[43] First, there is a wave of product innovations followed by a wave of manufacturing process innovations. Currently, the regenerative medicine field is nearing an inflection point of maximal rate of manufacturing process innovation (*arrow in **Figure 1***). For non-MSC therapies, an analogy of that process might first involve the isolation and expansion of tumor infiltrating lymphocytes and then, gene-edited T cells with improved post-expansion bioenergetics for a memory phenotype. Another parallel example might be first, creation of induced pluripotent stem cells (iPSCs) and then, gene-edited daughter cells that selectively report on differentiation fate for isolation via industrially scalable, off-the-shelf MACS reagents.

As products approach commercialization, manufacturers need to make products at commercially relevant scales and with cost structures that will support a business model. Life sciences is entering a stage of massive bioprocess innovation at which the uniform cellular starting material cell type can be used as a "chassis" to be industrialized for many different therapeutic indications. That progression from therapeutic discovery to a biologics drug assembly line is being supported by organizations such as the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)[44] and BioFabUSA, part of the Advanced Regenerative Manufacturing Institute (ARMI).[45] Such government-funded agencies are supporting public-private ventures in manufacturing sciences, specifically for cellular medical technologies. Their aim is to clear a path for rapid developments in the fields of biofabrication and regenerative medicine manufacturing, as competitive engines of long-term regional economic growth. Pursuant to this large-scale initiative, MSCs can play a pivotal role.

**Figure 2.** Manufacturing Platform Evolution Will Drive the Economics of Stem Cell Supply for Years to Come [10][53]




▲ **Fig. 2** (*above*) compares MSC productivity across different two- and three-dimensional systems. Fostering a "bioprocess approach" toward adherent cell production is helping manufacturers make important steps toward standardization in regenerative medicine.

MSC process innovation delves into technical problems less visible to product innovators, but no less significant. MSCs are primary cells and hence cultured "ex vivo." This necessitates MSCs' attachment to a surface with integrin ligands, and the need for empirically optimized media ingredients to approximate these cells' in vivo biological "nursery" of origin.[46] Thus, production systems for reduced COGS must be commensurately developed. Supporting media systems need to be engineered for productivity and efficiency, because culture medium is a major cost driver of cell manufacturing.[47] Surprisingly, despite higher ostensible per mass raw material cost, hyper-efficient and potent media can propel lower-cost regenerative medicine cell products in the final reckoning. This is due to factors such as minimized volume, reduced human error, and lower microbial contamination risk and waste.[48] Standardization of manufacturing processes and starting materials also reap newfound efficiencies for living cell technologies. The end goal is to democratize the availability of what traditionally had been a scarce resource (cells), available only to the best-funded companies or laboratories.

Attention to key input parameters like (i) attachment modality and (ii) media not only drives the scalable transition from flasks into multilayer vessels and then into bioreactor production platforms; they also impel sophisticated engineering of media systems to deterministically enhance cellular productivity.[49] Tweaks of metabolite flux and outside-in signal transduction must point to the important output readout. Analogous to grams per liter used in the monoclonal antibody (MAb) space, the key media productivity metric for MSCs is *millions of cells produced per liter of media consumed*. Until recently, this metric was not promoted for cell therapy manufacturing, but it is required to move the field toward a commercial mindset. As relating to the next section "*A Technology Roadmap*," a focused end metric helps highlight and then remedy where the interim technology gaps lie.

## A Technology RoadMap

Technology roadmaps are flexible (but detailed) plans to direct long-term strategy along a path of nearer-term goals to fill specified capability gaps where innovation is needed.[50] The National Cell Manufacturing Consortium is a NIST-driven cell manufacturing group, for which the Georgia Institute of Technology took the leadership role. This collaborative group devised one such upstream and downstream technology roadmap, *Achieving Large-Scale, Cost-Effective, Reproducible Manufacturing of High Quality Cells*.[51] From this effort, it was determined that cell manufacture processes needed to scale with product comparability across discrete stages. Figure 2 shows the evolution of manufacturing platforms from multilayer vessels into bioreactors, a process that will propel the economics of adherent-cell supply for years to come. Lot size (y axis) is on a log scale, and the x axis shows the technology platform. The technology S-curves in **Figure 2** show where adherent cell production constraints are for each platform.[10,42,52] Each technology production platform usually is good for a log or two of productivity, after which one advances to the next technology platform to reach desired lot sizes. To the left of the curve with T-flasks, initial capex is low but labor and reagent costs are high. To right of the curve, capex investment is high, but labor and reagent costs allow cost per cell to reach inexpensive, industrial levels of mass production. This is the classic journey of product development from "lab coat to hardhat," though it is not always an easy one.

For MSCs, and other adherent cells, production in T-flasks easily can make 5–100 million cells. But with this platform, generating a larger number of cells is much more difficult if one's need approaches a billion cells. The next platform is a multilayer vessel system that can produce 1–15 billion cells easily, but many tens of billions would be difficult. At that point, suspension bioreactors are required to achieve lots of 50–500 billion cells. Although a small-scale bioreactor can produce a billion cells, using multilayer vessels is more efficient at that scale. For producing 50 billion cells or more, multilayer vessels are no longer feasible, at least for MSCs. Single-use suspension bioreactors currently are enabling scale-up to hundreds of billions of cells, likely topping out at about a trillion cells.[10]

## Economics of Cell Production

The development of efficient, scalable technologies for producing large volumes of cells drives bioprocess economics. As we and others emphasize, the main bioprocess economic metric for cell therapy manufacturers to follow for the past several years (at least for MSCs) has been the "cost per million cells." Yet that varies according to cell type. For MSCs, cost varies with lot size and process engineering. Depending on scale and level of streamlining, the cost per million MSCs produced can range from hundreds of dollars per million cells when lot sizes are in the tens of millions, to tens of dollars when lot sizes are in the low billions. Costs of <$10 per million MSCs can be achieved as lot sizes surpass 100 billion cells/lot.[42]

The impact of lot size and process engineering on MSC economics is consistent with Moore's law in the microprocessor field's— just extended to stem cells. Moore's law can be applied to nearly any technology, including biotechnologies and directly to adherent cells. [53] Like many new technologies, stem cells will continue to decline in cost as the need to supply them strives to keep up with the demand for the world's next-gen cures in regenerative medicine.

Cost reduction of adherent cells into the tens of cents per million cells range will require future technology innovation step changes, but the authors expect the field to embrace those changes within 10 – 15 years. The industry is poised to deliver MSCs in the range of $1–5 per million cells (the next log – reduction level), but the demand for those cells must be sufficient to support that lot size. As the first MSC products achieve blockbuster status (>$1 billion in revenue, likely treating >100,000 patients per year), the economies of scale with automation will drive their costs to single-digit dollars per million cells. So, achieving such efficiencies is both technology and demand driven.

RoosterBio was founded specifically to drive rapid adoption of MSCs produced in large volumes with scalable processes, consistent with favorable economics. Under normal circumstances, making the approximate human-sized dose of 100 million cells takes well over a month, and incurs expenses for cell culture media that drive up the cost of goods. RoosterBio's contrasting approach is to provide one bottle of media, such that in only five days, the volume could be expanded to 100 million high quality MSCs—and with further passaging — to more than a billion cells.

Friction-free and low-cost bioprocess to a billion or more cells allows a company to develop and innovate on a number of products rapidly, thus helping to cultivate a whole ecosystem of MSC-based products and services. With optimization-friendly platforms that can generate cells with increasing efficiency (in millions produced per liter of media consumed), the biotech sector can start modeling productivity parameters for empirically regenerative medicine, much in the same way it did for biotherapeutics (in g/L). That approach enables 10× productivity enhancements in much shorter time frames than those mentioned above.

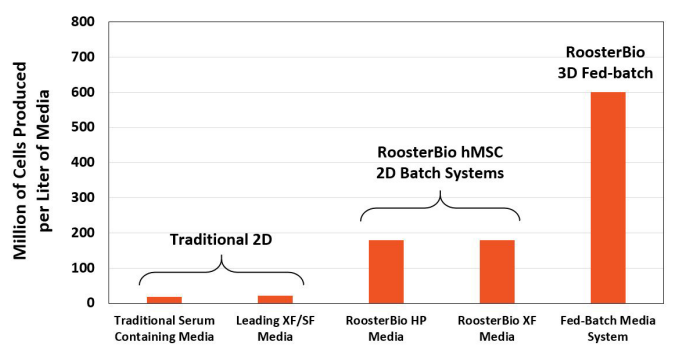
## *A Novel Strategy*

With a closed-system platform that includes xeno-free (XF) cell culture media and XF cells, RoosterBio laid the groundwork for its first current good manufacturing practice (cGMP) product which was branded under CliniControl™ and launched early 2018. Since then, RoosterBio has been developing the CliniControl™ product portfolio. It is the world's only commercially available cGMP MSC bioprocess system to accelerate cell and gene therapies and continue to drive unparalleled efficiencies and economies of scale in clinical and commercial MSC manufacturing.

RoosterBio was granted funding through the Department of Defense (DoD)-funded Medical Technology Enterprise Consortium (MTEC) in 2016, in collaboration with BioBridge Global, GenCure, and the United States Army Institute of Surgical Research to establish a 50-L XF MSC manufacturing process, capable of yielding tens of billions of MSCs.[54] That effort consisted of developing a seed train and a suspension culture process that scales up to an 80-L PBS bioreactor, a scale that is critical to generate the cell numbers necessary for clinical therapies. The process incorporated XF cells, XF microcarriers, and a XF fed-batch bioprocess media system. The downstream process successfully integrated microcarrier separation, continuous centrifugation for volume reduction and cell washing, vial filling, controlled rate cryopreservation, and storage, with each unit operation platform identified by the process scale.

With collaborators, RoosterBio successfully developed and transferred this process technology to BioBridge Global's GenCure business unit, achieving the goal to standardize bulk cell expansion of the process, which can be used by any company to grow a vast quantity of MSCs. These MSCs then can be used by future therapeutic developers for different downstream applications, including exosome/EV production, bioprinting of engineered tissues and organs, or gene-engineered cell therapies.

**Figure 3.** Fed-batch Bioreactor Expansion Enhances hMSC Media Productivity




▲**Fig. 3** Process innovations in cell manufacture via optimized media and adoption of 3D fed-batch culture can lead to dramatic improvements in productivity, reducing costs.

Empowered by a standardized MSC production platform for further engineering, the company is hoping to achieve an impact similar to what Lonza experienced with its GS-CHO cell line for protein biotherapeutics.[55,56] When that cell line came to the market, Lonza scientists knew how to scale it quickly to 10,000–20,000 L bioreactors to make a wide variety of proteins – bringing newfound efficiencies to biotherapeutic product development.

RoosterBio offers its customers a similar approach to MSC production: a standardized cGMP manufacturing process that anyone can access for any application — or for transfer to a CMO alliance partner to reduce the time needed for process development. The company starts by showing that a production process first works correctly in a 0.1-L bioreactor. Then it scales up the process to a 3-L bioreactor system. The MSC seed train ends with its 2-dimensional (2D) adherent growth on microcarriers expanded in stirred 3D chambers with XF media. Then a XF bioreactor feed is added three days later, and the cells are harvested for applications after five or six days in 3D culture.

The process has been shown to work via multiple donors and is repeatable, with good comparability of quality attributes. Cell surface marker expression coming out of the bioreactor is highly similar with that from flask expansion, and the cytokine secretion profile and immunomodulatory functions of the MSCs between 2D culture and 3D culture also is comparable.

The transition from traditional media exchange flask culture to a batch flask culture and then to fed-batch bioreactor expansion demonstrates increasing media efficiency as measured by cells produced per liter of media consumed. Traditional 2D cultures provide tens of millions of cells per liter, and batch 2D systems demonstrate enhanced productivity (**Figure 3**). Bioreactor expansion using fed-batch media systems are much more efficient in media volume use, tripling culture productivity. Because media are among the most significant cost drivers, overall production costs are lowered as a process moves to more efficient production platforms.

## *A Boost for Innovation in Clinical Manufacturing*

If a company wanted to start "traditional" clinical development with an adherent-cell therapy, it would need at least three years to develop and produce master and working cell banks from which production would begin. Nevertheless, a standardized platform that can generate fully tested, ready-to-go cGMP cell banks could dramatically reduce regenerative medicine product development costs and time en-route to clinical trials.

Using such a platform, several companies can work out of one cell bank for early clinical testing, allowing the entire industry to operate much more efficiently, and driving it toward improved bioprocess economics. Costs of $1–5 per million cells cannot be reached by making merely 10 billion cells. Instead, such cost efficiencies are to arrive with hundreds of billions of cells per production run. But the regenerative medicine industry is well on its way to increasing cost efficiencies, and systems such as those championed by RoosterBio are stoking the engine for Moore's law of stem cells. As demand for such products increases, the industry will have finally achieved truly cost-effective cellular therapies.

Considering the rate of innovation for an industry (**Figure 1**), as manufacturers determine solid product specifications and invest in the manufacturing sciences, they engineer the technology foundation for commercial supply of cost-effective regenerative medicines. RoosterBio believes that next-generation product innovation is going to increase dramatically when such new cellular therapy "apps" can be plugged into medical unmet needs, at much lower costs than are currently possible.

Cell therapy innovations can follow the same path as other technologies. For example, cell phones have benefited from convergent innovations, including touch-screen technologies, GPS, and camera systems — all in one small package. Such advances all benefited from decades of individual development out of several different fields. That is essentially what companies such as RoosterBio are working toward, with "standardized" platforms and dedicated teams of individuals who are focused on driving a new renaissance for MSCs.

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RoosterBio, Inc. is a leading supplier of high volume, affordable, and well-characterized adult human mesenchymal stem/stromal cell (hMSC) biomanufacturing systems designed to rapidly accelerate the commercialization of scalable regenerative cures. RoosterBio has simplified and standardized how living cells are purchased, expanded, and used in development, leading to marked time and cost savings for customers and rapid integration into cell and gene therapy product development.

Our mission is to fuel the rapid commercialization of scalable regenerative cures and further our vision of a world where safe and effective regenerative medicines are rapidly developed and widely available on a global scale, ultimately benefiting the lives of patients in need. For more information, resources, and publications like this one, please visit RoosterBio's Knowledge Center at www.RoosterBio.com and follow us on social media.

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