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PERSPECTIVES

An Industry-Driven Roadmap for Manufacturing in Regenerative Medicine

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SUMMARY

Regenerative medicine is poised to become a significant industry within the medical field. As such, the development of strategies and technologies for standardized and automated regenerative medicine clinical manufacturing has become a priority. An industry-driven roadmap toward industrial scale clinical manufacturing was developed over a 3-year period by a consortium of companies with significant investment in the field of regenerative medicine. Additionally, this same group identified critical roadblocks that stand in the way of advanced, large-scale regenerative medicine clinical manufacturing. This perspective article details efforts to reach a consensus among industry stakeholders on the shortest pathway for providing access to regenerative medicine therapies for those in need, both within the United States and around the World. STEM CELLS TRANSLATIONAL MEDICINE *2018;00:000–000*

SIGNIFICANCE

Technical advancements in the seven clinical manufacturing impact areas identified in this perspective article will accelerate clinical translation of regenerative medicine-based therapies, and facilitate the scale-up in clinical manufacturing capacity required for deployment of these therapies to the vast number of patients that would benefit from them.

THE BENEFITS OF REGENERATIVE MEDICINE

According to the US Department of Health and Human Services Report, *2020: A New Vision—A Future for Regenerative Medicine*, "Regenerative medicine will be the standard of care for replacing tissue/organ systems in the human body." Regenerative medicine represents a potentially disrupting new field of medicine that promises to deliver therapies that repair, replace, or regenerate organs and tissues rather than simply alleviate symptoms or prolong a reduced quality life. Regenerative medicine therapies use a combination of cells, biomaterials, and enabling technologies to provide engineered tissue or regeneration promoting substrates that restore function to compromised tissues. These therapies offer the potential to at times permanently cure—rather than treat injuries or diseases. Regenerative medicine therapies also provide significant cost advantages over the long term. For instance, a definitive cure for heart-valve disease in the U.S. alone would provide an annual cost savings of $23.4 billion. Considering the aging population of the U.S., these savings would increase significantly over the coming decades [1]. With the total global market for regenerative medicine products estimated at $3 billion currently, significant economic benefits are already being realized [2]. As more advanced regenerative medicine therapies are approved for human use, revenues will continue to climb. Indirect economic benefits will also be enjoyed as the field of regenerative medicine grows. The most prominent of these will be the growth of the large workforce of highly skilled and handsomely paid laborers that will be required for industrial scale clinical manufacturing [3]. All of these economic benefits rely on retention of the regenerative medicine manufacturing base within the U.S., and that retention is dependent on the development of advanced clinical manufacturing strategies that provide a competitive advantage for U.S. manufacturers.

CURRENT STATE OF MANUFACTURING IN REGENERATIVE MEDICINE

In its current, nascent form, regenerative medicine clinical manufacturing is often specialized among a collection of therapies based on a single cell type that undergo little ex vivo manipulation. Raw materials and manufacturing processes are customized for each product. There are few standards across the field, and each product is scrutinized from the ground up by the Food and Drug Administration (FDA). However, a bevy of products comprised of multiple cell types and a variety of biomaterials are beginning to enter clinical trials. As these therapies begin to advance toward widespread clinical use, efficient manufacturing

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Table 1. Summary of manufacturing challenges in regenerative medicine

Highlighted manufacturing challenges	Highlighted solution(s)
Need for scale up (e.g., expand to billions of cells)	Develop scalable bioreactor technology
High costs of manufacturing regenerative medicine product	Develop synthetic/defined media (defined media means that every component in the media is known. This will help reduce variability)
Lack of sufficient quality control systems for in line sensing	Develop in line systems for monitoring manufacturing processes that are nondestructive. In line systems allow for data to be gathered in real time throughout a manufacturing process. This is important for ensuring that your clinical product being manufactured has the necessary favorable attributes necessary for it to be a safe and efficacy therapy.
Lack of automation	Automate end to end manufacturing processes
Lack of closed and modular systems	Develop closed, modular manufacturing systems that reduce the risk of contamination
Lack of standards for regenerative medicine	Develop both reference standards and procedural standards

processes that provide a scale-up solution will become increasingly necessary [4]. Efficiency in manufacturing is partially derived from reducing variability in raw materials and manufacturing processes. When materials and processes are standardized, manufacturing may be optimized for a specific task, which improves production efficiency. This principal is well exemplified by the reliance of advanced regenerative medicine therapies on the expansion of hundreds of millions of cells from a surgical tissue sample. Any degree of standardization within the field, perhaps a standardized medium for expanding these cells, would reduce process development time, accelerate clinical translation, and contribute to an economical commercial manufacturing strategy [5–10].

Industry stakeholders have agreed on a list of primary challenges that limit the integration of regenerative medicine into standard health care (Table 1).

Identification of Manufacturing Impact Areas

In 2012, our group at the Wake Forest Institute for Regenerative Medicine initiated an effort to advance manufacturing innovation in the regenerative medicine space. The Regenerative Medicine Foundation in May of 2014 sponsored a conference in Berkeley, California, during which Industry participants drafted a white paper introducing the concept of a regenerative medicine clinical manufacturing road map that would provide a competitive manufacturing landscape for tissue engineered and regenerative medicine products [11]. The road map detailed efficient manufacturing workflows for tissue engineered and regenerative medicine products, and identifying roadblocks to expansion of the industry. The road map proposed in this white paper consisted of five elements: (a) Create an industry-driven consortium to develop infrastructure and resources to accelerate the advancement of clinical manufacturing reliability and capacity; (b) Create a series of standards for cell therapies, tissue-engineered products and combination products that would simplify and accelerate clinical translation and commercialization; (c) Incentivize innovation within the precompetitive space that would elevate efficiency in manufacturing across a broad range of clinical products; (d) Develop workforce training programs to supply quality labor for the expanding field; (e) Integrate regenerative medicine principals into all levels of the National education system.

Subsequent to the Berkley meeting, an industry consortium composed of 30 members was created, the Regenerative Medicine Manufacturing Innovation Consortium (REGMIC). This consortium operates in precompetitive space where all industry partners supporting these projects share in the data/knowledge gained. All of the members were united in the goal to promote innovation in area of regenerative medicine clinical manufacturing. Over the course of 2 years, and through hundreds of conversations among consortium partners, several national meetings, and multiple industry surveys, seven specific facets of the clinical manufacturing landscape were identified as areas in which a major impact could be made (Table 2). This list of impact areas is not comprehensive, but does reflect the consensus on the aspects of clinical manufacturing in most critical need of improvement. Each of the seven impact areas are described below.

Fully End to End Automated Manufacturing

The field will benefit from any technologies that contribute to full biofabrication that handles all aspects of the manufacturing process from tissue sample processing through product validation. The goal would be to develop an automated and modular platform, similar to current point-of-care cell processing devices, that would handle all aspects of generating a tissue engineered or regenerative medicine product. Such a platform would provide a clear means of scale-up, and would ensure reliability and reproducibility in manufacturing; resulting in fewer regulatory complications.

There are many existing technologies that could be adapted for application in regenerative medicine clinical manufacturing. Techniques used in apheresis might be combined with simple cell sorting methods for enriching specific cell populations. Automated cell culture technology may be harnessed for autologous cell expansion. Modern robotics are becoming increasingly adept at delicate work such as will be needed in generating custom tissue engineered products. Cutting edge biosensors and in vivo imaging may provide solutions for in line product quality assurance. Industry leaders working with these technologies should be incentivized to explore opportunities in the field of regenerative medicine.

The ultimate goal would be a platform comprised of modules dedicated to specific processes in clinical manufacturing. A hypothetical configuration of this platform might include a module for accepting and processing a surgical sample. In this module, tissue might be disassociated into single cells and fractionated based on a physical property such as size or density. A desired cell population could be transferred by simple fluid handling to a secondary module that would use an immune-sorting method for enriching one or more desired cell populations. These cell populations would be handed off to a third module that would provide automated cell population expansion using advanced high surface cell substrates. Other modules could house automated biomaterial fabrication to produce a customized scaffold. Cells and scaffold could be combined in a final module, and a matured construct

Table 2. Attributes of manufacturing impact areas

Manufacturing impact areas	Attributes
Fully integrated/modular/closed/sterile/ automated system for manufacturing	• Closed, integrated purification, formulation and vial-fill • Seamless media to bioreactor/cell culture vessel transition; • Cell concentration standard method to maintain viability/potency. • Customizable modules for expansion and cell retrieval • Automated cell handling in a fully controlled aseptic environment. Scale-up for mass production. • Passaging and layering multiple types of cells in a closed system • Automatic monitoring of glucose utilization/lactose production to adjust nutrient supply for continuous feed. • Off the shelf closed systems that can be easily upscaled.
Synthetic, defined serum (universal media)	• Synthetic serum for human immune cells and mesenchymal stem cell (MSCs) first. Following its success, it can be extended to other tissue sources in human body • Universal "basal" media • Synthetic, defined serum substitute "panel"
Storage and Shipping Platform Technologies	• Platform technologies for shipping human stem cells and mesenchymal stem cells should be initially tested. • Further development of formulations and methods for extending liquid storage stability • Further development and optimization of formulations and methods for freeze drying
Enabling technology using biomaterials for tissue engineering, therapy, and biosensing	• 3D printer for engineered tissues • Microfabrication of biosensors • Better oxygen and glucose monitoring sensors. • Improved material for adherent cells to maximize cell seeding and harvesting.
Nondestructive quality control strategies	• One area is lactate and ammonia management. • In line measurement of cell density. Providing in line measurement of cell density will enable real time assessment of how the cells are expanding during a manufacturing process. • Real-time cell "state"/phenotype monitoring • Data capture and mining/correlation. • Microscope with software for recognizing and quantifying cellular structures. • Disposable sensors or built in sensors for growth factor levels in addition to pH, osmolarity, oxygen (O_2), and carbon dioxide (CO_2). Glucose, ammonia, and potassium ($K+$) would be great as well. • There will not be a path to a universal solution—too divergent. Pick either most universal quality control spec/parameter (most therapy agnostic) or highest impact upcoming therapy—propose and prototype a solution
Automated and closed patient-specific-processes	• Integrated, standardized disposables for product types • Processes that are patient-specific and would involve modeling the patient's anatomy • Easily adaptable semi-universal automated system. • Semi-universal disposables. • Automated injector with detailed process control. • Novel single use sensors/sensing approaches for noninvasive monitor/control of process parameters and detection of microbial contamination • Disposable single use bioreactors that will support all steps from seeding through harvest. • Instrumentation that can provide carbon dioxide (CO_2) and temperature control without traditional incubators
Bioreactor technology (suspension, adherent, tissue); convergent or divergent platforms	• Determine if same system could be scaled to fit both small and large-scale needs • In-line cell "state"/phenotype monitoring. In-line cell state will provide in real time what is the phenotype of that cell type being expanded. This is important to ensure that you are expanding the appropriate cell types. • Scalable bioreactor technology • Novel single use format for high density cell culture (adaptable adherent or suspension, or tissue constructs) • Design for scale-out strategy for autologous

could be delivered in a sterile hermetically sealed package, ready for delivery to a waiting patient.

Standardized, Chemically Defined, and Xeno-Free Cell Culture Media

Commercially available cell culture media for most human primary cells contain a variety of "black box" biological extracts and suffer from lot to lot inconsistency and the potential for disease transmission. In addition, a myriad of media formulations, each optimized for a specific cell type, have been independently developed. A chemically defined and xeno-free cell culture medium would provide a common backbone, on which, media for clinical manufacturing could be built.

Industry leaders from large, medium, and small companies with expertise in cell therapy and regenerative medicine proposed the development of a chemically defined base medium, comprised of synthetic substitutes for many bioregulatory factors that would promote the expansion of a maximum number of cell types. This media formulation would limit the production of toxic metabolites, and eliminate the possibility of donor source pathogens. Many biological extracts including serum, platelet lysate, and

pituitary abstract are well characterized, and could provide guidance in producing a more defined medium for clinical manufacturing. Supplementation of a standard medium with additional bioregulatory molecules would be required for many human cell types. However, only these additional factors would need to be considered in process development and FDA review.

Efforts have already been initiated by members of the consortium to produce a series of chemically defined human primary cell media. These efforts are currently focused on producing media tailored to cells derived from each of the three embryonic germ layers. Media constituents include synthetic, recombinant, and human sourced molecules that may be combined into well-defined formulations. Should these formulations be adopted by the clinical manufacturing community, they will become familiar to the FDA and approval of clinical manufacturing processes will be simplified.

Noncryogenic Storage Methods

Storage of cells is currently accomplished through cryopreservation. This method is costly, unreliable, requires bulky equipment, and induces major stress to cells during both freezing and thawing. Current research is being conducted toward the development of cell stabilization media that preserve cell viability over extended lengths of time. The problem of cell and product storage can also be addressed by improving transportation and moving production to point of care where long-term storage becomes less of an issue.

Alternatives to dimethly sulfoxide (DMSO) for stabilizing cell membranes during long-term cryopreservation might increase cell viability following recovery. Cutting edge lyophilization might offer methods for dry-storage of cells that may be reconstituted years later. Shipping platforms must also be developed that would include media and physical assets that would slow the metabolic rate of cells and provide sufficient oxygen/nutrient delivery while eliminating toxic cellular waste. This would require control of temperature, fluid flow media formulation and vessel geometry. Transportation would also need to incorporate advanced biomonitoring to ensure that the transported product was maintained under appropriate conditions during shipping.

Biomaterials in Regenerative Medicine

Significant challenges remain in the biomaterial component of regenerative medicine clinical manufacturing, Chief among these are the need for standard reference materials, standard quality assurance metrics, and sterility verification.

Cell scaffolds represent the backbone upon which the cellular component of a regenerative medicine product is built. Consistency in this backbone is the foundation of a consistent product. Industry standards, and the metrics by which these standards are applied, must be developed for a variety of natural and synthetic cell scaffolds. A recent working group formed with the American Society for Testing and Materials (ASTM) is developing fundamental assays for assuring biomaterial composition and structure. Sterility of biomaterials remains a significant concern. Modern molecular based pathogen detection techniques must be incorporated with biomaterial manufacturing and regenerative medicine clinical manufacturing.

Non Destructive Quality Control Strategies

There are many challenges associated with clinical product quality control, and these challenges increase exponentially with the complexity of the clinical product. First generation quality assurance in regenerative medicine clinical manufacturing relies on sacrificial constructs that are processed in parallel with clinical products. These materials are evaluated as an indication of the product that is intended for therapeutic application. The use of these sacrificial constructs diverts resources from the therapeutic product and may not accurately reflect the quality of the product on which they are meant to inform. Advanced strategies for in-process quality assessment are needed.

A promising solution currently under development is an array of biosensors that continuously monitor protein biomarker in the media. The most recent of the biosensors are sensitive to the femtomolar range and offer real time data regarding both the function and viability of tissue constructs. Many cells secrete specific protein biomarkers that indicate a functional phenotype. Additionally, many cell types contain discriminate proteins within the cytoplasm that are released into the media upon cell death. This provides the opportunity to monitor specific cells within a multicell type construct in a noninvasive and nondestructive manner. Development of standardized and multiplex biosensors for specific cells and tissues would represent a powerful tool in automated biofabrication. Additionally, biosensors for microbial pathogens could be used to ensure product sterility in real time.

In vivo imaging represents another potentially powerful in-process quality assurance tool. These imaging technologies could be based on fluorescence, refraction index beam scatter, or any number of additional imaging techniques. Nondestructive gross visual inspection clinical products during manufacture may also provide critical quality assurance data. Improvements in quality control in clinical manufacturing would assure product consistency, save money by identifying deficient products early in the production process, and assure product safety in terms of sterility.

Automated and Closed Patient-Specific-Processes

Some regenerative medicine therapies may be accomplished over a short time frame and at the point of care. These therapies would avoid many of the roadblocks alluded to within this document. Small and mobile processing stations would provide a large economic strategy for delivering regenerative medicine therapies. Because these therapies are generally autologous, any strategy that maintains product manufacture at the site of tissue harvest would be most efficient. This impact area is dependent on several others, but combines those technologies into a mobile, and self, sufficient platform that may be deployed at a patient's hospital. Point of care clinical manufacturing eliminates the need for tissue or construct transport and reduces time between tissue harvest and product implantation. In comparison to centralized clinical manufacturing facilities, point of care processing stations would foster competition and innovation.

Bioreactor Technologies (Suspension, Adherent, Tissue); Convergent or Divergent Platforms

Bioreactor technologies have advanced significantly over the past 15 years. Unfortunately, current cell expansion technologies have not given consideration to the expansion of most human primary cell types. Many of these cell types expand preferentially under dynamic physical conditions provided by a perfused bioreactor. Emerging techniques for increasing bioreactor surface area may

increase cell expansion capacity significantly. Recent advances in the culture of cell aggregates suggest that a three-dimensional (3D) cell culture strategy might be appropriate for large-scale cell population expansion. Bio-supportive 3D hydrogels containing stabilized bioregulatory molecules or natural, tissue specific extracellular matrices have been shown to promote significant proliferation of primary cells. Any bioreactor strategy for primary cell expansion would need to consider scalability, and bioreactor geometry will need to change across a range of scales. Optimization of bioreactor design will need to be accomplished at 10-L, 50-L, and 100-L scales in order to accommodate all of the needs of the regenerative medicine field.

Summary and Conclusion

Seven impact areas have been identified by regenerative medicine stakeholders for targeted technical advancement. These areas represent the consensus opinion of a large industry consortium. Addressing these impact areas supports the health of the regenerative medicine field, and helps broaden the regenerative medicine clinical manufacturing base around the world. While different countries will have specific regulatory considerations, industry can build off of these platform technologies to manufacture their regenerative medicine technologies at a global scale. The development of methods for automated biofabrication and methods for ensuring product efficacy and safety will enable regenerative medicine products to be manufactured at commercial scale. Ultimately, meeting these manufacturing challenges will help to accelerate the transfer of regenerative medicine therapies to patients by developing solutions to scale up these therapies, reduce their costs, and make them widely available.

Author Contributions

JH, TS, AA wrote and conceived of manuscript.

Disclosure of Potential Conflicts of Interest

None.

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