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ARTIFICIAL TISSUES (A ATALA AND JG HUNSBERGER, SECTION EDITORS)

Five Critical Areas that Combat High Costs and Prolonged Development Times for Regenerative Medicine Manufacturing

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Abstract
Purpose of Review The purpose of this review is to examine five stages of process improvement in bioengineering of cellular products that could facilitate standardization and accelerate progress through the regulatory pathways and together make such treatments more widely available.
Recent Findings We present solutions to reduce costs, promote standardization, and enable acceleration through the required regulatory pathways.
Summary Regenerative medicine-based technologies and products have the potential to revolutionize the practice of medicine and become the next standard of care. We identify current barriers that are limiting the widespread availability of these potential life-saving treatments and platform technologies. One central barrier is the cost of manufacturing these regenerative medicine-based technologies and products at commercial scale.

Keywords Precision medicine · Tissue engineering · Stem cells · 3D printing · Body-on-a-chip

Introduction

Regenerative medicine seeks to develop therapies that can replace, repair, and regenerate injured or diseased cells, tissues, and organs. It encompasses tissue engineer and cell therapies, as well as enabling technologies. A recent perspective article focuses on tissue engineering approaches, and presents the possibilities of achieving 3D printing of human hollow organs including arteries, trachea, larynx, urethra, bile duct, and facial reconstruction of ears and nose [1]. In addition to the technical limitations to advancing our current technologies to more complex solid organs, the costs to manufacture these regenerative medicine-based therapies are too high to make such treatments widely available. There are few reported studies that document these costs. A group from the UK published for the first time a cost analysis for a tissue-engineered organ. Specifically, they determined that the costs for stem cell-based tissue-engineered airway transplantation ranged from $174,420 to $740,500 per patient [2••]. Clearly more of these types of studies and cost analysis are needed. This perspective article addresses five critical areas outlined in Table 1 including (1) cell dynamics, (2) cell expansion and maturation, (3) 3D bioprinting, (4) preservation and shipping, and (5) quality control. We believe strategic advances in each of these five critical areas will mitigate the current high costs of regenerative medicine manufacturing, which is required to enable these exciting new regenerative medicine-based therapies to become the next standard of care.

Cell Dynamics

Cell processes are dynamic. These multitudes of processes require support media to maintain the viability, function, and phenotype of cells in ex vivo conditions. Cells are also the building blocks for regenerative medicine in three main applications. The first application is the development of cell type-specific screening platforms commonly known as "tissue-on-a-chip" or "body-on-a-chip" platforms. These devices are being developed for personalized medicine approaches. The second application is cell-based therapy. The

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Table 1 Regenerative medicine manufacturing landscape

Key areas	Challenges	Solutions
Cell dynamics	Developing commercial grade media to support cell viability that is cost effective, standardized, and defined	Develop a universal, defined media formulation that can support many different cell types, tissues, and organs with cell-/tissue-specific supplements that is cost effective
Cell expansion and maturation	Expand cells to sufficient number for cell therapies or to be seeded on a scaffold for tissue-engineered products. Both cells and bioengineered products will need to be matured before their clinical use	Develop modular universal bioreactors to provide cell and tissue specific expansion and maturation environments to direct cells and bioengineered products to be ready for clinical use
3D bioprinting	Lack of standards for enabling 3D bioprinting of bioengineered products such that each facility must develop their own set of parameters, cell types, and biomaterials for each application	Develop a universal bioink formulation
Preservation and shipping	There are not sufficient technologies for preserving viable tissues and transporting them between hospitals and clinical manufacturing facilities	Development of tissue and cell preservation transport systems that can support the viability of bioengineered tissues and organs as well as providing quality assurance to its integrity and viability
Quality control	There is need for development of in-line biosensors to assure the quality of the clinical manufacturing product along the entire manufacturing process	Development of non-destructive quality control sensors that can integrate all along the regenerative medicine manufacturing process to ensure sample integrity and quality

Challenges and solutions are presented along 5 key areas: (1) cell dynamics, (2) cell expansion and maturation, (3) 3D bioprinting, (4) preservation and shipping, and (5) quality control

third application is a tissue-engineered organ that is constructed with many different cell types.

Each of these applications requires media to support the dynamic cellular processes. Currently, there is a lack of universal, defined culture media. This is one of the major roadblocks to regenerative medicine prototype development and product manufacturing. Presently, there are numerous challenges in the formulation of media for all phases of cell-based regenerative medicine clinical manufacturing. A recent review addresses some of the challenges in developing a defined, serum-free medium for cellular therapies and is encouraged for readers wanting a more in-depth discussion of this topic [3].

Broadly speaking, each human cell type has specific requirements to maintain both viability and function. In in vivo conditions, these requirements include cell-to-cell interactions, mechanical stimulation, access to nutrients, etc. Recapitulating this environmental milieu in vitro is a challenge. However, in order for regenerative medicine products to thrive, commercial grade media formulations that support clinical manufacturing are required.

In many cases, media for the support of cell isolation, expansion, differentiation, and maintenance, as well as media used in the generation and maturation of engineered tissues, consist of a minimum essential medium supplemented with serum. While the essential medium provides nutrients and other factors necessary for meeting the metabolic requirements of cells, the serum provides the complex array of bioregulatory factors required for the long-term maintenance of cell viability, function, and phenotype. There are thousands of combinations of media and additives in the literature. These different media can vary widely, both for a specific cell type and among varying cell types. The choice of media can be focused on the specific requirements of a given cell type but can also vary depending on the media manufacturer, international geographic regions, and cost.

Additionally, different media may contain a wide array of additives, depending on the cell type targeted. The presence of various cell types within the same therapeutic product, such as in engineered tissues, presents additional challenges for media selection. As a result, the manufacturing of cell-based products in the field of regenerative medicine is complicated by the lack of standards in the use of media.

Various entities within the clinical manufacturing industry formulate specific cell media that meet each of their specific needs. The cell media are formulated from the ground up, to meet the specific metabolic requirements of human cells within the clinical product. Each of these media formulations needs to be evaluated and approved by the FDA and/or other regulatory agencies in countries outside of the USA, before manufacturing can be initiated.

Unfortunately, variations in base media composition and lot-to-lot variability in cell culture sera often produce inconsistent effects on cells. In addition, the use of xenogeneic media components increases the potential for contamination with microbes or prions and introduces additional inconsistencies in clinical manufacturing that can complicate process development and navigation of the regulatory pathway.

These challenges have created a need for the development of a universal, xeno-free basic medium that meets the

biochemical, physical, and nutrient requirements for a wide array of human cells.

Recent advances have reported success in maintaining the viability of multiple micro-engineered tissues comprised of several human cell types using a common medium. With these recent advances, it may be possible to develop a single universal medium for cell maintenance, with sets of specific defined additives that could be used to support the expansion of cells derived from all three germ layers: ectoderm, mesoderm, and endoderm.

Every cell in the human body can be traced back to one of these three germ layers. Also, the target for cell expansion is the non-terminally differentiated cell population that is phenotypically similar to undifferentiated cells from each human germ layer. As a result, the development of three core media formulations that can be used for the expansion of most all cell types used for regenerative medicine manufacturing has been noted by regulatory and industry experts to be a feasible strategy.

A universal medium would create a common starting point that could be supplemented with additional factors tailored to the support of specific human cell types. This universal medium would contain well-characterized components that would simplify review by regulatory agencies. The universal media would be produced at a large scale, reducing production costs that would reduce the price of regenerative medicine clinical manufacturing.

Development of a Modular Universal Bioreactor

Bioreactors are an essential component of the regenerative medicine manufacturing landscape. Regenerative medicine clinical products are manufactured in multiple steps and require cells during the process. Cell-based products, as living constructs, often require specialized bioreactors for expansion and/or maintenance. For engineered tissues, a biomaterial is shaped into a scaffold with the morphology of the desired construct, and human cells are applied to the scaffold in the cell-seeding phase of production. Alternatively, tissue constructs may be bioprinted into desired morphologies using cell-laden bioinks. With these and other regenerative medicine manufacturing strategies, cell-based products are conditioned and matured over a period of time, in vitro.

Over the past two decades, it has been noted by both the regenerative medicine industry and academic researchers that the physical conditions within bioreactors during all phases of engineered tissue production have a major impact on the cellular characteristics of the product, including micro-architecture, functionality, and durability. Therefore, bioreactors have been deemed critical for the efficient manufacturing of regenerative medicine products. However, there are challenges in terms of the standardization of these bioreactor systems, as each tissue product has unique requirements in terms of its specifications. Different types of tissues need different shapes and sizes of bioreactors. Also, during the maturation phase of producing an engineered tissue, mimicking the physical conditions that the construct will experience, in vivo, following implantation can stabilize the tissue to tolerate these conditions. For example, engineered vessels and valves are stabilized by pulsatile flow; engineered muscles are fortified by cyclic uniaxial stretching; the pliability of engineered skin is increased by cyclic biaxial stretching; and the elasticity of hollow, non-tubular organs, such as bladders, may be bolstered by cyclic expansion/contraction.

Currently, the regenerative medicine industry (including both academic centers and industry) designs and fabricates these bioreactors independently. The cost associated with the engineering and fabrication of these custom-built bioreactors can be extremely high. There is also considerable time investment associated with the development of these bioreactors, further lengthening the timeline for clinical translation. Additionally, when engineered regenerative medicine clinical products are translated from preclinical to clinical testing, all of the hardware involved in the clinical manufacture of the product must be validated and must be deemed acceptable by regulatory agencies. If problems are identified in either the materials used for bioreactor fabrication or the mechanical operation of the system, the bioreactor must then be reengineered to conform to regulatory standards. A key challenge is the development of a modular universal bioreactor that can be configured to produce optimal conditions for the maturation of a variety of tissue-engineered clinical products. A recent publication [4] provides insights on bioreactors for cell therapies and covers the current status and future advances in this rapidly growth area.

Therefore, the development of a standardized, scalable, modular, and configurable bioreactor platform for the production of regenerative medicine cell-based clinical products will greatly help in making the process economical and efficient. Depending on the specific organ, the bioreactor manufacturing facility will have ready-made available components that would be easy to assemble with a rapid turnaround time. If such a system can be scalable, with modular shapes, many different tissue constructs could be accommodated. The bioreactor can then be configured to optimally position and stimulate the tissue-engineered construct which will prepare the construct to be physiologically active and ready for implant. All the bioreactors would have the same available software that would accommodate varying conditions and recordings. Additional features can also be standardized and incorporated, such as interchangeable spinners, agitators, tissue anchors, tissue actuators, cannula, electrodes, etc. Tissue anchors and brackets will be designed that can interface with push/pull rods and actuators for applying mechanical force in multiple axes. Integrated sensors for measuring environmental parameters will also be incorporated.

The materials used for the fabrication of platform components, especially those which come in contact with cells, biomaterials, or fluid, should be evaluated in terms of safety as determined by molecular stability, reactivity, and a history of use in approved clinical manufacturing equipment.

This will allow the bioreactors to be readily configured for any desired application and rapidly delivered based on the needs of the industrial entity, thus greatly simplifying and reducing the cost and time associated with the development of processes for the manufacture of engineered tissues.

Development of Universal Bioinks

Regenerative medicine therapies are entering the clinical translation pipeline at an ever increasing rate. Due to its capability to rapidly assemble biological materials, additive manufacturing or 3D bioprinting, using bioprinters, is expected to become a cornerstone of regenerative medicine clinical manufacturing. But technologies that facilitate the implementation of 3D bioprinting in regenerative medicine clinical manufacturing are one of the roadblocks. Bioprinters are intricate devices that are generally based on three deposition modalities: inkjet, micro-extrusion, and laser assisted. All of these approaches, though technically distinct, require cells to be suspended in a bioink during the printing process. The bioink, which is a bioactive material used in the 3D bioprinting process, is a combination of cells and biomaterials. The bioink serves as the environment that supports the cells and gives structure to the overall 3D bioprinted construct. The utility of these bioinks could be used in numerous applications ranging from creating an acellular 3D construct to a tissue-engineered construct with embedded cells for either temporary or permanent replacement. What is needed is a bioink toolkit that can facilitate the development of 3D-printed naïve tissue constructs [5••]. These bioinks must be formulated to an appropriate elastic modulus based on the following:

1. The particular bioprinting technique being employed
2. The speed that the construct is bioprinted
3. The cell type(s) that are suspended in the bioink
4. The resolution at which the construct is printed

Beyond the mechanical properties of bioinks, bioprinted tissues also have different final optimal elastic moduli that must be considered if maximum tissue function is to be achieved. This optimal stiffness will take into consideration both direct cellular support as communicated by mechanosensors intrinsic to the cells and the mechanical properties necessary to support the structure directly following implantation. Often, this optimal stiffness will be quite different than that which is required for bioprinting the construct.

In order to meet all of these requirements, bioinks are currently custom formulated for each specific application, resulting in a wide array of bioink components and formulations, with little, if any, standardization. Each of these bioink formulations will need to be evaluated by regulatory agencies for approval in clinical manufacturing, adding significantly to process development time. Therefore, the development of a universal, standardized bioink for the bioprinting of tissues and organs for patients should be deemed as an area of the highest importance. Such universal bioink should contain well-characterized components that are accepted as safe by regulatory agencies. These components can be combined in a modular fashion at various ratios in order to produce bioinks with the specific mechanical properties that meet the requirements for almost any bioprinting application. The tuning of mechanical properties of bioink also allows for further adjustment of stiffness once the construct has been produced, thereby aiding in optimization of the construct stiffness in order to best support tissue structure and function. For additional reviews on bioinks, these references are suggested [6–8].

The overarching goal would be to develop a standardized hydrogel bioink that meets regulatory standards and has adjustable stiffnesses for both bioprinting and for setting the elastic modulus of the final product. This will provide a valuable tool for ensuring cost-efficient manufacturing of bioprinted regenerative medicine products.

Development of a Tissue and Cell Preservation Transport Support Platform

The lack of a method for maintaining healthy, viable tissue during potentially long transport times which could include from manufacturing facility to the patient or from hospitals and clinics to clinical manufacturing facilities. Both of these transport processes represent critical roadblocks to manufacturing regenerative medicine products. Many regenerative medicine clinical products start with human tissue retrieval. This includes routinely obtained samples (e.g., from cord blood, bone marrow, fat, and amnion) for harvesting stem cells, as well as primary organ-specific biopsies for cell isolation. From these human samples, cells are isolated and cultured for cell therapies or incorporation into engineered tissues. All of these rely on optimized methods for shipping samples across long distances. The potential for delays in shipping raises the possibility of tissue quality deterioration between the site of harvest and the manufacturing facility. This can present a major challenge for cell-based therapies, where cell survival is critical for manufacturing and clinical success. The human sample preservation during shipping is one of the major challenge areas that need to be addressed in order to facilitate regenerative medicine industry manufacturing success.

Therefore, it is necessary to develop a tissue and cell preservation transport support platform for shipping human samples. The platform should incorporate a stabilizing liquid medium, dynamic fluid movement, an onboard temperature control regulator, and a reporting system. The goal is to lower the metabolic activity of the tissue/cell samples and to stabilize the product to prevent significant loss of viability over a 7-day period. This would greatly reduce the chances that production of the clinical product would need to be aborted. It would also ensure maximal preservation of tissue and cell quality to provide the best possible material for regenerative medicine (REGENMED) clinical manufacturing.

Living tissue begins to deteriorate the moment it is removed from an organism with preservation times for most vital organs ranging from 4 to 12 h [9]. Without constant perfusion with blood, cells lose access to oxygen and other metabolic requirements. Toxic waste products begin to accumulate, and ion concentrations change. These processes eventually lead to cell death. The development of a tissue and cell preservation transport support platform would provide onboard solutions for mitigating the factors that allow tissue deterioration to proceed. Reduction of temperature will be used to slow cellular metabolic rates as has been done with freezing and storing living cells decades now [10]. This thermal control system will use chemical cooling combined with high-performance insulation to maintain desired temperatures. A liquid stabilization medium will be selected from commercial sources, or specially designed, to support tissue for several days. Engineered holding brackets can maximize the sample surface area during transport, allowing dynamic flow conditions to perfuse the tissue/cell samples to the greatest degree possible. Upon receipt, a thorough evaluation of the tissue/cell is required but an onboard biomarker unit can be engineered which provides an initial indication of cell viability upon receipt.

Given the resources consumed in preparation for cell-based therapies, the economic benefit of dependable shipping is significant. Ethically considering, all precautions should be taken to preserve the quality of transported tissues in order to maximize the chances that a high-quality and effective clinical product will be produced. The possibility of needing to perform another procedure to obtain a second sample, even if that was possible, should also be kept to a reasonable minimum. For these reasons, creating a mobile tissue and cell support platform will improve regenerative medicine clinical manufacturing and accelerate the rate that regenerative medicine clinical products will become available in the clinic.

Development of In-process, Real-Time Quality Assurance System

Quality assurance and evaluation of product release criteria represent significant components of any clinical manufacturing process. Tracking the quality of regenmed clinical products as they move through production is a critical aspect of clinical manufacturing. To address this challenge, there is a need for creation of an in-line electrochemical biosensing approach for monitoring secreted protein biomarkers and metabolic factors from cells and engineered tissue constructs in a non-destructive manner are proposed. While currently not at this point for regenerative medicine manufacturing, the pharmaceutical industry is developing technologies to ensure quality of the final product by measuring critical quality attributes of the product in real time by non-destructive and non-contact methods during the manufacturing process [11]. Regenerative medicine-based therapies offer an additional level of complexity beyond a small molecule product. Some of these challenges include rapid expansion of several cell types, tissue engineering processes for developing a complex 3D architecture, and then maturation of this tissue complex. Therefore, developing non-destructive methods for monitoring quality control and assurance for regenerative medicine-based products is needed to facilitate manufacturing and ensure a safe and effective product.

This biosensing approach to monitor secreted protein biomarkers from cells and engineered tissue constructs would reduce the number of times that cells or tissue constructs would need to be sampled. The method for biomarker detection is not destructive to cells or tissues. The biosensors may be multiplexed to provide real-time quantitative and qualitative information for several cell types, simultaneously. Such systems are already being developed to provide real-time sensors for organ-on-a-chip devices [12••]. Automated monitoring of such biophysical and biochemical parameters would also reduce labor associated with off-line testing. Such sensors can be well aligned with regulatory requirements, produced inexpensively, and may be rejuvenated multiple times before replacement. The sensitivity and specificity of these sensors are exceptional, offering highly discriminate quantitation of biomarkers down to picogram per milliliter levels. This system would provide inexpensive, non-destructive, in-line, and real-time quality control for both the cell expansion and tissue construct maturation phases of regenmed clinical manufacturing. It has several advantages over direct sampling of cells and engineered tissues:

1. In-line monitoring eliminates the need to access cells or tissue construct within bioreactors, thus minimizing opportunities for introducing contamination
2. Secreted protein biomarkers may be selected for certain cell types that offer information on not only viability but also the functionality of cells
3. Protein biomarkers can be used to report on the functionality of tissue constructs without destructive tissue sampling
4. Multiplexed electrochemical biosensing can provide real-time quantitative and qualitative information for many cell types, simultaneously

5. Labor associated with off-line testing is markedly reduced from the manufacturing process.

In all, such sensor suite will provide real-time feedback on product safety, purity, and potency throughout the cell expansion phase of manufacturing. Successful development of in-process non-destructive technologies would reduce manufacturing costs, increase product consistency, and simplify review by regulatory agencies, which would bring down the overall price of regenmed clinical manufacturing.

Conclusion

We have covered five critical areas in this review: (1) cell dynamics, (2) cell expansion and maturation, (3) 3D bioprinting, (4) preservation and shipping, and (5) quality control. Developing solutions as we have suggested in each will help ensure regenerative medicine-based therapies can be scaled up and manufactured to ensure wide spread availability.

Compliance with Ethical Standards

Conflict of Interest Joshua G. Hunsberger, Sandeep Goel, Julie Allickson, and Anthony Atala declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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