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Bioprocessing of human mesenchymal stem/stromal cells for therapeutic use: Current technologies and challenges

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ABSTRACT

The long-term outlook for regenerative medicine predicts an increased need for scalable cell expansion technologies that utilize non-animal derived materials and are compatible with the limited number of downstream processing steps required for cell-based therapies. As more stem cell therapeutics progress through clinical testing, current *in vitro* culture methods using planar vessels are proving cumbersome to scale. Therefore, alternative processes are under investigation. Many human mesenchymal stem/stromal cell (hMSC) bioreactor-based manufacturing processes, in particular, are complicated by the requirement to separate cells from microcarriers with high cell yield and viability whilst maintaining target phenotypic and functional characteristics. Here we review currently available technologies and ongoing development for the expansion of cellular therapeutics, with focus on allogeneic hMSCs and microcarrier-based processes. Upstream challenges include the interplay between the cell culture substrate and media formulation, sourcing of high quality animal-free reagents, and considerations for the use of microcarriers in stirred-tank systems. Complications in downstream processes include harvest approaches for separation of cells from microcarriers and volume reduction.

1. Introduction

Stem cell-based therapies are distinct from traditional biopharmaceuticals in that the cell itself is the final treatment product rather than simply the means by which to produce a drug substance. Stem cells injected into a patient may engraft and/or secrete molecules that elicit an endogenous response. The injection is a complex therapeutic that must be administered in a certain functional state and can be influenced by the microenvironment or niche [1,2].

There are two categories of cellular therapeutics: autologous and allogeneic which can also be described as patient-specific and universal donor, respectively. For an autologous therapy, the individual donor is also the recipient of the treatment termed a "one-to-one" therapy. In the allogeneic approach, a single donor provides primary cells used to produce the therapeutic cells that will be administered to many recipients; thus considered a "one-to-many" therapy. Autologous and allogeneic therapies each require distinct criteria in order to meet manufacturers' and patients' needs. Autologous applications may require expansion of cells prior to administration; thus the scaling of these technologies is considered "scale-out" where many donors' cells may be processed in parallel. Key needs for autologous expanded cells are automated and closed manufacturing, as well as faster testing of the drug substance. Cell expansion is required for allogeneic therapies in order to generate ready-to-use doses for multiple patients; these are considered "scale-up" applications, calling for larger volumes to meet lot size needs. Key needs for allogeneic expanded cells are scalable expansion vessels up to 1–2kL with similar downstream processing capabilities [3,4]. There are examples of cell therapies that cross these commonly used descriptors, such as a cord blood transplants that are one-to-one, but in fact can be used in non-matched recipients. In all instances, cGMP grade raw materials, including serum replacement and ideally chemically-defined media, are important for ensuring that the highest quality product is manufactured.

There are a growing number of clinical and commercial activities in the cell therapy space including hundreds of global trials and

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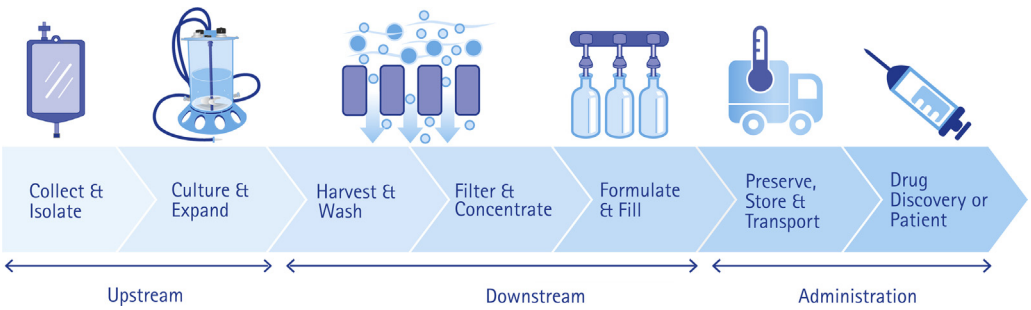

Fig. 1. A process for the manufacturing of a cellular therapeutic. Major challenges remain in all stages; in particular harvest, formulation and transportation.

marketed products for "stem cells", excluding gene modified therapies [5]. The vast majority of these employ adult, multipotent cells, such as human mesenchymal stem/stromal cells (hMSCs). Whereas many of the first cell therapy applications treated musculoskeletal and skin diseases, some of the most promising applications include those for acute diseases, such as cardiovascular or stroke events, and immunological dysfunction. Celyad (formerly Cardio3 Biosciences) is using hMSCs differentiated to a cardiac progenitor lineage in a phase 3 clinical trial to treat congestive heart failure [6]. Osiris Therapeutics recently divested their culture-expanded hMSCs to Mesoblast, and continues to investigate autologous applications in cell therapy [7], while Mesoblast has a phase 3 trial application for graft versus host disease using the expanded hMSCs [8]. Although a joint Athersys-Pfizer phase 2 trial did not find a significant effect of undifferentiated bone marrow-derived hMSCs on ulcerative colitis at midpoint outcomes, the safety profile of first round dosing was favorable and the trial continued to second round dosing and later time points [9,10]. Athersys is also evaluating these cells as possible treatment for other indications including ischemic stroke [11].

Challenges in generating cells for therapeutics include the lack of scalable, cost-effective systems utilizing processing equipment in which the product is not exposed to the immediate room environment (*i.e.,* closed systems). In order to affect this sort of system, solutions that ensure patient safety, operator ease of use and lot-to-lot reproducibility in the final product must be implemented. There has been intense focus on increasing scale and yield in order to drive down costs [3]; however, another avenue that can be investigated is to increase the potency of the cells produced. With a true understanding of the mechanism of action, the production of cells with specific quality attributes can be directed, thus requiring fewer cells for the same treatment. The result is a lower cost of goods and smaller batch size requirement. Both development of scalable manufacturing solutions and improvement of potency of the cells should be pursued in order to ensure the successful implementation cell therapy treatments.

The large-scale industrialization of stem cell production for their use as therapeutics (Fig. 1) presents several opportunities for manufacturers to develop regulatory-compliant, cost-effective processes. As discussed herein, it is clear that movement away from planar culture and towards stirred tank bioreactors where suspension culture using microcarriers is enabled, will be a requirement [4]. The bulk of current work to transition hMSCs to stirred tank bioreactors leverages microcarriers, however the growth of hMSCs as aggregates has also been described [12,13]. More recently, Alimperti et al.demonstrated the culture of hMSC spheroids in small-scale suspension culture [14]. Although this approach will not be discussed in detail, many of the concepts herein concerning reagent and process optimization may also be relevant to hMSC expansion in stirred systems without microcarriers. A complete single-use, end-to-end, manufacturing process is attractive due to its lower overall start-up cost versus traditional stainless steel processes, its inherent flexibility and adaptability to the emerging requirements of cell-based therapeutics, and the scale required. Some of the difficulties to this approach include ensuring that all components interacting with the process stream are low in extractables, do not contain materials derived from animal sources or at least comply with established regulatory guidance on minimizing the risk of transmitting animal spongiform encephalopathy as described in EMEA/410/01, while at the same time meeting the requirements of cell yield and performance. It should be noted that these are of critical importance as the cells undergo minimal processing before introduction into patients.

2. Scale-up approaches

2.1. Transition from planar to microcarrier-based systems

Adherent cell expansion has traditionally been performed on planar surfaces such as well-plates and tissue culture flasks for simplicity and easy handling when large numbers of cells are not required. For larger scale expansion, multi-layered flasks, spinner flasks and bioreactors offer higher capacity (available technologies summarized in Table 1); however, optimal culture conditions for stem cells are still under investigation [15] and will certainly depend on each particular cell type. In addition, there is concern regarding the population doublings required for expansion of cells to sufficient quantities to meet target dose requirements [16]. There are challenges with growing stem cells in multi-layered flasks as these vessels are cumbersome and time-consuming to handle, have limited scalability and typically limit the user's ability to monitor cell health or marker status during cultivation. Transitioning from planar-based culture to microcarrier-based systems not only allows for higher density culture, and thereby cost of goods reduction, but also for more stringent culture control and monitoring.

The transition to bioreactors allows for greater process control since samples can be collected during and following the expansion process and characterized by off-line analytics such as flow cytometry which aids in optimizing the process. In order to retain the functional characteristics of hMSCs, the passage numbers and cell doublings are monitored and minimized [17]. MSCs expanded both in planar and suspension formats are commonly evaluated for cell identity and purity by criteria outlined by the International Society for Cellular Therapy [18] as well as the International Federation of Adipose Therapeutics and Sciences [19], yet these criteria may not always be indicative of function [20]. Apart from these recommended criteria, there are additional markers which are used by several researchers in the stem cell field, often with the goal of isolating or defining a more homogeneous population of cells [21–23]. Moreover, a broad range of functional assays are employed to determine whether expanded hMSCs have maintained differentiation potential, cytokine release, immune modulation, migration capacity and/or angiogenic potency, as examples [23].

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