Facilitating scale up: controlled stem cell cultivation in stirred suspension bioreactors

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Stem cells have been envisioned as becoming an unlimited cell source for regenerative medicine. However most applications require a large number of stem cells and it is difficult to translate recently applied cultivation methods into the larger scale desired. Stirred suspension bioreactors in particular offer a suitable option. These bioreactors have been successfully introduced at the Hannover Medical School, Germany.

Basic stem cell research is primarily concerned with biological aspects, such as the influence of growth factors and media composition on cell growth and differentiation, whereas current studies also focus on the optimisation of cultivation processes. The main purpose of these efforts is to enable a larger scale process. Traditionally stem cells are cultivated in static 2D culture devices and a simple transfer of this cultivation technique to a larger scale is not possible. State-of-the-art cultivation methods, such as hanging drops or plating upon non-tissue culture-treated plates can also hardly enable a larger scale process.

The only established procedure is to increase the number of batches, but a 500-fold petri dish approach would be needed to provide the number of cells necessary for the therapy of one myocardial infarction patient [1]. The main problems occurring with this procedure are the high possibility of user-to-user errors and the intense lab effort which makes the procedure very unprofitable. Traditional techniques and devices for stem cell cultivation have to be adapted, not only to enable larger scale production, but also to meet the demands of present and future cell therapy requirements.

Large-scale production would be facilitated by the use of stirred tank bioreactors, in which culture parameters such as dissolved oxygen tension, pH, and fluid shear are steadily controlled. Using stirred tank bioreactors, which are the standard tool for the development and commercial production of biopharmaceutical material, also greatly decreases the costs incurred with large-scale cultures by reducing maintenance costs per production unit [2]. These well established tools would appear to be the ideal option for achieving the next level of stem cell cultivation.

The approaches used currently attempt to cultivate the anchorage-dependent stem cells in suspension cultures attached to micro-carriers, spherical beads or hollow beads, all of which float in suspension; or cells cling to each other and float as cell clumps,

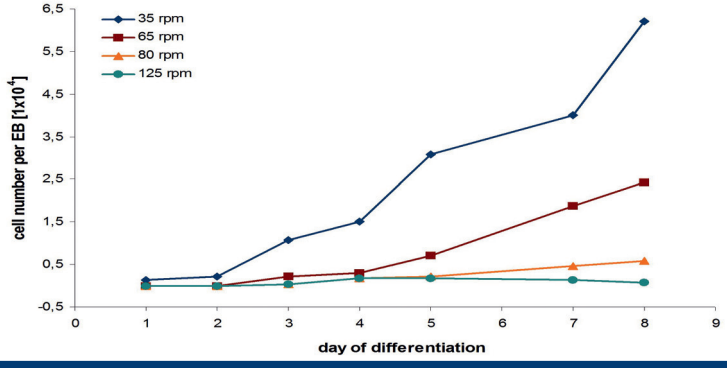

Figure 1. It has been shown that embryoid body (EB) growth is strongly affected by stirrer speed [according to 2]. While large cell aggregates were rapidly obtained when stirring at 35 rpm, EB growth was obviously hampered in the 80 and 125 rpm process. The most uniform EB growth was observed with stirring at 65 rpm.

so-called aggregates, in suspension. Another method used is encapsulation. The cells grow in suspension encapsulated in hydrogel beads consisting of a defined mixture of e. g. collagen and agarose polymers.

Challenges of stem cell suspension cultures

When adapting these cultivation methods and techniques to a common bioreactor environment, a few serious challenges had to be faced. Higher cell densities lead to limitations in oxygen and nutrient delivery, which results in fluctuations during static cultivation and therefore makes reliable scale-up difficult if not impossible. The main challenges were how to achieve a high cell density, and how to modulate cell differentiation and dictate the pathway of differentiation.

To overcome such difficulties, several changes have been made to static cultivation methods with the move toward dynamic cultivation techniques in order to produce higher cell masses. These changes have demonstrated significant improvements in cell expansion and differentiation [3], but none of them are suitable for evaluating the precise influence of a single parameter. However, this is a fundamental requirement for a reliable scale-up. If one variable changes during cultivation, it is essential to accurately monitor the influence on the other parameters. The critical parameters to control are [according to 3]:

Figure 2. DASGIP Parallel System for Stem Cell Research and Development provides stirred bioreactor solutions (from 2 to 250 rpm) for dynamic stem cell cultivation guaranteeing homogenous conditions during the entire cultivation process. Minimal working volumes (from 35mL) as well as high precision monitoring and control help saving cost-intensive resources while yielding reproducible results.

• Agitation

A major challenge to be faced is that stem cells tend to agglomerate to produce bigger clumps of cells. The size of these aggregates is an important factor for cell proliferation and differentiation. Different sizes lead to different, and thus not necessarily optimal, microenvironmental conditions. Agitation, involving impeller design and agitation speed, can be used to control the size of aggregates. Agitation also prevents cells from settling on the bottom of the bioreactor, where they can form uncontrolled sheets that deplete cells from the culture. However, the shear force needs to be low enough not to affect the viability of the stem cells [Figure 1].

• Oxygen

When stem cells are cultivated at lower, physiological oxygen tensions, the proliferation rate is significantly higher compared to cells cultivated in traditional incubators supplied by room air. However, it has also been shown that at such low oxygen tensions, stem cells sometimes respond by not expanding at all, whereas at certain higher concentrations their proliferation is accelerated. Thus considerable research still needs to be carried out in this area.

• pH

pH considerably affects the metabolic activity of cells, and is carefully regulated *in vivo* by complex mechanisms, which can be influenced by carbon dioxide, lactate production and many substances present in serum. Growth and differentiation of stem cells is also strongly pH-dependent.

What was realised so far?

These are the key factors in influencing stem cell proliferation and differentiation during cultivation. To prevent inhibition, it is useful to look at modern techniques for the culture of mammalian cells. The performance of state-of-the-art bioreactors for the cultivation of bacterial and phototrophic cells, as well as mammalian cells on a bench-top scale is well known. All relevant process parameters can be monitored and controlled very precisely [Figure 2].

A closed feedback loop allows tight control of pH within the closest ranges. Adjusting the amount of CO_2 flushing through a reactor can control pH, but this requires advanced gas-mixing technology. The resulting high precision in concentration and mass flow makes this perfectly suitable for stem cell cultivation. Additionally, it is possible to stir the suspension with minimal rotations, which ensures the necessary motion while preserving the viability of the stem cells. Within such bioreactors, adapted impellers agitate the culture medium and maintain cells in suspension, providing a homogeneous environment with controlled pH, oxygen concentration, temperature and other parameters. Suspension cultures are thus much more suitable for scale-up because these bioreactors allow cell density and culture volumes to be increased without it being necessary to change the cultivation system [4].

The suspension culture of human embryonic stem cells (hESC) has already been demonstrated by several groups, but they focused on aspects of cell differentiation rather than on expansion of undifferentiated cells. Others reported culture of undifferentiated hES cells on microcarriers, even though such approaches generally require removal of microcarriers from clinical-grade cell preparations prior to use.

Research at the Hannover Medical School (MHH)

Embryonic stem cells are a promising source of cardiomyocytes suitable for multiple applications, including development of cell transplantation therapies, cardiomyocyte-specific drug discovery screens and assays for drug toxicity. The generation of sufficient quantities of cardiomyocytes would enable therapy for both damage from cardiac infarction and congenital anomalies of the heart. Within the Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Germany different strategies for regenerative medicine and organ transplantation are being investigated.

Recently, Robert Zweigerdt's and Ulrich Martin's research groups have independently demonstrated the expansion of undifferentiated hESC and induced pluripotent stem (iPS) cells as floating aggregates. The researchers have established similar, highly reproducible cultures of several hESC, human iPSC (cord blood-derived lines established in the Martin lab [8]) and a cynomolgus monkey ESC line using both static and stirred culture platforms.

Such success in two independent labs provides strong evidence for the robustness of this novel method. Key features of the technology include a fully defined serum-free culture medium and the use of a Rho-associated coiled-coil kinase (ROCK) inhibitor (RI) enabling defined, single cell-based culture inoculation and cultivation in aggregates independent of any extracellular matrices or scaffolds [5-7]. Ongoing studies have revealed that culturing pluripotent hES /hiPS cells in controlled, stirred suspension bioreactors is equally important for achieving significant long-term expansion and especially for producing high numbers of cell [Figure 3].

Promising results

Mass expansion of human ES cells was readily achieved within the described investigations. The up-scaling strategy has significant potential to provide pluripotent cells on a clinical scale. Nevertheless, data obtained by Robert Zweigerdt also highlights a significant line-to-line variability and the need for a critical assessment of novel methods with numerous relevant cell lines.

The research has also shown that culture conditions have an enormous influence on the proliferation and differentiation of hematopoietic stem and progenitor cells. Even small changes in oxygen tension, pH, or medium composition caused significant changes in differentiation patterns and the proliferation potential. Attempts to untangle the effects of acid production, pH, medium use, and differences in oxygen tension on stem cell cultures have proven that each parameter seems to influence cell response.

Suspension culture in fully controllable bioreactors is the method of choice to generate mammalian cells at the clinically relevant scale. The modular DASGIP system offers researchers comprehensive equipment to find the perfect match for their studies. The flexible cultivation system offers users the opportunity to adapt the system to the requirements of each stem cell line – with minimum medium usage, high precision and reliable measurement. Clearly, being able to expand undifferentiated human iPS and ES cells in suspension culture avoids the limitations of adherent cell cultures and represents an important step towards controlled bioprocessing and industrial manufacturing of stem cells for clinical application and biopharmaceutical drug development.

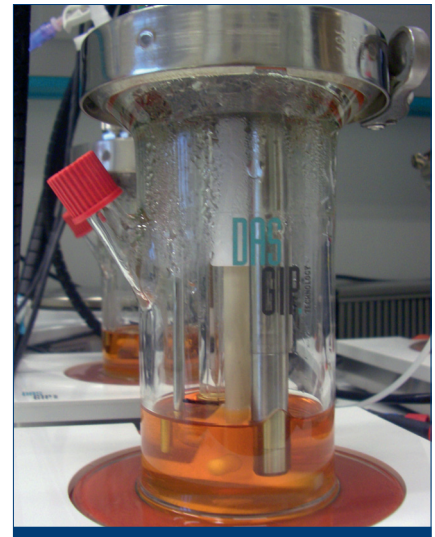

Figure 3. For bioprocess development and for further scale-up, researchers in DASGIP Parallel Bioreactor Systems have developed beneficial solutions combining small working volumes with individual control of agitation, oxygen tension, and pH for dynamic cultivation.

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