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Controlled Microcarrier Based Cultivation of Adherent Mesenchymal Stem Cells in a Stirring Reactor

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Objective

For the most effective application of primary mesenchymal stem cells (MSC) as a cell source in regenerative medicine, obstacles regarding (1) their cost and labor intensive expansion and (2) regulatory hurdles because of their non-controlled culture in monolayer have to be overcome. To develop a controlled, cost-effective and scalable process, a microcarrier based stirring reactor cultivation system for MSC was used.

Methods

To establish bioreactor cultures, MSC were isolated from porcine bone marrow and expanded up to P3 in monolayer according to standard protocols (Ringe et al., Cell and Tissue Research, 2002). Subsequently, they were seeded on commercially available porous Cultispher-G microcarriers as described earlier (Frauenschu et al., Biotechnology Progress, 2007). Cell seeded carriers were then cultured in the cellferm-pro parallel bioreactor system (Fig. 1; DASGIP Inc., Germany).

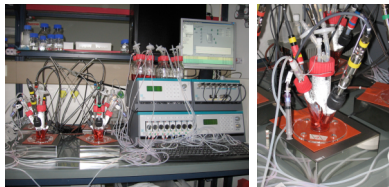


Figure 1: (Left) DASGIP cellferm-pro parallel bioreactor system; (Right) Spinner flask with DO and pH electrodes, temperature sensor, feed line (in/out), sampling valve, heating blanket and pitch blade impeller agitator.

In more detail, initially, reactor parameters like oxygen mass transfer coefficient (kLa) and oxygen uptake rate (OUR) were investigated. To evaluate the seeding procedure, the average number of adhered MSC was determined. Subsequently, MSC seeded carriers were cultured for up to 14 days. Samples were taken and analyzed for cell viability, morphology and distribution. Culture parameters, such as dissolved oxygen (DO; 50% vs. 100%), agitation speed (60rpm vs. 100rpm) were varied and the influence on growth kinetics was determined. To study whether MSC are able to recolonize freshly added carriers, during culture the carrier amount was increased with unseeded Cultispher-G carriers and analyzed for colonization. Finally, MSC were harvested from carriers, and evaluated for their quality.

Results

The amount of MSC in bone marrow is very low. Thus, in this initially study, cells were expanded in a preparatory culture up to P3, before seeding on Cultispher-G carriers. Cell seeding was determined at 0.5h, 1h, 3h and 6h. After 3h, optimal adhesion with a maximum of 70% adherent cells was observed (Fig. 2).

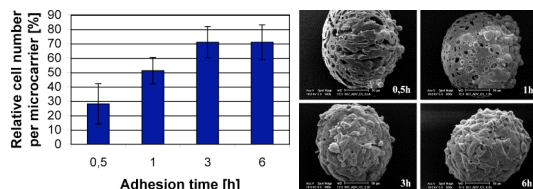


Figure 2: (Left) Optimal adhesion time of MSC on Cultispher-G microcarrier is 3h; (Right) Cultispher-G microcarriers, seeded with passage 3 MSC for up to 6h. Porous carriers are 130-380µm in diameter and consist of crosslinked gelatin.

MSC proliferation was examined on this carriers over 14 days. Here, 75.000 cells were seeded on 1mg carrier. Under standard conditions (100% DO, 60rpm, pH 7,4), the growth rate (μ) was 0.0055/h and the doubling time (t_d) 126h. The doubling time in monolayer (P2) was about 97h. A decrease in the oxygen concentration from 100% to 50% resulted in a higher cell number per time (Fig. 3). The percentage of seeded carriers was 100% in-

dependent of the dissolved oxygen concentration (Fig. 3). Figure 4 shows single carriers and carrier aggregate formation over the culture time.

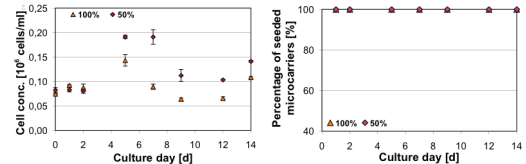


Figure 3: (Left) MSC growth curves for 50% and 100% DO. (Right) Percentage of seeded Cultispher-G carriers for 50% and 100% DO.

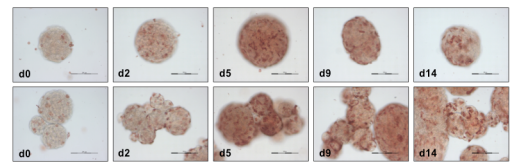


Figure 4: MSC culture on Cultispher-G carriers (50% Do, 60rpm).

Porous Cultispher-G carrier allow a high agitation speed up to 100rpm, which is important to reduce aggregate formation (Fig. 5). More important, even at 100rpm, the addition of 50% fresh unseeded carriers at day 9, resulted in almost 100% seeded carriers at day 14 indicating a carrier-to-carrier transfer that allows a continuous scale-up without cell passaging.

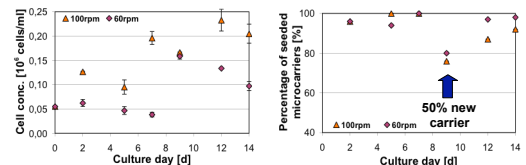


Figure 5: (Left) MSC growth curves for 60rpm and 100rpm agitation speed. (Right) Carrier-to-carrier transfer after addition of 50% unseeded carriers at day 9.

The specific oxygen uptake rate (q_{O_2}) and OUR are important parameters to monitor cell growth. Thus, reactor parameters like kLa and response and dead time of the O_2 electrode were gained to calculate q_{O_2} and OUR (Fig 6).

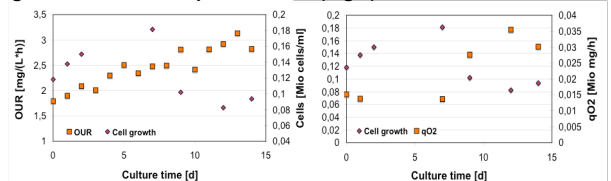


Figure 6: (Left) OUR vs. cell concentration. (Right) q_{O_2} vs. Cell concentration.

During the first 7 days (exponential growth phase), the OUR correlated with cell growth. Since μ is constant during log phase, q_{O_2} was also constant. Finally, cells were harvested using enzymes, resulting in a very high amount of dead cells (80%), or by outgrowth curve. Preliminary data indicate that these harvested cells still differentiate into the osteogenic and adipogenic lineage (Fig 7).

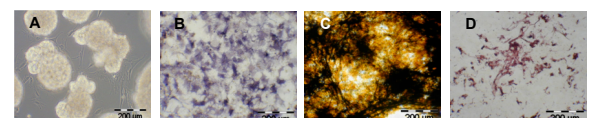


Figure 7: (A) MSC outgrowth culture, (B) ALP-, (C) von Kossa-, (D) Oil Red O staining.

Discussion

We have shown that the expression of MSC on Cultispher-G carriers in a stirring bioreactor could be a good alternative to monolayer culture. Major problems that have to be solved are high cell doubling times and the cell harvest from carriers.