Stem cell cultivation in single-use stirred tank bioreactors

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INTRODUCTION

The study of stem cells has been marked as prevalent research topic due to the therapeutic potential of these undifferentiated cells to treat otherwise difficult-to-treat diseases.

Human mesenchymal stem cells are multipotent adult stem cells that can be harvested in vivo and used towards cell therapy and tissue repair applications because of their differentiation, self renewal and immunomodulatory properties. In Figure 1 some of the research lines of adipose tissue-derived human mesenchymal stem cells (AT-hMSC) are shown in clinical applications.

To achieve clinically relevant cell numbers, the engineering of a GMP-compliant large-scale platform is essential.

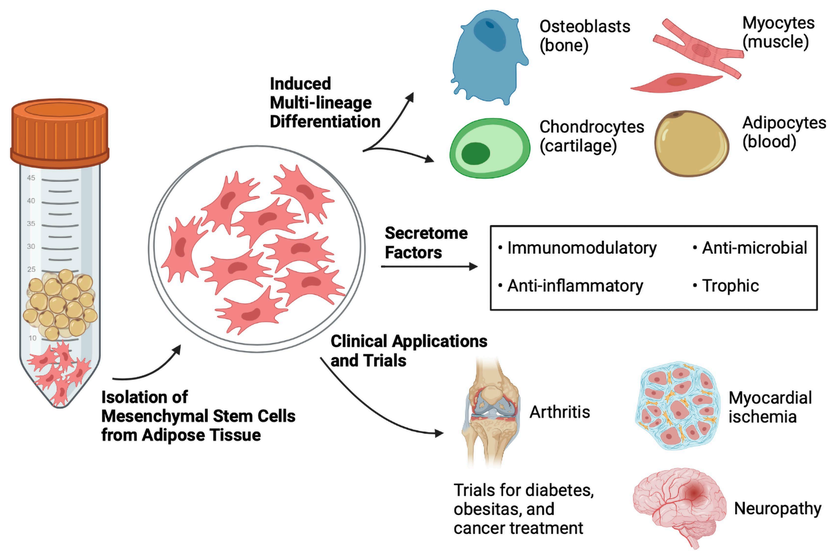

Figure 1. Method of AT-hMSCs isolation using density-based centrifugation and cell-adhering qualities of the stem cells and therapeutic characteristics for clinical applications (illustration created using biorender.com)

AIM

The aim of the present work was to analyze whether dissolvable microcarriers (DMCs) in combination with a single-use stirred tank bioreactor could serve as an expansion platform for the successful and controlled expansion of AT-hMSC.

MATERIALS AND METHODS

- Adipose tissue-derived human Mesenchymal Stem Cells (AT-hMSC) (C-12977, Promocell)
- Mesenchymal Stem Cell Growth Medium XF (C-28019, Promocell)
- Inverted microscope and the CellSens image software (Olympus)
- Image analysis for determining cell attachment distribution onto microcarriers
- DMCs (Dissolvable MC Synthemax™ II, Corning)
- Single-use AppliFlex-ST 0.5 L bioreactor with a capped L-type sparger (Getinge) (Figure 2)

Figure 2. AppliFlex ST 0.5 L bioreactor (cell culture configuration)

- my-Control biocontroller to control pH at 7.3 (down-control with CO2);
  DO above 20% (up-control with air) and temperature at 37oC (up- and down-control with a Peltier element)
- Batch mode of operation with a working volume of 250 mL, 1.6 g L^{-1} of initial microcarrier concentration and a total cell number seeded of 8*106 cells which corresponds to 4,000 cells cm^{-2} microcarrier surface area
- The Trypan Blue exclusion test was used to determine the cell viability
- The overview of the workflow is shown in Figure 3

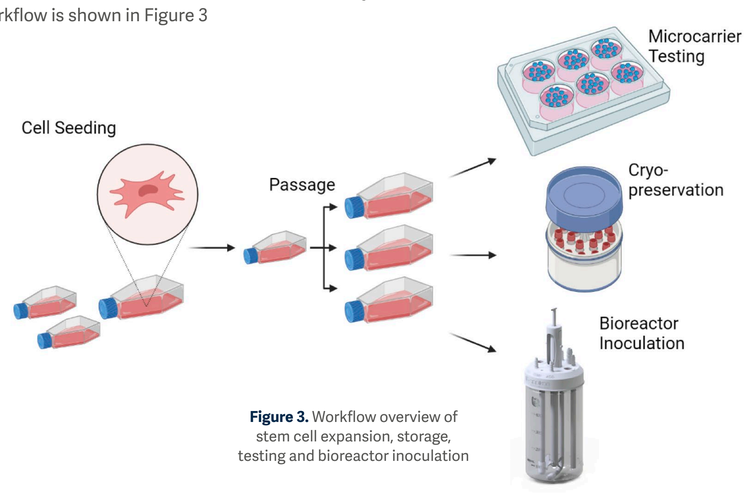

Figure 3. Workflow overview of stem cell expansion, storage, testing and bioreactor inoculation

RESULTS

Attachment and cell expansion

The successful cell-to-microcarrier attachment and distribution in the bioreactor is shown in Figures 4A and 4B. After 4 hours, 62 % of cells had successfully attached. On day one, 87 % of the DMCs had cells visibly attached.

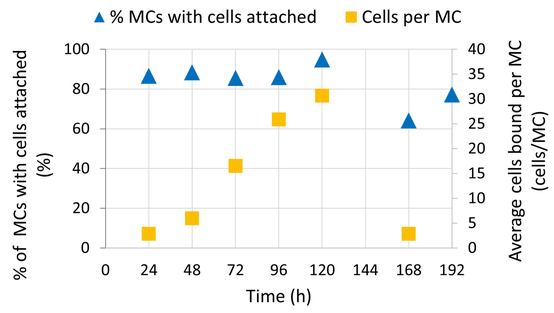

Figure 4A. Percentage of microcarriers with attached cells and number of cells per microcarrier

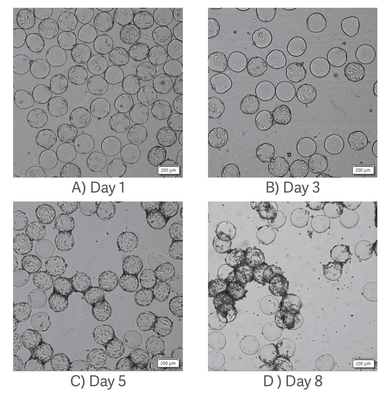

Figure 4B. Microscope images of the DMCs with cells attached during 8-day batch run (scale bar 200 um)

During the batch culture, a 5.5-fold expansion was achieved, with maximum measured cell concentration of 1.8*105 cells mL^{-1} and 97 % viability on day 5 of the batch (Figure 5). The cell doubling time in the bioreactor was found to be 30 hours compared to 27 hours measured in the planar flasks. The total number of cells measured in the entire batch was 45 M cells, which is nearly half of the required cell numbers for clinical application.

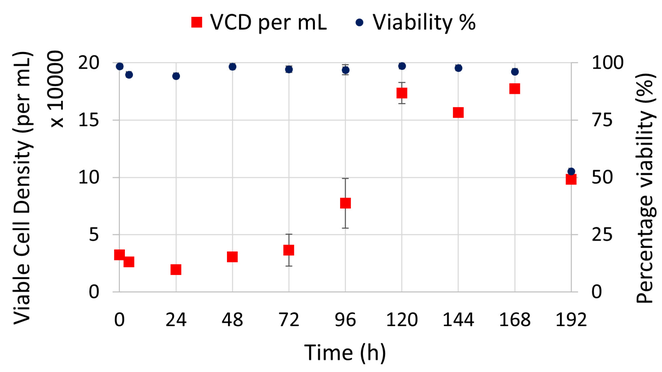

Figure 5. Viable cell density and viability (%) of stem cells along the run

Metabolite analysis

The levels of glucose, lactate, glutamine and glutamate were analyzed in the medium over time. Figure 6 shows increasing lactate concentrations (reaching a maximum of 0.85 g L^{-1} coupled to the respective decreasing glucose concentrations, starting at 0.9 g L^{-1} and reaching 0 g L^{-1} by day 7). The decreasing curve for the glutamine concentration did not reach a 0 g L^{-1} concentration by day 8 (Figure 7) as observed for glucose (Figure 6). Therefore, one could conclude that glutamine was not the limiting factor for the further expansion of AT-hMSCs in the batch.

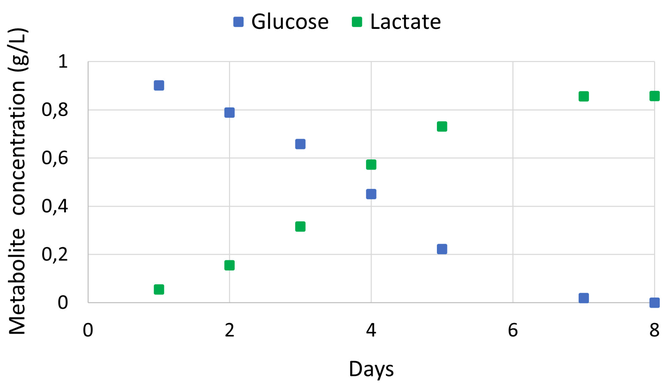

Figure 6. Extracellular glucose and lactate concentration over time

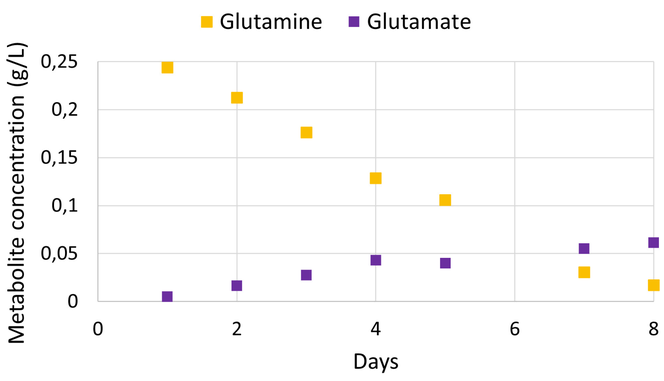

Figure 7. Extracellular glutamate and glutamine concentration over time

CONCLUSIONS

The successful expansion of the AT-hMSC cell line with dissolvable microcarriers in the controlled single-use 0.5 L bioreactor was achieved using Batch as a mode of operation.

The results were similar to the ones obtained with an analogous experimental set-up in the multi-use bioreactor (Moreira et al., 2020), where up- and down-control of DO was used. The comparison shows that DO up-control alone is suitable for the expansion of these cells in the bioreactor.

Future experiments could be oriented towards the Fed-batch as a mode of operation to test the importance of glucose as limiting factor and the use of a larger bioreactor volume in order to study the scale-up process.

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