EX VIVO EXPANSION AND DIFFERENTIATION OF ERYTHROBLASTS: FROM CULTURE DISHES TO STIRRED TANK REACTORS FOR THE PRODUCTION OF RED BLOOD CELLS

C. Bernal, Getinge Applikon, Heertjeslaan 2, 2629 JG, Delft, The Netherlands
cristina.bernalmartinez@getinge.com
J. Gallego Murillo, Delft university of Technology, Department of Biotechnology; St. Sanquin Bloedvoorziening, The Netherlands
T. van Arragon, Getinge Applikon, Heertjeslaan 2, 2629 JG, Delft, The Netherlands
L. van der Wielen, Delft university of Technology, Department of Biotechnology, Van der Maasweg 9, The Netherlands
E. van den Akker, St. Sanquin Bloedvoorziening, Plesmanlaan 125, The Netherlands
A.Wahl, Delft university of Technology, Department of Biotechnology, Van der Maasweg 9, The Netherlands

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Transfusion of donor-derived red blood cells (RBCs) is the most common form of cell therapy. Nevertheless, it faces challenges such as emerging blood-borne diseases, and supply limitations; for instance, in low-income countries, or for chronically transfused patients requiring special blood groups. Production of cultured RBCs (cRBCs), in which erythroid precursors (erythroblasts) are cultured from hematopoietic progenitors and subsequently differentiated into transfusion-ready erythrocytes, is a potential alternative. However, the large number of cRBCs required for a single transfusion unit requires major innovations in the culture process.

Stirred bioreactors are conventionally used for other mammalian cell cultures. In this culture system, turbulence is a critical parameter, due to the reported detrimental effects of shear stress and energy dissipation due to mixing in the growth of some mammalian cell lines [1]. Furthermore, the bioreactor materials can also influence the performance of the cell cultures, as the conventionally used stainless steel can leach metal ions that impact cell growth and viability [2]. The aim of our work is to scale-up the expansion and differentiation of erythroblast for the production of cRBCs using stirred bioreactors. In addition, we aim to compare the performance of two stirred bioreactor types (a conventional glass & stainless steel autoclavable bioreactor, and single-use plastic bioreactors).

We have successfully performed the expansion of erythroblast cultures using Applikon MiniBio 500 mL and single-use Applikon AppliFlex ST 0.5 L stirred tank bioreactors, following a repeated batch cultivation strategy. Erythroblasts produced in these systems have shown the same proliferation potential and similar expression of erythroid surface markers (CD235, CD71) as those cultured in conventional static conditions, while maintaining a high viability during the cultivation, suggesting that the potential negative effects of excessive shear rates and turbulence have been avoided.

In addition to proliferation, the differentiation of cultured erythroblast into mature reticulocytes was also tested in the stirred bioreactors. Slightly lower cell yields were observed in the stirred cultures compared to static conditions, in agreement with previous reports on the effect of shear stress on the differentiation dynamics of erythroblast cultures. During erythroblast differentiation, cells lost expression of CD71 and CD49d at a comparable rate to cells kept under static conditions (culture dishes). These results show that the expansion and differentiation of erythroblast cultures is feasible in stirred bioreactors and indicate that the single-use AppliFlex ST platform is adequate for the transition to GMP production of cRBCs.