AQUEOUS TWO-PHASE SYSTEMS FOR THE POTENTIAL RECOVERY OF STEM CELLS

Mirna González-González and Marco Rito-Palomares, Centro de Biotecnología-FEMSA, Tecnológico de Monterrey Campus Monterrey, Monterrey 64849, mrito@itesm.mx.

Keywords: Aqueous two-phase systems, stem cell isolation, immunoaffinity partitioning.

Introduction. A latent niche exists for the development of efficient, cost effective and fast separation systems for large scale stem cell isolation that can meet future demand of stem cells for clinical applications (1). Of particular interest is the purification of stem cells that express CD133 due to multiple reported therapeutic applications (2).
ATPS is a liquid-liquid extraction technique that exhibits several advantages including: biocompatibility, economical attractiveness, scalability, and low processing time (3). Moreover, if this methodology is complemented with the use of antibodies (known as immunoaffinity ATPS (4)), a novel strategy for the purification of $CD133^+$ stem cells can be achieved.
The objective of this research is to establish the basis for the development of a novel and scalable purification bioprocess for the selective recovery of $CD133^+$ stem cells employing ATPS.

Methods. The proposed bioengineering strategies include the implementation of traditional (PEG, Dextran and Ficoll) and novel (UCON) immunoaffinity ATPS in its multiple variants in order to prove the viability of $CD133^+$ stem cells and with the aim of concentrating contaminants and the stem cells of interest in opposite phases.
A pre-enrichment step exploiting a density gradient is performed to "debulk" the HUCB sample and to drastically reduce the sample volume. The sample is introduced into the selected traditional or novel ATPS as illustrated in Figure 1. The selected measurement system is flow cytometry employing the specific marker for CD133 and the 7AAD (7-Amino-actinomycin D) staining solution to monitor cell viability.

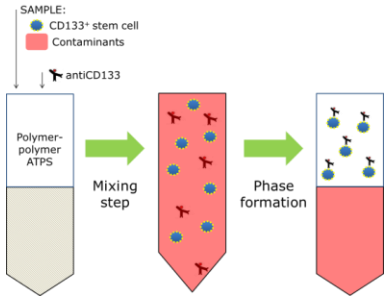

Fig. 1. Construction of immunoaffinity ATPS.

Results.

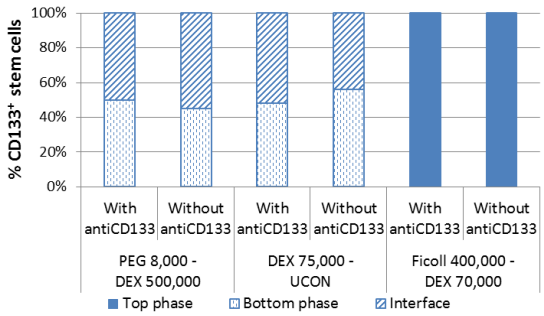

Fig.2 Percentage of $CD133^+$ stem cells in the top, bottom and interface of each of the studied ATPS.

Conclusions.

*Cells viability is at least 98%.
*ATPS are stabilized in less than 20 min., thus processing time of the separation process is less than 35 min.
*Results are reproducible in ATPS of 15 g (scalable process).
*$CD133^+$ stem cells can be concentrated in different phases, depending on the ATPS selected:
- Top phase: Ficoll 400,000-DEX 70,000
- Bottom phase (considering interface):
  PEG 8,000-DEX 500,000 and DEX 75,000-UCON.
*Further work in progress to justify immunoaffinity partitioning.

Acknowledgements. The authors wish to acknowledge the financial support of Tecnólogico de Monterrey, Bioprocess research chair (Grant CAT161), the Zambrano-Hellion Foundation and of the CONACyT for the fellowship of M. González-González No. 223963..

References.

1.González-González M, Vázquez-Villegas P, García-Salinas C, Rito-Palomares M. (2012). *J. Chem. Technol. Biotechnol*. 87 (1), 2-10.
2.Martinez HR, Gonzalez-Garza MT, Moreno-Cuevas JE, Caro E, Gutierrez-Jimenez E, Segura JJ. (2009) *Cytotherapy*. 11 (1), 26-34.
3.Benavides J, Aguilar O, Lapizco-Encinas BH, Rito-Palomares M. (2008). *Chem. Eng. Technol.* 31 (6), 838-845.
4.Ruiz-Ruiz F, Benavides J, Aguilar O, Rito-Palomares M. (2012). *J Chromatogr A*. 1244, 1-13.