Poster #5

Characterization of the acoustic contrast factor: a new approach for a label-free separation of the stromal vascular fraction

Hugo R. Sugier[1,2], Ludovic Bellebon[3], Jérôme Larghero[4], Juliette Peltzer[1,5], Jean-Luc Aider[3], and Christophe Martinaud[6]

[1] Aenitis Technologies, Paris, France
[2] INSERM UMR-MD 1197, Villejuif, France
[3] Laboratoire de Physique et Mécanique des Milieux Hétérogènes (PMMH), UMR 7636 CNRS, Ecole Supérieur de Physique et de Chimie Industrielle (ESPCI), Université Paris Sciences et Lettres (PSL), Sorbonne Université
[4] Université de Paris, AP-HP, Hôpital Saint-Louis, Unité de Thérapie Cellulaire, INSERM Centre d'investigation clinique de Biothérapies CBT501 et U976
[5] Institut de Recherche Biomédicale des Armées (IRBA), Clamart, France
[6] Centre de Transfusion Sanguine des Armées (CTSA), Clamart, France

Introduction

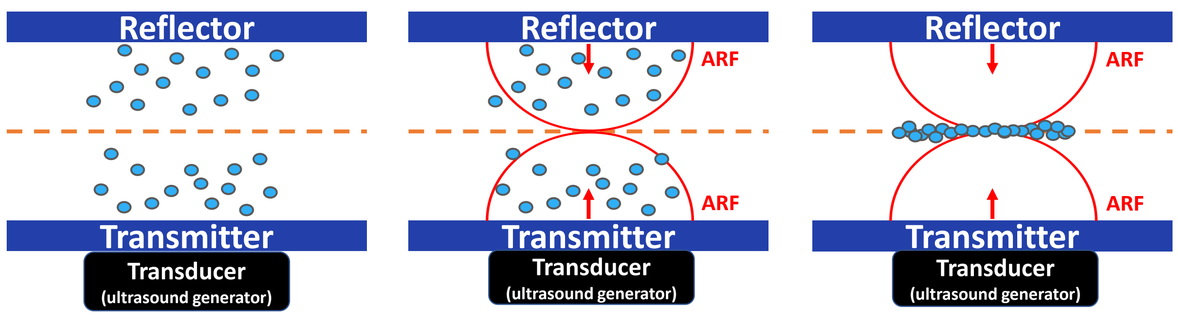
Acoustophoresis is an emerging technology allowing cell separation based on their physical properties via ultrasound. The compressibility and density of the cell and its surrounding medium define an acoustic contrast factor (ACF), which governs the migration direction of the cell. Different cell types with distinct ACFs would be separable by acoustophoresis. This innovative method has the advantages to be non-contact, label-free, and working in a controlled flow. Those features make it a promising solution for any bioproduction process requiring isolation and processing of cells without the disadvantages of centrifugation or immunomagnetic separation. It has already been successfully used in various clinical applications: isolation of blood components, circulating tumor cells, and exosome (1).

The Stromal Vascular Fraction (SVF) is an heterogenous cell population contained within adipose tissue and composed of Adipose tissue-derived Mesenchymal Stromal Cells (AD-MSCs), endothelial cells, Leukocytes, and pericytes. Although being increasingly used in regenerative medicine for various clinical indications including orthopedic disorders, and wound healing, the SVF therapy is not fully understood. The main mechanisms and the role of each cell type are not well defined yet (2).

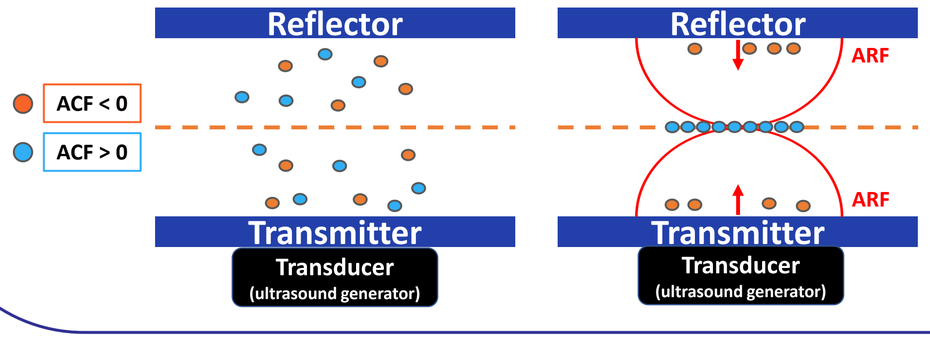
Objective: In this study, we developed a method to measure the ACF of any cells. We measured the ACF of AD-MSCs and cell lines similar to the SVF.

Theory

The Acoustic Radiation Force (**ARF**) induce the migration of the cell at the pressure node at the center.

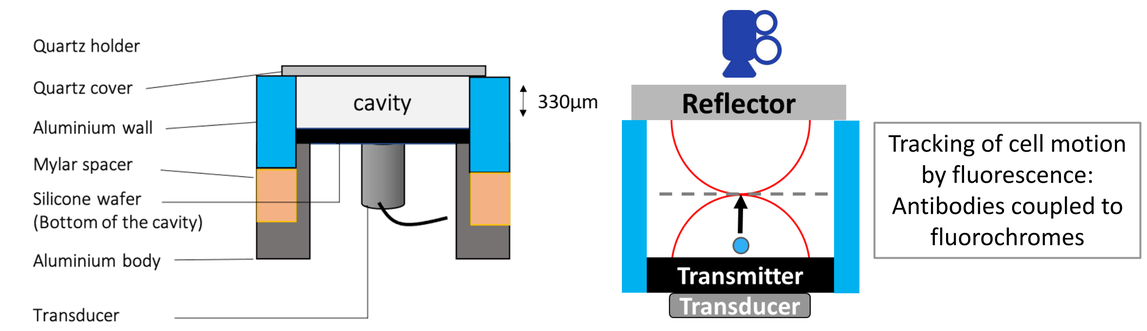


The Acoustic Contrast Factor (ACF) of cells depends on the density and compressibility of the cell and its surrounding medium. Cells with **ACF > 0** migrate toward the pressure node (center) while cells with **ACF < 0** migrate towards the antinode (walls).



Methods

The ACF is calculated with the focalization speed of the cell while undergoing the ARF into an acoustic cavity

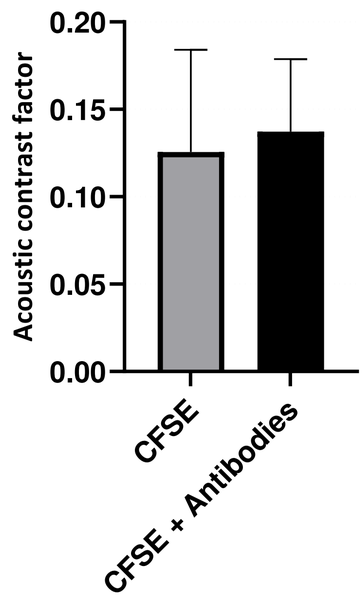


Focusing velocity → Acoustic contrast factor

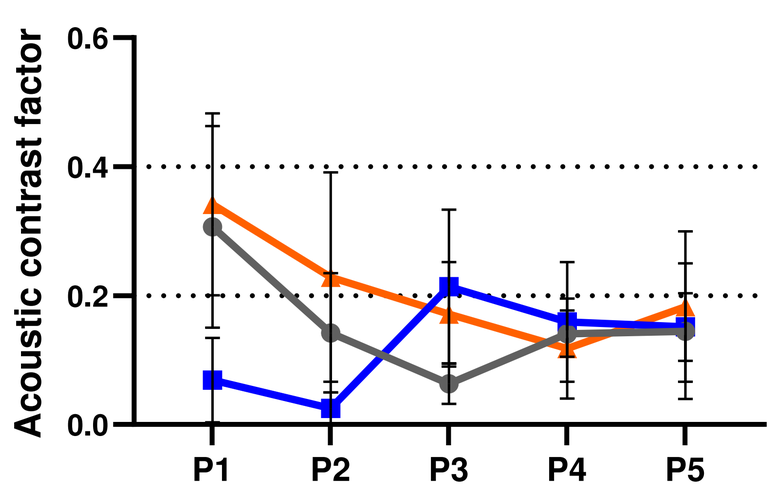
Cells

- **AD-MSCs** isolated from liposuction by a classical enzymatic method (n=3)
- **THP-1**: Monocyte-like cell line (n=6)
- **Jurkat**: Lymphocyte-like cell line (n=7)
- **EPC**: Endothelial Progenitor Cells cell line (n=3)

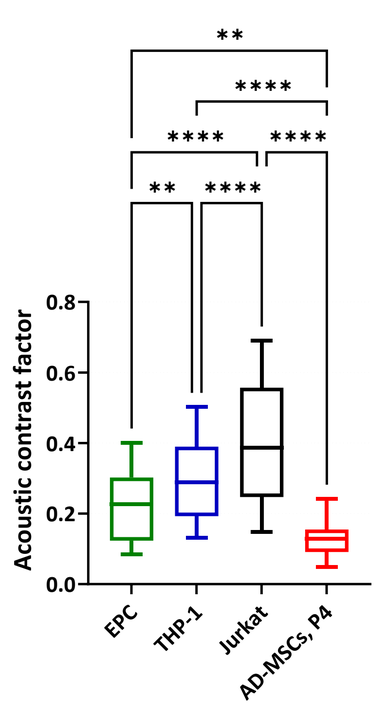
Results



AD-MSCs stained with CFSE or CFSE + antibodies (n=3)



AD-MSCs isolated from 3 donors cultivated from passage 1 (P1) to P5



Conclusion

In this study, we developed a method to measure the ACF of any cells using an acoustic cavity. We calculated the ACF from the acoustic focusing velocity of the stained cells. We confirmed that the staining with antibodies does not impact the ACF of the cells. We observed that the ACF of MSCs is heterogenous at the first passages and converged to 0,15 at P4. The mean of ACF of MSCs, EPC, THP-1, and Jurkat are significantly different. We noticed an overlap of the measures suggesting an acoustic cell sorting with high purity would be complicated and rather be an enrichment.

We are currently investigating the ACF of the different cell types of the SVF, sorted by FACS.

From a general point of view, this methodology can be applied to any cell type to investigate the relevance of the acoustic-based cell separation.

(1) Wu M, Ozcelik A, Rufo J, Wang Z, Fang R, Jun Huang T. Acoustofluidic separation of cells and particles. Microsyst Nanoeng. 2019;5:32.
(2) Nguyen A, Guo J, Banyard DA, Fadavi D, Toranto JD, Wirth GA, et al. Stromal vascular fraction: A regenerative reality? Part 1: Current concepts and review of the literature. J Plast Reconstr Aesthet Surg. févr 2016;69(2):170-9.

Contact: hugo.sugier@aenitis.fr
Aenitis technologies, Bâtiment Jean-Bernard, 16 Rue de la Grange aux Belles, 75010 Paris, France
www.aenitis.fr