# Exploiting elastic modulus of human cells for separation purposes

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**Summary**

In this work, we identified differences in the elastic modulus for pluripotent stem cells versus mesenchymal progenitor and osteoblast-like derivatives and fibroblasts using atomic force microscopy (AFM) and data processing algorithms. We exploited this information to develop and exemplify a label-free scalable segregation platform using microfluidic, cross-flow separation devices permitting a throughput of $10^6$-$10^7$ cells per min and high levels of removal of specific cell types per single pass. Our results demonstrate the principle of a scalable and automatable solution for high-throughput cell separation in the context of human pluripotent stem cell manufacturing.

**Introduction**

Human pluripotent stem cells are the primary template cells for nearly all the cell types within the body. The exclusivity of these cells arises from their unmatched capability to remain in an unspecialised state until they are induced to differentiate (change from being a stem cell to a cell that has a specific function in the organism). A major challenge of regenerative medicine is to exploit these twin properties to provide wide-ranging novel therapies through the manufacture of beneficial cell types at scale. To date, one of the principal bottlenecks in the development of large scale cell therapies has been the lack of manufacturing-suitable cell separation and purification technologies, effectively limiting the production of products pure enough for wide scale human trials.

This bottleneck is caused by the need for safety and efficacy in sorting cells to high purity without adding antibodies or other modifying agents, in order to remove residual pluripotent cells and also for the identification and definition of the cells in the final product. Hence what regenerative medicine needs in order to be translated into a manufacturing process is a scalable label-free sorting technology. This implies passive sorting which can both be done without labelling and in a highly parallelised manner. In this work, we looked to identify variations in exploitable physical properties of cells – in this case variation in elastic modulus of living, differentiating cells using specialized biological AFM – and then utilize these variations to develop a device capable of separating pluripotent and differentiated cells based upon elastic modulus

**Methods**

Living human embryonic stem cells (hESCs), terminally differentiated human dermal fibroblasts (HDFs) and cells undergoing a 21-day directed differentiation from hESCs to an osteoblastic lineage were mapped using an AFM response curve method (n in excess of 1000 in most cases). Data analysis algorithms based around a modified Hertz model fit were used to calculate local elastic modulus values for cells. From this, a tangential flow separation technique based on cell microsieve-type membranes was developed to exploit the ability of cells with a lower elastic modulus to reversibly deform more easily and pass through narrow perforations.

**Results**

AFM analysis of different cell types showed a significant variation in cell elastic modulus, correlating to state of differentiation or pluripotency. Figure 1 shows the elastic modulus variation for

For the model HDF/hESC system, up to 97% removal of HDFs was achieved from a mixed hESC/HDF population in a single pass. The validity of the elastic modulus-based approach was further demonstrated by the separation of differentiating osteoblasts from a population including hESCs at a separation efficiency that correlates with elastic modulus variation during the differentiation process (figure 2)

**Conclusions**

Variations in the stiffness of cells, measured using atomic force miscroscopy in a variety of cell types are

various stages of differentiation (pluripotent human embryonic stem cells, derivative mesenchymal progenitor cells, osteoblast-like cells and fibroblasts) hava been used to develop and demonstrate a novel scalable label-free separation device showing clear promise for the separation of mixed cell populations for industrial and clinical use.

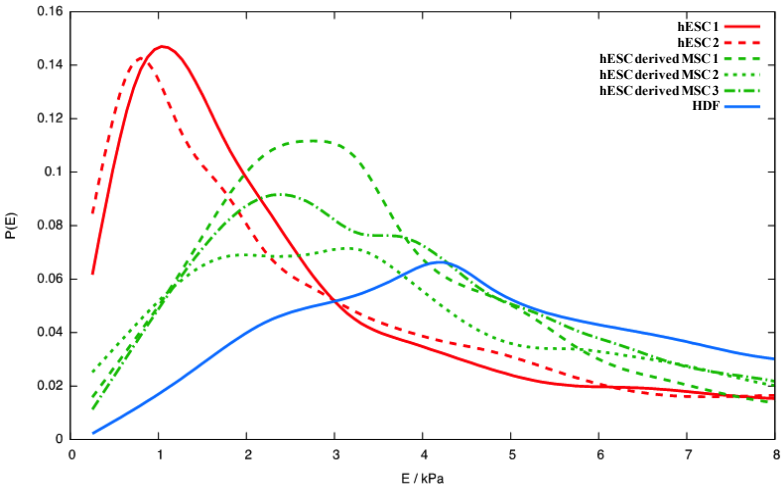


Figure 1 – Variation in elastic modulus as a probability distribution. Red data refers to two different pluripotent cell types, green to three different partially differentiated multipotent cell types (germ layer specified) and blue to terminally differentiated cells. All plots from analysis of >800 force response curves. Data clearly indicates increasing elastic modulus and therefore cell stiffness with cell differentiation, therefore with cell specificity.

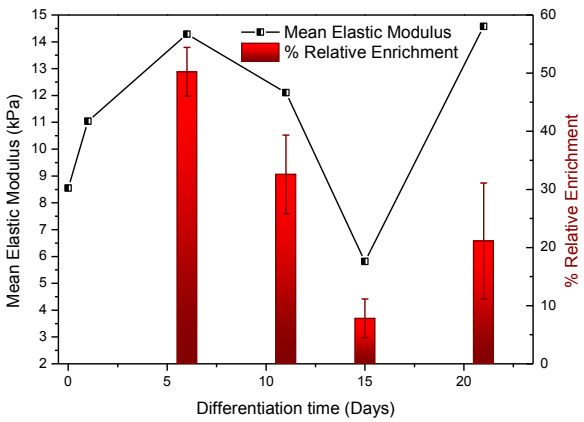


Figure 2 – Variations in mean elastic modulus (Y-axis on the left) of H1-MP cells during osteogenic differentiation (X-axis) are plotted in relation to the percentage depletion obtained in the permeate for an H1-hESC/osteocyte mixed cell population run once through our elastic-modulus based separation device, at the indicated days during differentiation.