Investigation of the Effects of Shear on Human Mesenchymal Stem Cells and How It Applies to Stirred Tank Bioreactors

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Introduction

With the continuing increase in number of approved clinical trails using human stem cells in patients, the demand for the efficient and consistent scale-up of stem cell production also increases. One of the most commonly used vehicles for cell culture expansion is the stirred tank bioreactor. When transitioning from flat (2D) tissue culture to microcarrier suspension culture there is an increase in the exposure to shear on the cells due to the different micro-environment. The research presented here investigates the effects of shear on human mesenchymal stem cells (hMSCs) as single cells in suspension or on microcarriers in single use stirred tank bioreactors. The following questions were asked:

- Can the shear in the bioreactor be modeled with a quantitative method using a fixed orifice device ("torture chamber") shown to measure shear?
- Can this model system be used to predict shear markers at the microRNA and mRNA levels?
- Do changes in agitation (shear) in stirred tanks have any effect on growth or function of hMSCs?

Method

Legend for different growth surfaces/conditions

Cells were sheared with torture chamber

2D Static gelatin coated flask

3D Static collagen coated microcarrier

3D Spinner flask collagen coated microcarrier

3D Mobius® CellReady 3L bioreactor collagen coated microcarrier

- hMSCs (Merck Millipore SCR108)
- miRNA analysis Sistemic
- mRNA analysis Precision Biomarker
- Collagen coated microcarriers (Solohill C102-1521)
- 125 mL Spinner Flasks (Corning 3152)
- Mobius® CellReady 3L bioreactors (Merck Millipore CR0003L200)
- Custom PCR Array plates (SA Biosciences CAPH10783)
- Guava® platform for flow cytometry (Merck Millipore 0500-4008)
- hMSC functional Identification kit (R&D systems SC006)

Results

The torture chamber: a model of shear

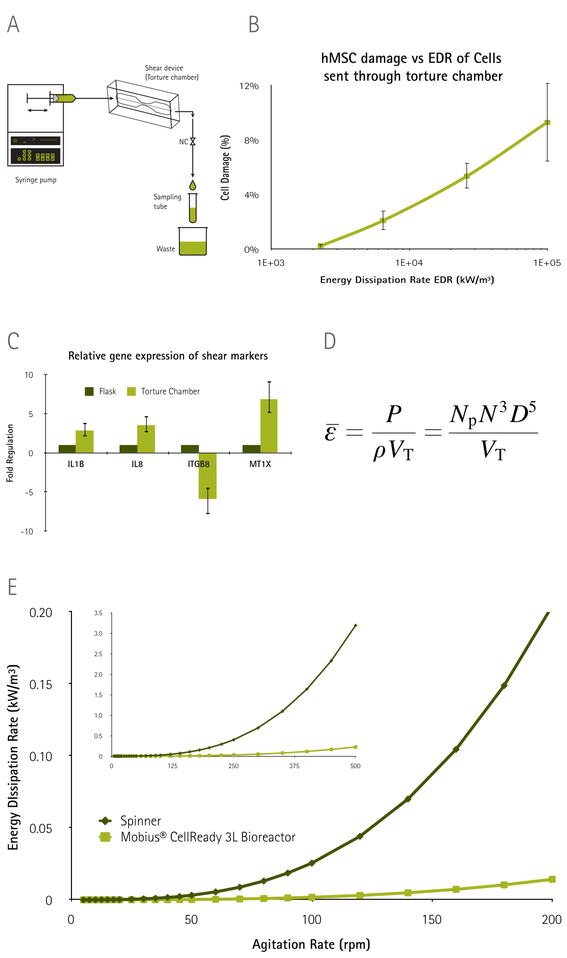


FIGURE 1. The Torture chamber (schematic **A**) was used to the shear cells at varying Average Energy Dissipation Rates and then the cells were assessed by qPCR with shear markers from literature. Using this fixed orifice device higher cell damage was observed at higher flow rates and energy dissipation rates **(B)**. qPCR was performed on some shear markers (from literature) and differences were observed **(C)**. According to the governing equation for Energy Dissipation Rate **(D)** a comparison of the EDR in the spinner flask and Mobius® CellReady 3L was generated for different agitation rates **(E)**.

Investigations of the miRNA

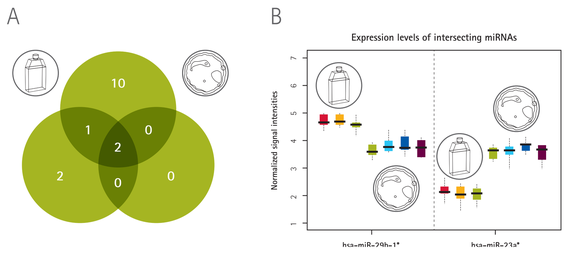


FIGURE 2. 1205 human microRNA (miRNA) expression levels were assessed for 63 samples. Three separate MSC lines were cultured on static collagen coated flasks, or on collagen coated microcarriers in static petri dishes, or on microcarriers in spinner flasks for 2 days then some were exposed to shear from the torture chamber. Venn diagram of overlap between differentially expressed miRNAs between two groups **(A)**. Two miRNAs were consistently different between samples cultured on tissue culture flaks and those grown on microcarriers, and the differences in the expression levels are shown **(B)**.

Analysis of mRNA expression

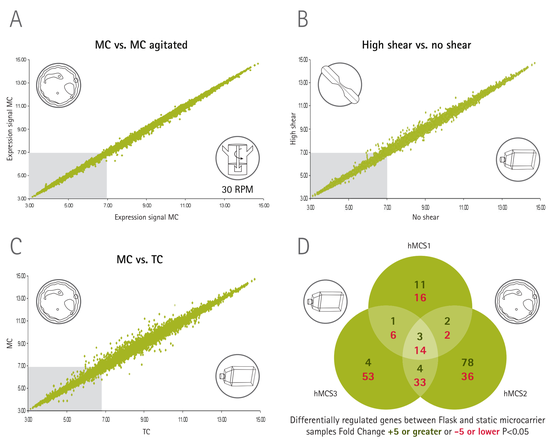


FIGURE 3. The same samples were also analyzed on the Affymetrix GeneChip® platform using Human Gene 1.0 ST Arrays. The agitation of the microcarriers **(A)** had the smallest effect compared to stress of the torture chamber vs no torture **(B)** or the change from microcarrier culture to flask culture **(C)**. The difference between flask culture and microcarrier generated 17 genes that were differentially regulated by 5 fold in 3 independent cell lines **(D)**.

hMSCs were grown in the Mobius® CellReady 3L bioreactor at different agitation rates

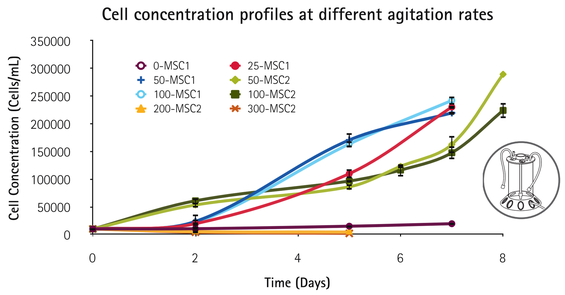


FIGURE 4. hMSCs from two independent lines were cultured for 7 or 8 days at different agitation rates in the Mobius® CellReady 3L bioreactor. The cell number increased to over 200 million for reactors that were agitated at 25, 50 or 100 rpm.

Quality and identity of the hMSCs from bioreactors

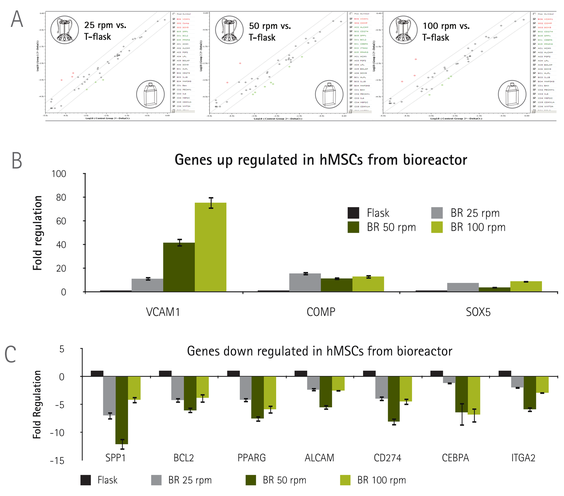
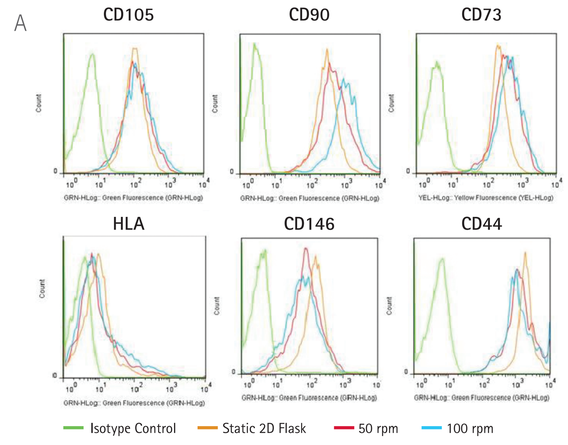


FIGURE 5. RNA was isolated from hMSCs that were cultured at 3 different agitation rates and qPCR was performed looking at 43 genes and this is shown as dot plots relative to 2D controls **(A)**. The 9 genes that showed a 5 fold expression difference relative to static 2D culture were then illustrated in bar graphs **(B, C)**.

Identity and functionality of the hMSCs from bioreactors



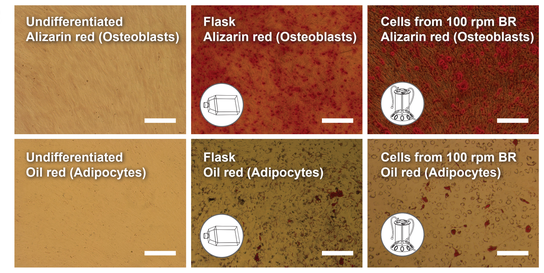


FIGURE 6. Flow cytometry histograms of hMSC markers showed similar expression to controls at 50 and 100 rpm **(A)**. The staining from cells that were differentiated towards osteoblasts and adipocytes also showed similar results to controls from the cells that were agitated at 100 rpm **(B)**. White Bars = 200 µm

Conclusion

- Shear can be modeled with a fixed-orifice device ("torture chamber") at high energy dissipation rates These rates were theoretically related to the spinner flask and Mobius® CellReady 3L bioreactor.
- Minor changes in miRNA were observed for hMSCs grown on microcarriers versus 2D culture. More differences in mRNA expression were observed between 2D flask culture and microcarrier cultures than between high and low shear.
- hMSC were expanded in the Mobius® CellReady 3L and agitated at different rates. Twenty-fold increases in cell number were observed at 25, 50, and 100 RPM. No significant differences in the characterization markers and functionality when compared to hMSCs taken from static 2D culture.

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