Determination of the Effect of Shear Stress on Hematopoiesis using two Shear Platforms

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Introduction

Current genetic blood disorder therapies are ineffective and hence require improvements. The discovery of stem cells has made the strides to treat these disorders advance rapidly. Stem cells when given the right stimuli are able to differentiate into various blood cells. This process is known as hematopoiesis. It has been reported that application of shear stress to embryo derived cells drives them towards the blood cell lineage. The current shear stress platform used to stimulate these cells however is very labor intensive and requires long shear exposure times. Hence, we developed a new shear platform that is able to promote hematopoiesis in the embryo derived cells within a short time. The two shear platforms were compared to determine the better enhancer of hematopoiesis

Experimental

Firstly, cells were taken from the aorta-gonad-mesonephros (AGM) region of mouse embryo. The AGM is the region of the mouse embryo that extends from the umbilical cord region to the front limb and contains the dorsal aorta. Hematopoietic stem cells develop in the AGM's dorsal aorta. The isolated AGM derived cells were taken and stimulated with shear stress using the two shear platforms. These platforms include the conventional parallel plate platform and the micro tube based shear platform. In the micro tube shear platform cells flow through tubes thereby experiencing shear stress in three dimensions. The outflow from these tubes was collected in tubes and gene expression analysis carried out on them. With the parallel plate platform cells were grown on the micro channel surface and media flowed over them hereby giving some degree of shear stress. The cells were then lysed for gene expression analysis or cultured in a colony forming unit assay where the blood cell colonies are enumerated.

Results and Discussion

Various degrees of shear stress were studied to determine its effect on hematopoiesis. These shear stresses include 1, 5, and 15 dyn/cm^2. The cells in the proximity of the circulatory system of the mouse experience a shear stress of 5 dyn/cm^2. Hence for these experiments we chose to interrogate a shear stress of 5 dyn/cm^2 along with a higher and lower shear stress than this. To evaluate whether hematopoiesis was occurring the pathways that are known to change during this process were interrogated by observing the genes from each pathway. The pathways studied included notch, hedgehog and wnt. Our results suggest that the notch signaling responds first followed by wnt and hedgehog under mouse physiological shear stress. This result is consistent

with what is reported in literature. Comparison of the two shear platforms studied at mouse physiological shear stress showed similar gene expression trends. To further compare the two shear platforms effect on hematopoiesis a colony forming unit assay was performed. Cells from either shear platform were taken and cultured in this assay which has various cytokines that allow cells to aggregate in respective colonies. Based on the number of colonies formed we were able to determine which shear platform was a better enhancer of hematopoiesis. The results from this assay can be observed in Figure 1 where the two shear platforms are compared at a shear stress of 5 dyn/cm^2. It can be concluded from this plot that the culture-based shear platform enhanced hematopoiesis more. The suspension-based system may not have proven to be better based on this assay however this result does show that 20 min exposure to shear stress in a tube can promote hematopoiesis. Hence, the significance of this work lies in our ability to promote hematopoiesis in embryo-derived cells in a short time with minimal pre-processing.

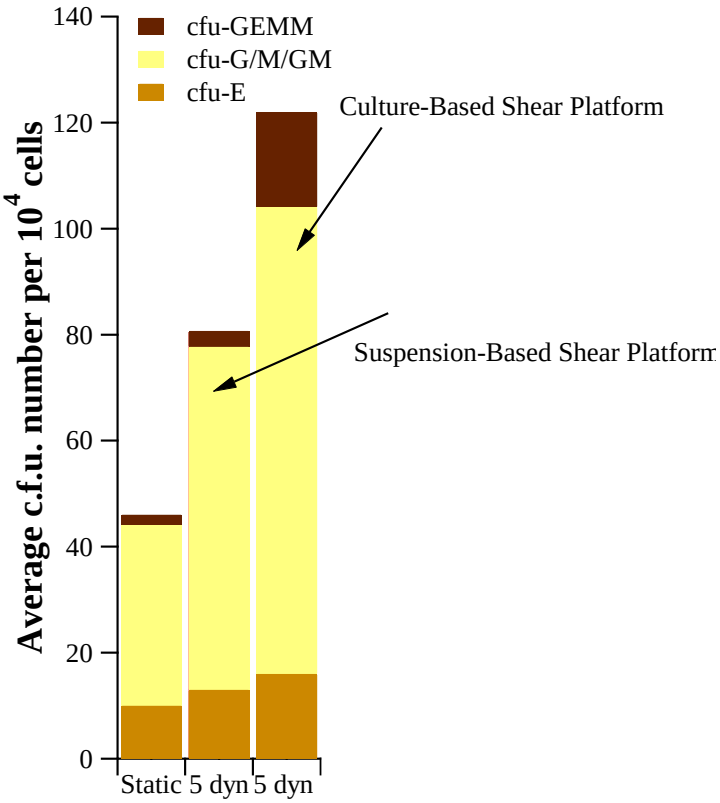

Figure 1: Methylcellulose hematopoietic colony forming unit (c.f.u) for both suspension-based shear and culture-based shear platform. Shear Stress increases the frequency of hematopoietic progenitors in complete M3434 methylcellulose. **GEMM, G/M/GM** and **E** are multi-potential granulocyte, erythroid, macrophage, megakaryocyte progenitors -granulocyte macrophage progenitors and erythroid progenitors colonies respectively.