Bioreactor Cultivation Enhances Human Embryoid Body Formation and Differentiation

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Background: Human embryonic stem (hES) cells can differentiate into any cell type in the adult body and consequently have the potential to provide an unlimited supply of different cells for tissue engineering and organ replacement. All these potential applications depend on the availability of controlled, scaleable processes for hES cell expansion and differentiation. We hypothesized that instigating hES cells differentiation under dynamic culture conditions would enable an efficient formation of differentiating embryoid bodies (EBs), by controlling the extent of initial hES cells agglomeration in the suspension.

Objective: To evaluate the effect of dynamic culture conditions on EBs formation and differentiation.

Methods: Undifferentiated hES cells were removed from their feeder layer and seeded in two rotating bioreactors (the High Aspect Rotating Vessel (HARV) and Slow Turning Lateral Vessel (STLV)) as well as in conventional static dishes, at the same initial cell concentration in differentiating mediumin. The vessels were set to rotate in a speed at which the suspended cell aggregates remained close to a stationary point within the reactor vessel. During cultivation, measurements were preformed at regular intervals for different cellular activities (glucose consumption, lactic acid formation, pH and lactate dehydrogenase levels), protein content and partial pressure of O_2 and CO_2 in the medium. The EBs were analyzed for their morphology and size using inverted microscopy and histological sections, and also for the generation of the three germ layers using RT-PCR and immuno-cytochemistry.

Results: The dynamic cultivation of differentiating hES resulted in a more efficient formation of hEBs, as revealed by the increased cellularity of the culture and the enhanced cellular activities compared to the static cultivation. Furthermore, we found the type of

rotating vessel to have a significant impact on the process of EBs formation. In HARV, two-five large cell clumps were observed within two days of seeding hES in the reactor and they developed into large EBs with an extensive necrotic center. In STLV, the EBs were smaller in size and with much smaller necrotic centers even after 1 month; they maintain high proliferative capacity and cell viability. The dynamically-formed hEBs preserved the differentiation course into the three germ layers, including primitive neuronal tubes formation, blood vessels sprouting, and specific-endocrine secretions.

Conclusions: We describe herein, for the first time, the dynamic formation of hEBs in rotating bioreactor systems, and show that the efficiency of the process depends on the geometry and properties of the vessel. Moreover, initial early developmental events involved in the generation of the three germ layers, are not altered in the dynamic-formed hEBs. Our study may pave the way for the development of scaleable bioprocesses for hES cell production for clinical and industrial applications.