Shear Alters the Proportion of Neural Crest versus Mesoderm-derived Osteoprogenitors in Embryoid Bodies

Alexander Seeliger*, Susanne Trettner*, Susann Horvat*, Julia Ast*, Beatrice Kuske†, Nicole I. zur Nieden*‡

*Fraunhofer Institute for Cell Therapy and Immunology, Department of Cell Therapy, Applied Stem Cell Technology Unit, 04103 Leipzig, Germany, †Department of Cell Biology and Neuroscience, University of California Riverside, Riverside, CA 92521

Tel: 951-827-3818, E-mail: nicole.zurnieden@ucr.edu

Summary: Murine embryonic stem cells were inoculated in suspension bioreactors and embryoid bodies were formed. Cells from embryoid bodies were then analyzed and further induced towards osteoblasts in static culture. Mature cultures were molecularly analyzed for presence of osteoblasts.

Due to the anticipated high demand for clinical supplies of skeletal tissue, it will be necessary to develop strategies for generating functional osteoblasts or osteoprogenitors in sufficient numbers. Embryonic stem cells (ESCs) represent a quasi unlimited cellular source and their differentiation to osteoblasts in static culture is established and well described. Typically, osteogenic differentiation is initiated through forced aggregation of cells in so-called embryoid bodies (EBs), which contain differentiating cells of all three germ layers. A labor-intensive protocol is used to generate those EBs, which typically involves the pipetting of droplets of cell suspension onto lids of petri dishes, which are then flipped into their regular position, so that the droplets are 'hanging'. Once EBs have formed, they are transferred into adherent cultures and cells are then pushed down the osteogenic differentiation path by supplementation of the culture medium with 1alpha,25 dihydroxy vitamin D3, ascorbic acid and glycerophosphate [1].

More recently, research veered toward the three-dimensional expansion and differentiation of ESCs in stirred suspension bioreactors (SSBs) in order to achieve quality controlled reproducibility of differentiation outcome and ease labor effort [2, 3, 4]. This current study was thus designed to compare the osteogenic differentiation capacity of EBs that were formed in SSBs (SSB EBs) to EBs made via the hanging drop method with the second step of the differentiation (osteogenic induction) carried out in static culture. We investigated the influence of various bioreactor parameters, among them impeller design, agitation rate and inoculation density on the size of EBs, expression of lineage marker mRNA and their osteogenic capacity.

Our data suggest that cell fate is influenced both by inoculation density and agitation rate. As such, an agitation rate of 90 rpm not only accelerated the osteogenic differentiation capacity, but the cultures were also characterized by a higher deposit of Ca2+ to their extracellular matrix as well as an increased expression of the bone-specific genes osteocalcin and bone sialoprotein. We followed this initial EB formation step up with a more detailed characterization of the identity of progenitors in the EBs. Expression analysis of genes associated with early neural crest and mesodermal fate as well as flow cytometry for the respective proteins allowed us to conclude that the majority of osteoprogenitors found in SSB EBs generated under low shear stress are of neural crest origin as documented by higher expression of p75 and snail. EBs that were formed under higher shear stress expressed markers characteristic for mesoderm. Taken together, our data suggests that formation of EBs in stirred suspension bioreactors may save time and labor, but seems to alter the differentiation path by which osteoblasts develop.