The Negative Effect of Time and Air Exposure on HSC/HSPC Populations

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Background: Hematopoietic stem and progenitor cells (HSC/HSPCs) inhabit niches within bone marrow that have significantly lower oxygen tension (1-4% O_2, LowO2) than ambient air (21% O_2) and provide signals regulating HSC signaling/function/self-renewal. Despite this knowledge, the majority of HSC/HSPC experiments are performed in ambient air, not replicating the endogenous environment. Our previous studies of HSC/HSPCs in LowO2 demonstrated enhanced phenotypic (CD150) marker expression, and frequency, of HSC/HSPC in live cells (~3fold LSK/LSKCD150 p=0.04) relative to historic data (fixed cells) as well as alterations in novel signaling/functional pathways.

Methods: Our novel technology facilitates the isolation/sorting, and analysis of rare HSC populations, in live cells, continuously at their native LowO2 environments (3% O_2). All assays have been adapted and validated in LowO2.

Results: Time course (0 min, 15 min, 40 min, 60 min, 180 min) analysis comparing phenotypic markers of HSC/HSPC populations at LowO2 and ambient air, showed HSC/HSPC enhancement at baseline in LowO2 that were significantly blunted by exposure to air (p-value =.00007) and time beginning at 40 min (p-value =.00003). Phenotypic expression, as shown by the marker CD150, was also diminished by exposure to air (p-value =.0008) and via time (p-value =.05). Analysis via mRNA sequencing showed enhancement in 324 and 73 (LSK, LSKCD150) pathways including calcium channel complex and calcium ion binding when HSC/HSPC remained in LowO2 compared to air. Analysis of calcium regulation via FURA staining correlated with these data confirming increased calcium influx in LowO2 HSC/HSPC populations.

Potential Impact: Our findings demonstrate significant negative impacts of ambient air, fixation, and time from harvest on HSC/HSPC phenotype and function. These findings suggest the critical importance of studying HSC/HSPC, live, quickly, and in their native environment, highlight potential caveats of current approaches, and rationale for lack of comparable results for studies of HSC/HSPC in the literature.