Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation.

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Public Summary:
The transplantation of blood-forming hematopoietic stem cells (HSCs) can used as a curative stem cell therapy for a range of blood diseases from immunodeficiencies to leukemias. However, while these cells are very important clinically, expanding this stem cell population ex vivo has remained challenging. Existing conditions only support HSCs short-term ex vivo, limiting the window for investigation and manipulation (e.g. for gene therapy). Here, we described the optimization of culture conditions to grow mouse HSCs for 1-2 months ex vivo, during which functional HSCs can expand up to 900-fold. Engraftment of donor HSCs following transplantation usually requires radiation or chemotherapy-based pre-conditioning of the recipient, which causes significant morbidity and mortality. However, by transplanting large numbers of HSCs generated by our culture system, we were able to achieve donor engraftment in non-conditioned recipients. These findings therefore have important implications for both basic HSC research and clinical hematology.

Scientific Abstract:
Multipotent self-renewing haematopoietic stem cells (HSCs) regenerate the adult blood system after transplantation(1), which is a curative therapy for numerous diseases including immunodeficiencies and leukaemias(2). Although substantial effort has been applied to identifying HSC maintenance factors through the characterization of the in vivo bone-marrow HSC microenvironment or niche(3-5), stable ex vivo HSC expansion has previously been unattainable(6,7). Here we describe the development of a defined, albumin-free culture system that supports the long-term ex vivo expansion of functional mouse HSCs. We used a systematic optimization approach, and found that high levels of thrombopoietin synergize with low levels of stem-cell factor and fibronectin to sustain HSC self-renewal. Serum albumin has long been recognized as a major source of biological contaminants in HSC cultures(8); we identify polyvinyl alcohol as a functionally superior replacement for serum albumin that is compatible with good manufacturing practice. These conditions afford between 236- and 899-fold expansions of functional HSCs over 1 month, although analysis of clonally derived cultures suggests that there is considerable heterogeneity in the self-renewal capacity of HSCs ex vivo. Using this system, HSC cultures that are derived from only 50 cells robustly engraft in recipient mice without the normal requirement for toxic pre-conditioning (for example, radiation), which may be relevant for HSC transplantation in humans. These findings therefore have important implications for both basic HSC research and clinical haematology.