

## Hematopoietic Stem Cells from Cord Blood were Expanded *Ex Vivo* in Various Cytokine Conditions

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### Abstract

Hematopoietic stem cells (HSCs) have two characteristics : Self-renewering and differentiating into various blood cells. Many kinds of growth factors play key roles in proliferation and differentiation of HSCs.<sup>1), 2)</sup> Although cord blood (CB) is a rich source of HSCs, the limited volume obtained from a single CB collection has resulted in it being used predominantly for transplantation of children and small adults. So successful expansion of HSCs *ex vivo* has increased not only for a part of stem cell researches but also for clinical applications. In this study, we used human cord blood to isolate HSCs, which is collected after informed consent. Mononuclear cells (MNCs) were separated by using Ficoll-paque. CD34<sup>+</sup> and CD34<sup>-</sup> cells were isolated by an immunomagnetic separation method using MidiMACS (Miltenyi Biotec, Germany). Various cytokines such as EPO, GM-CSF, G-CSF, SCF, TPO, Flt3/Flk2, IL-3 and IL-6 were used without serum supplementation. Basal medium was IMDM and various cocktails were used ( Coc-I : EPO, GM-CSF, SCF, IL-3 ; coc-II : SCF, G-CSF, TPO, Flk3/Flk2, IL-6 ; coc-III :EPO, G-CSF, SCF, IL-3 ; coc-IV : SCF, GM-CSF, TPO, Flk3/Flk2, IL-6 ). 2-mercaptoethanol and BSA were added at final concentration of 0.1mM and 1% (w/v), respectively. Conventional 6 well plates were used and culture time reached up to 35 days. When different populations with MNCs/CD34<sup>+</sup>/CD34<sup>-</sup> cells were inoculated, total nucleated cell expansion reached to 7 fold/5,000 fold/70 fold with coc-I, 5/1,600/15 with coc-II, 6/3,800/27 with coc-III and 9/1,500/25 with coc-IV. BFU-E expansion reached to 3/0/0, 48/120/24, 20/0/4 and 67/80/20. CFU-GM expansion reached to 6/50/2, 400/5,300/190, 110/600/20 and 600/3,700/170. Adherent cells were visually observed whencoc-IV was used. Viability and colony forming units were maintained or increased

continuously during the culture in coc-II and coc-IV conditions. These results showed that HSCs or progenitor cells could expand continuously in coc-II and coc-IV conditions. These highly effective expansion conditions seem to be useful for cell therapy applications using HSCs.

#### References

1. Daniel R. Marshak, Richard L. Gardner, David Gottlieb, "Stem Cell Biology", CSHL Press, Cold Spring Harbor, New York, 2001, pp 289 ~ 306.
2. Roland Mertelsmann, Friedhelm H, "Hematopoietic Growth Factors in Clinical Applications", Marcel Dekker, Inc., New York, 1995, pp 227 ~ 239.