***Ex vivo* expansion of umbilical cord blood HSCs by supporting of osteoblasts under low oxygen condition**

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Objective This study was aimed to construct a three-dimensional static low oxygen culture system by using encapsulated murine osteoblasts (OBs) and to investigate its support effect on expansion of hematopoietic stem/progenitor cells (HSPCs) for umbilical cord blood *ex vivo*. **Methods** First of all, the murine osteoblasts were isolated by the enzyme digesting method and were cultured in HG-DMEM containing 10% newborn bovine serum. Then the osteoblasts were encapsulated in gelatin-alginate-chitosan (GAC) microcapsules by the polyelectrolyte-complexation method to create the three-dimensional culture system, then the microcapsules were seeded in the 6 well cell culture clusters. Hematopoietic mononuclear cells (hMNCs) were isolated from fresh umbilical cord blood by density gradient centrifugation and seeded into the three-dimensional co-culture system to culture *ex vivo* in Galaxy R CO₂ Incubator under 5% oxygen condition. The growth of hMNCs cells was determined by counting the cell number, and the glucose, lactic acid, and pH value were detected everyday. By using colony-forming assay and CD34+ flow cytometric analysis, the ability of the three-dimensional system to culture HSPCs was observed. The results were compared with the results of normal oxygen three-dimensional co-culture system and control groups under hypoxic and normoxic condition respectively. **Results** After 7 days' culture, the expansion of hMNCs co-cultured with encapsulated osteoblasts under hypoxia condition is 18.7±1.60 fold, the percentage of CD34+ cells increased from 2.0% to 2.5%, and the CD34+ cells expansion fold is 23.4. It displayed more significant support effect compared with other culture systems, especially on long-term HSPCs survival *ex vivo*. **Conclusion** It is concluded that the supporting capacities of microencapsulated osteoblasts in *ex vivo* expansion of umbilical cord blood HSPCs is notable under hypoxia condition. It has also been proved that hypoxia and osteoblasts play a crucial role of synergistic action in regulation of HSPCs proliferation.

Keywords: hypoxia condition, hematopoietic stem/progenitor cells (HSPCs), umbilical cord blood, microencapsulation, osteoblast, co-culture

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