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Ex vivo expansion of hematopoietic stem/progenitor cells supported by microencapsulated osteoblasts under a hypoxia environment

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Osteoblasts play an essential role in the construction of the hematopoietic stem/progenitor cell (HSPCs) niche, also characterized by its hypoxic environment *in vivo*. In order to successfully reconstruct *in vitro* the hematopoietic microenvironment, gelatin-alginate-chitosan microencapsulated osteoblasts and hypoxia conditions were used for the expansion of HSPCs from umbilical cord blood. The different oxygen concentrations, suitable diameter of microencapsulated beads and cell number ratio between umbilical cord blood mononuclear cells (UCB-MNCs) and rat or human osteoblasts (rOB or hOB, respectively) were determined. The microencapsulated osteoblasts and UCB-MNCs were co-cultured in serum-free medium supplemented with relatively low doses of purified recombininant human cytokines in hypoxic and normoxic incubators, respectively, whilst the self-cultured UCB-MNCs in hypoxic and/or normoxic conditions were operated as the control groups. The expansion of HSPCs was evaluated by counting the UCB-MNCs, colony-forming unit (CFU) assay and $CD34^+$ flow cytometric analysis. After 7 days of culture, the expansion of UCB-MNCs co-cultured with microencapsulated osteoblasts with 0.5 mm diameter and cell ratio of 2:1 osteoblasts to UCB-MNCs under hypoxic conditions (5% oxygen tension) was 49.0±4.6 fold ($P<0.01$, when compared to the control group). The population percentage of $CD34^+$ cells increased from 1.9% to 3.4% and achieved a fold-expansion of 87.6±8.3 fold. The CFU-Cs also obtained 9.8±0.8 fold expansion. In conclusion, it was demonstrated that the presence of osteoblasts had an extremely significant effect on the expansion capacity of the HSPCs under hypoxia conditions *in vitro*.

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