

## 3-D long-term bone marrow culture to analyze stromal cell biological function

November 2 2011

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Stromal cells, as distinct from hematopoietic cells, are an essential component of the bone marrow microenvironment and are necessary for the long-term maintenance of hematopoietic stem cells (HSCs) in vitro. Previous studies have shown that stromal cells regulate the proliferation and differentiation of HSCs through the production of diffusible hematopoietic regulatory factors and extracellular matrix, and through physical cell-cell interactions involving adhesion molecules and gap junction-mediated cell communication. However, the ability of stromal cells to support the expansion of HSCs and to maintain their self-renewal potential has generally been investigated in long-term, two-dimensional (2D) bone marrow culture systems (BMCS), and most of the reports have shown a decline in HSCs within 4-6 weeks in culture.

In work published in the November 2011 issue of *Experimental Biology and Medicine*, Hirabayashi and co-investigators from Nihon University School of Medicine, Osaka Prefecture University and the Norwegian University of Science and Technology have developed a new three-dimensional (3D) BMCS. As stated by co-author Isao Tsuboi, "2D BMCS can't maintain HSCs for a long time, which does not enable us to analyze stromal-cell function. Therefore, we developed a new 3D BMCS and succeeded to maintain HSCs for much longer time."

This new 3D BMCS is based on unique particles. As explained by co-author Yukio Hirabayashi, "The polymer particle with grafted epoxy-polymer-chains is most important in our 3D BMCS. We selected the particle most suitable for cell culture from more than 20 types of

particles with various grafted polymer chain lengths and its surface density, the composition of base polymer network and graft polymer chain. We named this particle G-02". Furthermore, co-author Tomonori Harada adds "Several kinds of cell lines, other than murine fibroblast cell line (MS-5 cell), such as an epidermal cell line (HeLa cell), osteoblast cell line (MC3T3E1 cell) and chondrocyte cell line (ch-8 cell), can adhere easily on the G-02 particle and proliferate rapidly on its surface. This advantage of the G-02 particle enables us to develop 3D BMCS, which can also be applied to other 3D organ cultures of central nerve system, heart and liver."

CD34 is well known to be a surface marker for human primitive hematopoietic progenitor cells, however, CD34+ cells can also differentiate into stromal cells. When CD34+ cells are co-cultured with human stromal cells, it is complex to clarify the biological function of the preestablished stromal cell layer. Prof. Shin Aizawa, group leader and pioneer in the study of stromal cell, says "In this study, we used a murine stromal cell line (MS-5) instead of human stromal cells to exclude the effect of CD34+ derived stromal cells. This co-culture system makes it possible to distinguish the function of MS-5 stromal-cell layer from that of CD34+ derived stromal cells. Now our group is studying gene-expression levels of various cytokines in [stromal cells](#) using specific primers and probes for the mouse and human".

Steven R. Goodman, Ph.D. Editor-in-Chief of *Experimental Biology and Medicine* said "this 3-dimensional [bone marrow](#) culture system, developed by Hirabayashi and coworkers, should be an outstanding tool for the study stromal cell function".

Provided by Society for Experimental Biology and Medicine

Citation: 3-D long-term bone marrow culture to analyze stromal cell biological function (2011,

November 2) retrieved 25 April 2024 from <https://medicalxpress.com/news/2011-11-d-long-term-bone-marrow-culture.html>

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