The mechanism of long-term functional maintenance of neural stem cells by 3D culture system.

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The hippocampus that includes neural stem cells (NSCs) is an important organ associated with memory and learning. As a simple retrospective assay to identify NSC activity, neurosphere culture is generally employed. However, it is difficult to expand stemness-possessing neurosphere from the adult hippocampus. To better understand the nature of adult hippocampal NSCs, establishment of the specified *in vitro* expansion system is needed.

Here, we tried the culture of NSCs on a polyimide sheet possessing pores ranging from 5 to 50 μm . The NSCs derived from the adult hippocampus efficiently proliferate and sparsely formed sphere-like aggregates on the sheet. Those aggregates detached from the sheet could differentiate into the three cell lineages in response to differentiating agents. According to this procedure, we enabled the adult hippocampus-derived NSCs to maintain their stemness at least over 400 days. To evaluate the molecular mechanism, we performed a DNA microarray analysis. Notably, there was a big difference in expression profiles of mRNAs from NSCs between the long-term 3D culture and the short-term spheroid culture. We will discuss how the stemness of NSC can be maintained by the culture with polyimide sheet.