

<sup>3</sup> Name of journal: World Journal of Stem Cells

ESPS Manuscript NO: 17168

Columns: ORIGINAL ARTICLE

Basic Study

<sup>2</sup> Simplified three-dimensional culture system for long-term expansion of embryonic stem cells

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#### Abstract

**AIM:** To devise a simplified and efficient method for long-term culture and maintenance of embryonic stem cells requiring less frequent passaging.

**METHODS:** Mouse embryonic stem cells (ESCs) labeled with EYFP were cultured in three-dimensional (3-D) self-assembling scaffolds and compared with traditional 2-dimensional (2-D) culture techniques requiring mouse embryonic fibroblast (MEF) feeder layers or leukemia inhibitory factor (LIF). 3-D scaffolds encapsulating ESCs were prepared by mixing ESCs with polyethylene glycol tetra-acrylate (PEG-4-Acr) and thiol-functionalized dextran (Dex-SH). Distribution of ESCs in 3-D was monitored by confocal microscopy. Viability and proliferation of encapsulated cells during long-term culture were determined by propidium iodide (PI) as well as direct cell counts and PrestoBlue

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