Title	Effect of substrate topography on osteogenic potential of mouse iPS-cell derived embryoid bodies out growing cells
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№7：Effect of substrate topography on osteogenic potential of mouse iPS-cell derived embryoid bodies out growing cells

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Purpose：The purpose of this study was to investigate the effect of culture on polystyrene substrates of differing topographies on the osteogenic potential of embryoid bodies derived from iPS cells.

Materials and methods：Polystyrene substrates were sandblasted with 25-, 50-, and 150-μm Aluminum oxide particles to obtain topographies with an average Sa value of 0.6, 1.2, and 1.8μm, respectively. Embryoid bodies derived from mouse iPS cells were then treated according to the protocol of Nakatsuji and Suemori. Briefly, the cells were seeded on the substrates and left for 5 days. They were then examined over the next 16 days by SEM, immunocytofluorescence（ICF）for vinculin, runx 2, and quantitative RT-PCR（qRT-PCR）using primers for *RUNX-2* and *Collagen type I*.

Results：The results of ICF and SEM revealed that the surface roughness of the substrates had caused the cells to elongate and assume an irregular shape. Vinculin staining demonstrated how the Sa value affected mode of cellular attachment to each substrate. The results showed that the flatter the surface, the more random the distribution of focal adhesion points, which were smaller but more concentrated on the podia of the cells The results of qRT-PCR revealed that *runx-2* expression was highest on day 8 on surfaces with an Sa value of 1.2μm and on day 16 on both the 0.6- and 1.2-μm substrates. Expression of *Collagen type I* on day 8 was highest on the 0.6- followed by the 1.2-μm substrates.

Conclusion：Surface topography affects cell shape and early osteogenic gene expression in growing cells, particularly on substrates with an Sa value of 1.2μm.

№8：Effect of EDTA-treated dentin on the differentiation of mouse iPS cells into osteogenic/ odontogenic lineage *in vitro* and *in vivo*

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Purpose：The purpose of this study was to investigate the effect of dentin treated with EDTA on the differentiation of mouse iPS cells into osteogenic/ odontogenic lineage.

Materials and methods：Dentin discs（17×6 mm）were prepared from bovine incisor and treated by 17% EDTA. Embryoid bodies (EB) formed from mouse iPS cells were transferred to DMEM containing retinoic acid (RA) and BMP 4 and seeded on EDTA-treated dentin discs. In vitro, qRT-PCR analysis and IF study was performed on day 6. *In vivo*, EDTA-treated dentin discs with EBs and their outgrowth cells were subcutaneously transplanted into SCID mice. Immunohistochemical staining was performed on day 14.

Results：*In vitro*, RT-PCR analysis revealed that osteogenic markers of *Bsp* and *Ocn* were significantly higher in the EDTA-treated group. IF study showed that BSP and DMP 1 were present in both groups. *In vivo*, cartilage and bone-like tissue were observed adjacent to EDTA-treated dentin.

Conclusion：The study demonstrated that 17% EDTA - treated dentin induces mouse iPS cells to differentiate into osteogenic lineage.