Videobioinformatics Tracking of Calcification in Osteogenic Cultures derived from Human Pluripotent Stem Cells

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Summary: Human embryonic and human induced pluripotent stem cells were differentiated into mineralized osteoblasts. Cell fate was analyzed with molecular methods and videobioinformatics.

Embryonic stem cells (ESCs) are a class of pluripotent cells of embryonic origin with the ability to differentiate into all cell types found in the body. We have previously developed a procedure that allows murine ESCs to be differentiated into mature calcified osteoblasts using an osteogenic induction medium [1]. The osteogenic differentiation process is characterized by four defining key events: loss of pluripotency, establishment of the three germ layer lineages, mesenchymal specification and finally progenitor maturation. Specifically, the inducers permit the cells to time and stage dependently express a variety of genes associated with the osteoblast phenotype. In addition to expressing the 'correct' mRNAs, these ESC cultures also calcify during the maturation step. This calcified matrix is positive for osteocalcin, a unique protein secreted into the bone matrix, and has a black coloration in static photomicrographs (Fig. 1), which can be quantified employing morphometric image analysis [2].

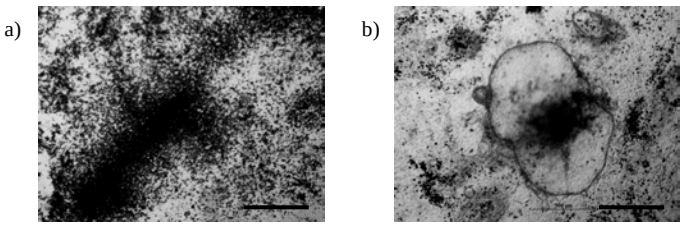

Fig. 1. Photographs of pluripotent stem cells induced with 1alpha, 25 $(OH)_2$ vitamin D_3, ascorbic acid and beta-glycerophosphate. a) Human embryonic stem cells. b) Human induced pluripotent stem cells. Bar = 500 µm.

Here, we focus on developing video bioinformatics tools, a new method of analysis to obtain data from images or videos, for the understanding of dynamic cellular processes that lead to the differentiation of human ESCs (hESCs) into osteoblasts taking advantage of the black coloration of the matrix once its calcified. Differentiation was induced from a confluent culture of hESCs with subsequent supplementation with 1alpha,25 $(OH)_2$ vitamin D_3, ascorbic acid, and beta-glycerophosphate. Emergence of mature osteoblasts was confirmed by assaying for Ca^{2+} content, alkaline phosphatase activity and by staining with the tissue-specific Alizarin Red S and von Kossa methods. Additionally, we have captured time-lapse videos of hESCs as they differentiated using the Nikon Biostation CT, an integrated cell culture observation system. Images were captured every 8h for a period of 15 days and analyzed to measure the degree of calcification during the differentiation process. The amount of calcification was estimated using a statistical image segmentation technique and compared to the biochemical assays, which allowed us to establish a 3D mineralization pattern (x, y, t).

Although these results are promising, the use of human ESCs (hESCs) faces a variety of ethical controversies, revolving around their embryonic origin, that deter the continuation of their investigation. In contrast, human induced pluripotent stem cells (hiPSCs) offer a less controversial path and are a great tool in the modeling of human diseases, since they are artificially generated from adult somatic cells [3]. Using videobioinformatic tracking of calcification, we also aimed to study the similarities found between hiPSC-derived osteoblasts and those differentiated from hESCs. This novel approach avoids the manual measurement of calcification and provides insight into the regions where differentiation occurs over time. In conclusion, video bioinformatics is a rapid and economical analysis method for determining temporal cellular changes in differentiating pluripotent stem cells.

[1] zur Nieden NI, Kempka G, Ahr HJ, "In vitro differentiation of embryonic stem cells into mineralized osteoblasts", *Differentiation*, **71**: 18-27, 2003.

[2] zur Nieden NI, et al, "Gene profiling of mixed ESC populations reveals a biphasic role for CatnB in osteogenic differentiation", *Mol Endocrinol*, **21(3)**: 674-685, 2007.

[3] Okita K, Ichisaka T, Yamanaka S, "Generation of germline-competent induced pluripotent stem cells", *Nature*, **448(7151)**: 313-317, 2007.