—Photogravure—

Chondrogenic and Osteogenic Differentiation of Adipose-derived Stem Cells Isolated from GFP Transgenic Mice

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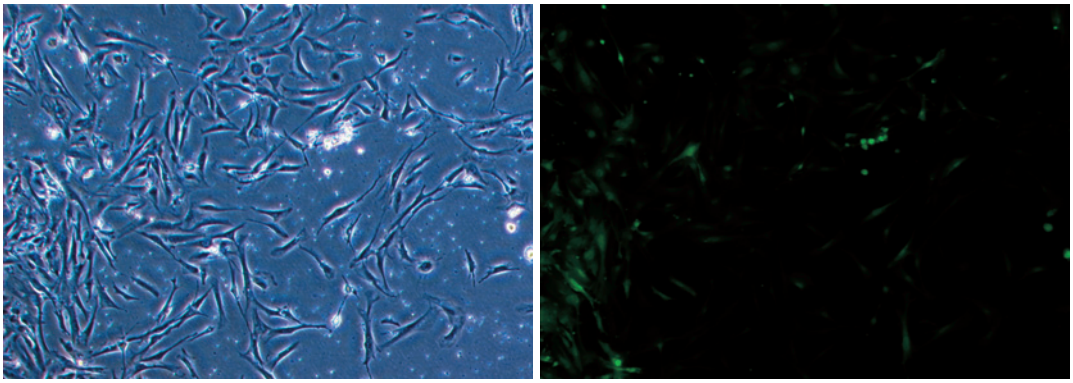
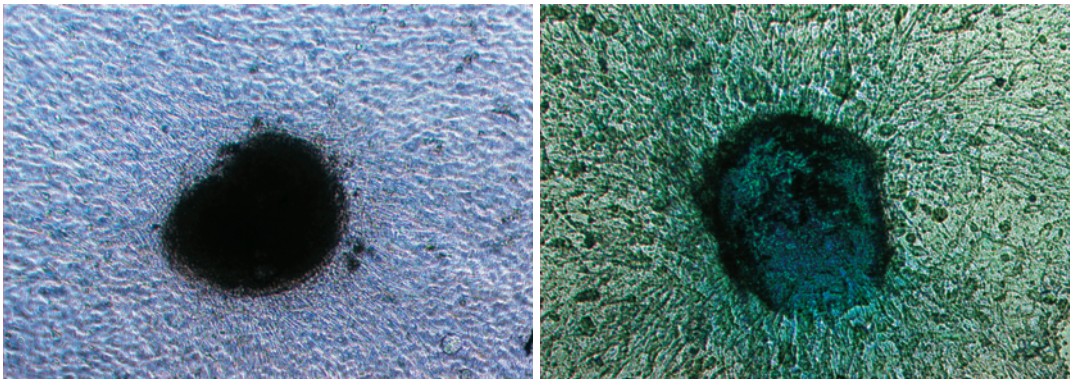
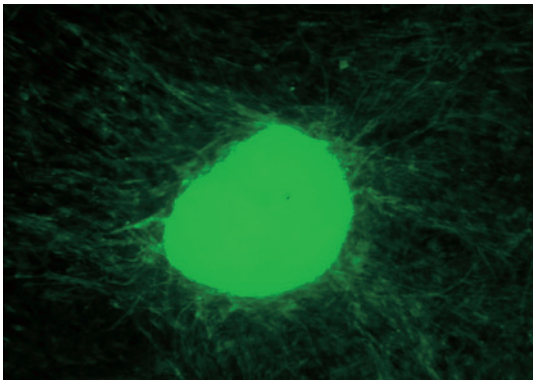


A. Light microscopic images B. Fluorescence microscopic images

Fig. 1



A. Light microscopic images C. Alcian blue staining



B. Fluorescence microscopic images

Fig. 2

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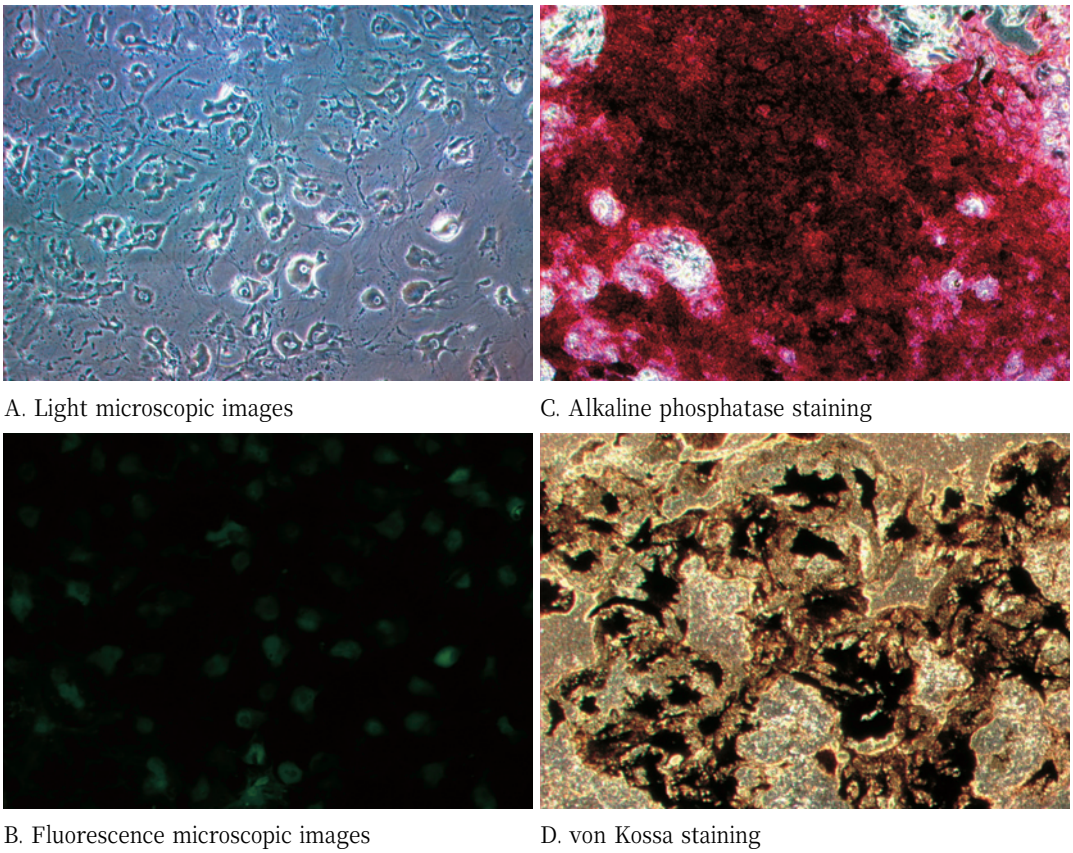

A. Light microscopic images C. Alkaline phosphatase staining

B. Fluorescence microscopic images D. von Kossa staining

Fig. 3

Abstract

Recent studies suggest that human adipose tissue contain pluripotent cells similar to bone marrow-derived stromal cells (BSCs)[1–3]. Taking advantage of homogeneously marked cells from green fluorescent protein (GFP) transgenic mice[4], we have previously demonstrated that BSCs differentiate into a variety of cell lineages both *in vitro* and *in vivo*[5]. In the present study, we extend this approach to characterize adipose-derived stromal cells (ASCs)[6]. These cells derived from human are sometimes called processed lipoaspirate (PLA) cells. ASCs were prepared from inguinal fat pads of GFP transgenic mice after extensive washing with PBS and treatment with collagenase. After the primary culture in control medium (DMEM+10% FBS), the cells were incubated in either chondrogenic medium (DMEM+1% FBS+insulin+ascorbate 2-phosphate+TGF-beta 1) or osteogenic medium (DMEM+10% FBS+dexamethasone+ascorbate-2-phosphate+beta-glycerophosphate) for two to four weeks. Chondrogenic differentiation was assessed by Alcian blue staining, while osteogenic differentiation was by von Kossa and Alkaline phosphatase staining. ASCs incubated in chondrogenic medium induced Alcian blue positive cells. Incubation with osteogenic medium became positive for von Kossa and Alkaline phosphatase staining. No osteochondrogenic differentiation was observed in cells incubated with control medium. This cell population can be easily identified through fluorescence microscope, it should be an ideal source of ASCs for further experiments of stem cell biology and tissue engineering.

References

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Fig. 1 Primary culture of ASCs–cultured for 4 days in control media–
The GFP transgenic mouse ASCs appeared as a fibroblast-like cells (A). GFP positive cells can be easily identified through fluorescence microscopy.

Fig. 2 Two weeks of " culture " in chondrogenic media
GFP transgenic mouse ASCs in the micromass culture supplemented with chondrogenic media formed small spheroids (A, B). The nodules stained positively for Alcian blue staining (C).

Fig. 3 Four weeks of culture in osteogenic media
GFP transgenic mouse ASCs in osteogenic media changed fibroblastic appearance to a rounder, more cuboidal shape (A, B). These cells stained positively in Alkaline phosphatase staining (C), and extracellular matrix stained positively in von Kossa staining (D).