International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:5, No:2, 2011

# Differentiation Capacity of Mouse L929 Fibroblastic Cell Line Compare With Human Dermal Fibroblast

Kasem Theerakittayakorn, Tanom Bunprasert

***Abstract*—**Mouse L929 fibroblastic cell line, which is widely used in many experiment aspects, was tested for their differentiation potency in osteogenic differentiation and adipogenic differentiation. Human dermal fibroblasts, which their differentiation potency are still be in confliction, also be taken in the experiment. The differentiations were conducted by using the inducing medium ingredients which is generally used to induce differentiation of stem cells. By the inducing media used, L929 mouse fibroblasts successfully underwent osteogenic differentiation and adipogenic differentiation while human dermal fibroblasts underwent only osteogenic differentiation but not for adipogenic differentiation. Human dermal fibroblasts are hard to be differentiated in adipogenic lineage and need specific proper condition for induction.

***Keywords*—**Adipogenic differentiation, Fibroblast, Inducing medium, Osteogenic differentiation

## I. Introduction

FIBROBLASTS are widely distributed in many types of tissues, such as tendon, ligament and skin. Fibroblasts are traditionally defined as the cells that produce collagens and are considered to be the primary source of most extracellular matrix components. They play a critical role in regulating the turnover of extracellular matrix and play an important part in wound healing. Fibroblasts are able to differentiate to myofibroblasts, specialized cells that possess a contractile phenotype with α-smooth muscle actin expression [1]. Myofibroblasts are responsible for the generation of the contraction forces that allow wound contraction during wound healing process [2].

The differentiation capacity of fibroblast to myofibroblast is obviously clear but its differentiation capacity to other lineages is still in controversy. Multipotency of human fibroblast was shown by Dan and colleagues [3]. They established 61 fibroblast clones from human foreskin, 21 clones of them exhibited adipogenic differention potential but only two clones, which showed neurogenic and hepatogenic potential, could be differentiated into islet-like cells [3]. In contrast, in the same year, Johan and colleagues stated that single-cell- cloned fibroblasts displayed similar differentiation potential as primary culture fibroblasts when adipogenic, chondrogenic and osteogenic differentiation were performed [4]. Steven and colleagues insisted the capacity of human dermal fibroblasts to undergo chondrogenic differentiation but the percentage of cells undergone differentiation was not shown [5]. Sorisky and colleagues investigated adipogenic differentiatin capacity of orbital fibroblasts from patients with or without Graves' ophthalmopathy and found that less than 10% of fibroblasts underwent differentiation [6]. While Sorisky and colleagues showed that dermal fibroblasts and perimysial fibroblasts from extraocular muscle failed to differentiate [6], Florence and colleagues showed that human fibroblasts from dermis and retroocular muscle were able to accumulate Oil Red O-positive droplets spontaneously without any differentiation induction when fibroblast were cultured on glass slides [7]. The differentiation capacity of mouse fibroblast has also been investigated. Spontaneous lipid droplet accumulation was found in L929 and NIH/3T3 mouse fibroblast cell lines when cultured on glass slides [7]. In contrast, Chaoxiang and Andre did not found progression of osteogenic differentiation in NIH/3T3 mouse fibroblasts when cultured in normal culture medium but progression of osteogenic differentiation was found when NIH/3T3 mouse fibroblasts were cultured in medium supplemented with 1α,25-Dihydroxyvitamin D3 [8].

Cell lines are useful models for doing research since they provide large amounts of consistent cells for prolonged used. Because most cellular characteristics are maintained in cell lines, they provide reliable in experimental results. Experimental results can be compared among other research reports, in which, the same cell lines were used. L929 cell line is usually used as tool in many standard testing. It is a cell line which popular in many experiment aspects such as material biocompatibility testing [9],[10],[11], drug cytotoxicity testing [12],[13] and cell biology studies [14],[15],[16].

This research is aimed to prove the differentiation potency of L292 mouse fibroblast cell line compare with human dermal fibroblast by conducting in frequently used differentiation inducing media which their ingredients different from the media used in the above reports.

## II. Materials and Methods

*Materials*

All chemicals and reagents in cell culture were purchased from GIBCO (Paisley, United Kingdom). All chemicals and

Kasem Theerakittayakorn is with Medical Science program, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand (corresponding author to provide phone: 66851203485; e-mail: kasemtheera@gmail.com)

Tanom Bunprasert is with Department of Otolaryngology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

reagents in differentiation and analytical experiments were purchased from (Sigma-Aldrich, St. Louis MO, USA). Plasticwares were purchased from Corning (New York, NY). Materials purchased from other sources mentioned above will be specified.

*Cell acquisition and culture*

L929 fibroblast cell lines were derived from commercial sources. Human dermal fibroblasts were established from facial reconstruction surgery remnants with informed consent. The specimens were washed three times with phosphate buffer saline and epidermis was separated by cutting off. The dermis was minced by surgical blades into small pieces (1-2 mm3). Then, the minced dermis was submerged in enzyme solution composed of 0.075% collagenase I and 0.1% trypsin for 1 hour. The digested suspension was passed through 70 µm cell strainers (BD Biosciences, Mississauga, ON, Canada). The flow though was centrifuged at 1000 rpm for 10 min. The obtained cell pellets were plated at 1×104 cells/cm2 in 100mm tissue culture Dishes. For proliferation, h-BMSCs were culture in α-MEM containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells were cultured in incubator at 37°C in a humidified air mixed with 5% CO2.

*Cell morphology and number determination*

Cell morphology was observed under microscope. Growth curve and doubling time were determined. Number of viable cells was assayed by MTT method. MTT solution (5 mg/ml MTT in DMEM without phenol red) was incubated with cells at 37°C for 30 min. MTT solution was removed and violet formazan dye entrapped in viable cells was dissolved by dimethyl sulfoxide. The absorbance measured at 570 nm using a microplate reader. Amount of cells was determined using a standard curve established from known number of cells. Doubling time was determined form equation obtained from the growth curve.

*Cell morphology and number determination*

Cell morphology was observed under microscope. Growth curve and doubling time were determined. Number of viable cells was assayed by MTT method. MTT solution (5 mg/ml MTT in DMEM without phenol

*Osteogenic differentiation induction*

Cells were seeded in 12-well plate at density 5000 cells in each well. Human fibroblasts from passage 3 were used. Osteogenic differentiation was induced by culture fibroblasts in osteogenesis inducing medium which was normal culture medium supplemented with 0.1 µM Dexamethazone, 10 mM β-Glycerol phosphate and 0.2 mM L-Ascorbic acid. Osteogenic differentiation was assessed by measurement of alkaline phosphatase activity and calcium deposition. Alkaline phosphatase activity was assayed by measurement the inversion rate of p-Nitrophenyl phosphate to p-Nitrophenol according to [17]. Calcium deposit was assayed by O-Cresolphthalein method according to [18]. Von Kossa staining followed the protocol from [19].

*Adipogenic differentiation induction*

Cells were seeded in 12-well plate at density 5000 cells in each well. Human fibroblasts from passage 3 were used. Adipogenic differentiation was induced by culture fibroblasts in adipogenesis inducing medium which was normal culture medium supplemented with 1 µM Dexamethazone, 100 µM Indomethacin, 0.5 mM IBMX and 10 µg/ml Insulin. Adipogenic differentiation was proved by Oil red O staining. Briefly, cells were fixed with 4% formaldehyde for 30 min and rinse twice with water. 0.36% Oil Red O Solution in 60% isopropanol was added and incubated for 50 min. Oil red O solution was removed and cells were washed three times with 60% isopropanol. Stained cells were assessed under microscope.

*Statistical Analysis*

All results are presented as the mean ± standard deviation of three samples. Significance was determined by student's t-test at $p<0.05$ and $p<0.01$.

## III. Results

Morphology of L929 mouse fibroblasts and human dermal fibroblasts at passage 3 visualized under inverted microscopes are shown in Fig. 1. All types of fibroblast are spindle-shaped cells but size difference was obviously observed. Human dermal fibroblasts are much larger than L929 mouse fibroblasts. Doubling time for L929 mouse fibroblasts was 14 hr and doubling time for human dermal fibroblasts was 26 hr.

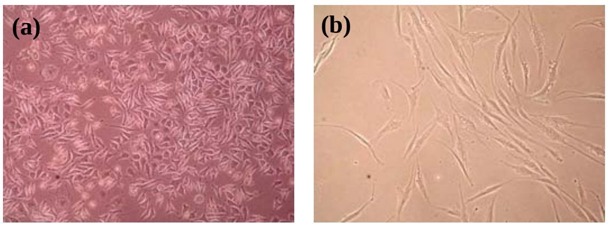


Fig. 1 Morphology of fibroblasts under microscope at 100X magnification (a) L929 mouse fibroblasts (b) Human dermal fibroblast at passage 3

In osteogenic differentiation experiment, changes in alkaline phosphatase activity and calcium deposition of the fibroblasts are shown in the Fig. 2. For the progression of alkaline phosphatase activity of L929 mouse fibroblasts, it shapely increased at day 7 with significant higher than that at initial date ($p<0.01$). After day 7, alkaline phosphatase activity of L929 mouse fibroblasts was slow down and then rapidly increased highest at day 28 with significant higher than that at day 7 ($p<0.01$). For the progression of alkaline phosphatase activity of human dermal fibroblasts, there was no significant difference from the initial date found until at day 28 which alkaline phosphatase activity was suddenly increase with significant higher than all previous dates ($p<0.01$). Significant difference between alkaline phosphatase activity of L929 mouse fibroblasts and human dermal fibroblasts was only found at day 7 which that of L929 mouse fibroblasts was significant higher than that of human dermal fibroblasts ($p<0.01$). For L929 mouse fibroblasts, difference in calcium deposit could not detect until day 21. At day 21, calcium deposit was significant higher than the initial and calcium deposit was significant highest ($p<0.01$). For human dermal fibroblasts, calcium deposit was little increase at day 28 which was insignificant different ($p<0.05$) from the previous dates. No significant difference ($p<0.05$) of alkaline phosphatase activity and calcium deposition was found in fibroblast, both L929 mouse fibroblasts and human dermal fibroblast, in normal proliferation culture medium.

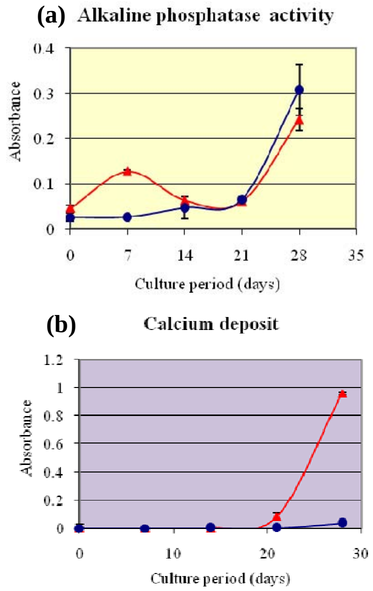


Fig. 2 Responses of L929 mouse fibroblasts (▲) and human dermal fibroblasts (●) in osteogenic differentiation inducing medium for (a) Alkaline phosphatase activity and (b)Calcium deposition

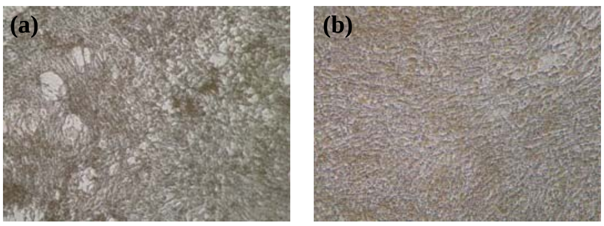


Fig. 3 Morphology of fibroblasts from osteogenic differentiation experiment at day 28 after von Kossa staining under microscope at 100X magnification (a) L929 mouse fibroblasts (b) Human dermal fibroblast at passage 3

Osteogenic differentiation of fibroblasts was visibly revealed by von Kossa staining (Fig. 3) and adipogenic differentiation was visibly revealed by Oil red O staining (Fig. 4). At day 28, the last day of osteogenic differentiation experiment, L929 mouse fibroblasts were stained much darker than human dermal fibroblasts which consistent to the results of calcium deposition determined by O-cresolphthalein method. By the condition used in adipogenic differentiation experiment, L929 mouse fibroblasts successfully underwent differentiation as confirmed by Oil red O staining (Fig. 4-a) but not for human dermal fibroblast which some of cells died and no cells was stained by Oil red O staining (Fig. 4-b). No accumulated lipid droplets were observed in adipogenesis-induced human dermal fibroblasts prior to Oil red O staining.

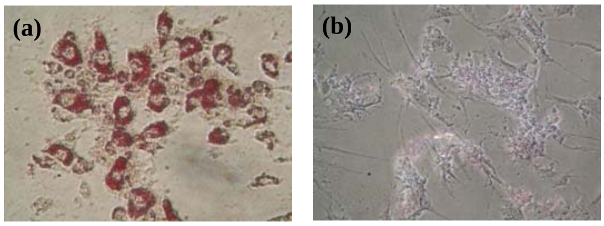


Fig. 4 Morphology of fibroblasts from adipogenic differentiation experiment at day 14after Oil red O staining under microscope at 400X magnification (a) L929 mouse fibroblasts (b) Human dermal fibroblast at passage 3

## IV. Discussion

L929 mouse fibroblasts and human dermal fibroblasts were quite different both in cell size and doubling time. By the condition used in this research, L929 mouse fibroblasts could be induced to undergo osteogenic differentiation and adipogenic differentiation while human dermal fibroblasts could be induced to undergo osteogenic differentiation but not for adipogenic differentiation. Our results consistent to Jääger and Neuman [20] whom stated that human fibroblasts are difficult to induced to undergo adipogenic diffentiation since they found that fibroblasts revealed a time lag in the induction of adipogenesis-related genes. Our results confirmed the osteogenic and adipogenic differentiation potency of L929 mouse fibroblasts when they were cultured in the medium with supplements as mentioned in the methods. This report and the accumulated results from other reports reveal the plasticity of L929 mouse fibroblasts that can be successfully induced to undergo differentiation by diverse culture conditions. Their revealed potency allows L929 mouse fibroblast cell line to become a useful cell line for various experiment aspects. Human dermal fibroblasts unsuccessful induced to undergo adipogenic differentiation this may be due to inducing medium in this research differ from those researches which special inducing media were used.

## V.Conclusion

By the inducing media used in this work, L929 mouse fibroblasts successfully underwent osteogenic differentiation and adipogenic differentiation while human dermal fibroblasts underwent only osteogenic differentiation but not for adipogenic differentiation. L929 mouse fibroblasts could replace mouse stem cells in experiment involved in differentiation process. Human dermal fibroblasts are hard to be differentiated in adipogenic lineage and need specific proper condition for induction.

## REFERENCES

[1] R. A. Evans, *et al.*, "TGF-beta1-mediated fibroblast-myofibroblast terminal differentiation-the role of Smad proteins," *Exp Cell Res,* vol. 282, pp. 90-100, Jan 15 2003.
[2] C. Helary, *et al.*, "Dense fibrillar collagen matrices: a model to study myofibroblast behaviour during wound healing," *Biomaterials,* vol. 27, pp. 4443-52, Sep 2006.
[3] D. Bi, *et al.*, "Differentiation of human multipotent dermal fibroblasts into islet-like cell clusters," *BMC Cell Biol,* vol. 11, p. 46, 2010.
[4] J. P. Junker, *et al.*, "Adipogenic, chondrogenic and osteogenic differentiation of clonally derived human dermal fibroblasts," *Cells Tissues Organs,* vol. 191, pp. 105-18, 2010.
[5] S. B. Nicoll, *et al.*, "Hyaluronidases and CD44 undergo differential modulation during chondrogenesis," *Biochem Biophys Res Commun,* vol. 292, pp. 819-25, Apr 12 2002.
[6] A. Sorisky, *et al.*, "Evidence of adipocyte differentiation in human orbital fibroblasts in primary culture," *J Clin Endocrinol Metab,* vol. 81, pp. 3428-31, Sep 1996.
[7] F. Jeney, *et al.*, "Cytochemical studies on the fibroblast-preadipocyte relationships in cultured fibroblast cell lines," *Acta Histochem,* vol. 102, pp. 381-9, Nov 2000.
[8] C. Shui and A. M. Scutt, "Mouse embryo-derived NIH3T3 fibroblasts adopt an osteoblast-like phenotype when treated with 1alpha,25-dihydroxyvitamin D(3) and dexamethasone in vitro," *J Cell Physiol,* vol. 193, pp. 164-72, Nov 2002.
[9] F. Bretagnol, *et al.*, "The effect of sterilization processes on the bioadhesive properties and surface chemistry of a plasma-polymerized polyethylene glycol film: XPS characterization and L929 cell proliferation tests," *Acta Biomater,* vol. 4, pp. 1745-51, Nov 2008.
[10] M. C. Serrano, *et al.*, "Transitory oxidative stress in L929 fibroblasts cultured on poly(epsilon-caprolactone) films," *Biomaterials,* vol. 26, pp. 5827-34, Oct 2005.
[11] R. Zange and T. Kissel, "Comparative in vitro biocompatibility testing of polycyanoacrylates and poly(D,L-lactide-co-glycolide) using different mouse fibroblast (L929) biocompatibility test models," *European journal of pharmaceutics and biopharmaceutics* vol. 44, pp. 149-157, 1997.
[12] G. Faria, *et al.*, "Chlorhexidine-induced apoptosis or necrosis in L929 fibroblasts: A role for endoplasmic reticulum stress," *Toxicol Appl Pharmacol,* vol. 234, pp. 256-65, Jan 15 2009.
[13] M. Nordin, *et al.*, "Effects of exposure period of acetylsalicylic acid, paracetamol and isopropanol on L929 cytotoxicity," *Toxicol In Vitro,* vol. 5, pp. 449-50, 1991.
[14] T. Taniguchi, *et al.*, "Hydrogen peroxide-induced arachidonic acid release in L929 cells; roles of Src, protein kinase C and cytosolic phospholipase A2alpha," *Eur J Pharmacol,* vol. 546, pp. 1-10, Sep 28 2006.
[15] B. Roelofs, *et al.*, "Acute activation of glucose uptake by glucose deprivation in L929 fibroblast cells," *Biochimie,* vol. 88, pp. 1941-6, Dec 2006.
[16] M. Cartwright, *et al.*, "The human nerve growth factor gene: structure of the promoter region and expression in L929 fibroblasts," *Brain Res Mol Brain Res,* vol. 15, pp. 67-75, Sep 1992.
[17] H. Declercq, *et al.*, "Isolation, proliferation and differentiation of osteoblastic cells to study cell/biomaterial interactions: comparison of different isolation techniques and source," *Biomaterials,* vol. 25, pp. 757-68, Feb 2004.
[18] J. Stern and W. H. P. Lewis, "The colorimetric estimation of calcium in serum with o-cresolphthalein complexone," *Clinica Chimica Acta,* vol. 2, pp. 576-580, 1957.
[19] T. J. Wu, *et al.*, "Studies on the microspheres comprised of reconstituted collagen and hydroxyapatite," *Biomaterials,* vol. 25, pp. 651-8, Feb 2004.
[20] K. Jaager and T. Neuman, "Human Dermal Fibroblasts Exhibit Delayed Adipogenic Differentiation Compared with Mesenchymal Stem Cells," *Stem Cells Dev,* Jan 16 2011.