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

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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 in controversy, 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. However, it is obvious that fibroblasts are able to differentiate into 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 differentiation 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 differentiation 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]. Compared with human fibroblast, L929 mouse fibroblasts have been used 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 L929 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
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.

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**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 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.

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 and human dermal fibroblasts was shown at day 28.
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.

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 Jäger and Neuman [20] whom stated that human fibroblasts are difficult to induced to undergo adipogenic differentiation 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 experiments 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


