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Isolation of Feline Adipose Tissue Derived Mesenchymal Stem Cells

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A B S T R A C T

The omental adipose tissue was collected from, the animals undergone ovariohysterectomy surgical procedures. The samples were digested with 0.1 per cent (W/V) collagenase type I and transferred to a beaker with magnetic stirrer at 600 rpm at 37°C for 30 minutes. The lesser time was given for enzymatic digestion as compared to other species. The viability of the cell was evaluated by trypan blue exclusion method using haematocytometer. Trypan blue had a high affinity to nuclear DNA, which traverse the membrane in a dead cell and dye it blue. The isolated cells were seeded at a density of $1x10^6$ cells per T25 culture flask and incubated in 5 ml of DMEM-HG at 37°C with 5 per cent CO_2. 70-80 per cent confluency was observed on day six and 100 percent confluency on day seven.

Introduction

Adipose tissue, as a stem cell source is ubiquitously available and has several advantages compared to other sources.

It is easily accessible in large quantities with minimal invasive harvesting procedure and the isolation of these Adipose-derived mesenchymal stem cells (AD-MSCs) yields a high amount of stem cells, which is essential for stem-cell-based therapies and tissue engineering. AD-MSCs are indeed multipotent somatic stem cells exhibiting growth kinetics, plasticity and proved to induce efficient tissue regeneration in several biomedical applications. Cultures of AD-MSCs were easier to generate because of their higher intrinsic proliferative rate and maintain their phenotypes. Hence, it is used for tissue engineering and regenerative medical applications (Bunnell *et al.*, 2008).

Materials and Methods

Collection of feline adipose tissue

The feline omental adipose tissue, collected from healthy animals undergone surgeries of ovariectomy and ovariohysterectomy (Figure

1). The sample was collection bottle which contained phosphate buffer saline (PBS) and processed within 30 minutes of collection.

Isolation of feline adipose derived mesenchymal stem cells (fAD-MSCs)

The weighed adipose tissue was rinsed with phosphate-buffered saline, to remove blood, small vessels and connective tissue. Cleaned adipose tissue was cut into small pieces by using sterile forceps and surgical blade. Samples were digested with 0.1 per cent (W/V) collagenase type I (SIGMA) and transferred to a beaker with magnetic stirrer and kept in stirrer with 600 rpm at 37^{O}C for 30 minutes (Kono *et al.,* 2014).

After digestion stromal vascular fraction (SVF) was pipetted out and neutralized with equal volume of Dulbecco's modified Eagle medium-high glucose (DMEM-HG) with fetal bovine serum (FBS) and antibiotic solution and non-essential amino acids was added to it (Al Nbaheen *et al.,* 2013).

Finally, it was centrifuged at 2500 rpm for 10 minutes, supernatant was discarded and pellet was resuspended in 1ml of culture media to get cell suspension (Aliborzi *et al.,* 2016) (Figure 2). Cell viability and total cell density were determined by 0.4 per cent Trypan blue exclusion test to check the viability of cells (Figure 3).

Seeding of feline adipose derived mesenchymal stem cells

Viable AD-MSCs from resuspended pellet were seeded at a density of $1x10^{6}$ cells per T25 culture flask and incubated in 5ml of DMEM-HG at 37^{O}C under 5 per cent CO_2 in CO_2 incubator (Beaulah *et al.,* 2016) (Figure 4). The spent medium was replaced initially with fresh DMEM-HG after two days of seeding. Then fresh medium was added daily until the cells reached a confluency of 70- 80 per cent (Vieira *et al.,* 2010).

Subculturing of feline adipose derived mesenchymal stem cells

After 70-80 per cent confluency the cells were detached from the culture flask by using 0.25 per cent trypsin / EDTA for 2 minutes at 37°C. DMEM-HG containing 10 per cent fetal bovine serum (FBS) was added and cells were kept in refrigerator to stop the action of trypsin.

Then the cells were disaggregated by pipetting the medium into culture flask gently. Cell pellet was prepared by spinning the cell suspension at 1500 rpm for 15 minutes.

Then the pellet was reconstituted with 1 ml DMEM-HG and seeding density was maintained after counting the cells for sub-culturing.

Cells were passaged subsequently on reaching the confluency (Zahran *et al.,* 2013)

Results and Discussion

Source of fAD-MSCs

In this study, the sterile omental adipose tissue was collected from healthy animals undergone surgeries of ovariectomy and ovariohysterectomy in line with the procedure followed by Webb *et al.,* (2011) and Kono *et al.,* (2014) in feline. In canine, adipose tissue was collected from inguinal fat, omental and subcutaneous fat (Neupane *et al.,* 2008).

In human, the most common sites for collection of adipose tissue were subcutaneous fat and omental fat as per the finding of Marappagoundar *et al.,* (2010), as they concluded that AD-MSCs from both the sites could be useful for regenerative medicine

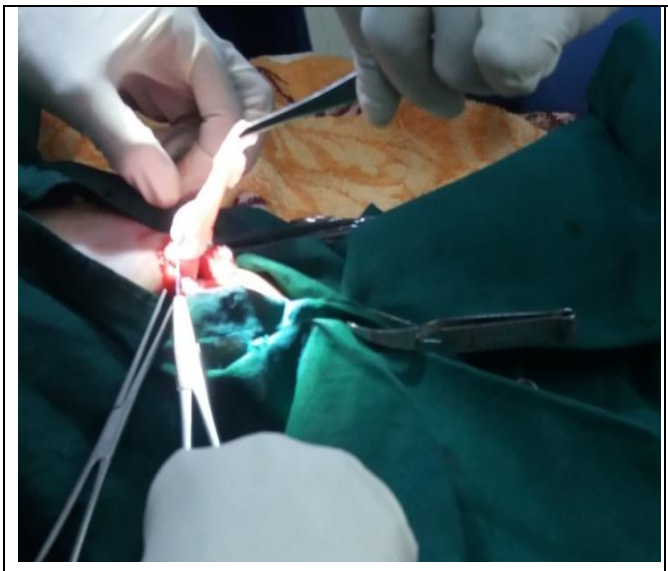

Fig.1 Surgical removal of feline adipose tissue

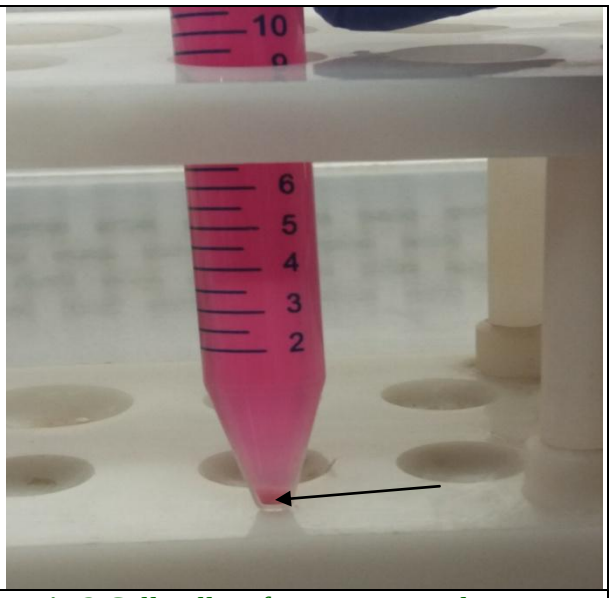

Fig.2 Cell pellet after enzymatic digestion

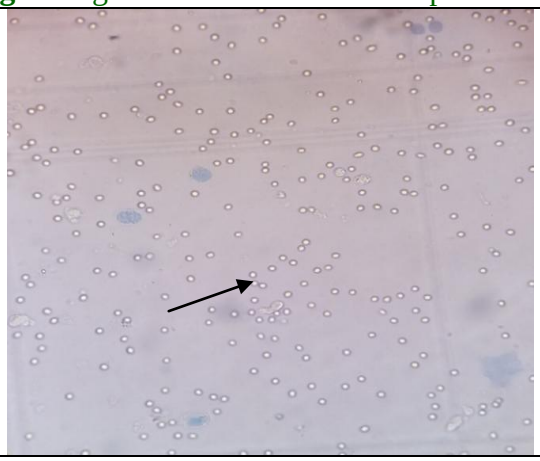

Fig.3 Photomicrograph showing viable fAD-MSCs (arrow) from omental adipose tissue Trypan blue exclusion test X 100

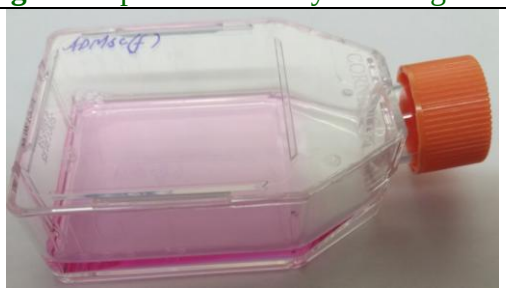

Fig.4 Photograph showing seeding of fAD-MSCs cells in T25 flask on day-0

Enzymatic digestion

The feline adipose derived mesenchymal stem cells (fAD-MSCs) were processed and subjected to collagenase type I (0.1 per cent W/V) treatment for isolation of stromal vascular fraction (SVF) as followed by Webb *et al.,* (2011) and Kono *et al.,* (2014) in feline. The cell viability was confirmed by Trypan blue exclusion test. The live cells were observed with transparent cytoplasm and intact nucleus. The cytoplasms of dead cells were stained with trypan blue. In the present study, lesser time was given for enzymatic digestion as compared to other species, because feline adipose tissue was soft which might be due to less collagen fiber content. According to Hepsibha *et al.,* (2011) AD-MSCs isolation from buffalo was processed by 0.1 per cent collagenase with 1 per cent bovine serum albumin (BSA). The yield of AD-MSCs was low when collagenase alone was used might be due to higher collagen fiber content in buffalo adipose tissue. In the present study, after enzymatic digestion, stromal vascular fraction (SVF) was pipetted out and neutralized with equal volume of DMEM-HG with fetal bovine serum and antibiotic solution and non-essential amino acids as described by

Al-Nbaheen *et al.,* (2013) in human. Than samples, was centrifuged at 2500 rpm for 10 minutes, supernatant was discarded and pellet was resuspended in one ml of culture media to get cell suspension as reported by Aliborzi *et al.,* (2016) in guinea pig.

Culture and expansion

Seeding of fAD-MSCs

Viable fAD-MSCs were seeded at a density of $1x10^6$ cells per T_{25} culture flask and incubated in 5 ml of DMEM-HG at 37^oC under 5 per cent CO_2 as per Beaulah *et al.,* (2017) in Madras Red Sheep. Density of mononuclear cells is one of the important factors to determine efficiency of MSCs in culture as this affects the adherence of MSCs, contamination by other cell types and initial growth of adhered MSCs as reported by Ikeba and Suzuki (2014). In the present study, the plastic adherence was observed after 24 hours which satisfied the first criteria of being mesenchymal stem cells proposed by Mesenchymal and Tissue Stem Cell Committee of the International society for Cellular Therapy (Dominici *et al.,* 2006). Similar finding was observed by Webb *et al.,* (2011) in feline. According to Beaulah *et al.,* (2016) and Fadel *et al.,* (2011), the plastic adherence of AD-MSC was observed after 24 hours in primary culture. Similarly in buffalo, adherence was noticed after 24 hours (Hepsibha *et al.,* 2011). Whereas Ren *et al.,* (2012) reported in rat and goat that adherence has been observed after two to four hours and Barberini *et al.,* (2014) reported the adherence of AD-MSCs in 32 hours in equine.

The presence of non-adherent cells were removed from the primary culture after three days to get rid of contamination as it contained some dead cells. Since adipose tissue was highly vascularized, higher proportion of hematopoietic cells were seen. Frequent media change in first two days of initial culture had significant importance for maintenance of culture which is also reported by Deans and Moseley (2000). The spent medium was replaced initially with fresh of DMEM-HG after two days of seeding. Then fresh medium was added daily until the cells reached of 70- 80 per cent confluency in accordance with Vieira *et al.,* (2010).

Subculture of fAD-MSCS

In the present study, the cultured cells from P0 on day seven post incubation with 100 per cent confluency were subjected to passage1. The cells were trypsinised and seeded into the new plates at 1:3 ratio for P1. On day two in P1, the cells started showing expansion with spindle-like morphology. On day three, the cells exhibited varied morphology such as stellate, triangular and polyhedral. The stellate cells were found to be more. On day five post incubation the cells attained about 60-70 per cent confluency at P1. According Beaulah *et al.,* (2016) in Madras Red Sheep, subcutaneous and omental AD-MSCs took six days to reach same percentage in P1. The initial culture contained heterogeneous cell population with round and fibroblastic cell, upon culture, the number of rounded cell decreased and fibroblastic cells started increasing.

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