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In vitro internalization of moldey ion Rhodamine B in ovine mesenchymal stem cell: A comparative study

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Abstract
Ovine adipose and bone marrow derived mesenchymal stem cells were isolated by Collagenase digestion and cultured upto passage 6. Cultured adipose and bone marrow derived mesenchymal stem cells (AD-MSCs and BM-MSCs) at passage 4, 5 and 6 were labelled with Moldey Ion Rhodamine-B (MIRB) at the concentration of 25 ul of MIRB in 1ml of culture medium. The internalization of iron particles was assessed by calorimetric technique after 72 hours of incubation. Internalisation did not require any induction method. The results indicated that 16.5 to 18.1 pg and 17.0 to 18.2 pg of Fe was inernalised within the cytoplasm of adipose and bone marrow derived mesenchymal stem cells respectively after 72 hrs of incubation at P4, P5 and P6. However, in both AD-MSCs and BM-MSCs, internalisation with MIRB particles did not affect the viability and proliferation of the stem cells.

Keywords: Mesenchymal stem cells, Ovine, MIRB, *In-vitro* internalisation

1. Introduction
Mesenchymal stem cells represented a promising tool in support of cellular therapy. Repair of major fracture, better outcome of major restructuring surgeries and implants such as hip replacement are of equal concern in both human and veterinary medicine [1]. Adipose tissue is an attractive source of mesenchymal stem cells because of its abundance and easy access with minimal donor site morbidity. Bone marrow mesenchymal stem cell therapy is a new, attractive theraupautic approach for treatment of intervertebral disc degeneration (IVD); however the leakage and back flow of transplantal cells in to the structure surrounding the disc may lead to the formation of undesirable osteophytes [2].
Use of MSCs in the treatment requires bio-scaffold and method to detect transplanted cells *in vivo* [3, 4]. Though, there is a potential demand for stem cell based therapy in recent years, researchers working on the development of suitable methods for post-transplant *in vivo* detection [5-9]. Currently, several iron based magnetic contrast agents are available in the market for cell tracking.
Molday ION Rhodamine-B (MIRB) is a new Superparamagnetic Iron Oxide (SPIO) contrast agent specifically formulated for cell labeling and internalized by non-phagocytic cells. It is visualized by beta MRI with fluorescence microscopy and assessed the potential for imaging and monitoring of MSCs transplantation. Magnetic core and hydrodynamic sizes of MIRB is roughly 8 and 35 nm, respectively, (http://www.biopal.com/Molday%20ION.htm) and is conjugated to Rhodamine-B (Rh-B) (2 flourophores per particle). The Rh-B excitation wavelength is 555 nm and the emission wavelength is 565–620 nm [10]. Hence, the present study was designed for quantitative analysis of MIRB internalised within the cytoplasm of ovine adipose and bone marrow MSCs by using calorimetric technique.

2. Materials and Methods
Ovine adipose derived mesenchymal stem cells and ovine bone marrow derived mesenchymal stem cells were isolated, cultured and expanded upto passage 6 as per the protocol followed by Archana Mohapatra [11] and Violet Beaulauh [12] using Collagenase enzymatic digestion. The cells from passage 4 to 6 were used for labeling. The cells were incubated with MIRB at the concentration of 25ul / ml for 72 hours using the standard protocol [13].
Average cellular iron uptake was determined by calorimetric iron assay [14, 15, 10]. The labelled ovine adipose derived mesenchymal stem cells were counted and resuspended in 200 ul of 10M HCl was then added to the cell. Suspension was left for 12 hr incubation to lyse cells and to reduce iron to Fe 3+ state [10].

400ul of 5% potassium ferrocyanide added and allowed to sit in the dark for 12 hours. Then it was transferred to cuvettes and absorbance was read with a cepil spectrometer at 700 nm. Correction value was obtained from unlabelled cell suspension of OADMSC and OBMMSC respectively. Absorbance curve was plotted by the solutions having known values concentration of MIRB (0.25 to 12 ug/ml) in 700 nm. Average iron intake per well was then determined by mapping on to standard Fe vs absorbance curve. Total iron per cell was calculated by dividing total Fe / well by number at cells/ well.

3. Result and Discussion

In the present study, both adipose and bone marrow derived MSCs could effectively labeled with MIRB at a concentration of 25ul / ml. Labeling did not require any induction method for internalisation of MIRB as reported by Arbab *et al.* [16], Frank *et al.* [17], Giesel *et al.* [18], Hsiao *et al.* [19], Hu *et al.* [20], Janic *et al.* [21]. It was observed that 16.5 to 18.1 pg of Fe was found to be internalised in labelled oBM-MSCs after 72 hours of incubation. Similarly, in OADMSCS, 17.0 to 18.2 pg of Fe was found to be internalised after 72 hours of incubation. The quantity of iron internalised in BM-MSCs and AD-MSCs at different passages were given in Table-1. There is no much difference in the iron uptake between ADMSCs and BMMSCs in ovine when the cells were labeled with similar concentrations of MIRB and incubated in the same environment. This is in accordance with the findings of Addicott *et al.* [10] with regard to BM-MSCs from cynomolgus monkey (*Macaca fascicularis*). This is comparable to previous studies with Feridex™, where the values from 10 to 20 pg/MSC have been reported with the use of high molecular weight poly-L-lysine as a transfection agent [22].

MIRB labelling did not affect the *in-vitro* viability and proliferation of MSCs in the study as it was localised within the cytoplasmic endosomes surrounding the nucleus [16, 24, 25, 8, 20].

Table 1: Quantification of Fe in adipose and bone marrow derived mesenchymal stem cells at different passage level

Passage number	Quantity of Fe/ml in BM-MSCs		Quantity of Fe/ml in ADMSCs	
	Number of cells per well	Quantity of Fe/BM-MSC (in pg)	Number of cells per well	Quantity of Fe/ADMSC (in pg)
4	$1.2x10^6$	18.0	$1.3x10^6$	17.5
	$1.5x10^6$	17.3	$1.5x10^6$	17.0
5	$1.1x10^6$	16.5	$1.1x10^6$	18.0
	$1.4x10^6$	17.2	$1.4x10^6$	17.2
6	$1.3x10^6$	17.4	$1.5x10^6$	17.0
	$1.5x10^6$	18.1	$1.5x10^6$	18.2

4. Conclusion

Results of the current in-vitro study with ovine adipose and bone marrow derived mesenchymal stem cells revealed that there is no difference in uptake of iron particles between the different sources of mesenchyaml stem cells when cultured in-vitro. Iron particles internalised within the cytoplasmic endosomes did not affect the viability and proliferative capacity of these cells. Hence, it is concluded that MIRB can be used in mesenchymal stem cell labelling and also its applicability in *in vivo* tracking of stem cells.

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