



*Original Research*

## Effect of Moldey Ion Rhodamine B on Viability of Adipose and Bone Marrow Derived Mesenchymal Stem Cells - An *In-vitro* Study

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### Abstract

The study was performed to access the effect of Moldey ion Rhodamine B on *In-vitro* labelled bone marrow and adipose derived derived stem cells in sheep. Perirenal adipose tissue and bone marrow from femur were collected from 28 male sheep. Adipose derived mesenchymal stem cells were isolated from perirenal fat using enzymatic digestion method by using type II collagenase. Bone marrow derived mesenchymal stem cells were separated from femur by density gradient centrifugation method using percoll. These mesenchymal stem cells from bone marrow and adipose tissue were cultured and expanded using Dulbecco's Modified Eagle's Medium with 10 per-cent of fetal bovine serum, one per-cent of antibiotic-antimycotic solution. Adipose and bone marrow derived mesenchymal stem cells from passage 4 to passage 6 were used for labelling with medium containing 25 µg of MIRB per ml. Cell viability was evaluated by Trypan blue exclusion test in both labelled and control (unlabelled) groups. There was no significant difference in the viability of bone marrow and adipose derived mesenchymal stem cells between the labelled and control groups. It was observed that *in-vitro* labelling of mesenchymal stem cells with MIRB at a concentration of 25 µg per ml of medium did not affect cell proliferation and viability.

**Key words:** *In-vitro* Labelling, MIRB, Mesenchymal Stem Cells, Sheep, Viability

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### Introduction

MSCs were ethical, practical and biologically appropriate agents for cell therapy. In view of greater potential for tissue regeneration in animal and human studies require biosafety and an effective method to detect transplanted cells both *in-vitro* and *in-vivo* (Bussolati and Camussi, 2006, Budde and Frank, 2009, Adler *et al.*, 2009). Mesenchymal stem cells (MSCs) represented a promising tool in the field of



regenerative medicine in veterinary science. Bone marrow is the first source reported to contain MSCs and an another promising source is found to be adipose tissue (Kern *et al.*, 2006).

To understand the mechanisms behind a successful stem cell-based therapy, monitoring of transplanted cell's migration, homing as well as the engraftment efficiency is one of the critical issue (Henning *et al.*, 2009). This can be accomplished by labelling the stem cells with super paramagnetic iron oxide (SPIO) particles (Arbab *et al.*, 2005 and Terrovitis *et al.*, 2006) and subsequent tracking of magnetic nanoparticles in labelled cells by magnetic resonance imaging (MRI) (Hoehn *et al.*, 2002; Guzman *et al.*, 2007 and Sykova *et al.*, 2007). An appropriate magnetic nanoparticle must be nontoxic, biocompatible, efficient for intracellular labelling, and highly sensitive to detection by imaging technique (Cai *et al.*, 2008 and Kim *et al.*, 2008). Molday ION Rhodamine-B<sup>TM</sup> (MIRB) is a newer super paramagnetic iron oxide (SPIO) contrast agent specifically formulated for cell labelling and is readily internalized by non-phagocytic cells. It is also visualized by both MRI and fluorescence microscopy and assessed the potential for imaging and monitoring of MSCs transplantation (Addicott *et al.*, 2011).

We therefore designed this study to determine the ability of MIRB on viability and proliferation of ADMSCs and BMMSCs in sheep.

## Materials and Methods

Samples of perirenal fat and bone marrow from long bones were collected from 28 numbers of male sheep for the study. Samples were collected from Corporation Slaughter House, Chennai in phosphate buffered saline (PBS) containing antibiotic-antimycotic solution.

## Isolation of MSCs

Adipose derived mesenchymal stem cells (ADMSCs) were isolated from perirenal fat using enzymatic digestion method by type II collagenase (SIGMA® Cat.No.-CO130) (Violet Beulah *et al.*, 2017). Bone marrow derived mesenchymal stem cells (BM-MSCs) were separated from femur by density gradient centrifugation method using percoll (SIGMA® Cat.No.-P1644). Both ADMSCs and BMMSCs were cultured *In-vitro* up to passage 6 (Archana Mohapatra *et al.*, 2015).

## Labelling of MSCs

Adipose and bone marrow derived MSCs from passage 4 to passage 6 were used for *In-vitro* labelling. Labelling medium was prepared with DMEM (GIBCO® Cat. No.-11320-033), 10 per-cent FBS (GIBCO® Cat. No.-10082-142), one per-cent antibiotics-antimycotic solution GIBCO®- Cat No.- 15240-062) containing MIRB (BioPAL®- Cat No.- CL-50Q02-6A) at the concentration of 25 µg MIRB/ml (Addicott *et al.*, 2011 and Ren *et al.*, 2011). Medium without MIRB was used for unlabelled cells (control group).

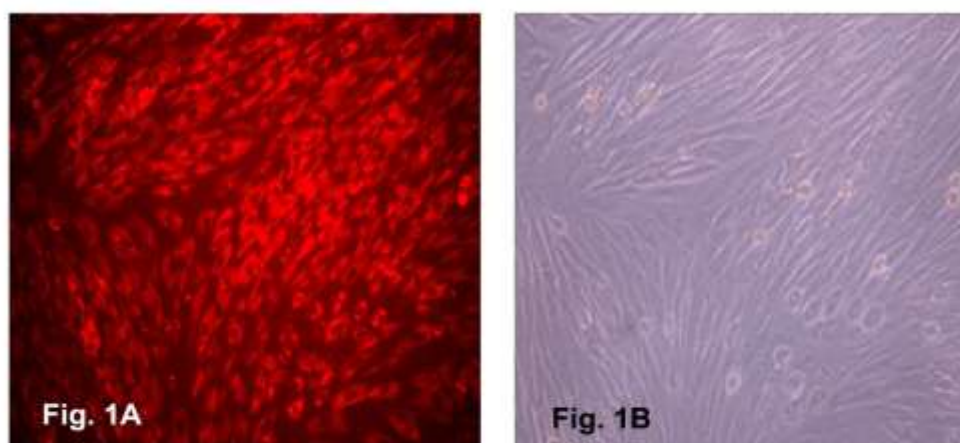
Cells from passage 4 to 6 were incubated with labelling solution at 37°C with 5 per-cent CO<sub>2</sub> and monitored for integration at an interval of 24, 48 and 72 hrs (Shen *et al.*, 2013).

### Viability Assessment

Cell viability and cell proliferation in labelled and unlabelled group was estimated using a Trypan blue (SIGMA®- Cat No.- T8154 ) (0.4%) exclusion test. Percentage of cell viability was evaluated by counting the cells using hemocytometer (Nan *et al.*, 2013). Viability per-cent of labelled and unlabelled cells was analysed by chi-square test statistically as per standard protocol (Snedecor and Cochran, 1994).

### Results and Discussion

In the present study, 98.4 per-cent of ADMSCs were viable after 72 hr incubation with MIRB in P-4. It was observed that 98.8 per-cent cells in P-5 and 98.9 per-cent cells in P-6 were viable in 72 hrs of incubation (Fig.1 and Table 1).



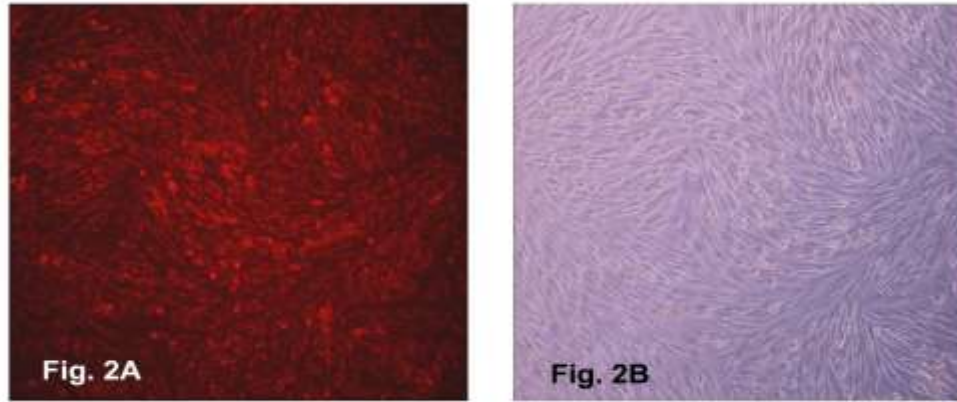
**Fig.1:** Photomicrograph showing MIRB labelled ADMSCs (1A) and unlabelled (control) ADMSCs (1B) x 100

**Table 1:** Viability per-cent in MIRB labelled and control ADMSCs

		Viable cells (x 10 <sup>6</sup> )	Non-viable cells (x 10 <sup>6</sup> )	Total cells (x 10 <sup>6</sup> )	Per-cent of viable cells
<b>P4</b>	labelled	2.94	0.046	2.99	98.46
	unlabelled	3.1	0.048	3.14	98.47
		(χ <sup>2</sup> )	<b>1.764272677<sup>NS</sup></b>		
<b>P5</b>	labelled	2.9	0.034	2.93	98.84
	unlabelled	2.95	0.034	2.98	98.84
		(χ <sup>2</sup> )	<b>0.105417701<sup>NS</sup></b>		
<b>P6</b>	labelled	2.6	0.026	2.62	98.99
	unlabelled	3.15	0.032	3.18	98.99
		(χ <sup>2</sup> )	<b>0.156252901<sup>NS</sup></b>		

NS – Non Significant at P≤0.05 level.

About 98.7 per-cent of labelled BM-MSCs were viable after 72 hrs incubation with MIRB in passage 4 (P-4). It was observed that 98.5 per-cent cells in P-5 and 99.0 per-cent cells in P-6 were viable, in the same hours of incubation (Fig. 2 and Table 2).



**Fig. 2:** Photomicrograph showing MIRB labelled BMMSCs (2A) and unlabelled (control) BMMSCs (2B) x 100

**Table 2:** Viability per-cent in MIRB labelled and control BM-MSCs

		Viable cells (x 10 <sup>6</sup> )	Non-viable cells (x 10 <sup>6</sup> )	Total cells (x 10 <sup>6</sup> )	Per-cent of viable cells
<b>P4</b>	Labelled	3.18	0.04	3.22	98.75
	Unlabelled	3.1	0.039	3.13	98.75
		( $\chi^2$ )	<b>0.000507241<sup>NS</sup></b>		
<b>P5</b>	Labelled	2.25	0.034	2.28	98.51
	Unlabelled	2.95	0.044	2.99	98.53
		( $\chi^2$ )	<b>3.215957114<sup>NS</sup></b>		
<b>P6</b>	Labelled	3.08	0.03	3.11	99
	Unlabelled	2.43	0.024	2.45	99
		( $\chi^2$ )	<b>0.907691248<sup>NS</sup></b>		

NS – Non Significant at  $P \leq 0.05$  level.

In mitotically active cells, uptake of iron (Fe) occurs through trans-membrane receptor mediated endocytosis. The endocytosed iron bound with endosomes, from where it is transferred into the cytoplasm where it forms intracellular labile iron pool ranging from low molecular weight iron complexes or high molecular weight intermediates (Terrovitis *et al.*, 2006). In the present study it was observed that on the whole, the internalization of the MIRB into adipose and bone marrow derived mesenchymal stem cells did not affect cell viability and its proliferation in medium containing 25 µg of MIRB/ml. This hypothesized that the endocytosed intracellular iron might be used for metabolic function of ovine adipose and bone marrow derived MSCs. A similar finding was observed by Rad *et al.* (2007) in human lymphocytes and rat gliosarcoma cells, Lee *et al.* (2009) in human BMMSCs, McFadden *et al.* (2011) in cancer stem cells, Nan

*et al.* (2013) in rat ADMSCs, Shen *et al.* (2013) in human neural precursor cells and Talaie *et al.* (2015) in rat platelet-rich plasma. However, Addicott *et al.* (2011) observed in BMMSCs of cynomolgus monkey, the viability was found to be decreased when the BMMSCs were cultured in labelling solution containing 30µg of MIRB/ml. There has been an evidence to suggest that increase concentration of iron in labelling solution might affect the cellular endosomes (Arbab *et al.*, 2005). In addition, increased concentration of MIRB in labelling solution might enhance the formation of reactive oxygen species (ROS) which also lead to cellular toxicity.

### Conclusion

The results of this study suggested that in-vitro labelling adipose and bone marrow MSCs with Moldey Ion Rhodamine B at the concentration of 25µg iron/ml did not affect the cell viability and its proliferation. It suggests that MIRB labelling is found to be non-toxic and hence it can be used for tracking adipose and bone marrow derived MSCs by MRI.

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### Competing Interest

The authors declare that they have no competing interests.

### Ethics Approval

The study was conducted in accordance with the approval of Institutional Ethical Committee for stem cell research, Tamil Nadu Veterinary and Animal Sciences University.

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