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Research Article

# ASSESSMENT OF INTERNALIZATION OF IRON IN MOLDAY ION RHODAMINE B LABELLED OVINE ADIPOSE DERIVED MESENCHYMAL STEM CELLS

**Gnanadevi R[1]., Geetha Ramesh[1]., *Kannan T.A[2] and Sathyan G[3]**

[1]Department of Veterinary Anatomy, Madras Veterinary College, Chennai-600 007
[2]Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Chennai-600 007
[3]Department of Radiodiagnostics, Govt. Stanley medical College, Chennai

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**ABSTRACT**

Ovine adipose derived mesenchymal stem cells (oADMSCs) are isolated, expanded and passaged up to passage 6. *In-vitro* cultured cells at passage 4, 5 and 6 were used for Molday Ion Rhodamine-B **(**MIRB) (25µl/ml concentration) labelling and the internalization of iron was assessed 72 hrs after labelling. Quantification was done by colorimeteric iron assay using 10M HCl and 5 per cent Pottasium Ferrocyanide. In labelled oADMSCs, the quantity of iron is 17-18.2pg of Fe/oADMSCs. In this concentration viability and differentiation potential of labelled oADMSCs was not affected. The results of this study infer that MIRB can be used for labeling the oADMSCs for tracking Mesenchymal stem cells without affecting its viability.



## INTRODUCTION

Stem cell therapy has emerged as a therapeutic option in several types of injury and disease. Mesenchymal stem cells (MSCs) are ethical, practical and biologically appropriate cell populations for cell therapy (Caplan, 2005). Central to the success of stem cell therapy is the ability of cells to migrate and engraft (Wimpenny *et al*., 2012). MIRB has been used to label the stem cells allowing researcher to monitor migration of labeled cells by using Magnetic Resonance Imaging (Addicott *et al*., 2011). In the present quantitative method, internalization of iron in the labeled oADMSCs was assessed by calorimetric iron assay.

## MATERIALS AND METHOD

oADMSCs were isolated, expanded and passaged up to passage 6 as per the standard protocol (Violet Beulah *et al*., 2017). oADMSCs from passage 4, 5 and 6 were used for MIRB (25µl/ml concentration) labelling and internalization of iron was assessed after 72 hrs post labelling (Gnanadevi *et al*., 2016(a)). Average cellular MIRB uptake was determined via a colorimetric Fe assay (Rad *et al*., 2007 (a); 2007(b), Addicott *et al*., 2011). Labelled oADMSCs (72hrs MIRB incubation) were counted and re-suspended in 200µl phosphate buffered saline. 200µl HCl (10 M) aliquot was then added to cell suspensions and left for 12 hr incubation to lyse cells and to reduce iron to $Fe^{3+}$ (Addicott *et al*., 2011).

**Corresponding author:* **Kannan T.A**
Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Chennai-600 007

400µl aliquot of five per cent potassium ferrocyanide was then added and allowed to sit, protected from light overnight (12 hr) and it was transferred to cuevettes and Absorbance was read with a Cepil spectrometer at 700 nm. Sample absorbance values were corrected for non-MIRB absorbance by subtracting absorbance value of unlabelled oADMSCs.

Average total iron per well was then determined from absorbance by mapping onto standard Fe *Vs* absorbance curve generated by measuring absorbance at 700nm for solutions similarly prepared with known amounts of MIRB concentrations ranging from 0.25 to 12µg/ml.

Total Fe per oADMSCs was calculated by dividing total Fe per well by number of oADMSCs per well. Data from six labelling experiments were averaged to establish a MIRB labelled oADMSCs loading curve.

## RESULTS AND DISCUSSION

In labelled oADMSCs (72hrs MIRB incubation), iron content was found to be 17.5 and 17 pg/cell in the wells with $1.3x10^6$, $1.5x10^6$ cells per well at passage 4. In passage 5, quantity of iron in $1x10^6$, $1.4x10^6$ cells per well was found to be about 18 and 17.2pg/cell, respectively. In passage 6, 17 and 18.2pg of iron/cell was found in $1.5x10^6$, $1.5x10^6$ cells per well, respectively (Table 1).

**Table 1** Quantity of Fe/ml in ovine adipose derived Mesenchymal stem cells

| Passage number | Number of cells per well | Quantity of Fe/oADMSC (in pg) |
|---|---|---|
| 4 | $1.3x10^6$ | 17.5 |
| | $1.5x10^6$ | 17.0 |
| 5 | $1x10^6$ | 18.0 |
| | $1.4x10^6$ | 17.2 |
| 6 | $1.5x10^6$ | 17.0 |
| | $1.5x10^6$ | 18.2 |

However, Gnanadevi *et al*. 2016(b) reported that, the iron concentration in MIRB labelled (25µl/ml concentration) ovine bone marrow derived mesenchymal stem cells ranged from 16.5pg to 18.1pg per cell. In both studies, viability and differentiation of stem cells didn't get altered. Hence, it can be concluded that MIRB at the concentration of 25µl/ml is safe for labelling the mesenchymal stem cells without affecting its viability *in-vitro*.

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