LOW OXYGEN CULTURE CONDITION ACCELERATES CELL EXPANSION OF MOUSE MESENCHYMAL PROGENITORS

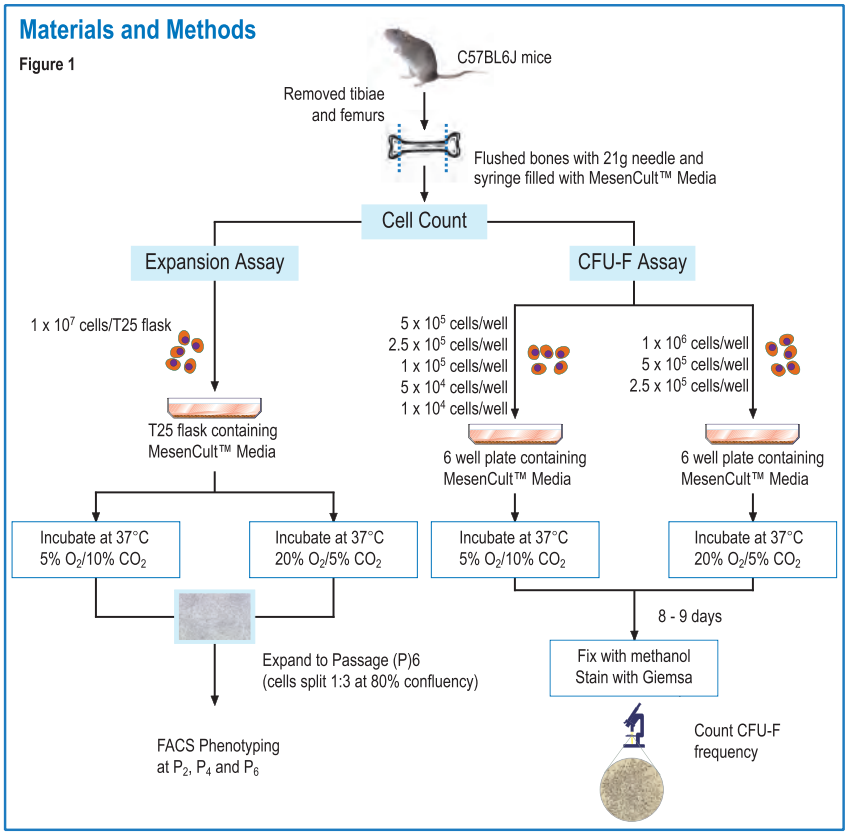
Ravenska Wagey PhD[1], Betty Hoac[1], Brenton Short PhD[1], Terry Thomas PhD[1], Allen Eaves MD, PhD[1,2], and Emer Clarke PhD[1].
[1]StemCell Technologies Inc., 570 W 7th Avenue, Suite 400, Vancouver, BC, Canada, V5Z1B3. Phone: 604.484.7098, Fax: 604.876.8440, email: venska@stemcell.com
[2]Terry Fox Laboratory, 675 W 10th Ave., Vancouver, BC, Canada, V5Z 1L3.

Introduction

A major limitation in studying the biology of mesenchymal cells derived from mouse marrow is the very low frequency of mesenchymal progenitor cells (MSC) and the slow proliferation of these cells when cultured in 20% oxygen. It has been shown that culture of MSC in low oxygen tension mimics physiological oxygen conditions for MSC, increases cell proliferation and contributes to the maintenance of these cells in an un-differentiated state. The objective of this study was to investigate the effect of culturing MSC in 5% and 20% oxygen tension, specifically the frequency and size of colony forming unit fibroblast (CFU-F), the expansion time and phenotype of these cells.

Materials and Methods

Figure 1



Results

Table 1: CFU-F-derived colony frequency and size (average from all seeding densities) in 5% and 20% Oxygen

CFU-F/10^6 cells x ± SD; n = 3		CFU-F Size (mm) Average diameter ± SD; n = 3* (Range)	
5% Oxygen	20% Oxygen	5% Oxygen	20% Oxygen
11.72 ± 3.53	3.05 ± 1.86	1.83 ± 0.64 (1 - 3.5)	1.63 ± 0.43 (1 - 2.5)

* n=3 replicate experiments; 30 – 60 colonies were measured per experiment.

Table 2: Mesenchymal cell expansion; Average number of days between passaging**

	Average # Days Between Passaging (x ± SD)	Range of Days Between Passaging	Average # Days to Reach P6 from P0
5% Oxygen	3.3 ± 1.5	1 - 5	20 days
20% Oxygen	10.6 ± 5.5	8 - 21	70 days

** Cells were passaged when they reached 80% confluency.

Table 3: Expansion Assay: Phenotype of mouse MSC at P2, P4, and P6

		Hematopoietic Markers					Putative Mesenchymal Markers					
		B220	CD11b	CD34	CD45	Ter119	CD90.2	CD44	CD49f	CD51	CD105	SCA-1
5% Oxygen	P2	-	-/+	+++	-/+++	+	-	-	-	-	+	++
	P4	-	-/++	++	-/+++	-	-	-	-	-	+	++
	P6	-	-/+	-	-/++	-	+	++	-	-	++	+++
20% Oxygen	P2	-	+	+	+++	-	-	-	+	-	-	-
	P4	-	-/+	-	+++	-	-	++	++	-	-	+
	P6	-	-	-	-/+	-	-	+++	+++	++	++	+++

Symbols & Expression Levels

+	Low expression within entire population
++	Medium expression within entire population
+++	High expression within entire population
-	Entire population lacks expression
-/+	Part of population has low expression, part of population lacks expression
-/++	Part of population has medium expression, part of population lacks expression
-/+++	Part of population has high expression, part of population lacks expression

Mouse bone marrow-derived MSC were stained at P2, P4 and P6 for mouse hematopoietic and putative MSC markers.

Cells stained for expression of hematopoietic and putative MSC markers were analysed and gated on Propidium Iodide (PI) negative (live) cells. Cells stained for expression of putative MSC were also gated to exclude $CD45^+$ cells to observe changes in putative MSC population only.

The frequency of cells expressing hematopoietic markers, CD11b, CD34 and CD45 declined over several passages in 5% and 20% oxygen conditions. At the same time expression of putative MSC markers, CD44, CD105 and SCA-1 increased in both oxygen conditions.

The decline of hematopoietic marker expression level over several passages was slower in 5% oxygen than 20% oxygen which may suggest that low oxygen culture condition also supported hematopoietic cell expansion.

Conclusions

- CFU-F derived colony frequency increased ~4 fold when mesenchymal progenitor cells were cultured in 5% oxygen compared to 20% oxygen.
- CFU-F derived average colony size was not significantly different between 5% and 20% oxygen. However maximum CFU-F colony size was greater when cells were cultured in 5% oxygen.
- Culture-expanded mesenchymal cells in both oxygen concentrations showed increased expression levels of putative mesenchymal markers and deceased expression of hematopoietic markers with increasing passage number.
- Expansion of mesenchymal progenitor cells was accelerated in 5% oxygen culture condition, indicating that low oxygen condition is important for culture of mouse mesenchymal cells.