The Effect of Low Oxygen on Culture of Mouse and Human Mesenchymal Progenitor Cells from Bone Marrow

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Introduction

Oxygen level is an important factor in culturing stem cells. At 5% O_2, rat mesenchymal progenitor cells had higher proliferation rates, higher colony numbers, and greater osteogenic potential than cells cultured at 20% O_2 (J. Cell. Phys. 187, 2001). As *in vitro* proliferation rates of mesenchymal progenitor cells (MSC) vary between species, the aim of this study was to examine whether oxygen level affects the colony forming unit-fibroblast (CFU-F) frequency, proliferation rate and phenotype of bone marrow (BM)-derived mouse and human MSC cultured in normoxic or hypoxic conditions.

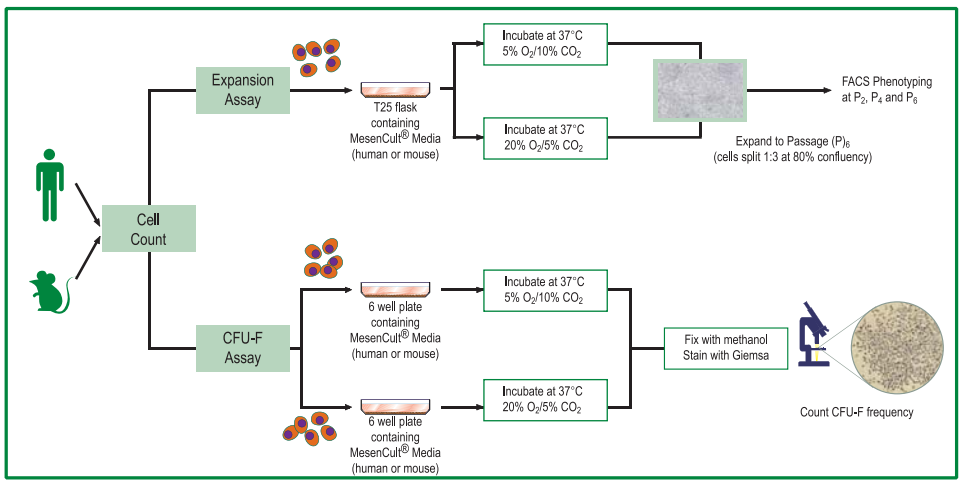
Materials and Methods

Mouse

BM-derived mouse cells were plated in Complete MesenCult® medium (Mouse). CFU-F assay was performed using 6 well-plates. Cells incubated at 5% O_2 were plated at concentrations of 1 x 10^4 to 5 x 10^5 cells/well, whereas cells incubated at ~20% O_2 were plated at 2.5 x 10^5 to 1 x 10^6 cells/well. Expansion assay was performed in T-25 cm flasks. Cells incubated at 5% or ~20% O_2 were both plated at concentrations ranging from 1 x 10^6 to 1 x 10^7 cells/flask.

Human

BM-derived human cells were plated in Complete MesenCult® medium (Human). CFU-F assay was performed using 6 well-plates. Cells incubated at 5% O_2 or ~20% O_2 were both plated at concentrations of 1 x 10^5 to 1 x 10^6 cells/well. Expansion assay was performed in T-25 cm flasks. Cells incubated at 5% or ~20% O_2 were both plated at concentrations ranging from 1 x 10^6 to 1 x 10^7 cells/flask.



Results - Mouse

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

CFU-F/10^6 cells mean ± SD; n = 3		CFU-F Size (mm) Average diameter ± SD; n = 3* (Range)	
5% Oxygen	20% Oxygen	5% Oxygen	20% Oxygen
11.7 ± 3.5	3.1 ± 1.9	1.8 ± 0.6 (1 - 3.5)	1.6 ± 0.4 (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 (mean ± SD)	Range of Days Between Passaging	Average # Days to Reach P_6 from P_0
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 passage (P) P_2, P_4, and P_6

		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 BM-derived MSC were stained at P_2, P_4, and P_6 for mouse hematopoietic and putative MSC markers.

Mouse 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 mouse 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 - Mouse

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

Results - Human

Table 4: Human Bone Marrow CFU-F derived colony frequency and size in 5% oxygen and 20% oxygen

CFU-F/10^6 cells mean ± SD; n = 3		CFU-F Size (mm) Average diameter ± SD; n = 3* (Range)	
5% Oxygen	20% Oxygen	5% Oxygen	20% Oxygen
18.8 ± 3.6	13.9 ± 3.7	3.5 ± 1.1 (2 - 6)	3.0 ± 1.0 (1 - 5.5)

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

Table 5: Mesenchymal cell expansion; Average number of days between passaging**

	Average # Days Between Passaging (mean ± SD)	Range of Days Between Passaging	Average # Days to Reach P_6 from P_0
5% Oxygen	4.7 ± 2.1	3 - 13	42 days
20% Oxygen	4.9 ± 2.1	3 - 13	42 days

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

Table 6: Expansion Assay: Phenotype of human MSC at P_2, P_4, and P_6

		Hematopoietic Markers			Putative MSC Markers			
		CD33	CD34	CD45	CD271	CD105	CD73	CD90
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

Human BM-derived MSC were stained at P_2, P_4, and P_6 for human hematopoietic and putative MSC markers.

Conclusions - Human

- Human CFU-F derived colony frequency and average colony size were not significantly different in 5% compared to 20% oxygen levels.
- *In vitro* culture of human Mesenchymal progenitor cells showed stable expression of putative MSC markers (CD105, CD73 and CD90) and lack expression of human hematopoietic markers (CD33, CD34 and CD45) over 6 passages in both oxygen levels.
- Unlike mouse Mesenchymal cells, the *in vitro* expansion of human Mesenchymal progenitor cells was not accelerated in 5% oxygen in comparison to 20% oxygen culture condition.

Final Conclusion

This study showed that low oxygen culture condition is effective for increasing colony frequency and expansion of BM-derived mouse mesenchymal progenitor cells, while it has little effect on expansion of BM-derived human mesenchymal progenitor cells.

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