Influence of Oxygen Tension on Osteogenic Differentiation of Encapsulated Stem Cells

Suchit Sahai, James O. Blanchette.
University of South Carolina.

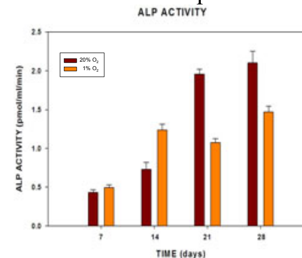
Introduction

Treatment of critically sized bone defects (CSDs) requires autologous bone grafting or implantation of bone substitute materials. An attractive alternative has been to engineer fully viable, biological bone grafts in vitro by culturing adult stem cells within 3-D scaffolds, under conditions supporting bone formation. Suprisingly however, until recently, no convincing successes have been achieved in humans. Osteogenic differentiation of adult stem cells into osteoblasts is a prerequisite for subsequent bone formation. Numerous studies have explored osteogenic differentiation under standard tissue culture conditions, which usually employ 20% of oxygen. However, bone precursor cells such as hMSCs reside in stem cell niches of low oxygen atmospheres. Furthermore, they are subjected to low oxygen concentrations when cultured on 3-D scaffolds in vitro for bone tissue engineering purposes, and even more so after transplantion when vascularization has yet to be established.

Understanding the cellular responses to hypoxia has grown primarily from study of individual molecular factors. The master regulator of adaptive responses to low oxygen availability is the nuclear factor, hypoxia-inducible factor-1α (HIF-1α) [1]. HIF-1 is a heterodimeric protein, stable below 6% O_2 condition in the nucleus and has been shown to play a role in angiogenic-osteogenic coupling. In the literature, there are conflicting reports on influence of hypoxia on osteogenesis, with some studies showing a inhibitory effect and some enhanced osteogenic potential under hypoxia [2]. This study seeks to identify the importance of hypoxia in stem cell differentiation potential and the role of HIF-1α in osteogenic differentiation. Better understanding of the roles of hypoxia will help prevent common problems and exploit potential benefits of hypoxia in engineered tissues.

Methods: The study uses adipose-derived stem cells (ADSCs), which are another source of osteoprogenitor stem cells that exhibit multi-lineage potential similar to bone marrow-derived mesenchymal cells (MSCs) upon induction. For 2-D experiments, ADSCs were seeded at 3×10^4 cells/cm^2 in 12-well tissue culture treated plates. For 3-D experiments, 2×10^5 cells were encapsulated in poly(ethylene glycol) (PEG) scaffolds in media containing osteogenic supplements. Cells were cultured in 1% and 20% oxygen regulated incubators for 4 weeks with media changes every three days. For encapsulation, cells are suspended in 40µl of PEG solution followed by exposure to UV light for 8 minutes. These photo-encapsulated cells in PEG have been shown to survive the exposure to UV light [3]. To check the osteogenic potential of ADSCs, ALP activity was determined quantitatively using a routine pNPP colorimetric assay and subsequently measuring the absorbance at 405nm. Alizarin red staining was performed to observe the extracellular mineral deposits under the microscope. Immuno-histochemical (IHC) staining was performed to monitor if the cells are expressing HIF-1α.

Results: ALP activity is considered a relatively early marker of osteoblast differentiation. The ALP activity in the 2-D showed 1.5-fold higher levels in 20% than 1% O_2 by the 4th week and a 5-fold increase relative to the 1st week. On day 14, the ALP activity at 1% was 1.6-fold higher than the cells at 20% O_2. The ALP activity of ADSCs in 3-D PEG scaffolds was monitored for 2 weeks, the activity was higher in 20% O_2 showing similar trends to 2-D. Mineral deposition is associated with the final differentiation phase. This qualitative assessment using Alizarin red staining confirmed significantly less mineral distribution in 1% compared to 20% O_2 after 4 weeks of culture. The possibility that HIFs participate in osteoblast differentiation and function is still unclear. For that, IHC staining was performed using HIF-1α antibody (green) and DAPI stain for nucleus (blue) on ADSCs that were cultured in both 20% and 1%. To see the onset of HIF-1α, IHC was performed at 2, 4, 8, 24 and 48 hours. At 48 hours in 1% O_2, expression of HIF-1α (green) was observed (Fig. 2), whereas for ADSCs at 20% O_2 there was no HIF-1α expression for any of the time points.



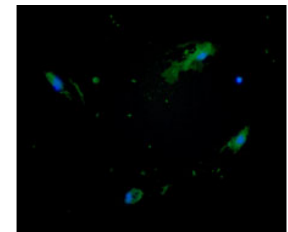


Fig.1: ALP Activity in 1% and 20% O_2 over a period of 4 weeks.	Fig.2: IHC Staining of ADSCs for expression of HIF-1α in 1% O_2 at 48 hours.

Conclusions

These studies show that the ALP activity and mineralization in 2-D and 3-D are higher in 20% than 1%, suggesting the osteogenic potential of ADSCs is lower in hypoxic condition. As seen on day 14, with higher ALP activity at 1%, it is a possibility that the influence of hypoxia on osteogenesis may not be a clear cut positive or negative factor but might depend on when cells are cultured in hypoxic conditions which encourages us to look at other periods of hypoxic culture in addition to 20% and 1% O_2 conditions. The IHC showed expression of HIF-1α in 1% oxygen. Further studies, up-regulating or silencing the expression of HIF-1α at both 20% and 1% O_2 condition will be helpful in determining its role or function in osteogenesis of the encapsulated ADSCs.

References

[1] (Semenza GL. 1992. Mol Cell Biol; 12, 5447–5454)
[2] (Ma T. 2009. Biotechnol Prog; 25, 32-42)
[3] (Anseth KS. Biomaterials. 2002; 23, 4315-4323)

©2011 Society For Biomaterials