EXTRACELLULAR HEMOGLOBIN OF XENOGENEIC ORIGIN MODULATES FUNCTIONAL CHARACTERISTICS OF MESENCHYMAL CELLS *IN VITRO*

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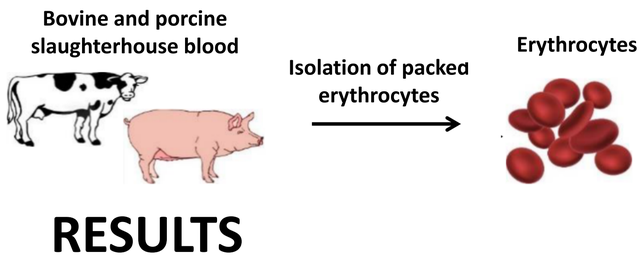
INTRODUCTION

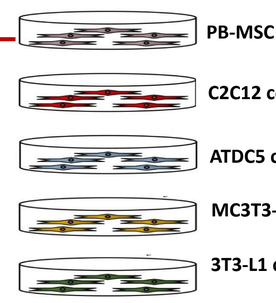
In addition to its highly conserved role in the transport of oxygen within erythrocytes, hemoglobin can also perform numerous functions when it is found in the extracellular environment.

This study aimed to obtain data on the influence of extracellular hemoglobin of xenogeneic origin on the functional properties of mesenchymal cells *in vitro*. Porcine (PHb) and bovine (BHb) hemoglobin isolated from slaughterhouse blood were used as abundant sources of vertebrate hemoglobin, which show a high degree of homology with human hemoglobin.



METHODS

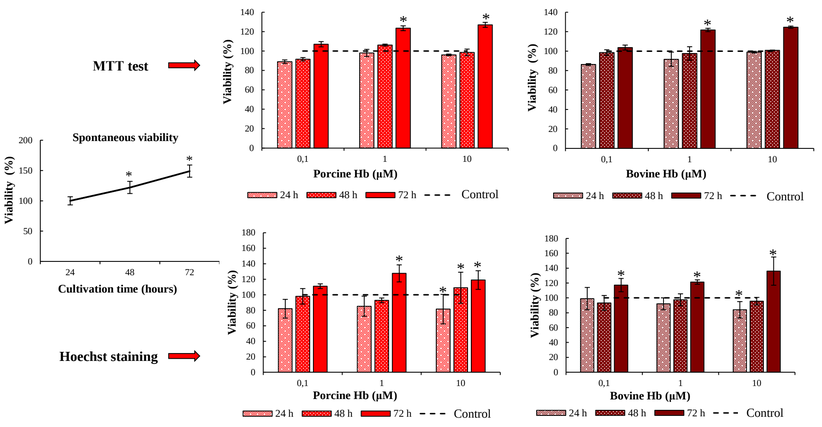
We have investigated the effect of PHb and BHb in the concentration of 0.1, 1, and 100 µM on cells' proliferation and migratory potential, by using MTT test and Hoechst 33258 staining. Human peripheral blood mesenchymal stem cells (PB-MSCs) were selected as adequate cell model systems since extracellular hemoglobin may encounter these cells during intravascular hemolysis. In addition, three cell lines ATDC5, MC3T3-E1, 3T3-L1 were tested as more uniform model systems compared to PB-MSCs to study chondro-, osteo-, and adipogenesis. The cells differentiation capacity in the presence of PHb and BHb was evaluated after induced differentiation, by histochemical staining and by RT-PCR analysis of the expression of specific genes.



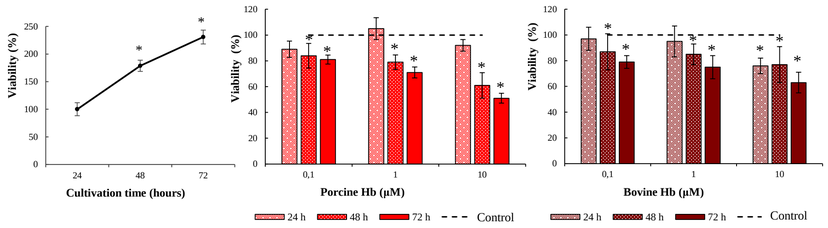
- **Potential dietary supplements or additives for cell culture media**
- **Understanding of the manifestation of extracellular hemoglobin on MSCs in *in vivo* intravascular hemolysis.**

RESULTS

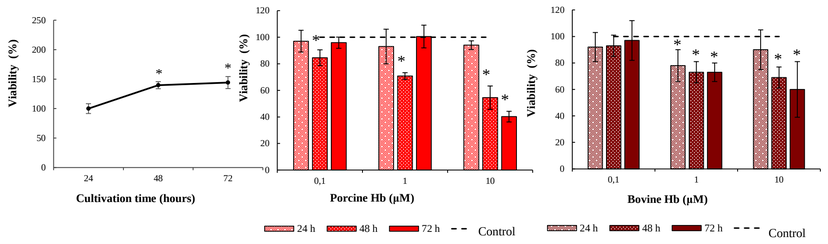
Influence of hemoglobins (Hb) on viability of PB-MSC



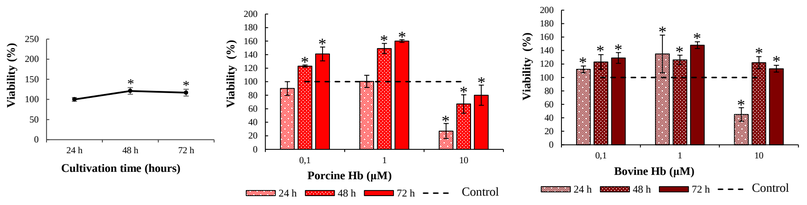
Influence of hemoglobins (Hb) on viability of ATDC5 cells



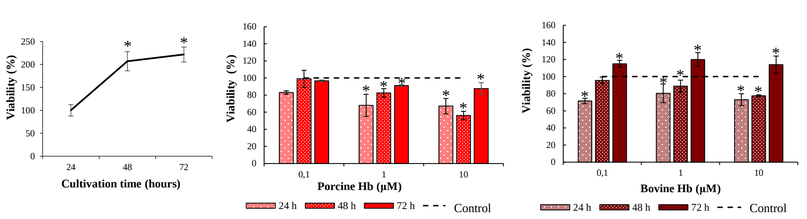
Influence of hemoglobins (Hb) on viability of MC3T3-E1 cells



Influence of hemoglobins (Hb) on viability of MC3T3-L1 cells

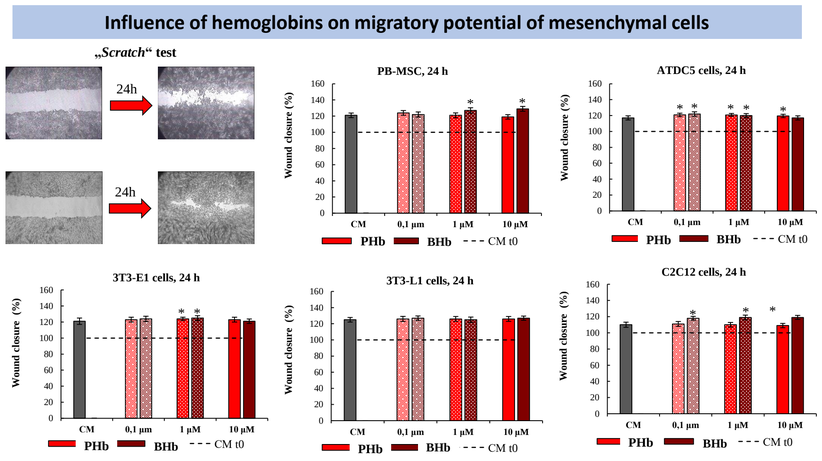


Influence of hemoglobins (Hb) on of C2C12 cells

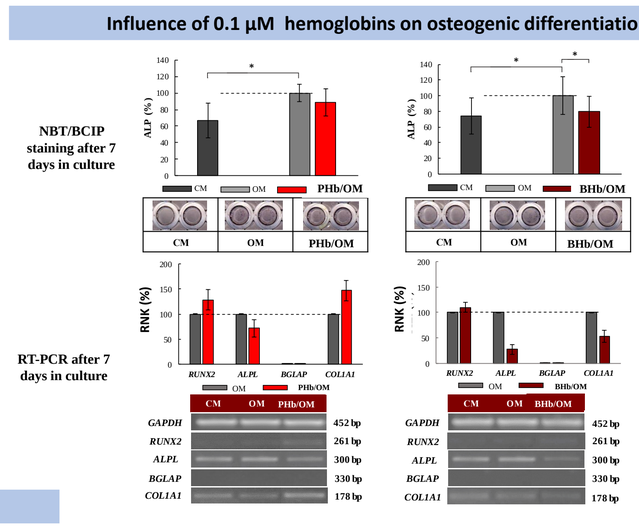


Extracellular hemoglobin modulate the viability and migration of mesenchymal cells depending on the cell type, hemoglobin concentration, animal species from which hemoglobin was isolated and incubation time.

Influence of hemoglobins on migratory potential of mesenchymal cells

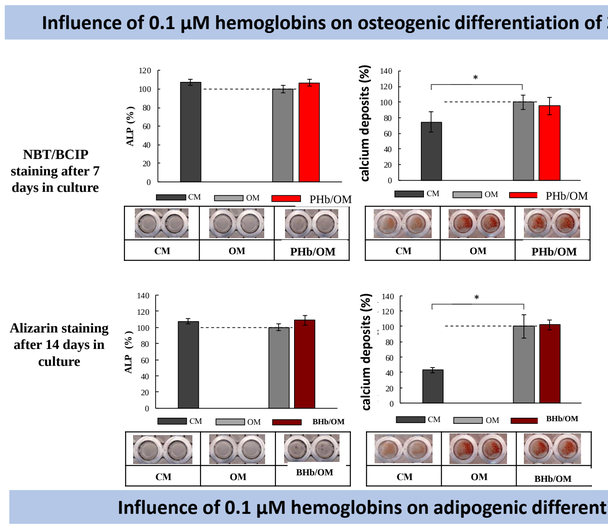


Influence of 0.1 µM hemoglobins on osteogenic differentiation of PB-MSC



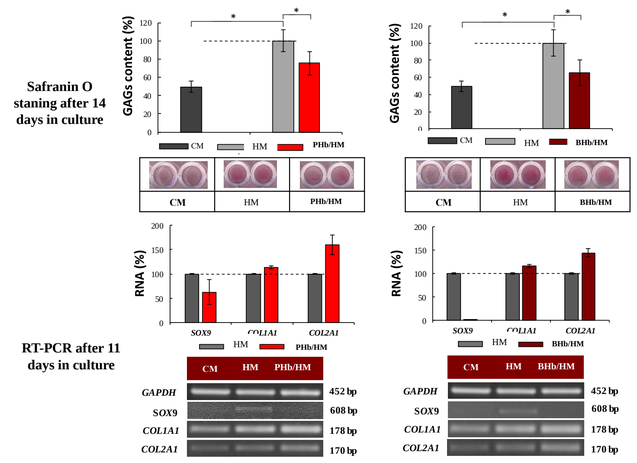
PHb and BHb after 7 days culture: reduce the activity of ALP enzymes; stimulate RUNX2 gene expression; decrease ALPL gene expression; PHb stimulates, while BHb decreases COL1A1 gene expression. BGLAP osteogenic marker expression was not detected.

Influence of 0.1 µM hemoglobins on osteogenic differentiation of 3T3-E1 (OM with Dex)



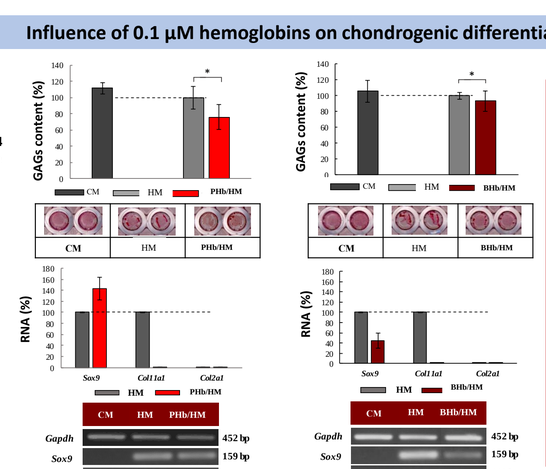
PHb and BHb had no effect on ALP enzyme activity in MC3T3-E1 cells after 7 days in culture in osteogenic medium with dexamethasone, while reducing the amount of calcium deposits after 14 days in culture in the same medium.

Influence of 0.1 µM hemoglobins on chondrogenic differentiation of PB-MSC



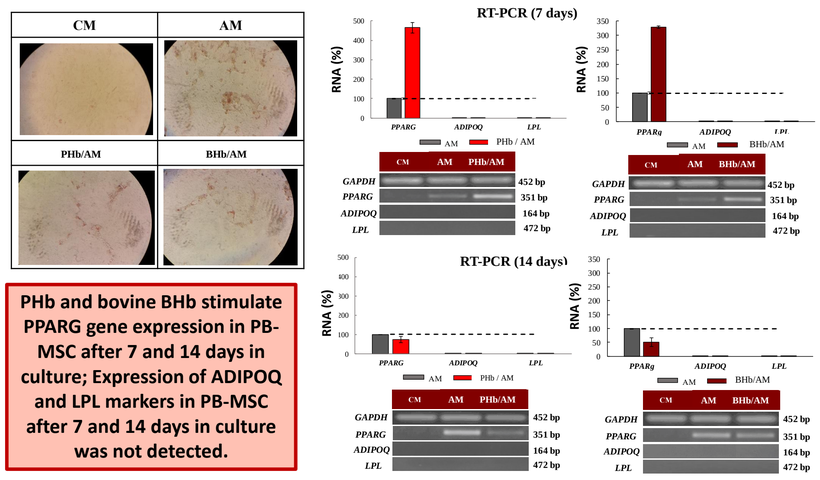
PHb and BHb reduce the GAG content in PB-MSC, decrease SOX9 gene expression and stimulate COL1A1 and COL2A1 gene expression in PB-MSC.

Influence of 0.1 µM hemoglobins on chondrogenic differentiation of ATDC5



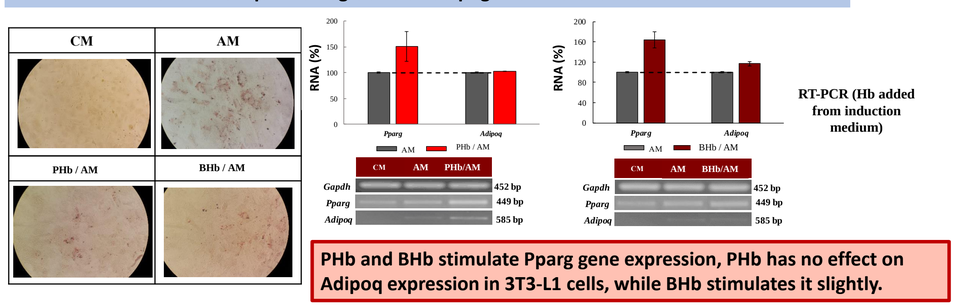
PHb and BHb reduce GAG content in ATDC5 cells. PHb stimulates, while BHb decreases Sox9 gene expression in ATDC5 cells; Hemoglobins of both animal species decrease the expression of the Col11a1 gene in ATDC5 cells.

Influence of 0.1 µM hemoglobins on adipogenic differentiation of PB-MSC



PHb and bovine BHb stimulate PPARG gene expression in PB-MSC after 7 and 14 days in culture; Expression of ADIPOQ and LPL markers in PB-MSC after 7 and 14 days in culture was not detected.

Influence of 0.1 µM hemoglobins on adipogenic differentiation of 3T3-L1



PHb and BHb stimulate Pparg gene expression, PHb has no effect on Adipoq expression in 3T3-L1 cells, while BHb stimulates it slightly.

Observed finely tuned differences in the effects of PHb and BHb on MSCs functional characteristics may be attributed to differences in primary protein structure, higher levels of protein organization or some differences in the level and type of contaminating proteins and phospholipids in isolated hemoglobin samples. These contaminants, although present in low amounts, represent an inevitable side component due to the preparation method used.

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