Evaluation of the contribution of a marine hemoglobin in the culture of mesenchymal stem cells in bi- and three-dimensional scaffolds for bone and meniscus tissue engineering.

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Introduction

Context:

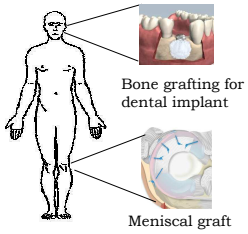
Mesenchymal stem cells (MSCs) are self-renewing multipotent cells that are currently broadly used in the development of **regenerative medicine** approaches. Although their capacity to generate a functional tissue after **colonization** of a porous three-dimensional (3-D) **scaffold**, which would facilitate its implantation and improve its long-term fate, has already been shown, scaffold **cellularization remains incomplete**.

A critical element that limits scaffold cellularization is the **lack of oxygen diffusion** in its innermost part.

Hypothesis:

Culturing cells with an oxygen carrier developed by the French company Hemarina (HEMOXCell®)* to improve the 3-D scaffold cellularization.

The evaluation program was initiated by using two experimental models: meniscus and bone.

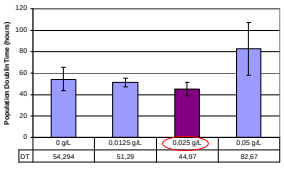


* ***The hemoglobin HEMOXCell® is a nonimmunogenic, nonallergenic biopolymer of 3.6 MDa composed of 156 globin chains (vs 4 in humans).***

Methodology & Results

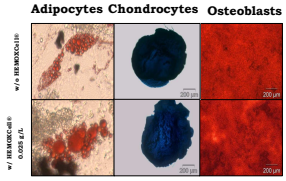
Analysis of MSC proliferation and phenotype in 2-D ± HEMOXCell®

Low concentration of HEMOXCell® improves MSC 2-D culture at 21% pO_2



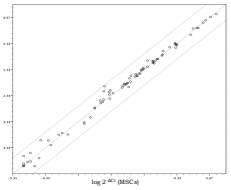
HEMOXCell® induces a dose-dependent increase in MSC growth in 5% PL-supplemented medium (n=4).

HEMOXcell® does not impact MSC differentiation potential *in vitro* (21% pO_2)



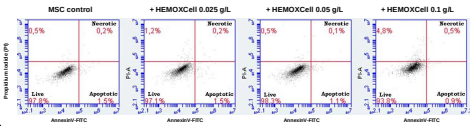
MSCs cultured in the respective differentiation media for 21 days.

Transcriptional profiles remains unchanged



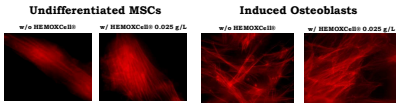
Scatter plot analysis of 84 MSCs related genes (RT² Profiler PCR Array, Qiagen) in MSCs *vs* MSCs + 0.025 g/L HEMOXCell® (n = 3). Cut-off ±4.

No increase in cellular apoptosis and necrosis



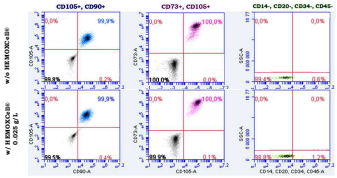
Annexin V-FITC Apoptosis Detection Kit (SIGMA)

HEMOXcell® does not disturb actin filaments during osteogenesis



(Actin staining: Alexa Fluor® 594 Phalloidin)

MSC phenotype remains unchanged



Flow cytometric analysis of MSCs *vs* MSCs + 0.025 g/L HEMOXCell® (n = 3) with MSC Phenotyping Kit (Miltenyi Biotec Germany). Coloured spots: double labeling; black spots: unmarked cells.

Preliminary 3-D culture of MSCs

Meniscus regeneration scaffolds

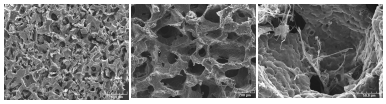
Synthetic meniscal substitute (Actifit®, Orteq® Sport Medecine)

MSC

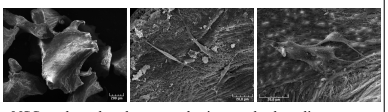
Bone regeneration scaffolds

Decellularized human bone (BIOBank)

• Morphological analysis of cells in Scanning Electron Microscopy after 6-week (Actifit®) and 4-day (BIOBank) cultures: cells exhibit a characteristic spindle shape

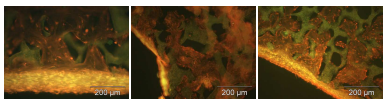


Culture in differenciation medium with growth factor TGFβ3.



MSCs cultured on bone powder in standard media.

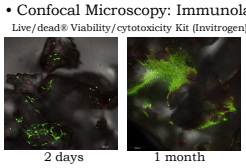
• Phenotypical analysis of cells in histological section of Actifit® after 3-month culture:



CD90 FITC CD29 FITC CD44 FITC

• Confocal Microscopy: Immunolabeling

Live/dead® Viability/cytotoxicity Kit (Invitrogen)



2 days 1 month

staining after 4 days culture

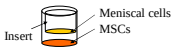
(Green: live cells, red: dead cells)

Potential of MSC-derived fibrochondrogenesis

Characterization of fibrochondrocytes

Induction of fibrochondrogenesis by MSC/fibrochondrocytes co-culture system

3-to-5 weeks in standard medium



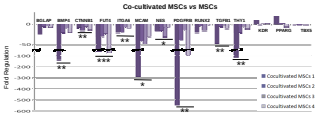
	Overexpressed genes (*vs* MSCs)	Underexpressed genes (*vs* MSCs)
Co-cultured MSCs	1	**40**
Fibrochondrocytes	5	7
Chondrocytes	7	6

Taking MSCs as references, more genes are underexpressed in co-cultured MSCs than in the differentiated cell types (n = 3 or 4).

⇒ Gene expression profile by RT-qPCR in MSC-related genes

(RT² Profiler PCR Array, Qiagen)

12 genes were selected on the basis of their similar expression profile with chondrocytes and fibrochondrocytes but exhibiting various expression ratios.



p-value comparing to control group: * = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.0005$

Data indicate similarities in gene expression profiles between cell types. They also suggest that co-culturing induces differentiation of MSC towards the cell type of interest

Conclusion

At 21% pO_2, a concentration of 0.025 g/L of HEMOXCell® was found to be optimal to promote MSC proliferation without impacting cell adhesion.

This concentration neither affects MSC multipotency nor actin cytoskeleton structure. Moreover, no significant change in MSC gene expression, expression of stem cell markers and cell viability has been observed.

Meniscal and bone scaffolds are well-suited for cell adherence, viability and proliferation.

MSCs co-cultured with fibrochondocytes differentiate, resulting in a gene expression profile similar to chondrocytes and fibrochondrocytes.

Prospects

Fibrochondrocyte characterization: A comparative proteomic study of MSC/chondrocyte/fibrochondrocyte membranes has been initiated to identify specific cell markers.

Perfusion culture system: Culture under perfusion to promote an homogeneous cellularisation and to ensure an overall nutrient diffusion.

Impact of HEMOXCell® on MSC 3-D culture: Assessment of cellularization in meniscal and bone substitutes in the presence of HEMOXCell® to evaluate its effect on oxygen diffusion in 3-D culture. Complementary studies have been initiated to confirm the phenotype of meniscus-differentiated MSCs.

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