Qualitative effects of hypoxia on the composition of extracellular matrix produced by a cloned mouse mesenchymal stromal cell

Andreia Ribeiro[1], Satbir Gill[2], Lokesh Joshi [2], Rhodri Ceredig[1]

[1] Regenerative Medicine Institute, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland
[2] Glycoscience group, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

Introduction

Extracellular matrices (ECMs) are important components in cell signaling as well as in defining the shape and stability of tissues. ECM promotes cell recruitment, adhesion, migration, proliferation and differentiation, thereby emphasizing the importance of the biological role of ECM[1]. It has been shown that ECM is capable of directing the differentiation fate of mesenchymal stromal (MSC) cells cultured on top of ECM. It was also shown that ECM prepared in normoxia (21% O_2) and hypoxia (5% O_2) conditions alters the differentiation of cells[2].

MS-5 cells represent a continuously growing clone of MSC. They have been extensively used in the literature as a model of MSC because a) their proteome is enriched in pro-angiogenic factors and b) their extracellular matrix (ECM) supports human hematopoietic stem and progenitor cell survival and differentiation. We have recently shown that a) the differentiation of MS5 and other continuously growing mouse MSC lines[3] and b) the DNA damage response of MS-5[4] are both influenced by hypoxia.

Aim of the study

To study the differences between two different methods to prepare ECM produced by MS-5, in the presence of 21% or 5% O_2. Method 1 is by cell lysis, Method 2 by inducing apoptosis. The long-term goal of these studies is to identify ECM molecules that signal mouse stromal cells to retain their stemness.

Methods

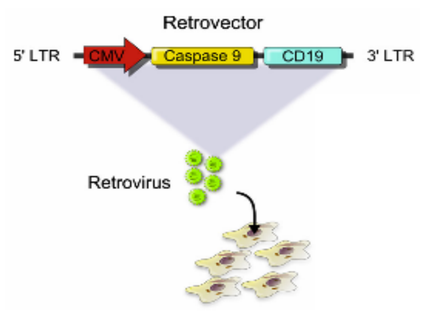
Methods to prepare ECM:

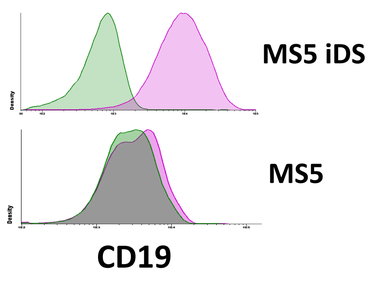
Method 1 - Lysis:

Seed MS5 21% or 5% O_2 → (3 days) → Mitomycin C treatment → (4 days) → Lysis by osmotic shock (Tris-EDTA buffer)

Method 2 – Apoptosis:

Seed MS5 iDS 21% or 5% O_2 → (3 days) → Change media → (4 days) → Induce Apoptosis





Quantitative characterization of ECM:

- Immunocytochemistry (ICC)
- Proteomic analysis
- RT-PCR

Qualitative characterization of ECM:

- Culture of Balb/c (mouse stromal cells) on top of ECMs or plastic
- Differentiation assays of Balb/c on top of ECMs or plastic

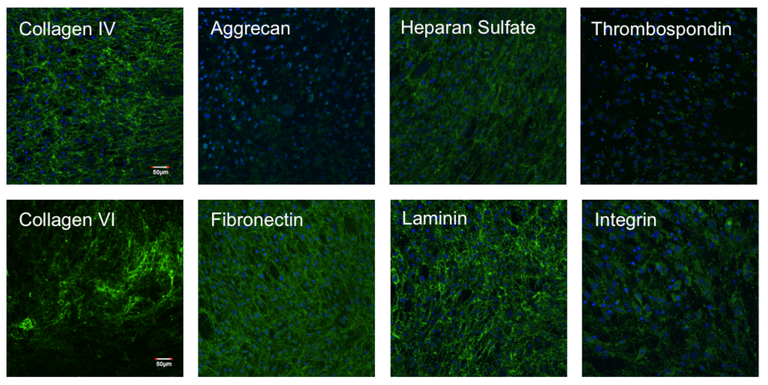
Acknowledgements

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 315902. Within the Marie Curie Initial Training Network DECIDE (Decision-making within cells and differentiation entity therapies).
A special thank you to Professor Ivan Martin and Sébastien Pigeot from Institute for Surgical Research, in Basel, Switzerland.

References

1. Bourgine, PE, Pippenger, BE, Todorov Jr., A, Tchang, L, Martin, I. Tissue decellularization by activation of programmed cell death. Biomaterials, 34, 6099-6108, (2013).
2. Tiwari, A, Lefevre, C, Kirkland, MA, Nicholas, K, Pande G. Comparative Gene Expression Profiling of Stromal Cell Matrices that Support Expansion of Hematopoietic Stem/Progenitor Cells. Stem Cell Research & Therapy, 3:4, (2013).
3. Prado-Lòpez, S, Duffy, MM, Baustian, C, Alagesan, S, Hanley, SA, Stocca, A, Griffin, MD, Ceredig, R. The influence of hypoxia on the differentiation capacities and immunosuppresive properties of clonal mouse mesenchymal stromal cell lines. Immunology and Cell biology, 92, 612-623, (2014).
4. Sugrue, T, Lowndes, NF, Ceredig, R. Hypoxia enhances the radio-resistance of mouse mesenchymal stromal cells. Stem Cells, 32, 2188-2200, (2014).

Results



The ECM prepared with both methods in presence of 21% or 5% O_2, present the common ECM proteins

Figure 1: Immunocytochemestry results. Representative pictures of proteins staining in ECM.

ECM prepared at 21% O_2 have higher expression of proteins

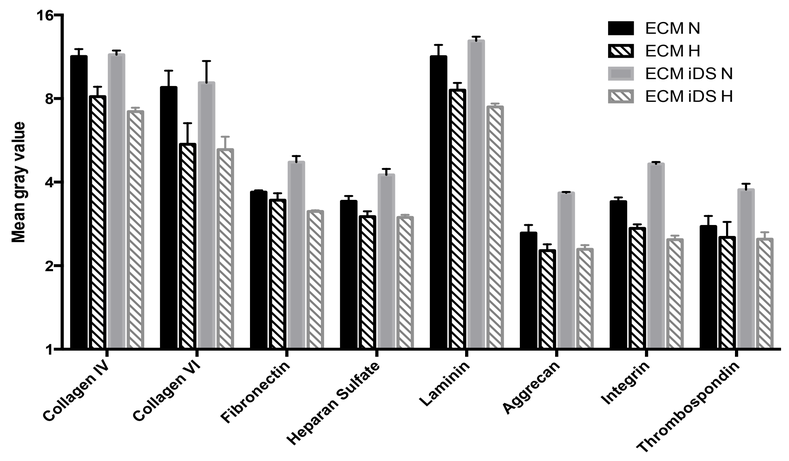


Figure 2: ICC results converted into intensity gray values. Results show the average of 5 pictures per protein per sample.

Proteins are expressed differently in both methods.

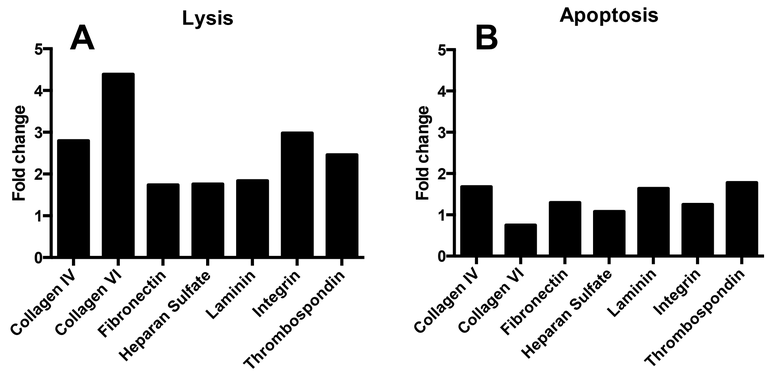


Figure 3: Proteomic results (n=2). PSM fold changes H/N. **A** ECM prepared with Method 1 and **B** with Method 2

Balb/c cells differentiate better into adipocytes in presence of 21% O_2

Balb/c cells differentiate better into osteocytes in presence of ECM and 21% O_2

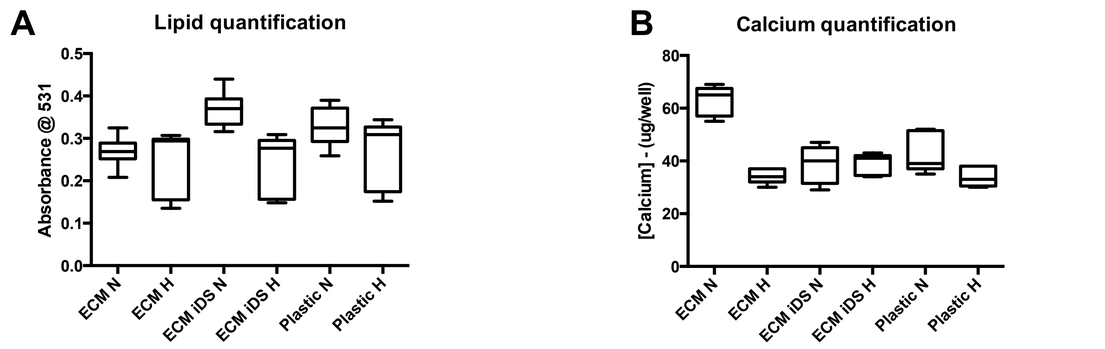


Figure 4: Differentiation assays results. **A**. Lipid quantification of Balb/c cells differentiated into adipocytes on top of ECM or plastic, in presence of 21% or 5% O_2 (n=3). **B**. Calcium quantification of Balb/c cells differentiated into osteocytes on top of ECM or plastic, in presence of 21% or 5% O_2 (n=3).

Transcripts for stem cell genes

Transcripts for HIF genes

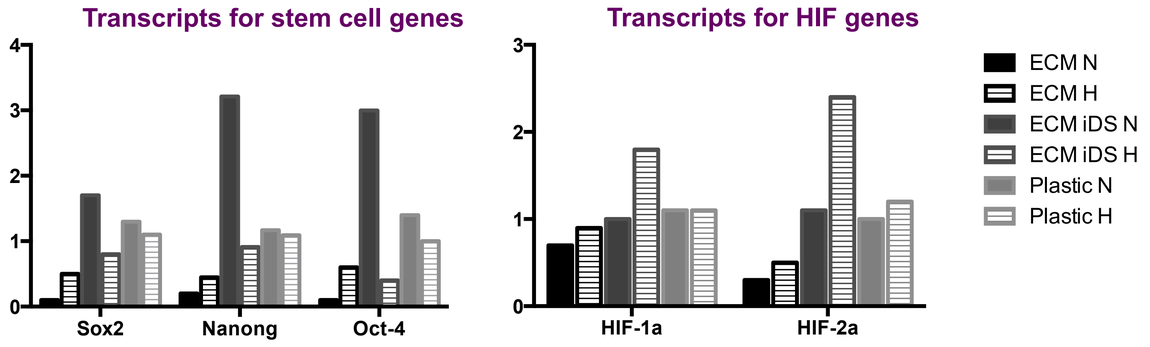


Figure 5 – RT-PCR results of Balb/c cells cultured on top of ECM or plastic in presence of 21% or 5% O_2, for 72h (n=1). Results normalized with respetively plastic conditions.

Conclusions

We are able to produce two types of ECM with different characteristics. They share many common ECM proteins, but at different ratios. By Immunocytochemistry, ECM prepared in normoxic conditions is enriched for many ECM proteins; however, this is not confirmed by proteomic analysis.
Mouse MSC differentiate preferably into adipocytes on top of ECM from apoptotic cells in normoxic conditions, whereas osteogenic differentiation occurs better in the presence of ECM from lysed cells in normoxic conditions. Preliminary results also show that MSC cultured on top of ECM from apoptotoc cells in normoxic conditions, increase stem cell gene expression.
In conclusion different methods of preparing ECM *in vitro* lead to different protein composition and different outcomes in cell differentiation.