Stem Cells Europe
1-3 September 2008 Amsterdam, The Netherlands

HUMAN FETAL BONE CELLS SEEDED IN HYLURONIC ACID GEL AS A DELIVERY SYSTEM FOR BONE ENGINEERING

Author(s): [1,2,3] D.Tenorio, [3]L.A.Applegate, [1]S.Jaccoud, [3]C.Scaletta, [1]D.P. Pioletti
1)Laboratory of Biomechanical Orthopedics-Ecole Polytechnique Fédérale de Lausanne; 2) Federal University of Alagoas – Brazil; 3) Orthopedic Cell Therapy Unit- CHUV

INTRODUCTION

Bone tissue engineering is a potential alternative to overcome many difficulties for bone loss replacement. Hydrogels have been tested as cells delivery for tissue engineering [1]. Hyaluronic acid is a molecule abundant in the human body and due to its biocompatibility it can be used as a matrix support and cell delivery system [2].

The aim of this study was to culture human fetal bone cells hyaluronic acid gel in (Mesolis®, Anties SA, Geneva, Switzerland) and to verify their biological behavior during one week in a 3D structure [3].

METHODOLOGY

Cell source - Human fetal bone cells were obtained from a cell bank of the University Hospital of Lausanne with permission from the ethics committee.

Culture - Cells were cultured within a hyaluronic gel and a DMEM medium supplemented with osteogenic factors (Montjovent et al, 2005). The complex of gel -cells were placed in an agar mold and incubated during one week (Fig A-B).

Freezing - After one week of culture, cells were frozen in liquid nitrogen. Frozen sections of 20 µm were obtained (Fig C-D).

Staining procedures - Before staining, sections were fixed with 4% formaldehyde.

- For detection of alkaline phosphatase (ALP) activity, we followed the procedure from Sigma-Aldrich (85L3R-1KT).
- Staining for von Kossa was applied to detect clusters of mineralization in the matrix.
- To confirm the presence of cells, nuclei were detected by DAPI fluorescense.

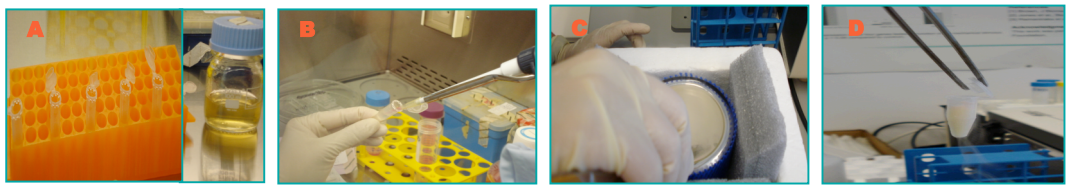
RESULTS

Human fetal bone cells (HBFC) were present within the gel of HA (Fig 1-3). These cells were positive for alkaline phosphatase (ALP) (Fig 4-5). No reaction for ALP was observed in gel without human fetal bone cells (Fig 6). Positive reaction for von Kossa staining was observed in the presence of HFBC within hyaluronic gel (Fig 7-8). In the absence of cells, no staning was observed (Fig 9-10).

CONCLUSION

Hyaluronic hydrogel can be used as a cell delivery system for tissue engineering. Human fetal bone cells have potential to synthesize a mineralized matrix and can provide an interesting cell type for efficient bone tissue engineering.

Our preliminary results show that human fetal bone cells can survive and proliferate within this gel. These cells are positive for alkaline phosphatase reaction and von Kossa staining, demonstrating the potential of human fetal bone cells to synthesize a mineralized matrix.



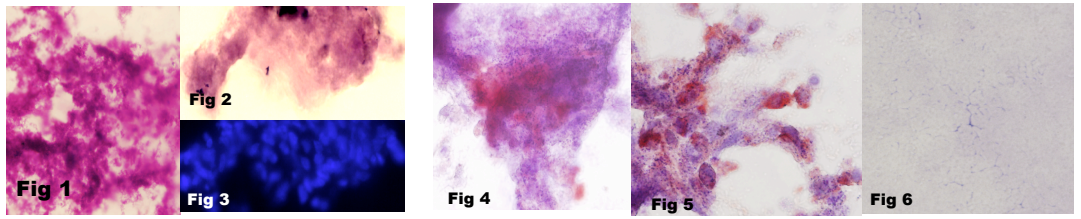


Fig 1. In dark HBFC, hyaluronic gel appears in rose (Giemsa) Fig 2. HBFC within a HA gel-HE. Fig 3. Clusters of nuclei confirmed with DAPI. Fig 4. ALP reaction shows clusters of HBFC. Fig 5. HBFC nuclei in blue and cytoplasm in red positive for ALP. Fig 6. Culture with HA gel only, no reaction for ALP.

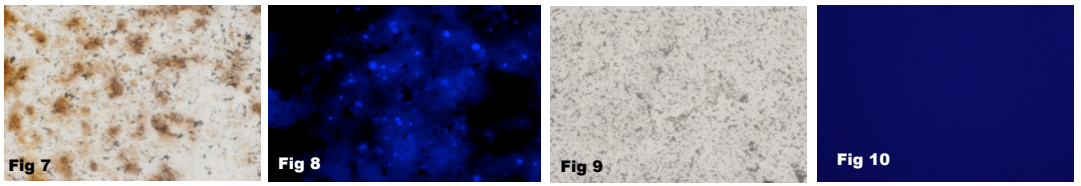


Fig 7. von Kossa staining shows clusters positive for mineralization. Fig 8. HBFC nuclei -DAPI. Fig 9. HA gel alone, in the absence of HBFC, no clusters of mineralization were observed with von Kossa staining. Fig 10. No HBFC nuclei were observed with DAPI.

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Supported by CAPES and EPFL; gel supplied by Anties