

PROLIFERATION OF HUMAN FETAL CELLS IN FIBRIN GEL

N. Ito¹, N. Burri², L. Applegate², E. Federici¹, M.O. Montjovent¹, P.E. Bourban³, J.A. Manson³, P.F. Leyvraz⁴, P.Y. Zambelli⁴, D.P. Pioletti¹

¹Center for Orthopaedic Research, EPFL, 1015 Lausanne, CH; ²Lab of Fetal Medicine, Dept of Obstetrics, CHUV, 1011 Lausanne, CH; ³Lab of Polymer & Composite Technology, Materials Institute, EPFL, 1015 Lausanne, CH; ⁴Hôpital Orthopédique de la Suisse Romande, 1005 Lausanne, CH

INTRODUCTION: For clinical bone transplantations, engineered tissues are being studied with great interest by many researchers. However, there are still no totally satisfying grafts because of possible immuno-rejection and/or mechanical weakness of the scaffold. If the grafts are large, cells in its central part tend to die because of nutrition shortage. To solve these problems, we are developing a new bone tissue engineering. The idea is to use cells for facilitating matrix production, gel for nutrition storage and cell carrier and polymer scaffold for the mechanical support. We use fetal cells that have advantage in immunological tolerance¹, fibrin gel that is biodegradable and may induce vascularization², and Poly-lactite-acid reinforced scaffold. In this study, we investigate the proliferation of human fetal cell in a fibrin gel.

METHODS: Fetal cells are cultured on 75 mm² culture flask, collected by trypsinization and centrifugation just before use. Cell suspension is adjusted to 3 different concentrations (3.2×10^6 cells/ml, 6.4×10^6 cells/ml, 12.8×10^6 cells/ml). Tisseel² fibrin sealant (Baxter AG) is used as gel material. Fibrinogen and Thrombin solutions are made referring to manufacturer's instruction. Aprotinin solution is added to the fibrinogen powder and diluted with equal volume of PBS. CaCl₂ solution is added to thrombin powder for slow solidification (4 IU/ml) and mixed with equal volume of each cell suspension. 30 µl of fibrinogen solutions are poured on to each well of 96 well plate. Then 30 µl of thrombin-cell suspensions are poured on the fibrinogen. It takes about 5 minutes to obtain a gel. Fibrin-cell gels are then removed from the 96 well plate and cultured in 24 well plate with 1 ml of culture. At day 0, 3, 6 of culture, 4 samples from each group are taken for measurement of proliferation using CellTiter (Promega). The same samples are used for image acquisition of viable cells. Samples are immersed in 100 µl of fluorescein diacetate to stain live cell into green and 100 µl of propidium iodide to stain dead cells into red. The fluorescent images are taken with confocal microscope.

RESULTS/DISCUSSION: During the 6 days of culture, the fetal cells in fibrin gel proliferate in all groups (Fig. 1). With the lower cell density, the proliferation was faster probably due to the fact that cells have more space to proliferate than with the higher cell concentrations.

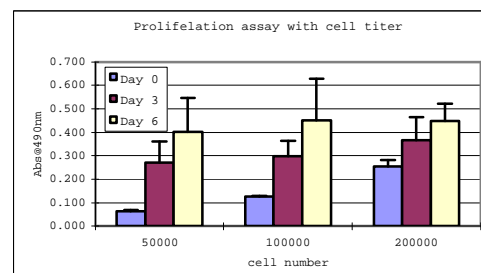


Fig. 1: Proliferation of different density of fetal cells in fibrin gel (mean \pm SD, n=4)

Fluorescent images show elongated fetal cells in gel after 6 days in comparison to round shape cells just after seeding (Fig.2). The fetal cells are then able to grow in the fibrin gel.

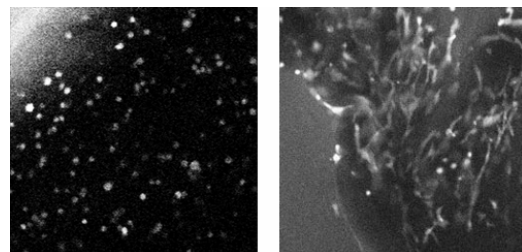


Fig.2: Live cells image in fibrin gel (Left: Day 0, Right: Day6 of 0.8×10^6 cells/ml)

In this preliminary study, we show that fibrin gel can be used as carrier for fetal cells at least from the proliferation point of view. We need to determine now the influence of fibrin gel on the gene expression of fetal cells, especially for osteoblastic markers.

REFERENCES: ¹Fauza DO (2000) Fetal Tissue Engineering in *Prin Tissue Eng 2nd Ed*, Academic Press: 353-68. ²Ameer GA, et al. (2002) *J Orthop Res* 20:16-9.

Acknowledgments: This work was supported by a grant PNR46 from the FNSR.