

Multidye coculture methodology to study fibroblast-osteoblast interactions

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INTRODUCTION: Current bone related implant surfaces are optimized empirically with respect to their *in vivo* performance. The performance of such implants is defined by the velocity and degree of osteointegration and their long-term performance. Integration of an implant depends on: 1) attraction, 2) proliferation of the appropriate cells and, 3) their differentiation into bone forming osteoblast cells. Current *in vitro* work to describe cell reactions in order to optimise implant surfaces is performed using one cell type. However, at the implant surface a variety of cell types are in competition with the capability to contact and colonize the implant surface, the most important of which are fibroblasts and cells of the osteoblast lineage. Furthermore, each of these cell types mutually affects the state of differentiation and functionality of the other cell types and these interactions play a defining role in the fate of the implant [1]. The aim of the present study is to establish a methodology to describe and monitor the interactions between fibroblasts and osteoblastic cells in coculture.

METHODS: Primary human fibroblasts (NHDF) were purchased from Cambrex and primary adult human bone cells (HBC) were obtained by cultivating spongiosa bone pieces from patients receiving hip prosthesis after washing under microscopic observation in culture flasks in proliferation medium. Cells growing out of the bone pieces were defined as HBC. Only HBC cells of the first passage were used for the experiments. To distinguish the two types, cells were labeled prior to seeding. NHDF fibroblasts were vitally labeled with Vybrant™ DiD solution at 16 µl/ml and the HBC were vitally labeled with Vybrant™ DiI solution at 16 µl/ml [2]. In addition, in order to recognize single cells both cell types were treated with DAPI (Sigma Aldrich) for nuclei visualization. Petri dishes (3.5 mm) were inoculated with 2 ml of modified αMEM (10% foetal calf serum, 1% penicillin/streptomycin, 50 µM ascorbic acid phosphate, 2 mM β-glycerophosphate and 10nM 1,25 dihydroxyvitamine D3) containing NHDF and HBC. Cell clustering and proliferation were monitored with fluorescence microscopy at days 1, 4 and 7. Two different locations were scanned for

each well and three wells were seeded for time point. Controls were used containing only fibroblasts or osteoblasts. After 7 days of coculture, cells were fixed with 2% paraformaldehyde containing 0.3% Tween 20 and immunostained for alkaline phosphatase.

RESULTS & DISCUSSION: A multidye coculture system was developed that allowed monitoring of individual cells over time. Membrane-bound dyes DiI and DiD permitted identification of fibroblasts and bone cells (Figure 1A). In addition all cells were stained with DAPI at day 4. With this dye combination we could account for all the cells and to assign them to one of the two populations at all times.

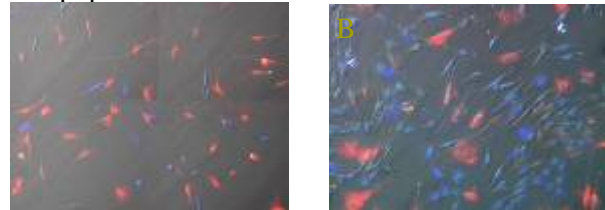


Fig. 1: Fluorescence image of NHDF fibroblast (DiD stained, blue) and human bone cells (DiI, red) after (A) 1 day and (B) 4 days in coculture.

The developed methodology is being used in ongoing experiments to investigate the cell-cell interactions of fibroblasts and human bone cells and how both cell types affect the proliferation and differentiation of the other.

REFERENCES: ¹B. Ogiso, F.J. Hughes, A.H. Melcher et al (1991) *J Cell Physiol* **146**:442-50. ²J.P. Kaiser and A. Bruinink (2004) *J Mater Sci: Mater Med* **15**:429-35.

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