

Fibrin as a substrate and carrier for human periosteum derived progenitor cells during osteogenic induction.

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INTRODUCTION: Fibrin is a widely used cell carrier in tissue engineering [1]. Although in vivo heterotopic bone formation and in vitro osteogenic differentiation of embedded progenitor cells have been reported previously [2,3], its role in osteogenesis remains unclear. Apart from the matrix material, the culture conditions also affect the cellular response: 3D systems are often very different from 2D cultures. In this study viability, proliferation and osteogenic differentiation of human periosteum derived progenitor cells (HPDCs) cultured in monolayer on fibrin substrates are compared to HPDCs encapsulated in fibrin carriers.

METHODS: HPDCs from five donors were pooled. Cells were plated at a density of 3000 cells/cm² in 12-well plates ('2D') or on a fibrin (Tisseel VH SD) gel ('2D+'). The next day, medium was replaced with osteogenic medium (StemPro MSC Serum Free Medium supplemented with 100 nM dexamethasone + 10 mM β -glycerophosphate + 0.050 mM ascorbic acid) with 0.5 mg/ml tranexamic acid (Exacyl). In addition, cell-gel constructs were prepared with 10⁶ cells/ml, 1 U/ml of thrombin and 33 mg/ml of fibrinogen ('3D'). The constructs were cultured in osteogenic medium with tranexamic acid for three weeks.

After one, two and three weeks of culture, cell behaviour was evaluated by live/dead staining, DNA quantification, RT-qPCR (ALP, Runx2, collagen I, BSP and GAPDH) and histology.

RESULTS: Cell growth, gene expression and mineralization in 2D were unaffected when tranexamic acid was added to the medium. Using fibrin as a substrate (2D+) did not significantly alter cell attachment, proliferation and osteogenic marker expression compared to plastic. Initially, the cell-gel constructs contained predominantly viable cells, homogeneously distributed throughout the carrier. However, after one week cell death increased towards the centre of the gel. In

addition, cell growth was almost twenty times lower in 3D compared to 2D+. Osteogenic marker expression was also reduced, except for collagen type I.

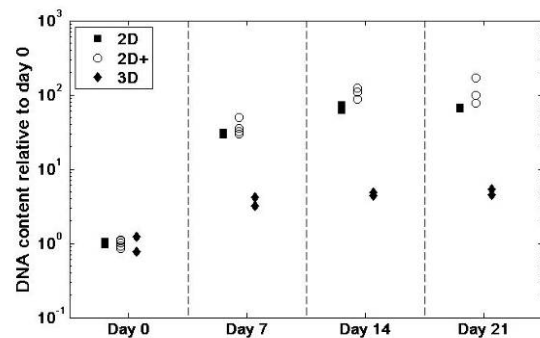


Fig. 1: Relative increase of DNA content normalized to day 0.

DISCUSSION & CONCLUSIONS: HPDCs differentiate similarly into the osteogenic lineage when cultured on fibrin substrate as on culture plastic. Encapsulating HPDCs in fibrin decreases cell growth and interferes with osteogenic differentiation. These results suggest that not the matrix material, but the 3D culture condition hampers normal cell behaviour as nutrient supply, cell-cell contact and cell morphology will vary significantly compared to 2D culture systems. The diminished cell viability in 3D culture is probably due to reduced nutrient and oxygen concentrations. This indicates the need for an active nutrient supply to the carrier centre together with an appropriate carrier design.

REFERENCES: ¹ Ahmed et al., Tissue Eng Part B Rev. 14, 199-215 (2008). ² Isogai et al., Plast Reconstr Surg. 105, 953-963 (2000). ³ Catelas et al., Tissue Eng. 12, 2385-96 (2006).

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