Fibrin Hydrogels as Substrate or Carrier and the Behaviour of Periosteum Derived Cells for Bone Tissue Engineering

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Introduction

Biomimetic approaches to tissue engineering often rely on hydrogels to support cell growth and tissue formation. To this end, protein-based hydrogels, such as fibrin, are commonly used.\textsuperscript{1} Besides the matrix material, culture conditions also affect cell function (e.g. 2D and 3D cultures are often very different).\textsuperscript{2} This study investigated viability, proliferation and differentiation of human periosteum derived cells (hPDCs) seeded in monolayer on fibrin substrates versus hPDCs encapsulated in fibrin carriers when cultured in vitro under osteogenic conditions.

Materials and Methods

Pooled cells from five donors were seeded at 3000 cells/cm\textsuperscript{2} in 12-well plates (2D) or on fibrin substrates (2D+). In addition, cells were encapsulated in fibrin hydrogels (Ø8x4mm) at 10\textsuperscript{6} cells/mL (3D). The constructs were cultured in osteogenic medium (OM, StemPro MSC SFM + 100 nM dexamethasone + 10 mM β-glycerophosphate + 0.05 mM ascorbic acid) with or without 0.5 mg/ml tranexamic acid (TEA). At day 0, 7, 14 and 21 cell behaviour was evaluated by Live/Dead staining, histology, DNA quantification, and RT-qPCR.

Results

In 2D, mineralisation, proliferation, and gene expression were similar with or without TEA. Microscopy analysis showed that in 2D+ cells attached to fibrin (Fig.1A) and formed a sheet of viable cells. In 3D, cells had a round shape (Fig.1B). After 2 weeks a viability gradient with increased death centrally was observed (Fig1C). Cell growth in 2D+ was twice as high as in 2D, however in 3D it was 20 times lower (Fig.1D). ALP, Runx2 and collagen 1 gene expression increased, while osteocalcin expression remained constant in 2D and 2D+. In 3D, expression levels were significantly lower (Fig.1E).

Discussion and Conclusions

The higher proliferation rate and the preserved differentiation potential of hPDCs in 2D+, are in contrast with the reduced cell viability, growth, and differentiation observed in 3D. Inadequate nutrient supply, high matrix density, altered cell morphology and cell-cell contact interfere with cell behaviour. This emphasises that besides a suitable biomaterial, an optimised and controlled 3D environment is essential to obtain the desired biological response.

References


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Disclosures

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