

## Effect of a serum free medium containing TGF- $\beta_3$ on osteocyte viability of cultured human cancellous bone explants

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**INTRODUCTION:** The replacement of Foetal calf serum (FCS) as a growth factor and hormone supplement in culture media would have major advantages in cell or organ culture. The factor concentrations within FCS are inconsistent and vary from batch to batch. This can cause unintended effects on cultured cell populations. Furthermore, the protein level in media with added FCS could cause problems, if investigation of secreted factors at low concentrations is intended. However, if using a serum free medium, problems arise in maintaining cell viability, which can be achieved by adding suitable factors. The aim of this study was to investigate the effect on osteocyte viability of a serum free medium containing 15 ng/ml TGF- $\beta_3$  during *ex vivo* culture of human cancellous bone explants.

**METHODS:** Human femoral heads (Ethic Commission Graubünden 18/02) from hip transplant patients were excised and cut into 7 mm thick sections with the use of an Exakt 300 band saw. Cores 9.5 mm in diameter were bored from the sections with Synthes drill bit (Ref: 387.661, Synthes, Bettlach, Switzerland) and cut parallel to 5 mm with a Leica annular saw. After insertion inside the culture chambers, cores were perfused with either DMEM + 10% FCS or DMEM serum free + 15 ng/ml TGF- $\beta_3$  (ITS and lipids supplemented). A daily load was applied with a complete jump wave form for 5 min (300 cycles, 1 Hz, 4,000  $\mu$ strain) using a Zetos bioreactor [1, 2]. After a culture period of 14 days cores were harvested and cut to 250  $\mu$ m sections down to the centre with a Leica annular saw. Surface and centre sections were assayed with LDH viability staining [3]. The sections were fixed in 4% buffered formalin and visualised using fluorescence and light microscopy (Axioplan).

**RESULTS:** Using the natural autofluorescence of the bone matrix, osteocyte viability of LDH stained sections was analyzed at 515-565 nm emission filter. Dark stained, viable osteocytes will efficiently block the autofluorescence, and viability quantification can be accomplished. In every case of performed three experiments, the use of DMEM serum free + 15 ng/ml TGF- $\beta_3$  (Fig.1B)

showed similar or enhanced maintenance of osteocyte viability in core centres compared to the use of DMEM + 10% FCS (Fig.1A).

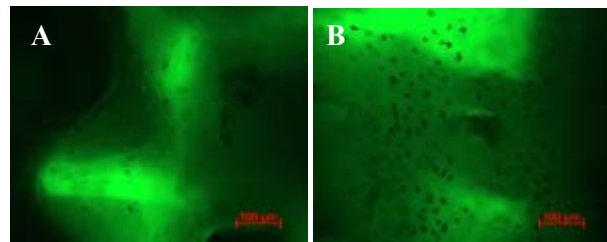


Fig. 1: Representative fluorescence pictures of LDH stained centre sections from human cancellous bone cores after 14 d culture.

**A:** Cultured in DMEM + 10 % FCS;

**B:** Cultured in DMEM serum free + 15 ng/ml TGF- $\beta_3$ .

An additional effect using serum free medium was a reduction in the number of surface fibrous cells seen on cores cultured in serum containing medium. Fibroblast-like cells were found on 68% of cores cultured within serum containing medium. On the contrary 61% of the cores cultured in serum free medium did not show the fibrous tissue.

**DISCUSSION & CONCLUSIONS:** DMEM serum free with added 15 ng/ml TGF- $\beta_3$  seems suitable to replace the serum containing DMEM during culture of human cancellous bone cores, when looking at osteocyte viability and surface fibrous tissue formation. The reduction of the fibrous tissue will provide an even more defined culture environment, as the fibroblast-like cells would compete for nutrients, and are also likely to secrete unknown factors.

**REFERENCES:** <sup>1</sup>D.B. Jones *et al.* (2003). *Eur Cell Mater* **5**: 48-60; <sup>2</sup>C.M. Davies *et al.* (2006). *Eur Cell Mater* **11**: 57-75 ; <sup>3</sup>M.J. Stoddart *et al.* (2006). *Eur Cell Mater* **12**: 16-25

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