

EFFECTS OF CULTURE CONDITIONS ON HUMAN BONE (MARROW) CELL PERFORMANCE

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Due to the increased life expectancy the need of optimal designed implants is steadily increasing. Since permanent implants have their clear-cut limitations regarding long-term functionality and biocompatibility it is nowadays a general goal in orthopaedics to substitute injured or not functioning tissues (and by that of latter implants) by engineered tissue using cells of the patient.

In case of cartilage and bone tissue engineering osteoblast and chondrocyte progenitor cells have to be cultivated and subsequently differentiated. Current culture conditions are far from optimal.

The present study was undertaken to investigate the role of different medium supplements on cell proliferation and differentiation. In order to be as close as possible to the *in vivo* implant situation we use human bone marrow (mesenchymal stem) cells of adult patients obtaining a total hip prosthesis.

MATERIALS AND METHODS: Bone marrow mesenchymal stem cells and bone cells were isolated from human femor trabecular bone. Cells were expanded using expansion medium containing fibroblast growth factor, dexamethasone and different sources of serum (fetal bovine serum, FBS, adult bovine serum, BS, or adult human serum, HS; with or without heat-inactivation). At confluency cells were isolated by trypsinization and seeded into well of 24-well plates in expansion medium. After 3 days medium was replaced by medium without additions, by medium with dental extracts [1] or particles (grinded teeth), or by differentiation medium containing β -glycerophosphate, dexamethasone ascorbic acid-phosphate (with or without vitamin 3OH-D₃). Cell performance was determined by measuring total culture DNA, protein and alkaline phosphatase (ALP) activity.

RESULTS AND DISCUSSION:

Serum effects

Generally, foetal calf serum-containing media are used in *in vitro* experiments. Although cells behave well in these media several aspects must be kept in mind, i.e., that latter addition is rather artificially. Our findings indicate that indeed serum source as well as pre-treatment (heat-inactivation) affect human bone (marrow) mesenchymal cell performance and substratum serum-protein

adsorption (Hälg, 2003). These data support our hypothesis that the choice of serum source and serum pre-treatment may greatly affect the outcome of the cell culture experiments. Furthermore, it could be shown that in case of human serum heat inactivation has an adverse effect on cell yield (Fig. 1). The yield using untreated human serum was comparable to that of FBS.

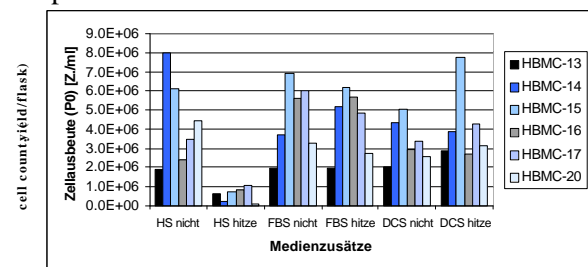


Fig 1. Effects of different sera and serum treatment on the cell yield of adult human stem cells at confluency (before the first passage) in 6 experiments (HBMC 13-17 and 20). Please note that heat inactivation of human serum specifically reduce the yield

Particles

Grinded teeth particles were found to have an adverse effect on human bone cell performance. However acid and guanidine extracts of these particles promote cell proliferation with simultaneous reduction of osteoblast differentiation. Furthermore, remnants of these extracts were found to attract cells.

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References

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