Effects of Heparin on MC3T3-E1 Osteoblast-like Cell Behavior

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Introduction : Heparin, a linear sulfated polysaccharide consisting of repeating units of 1→4 linked pyranosyluronic acid and 2-amino-2-deoxyglucopyranose residues, has long been in wide clinical use as a powerful anticoagulant to prevent thrombosis[1]. Heparin has a high negative charge density, which renders it the ability to bind with certain growth factors containing heparin-binding domains such as fibroblast growth factors (FGFs), transforming growth factor–β (TGF–β), and bone morphogenetic proteins-2 and 4 (BMP-2 and 4), etc[2]. Therefore, heparin can be potentially used to modify bone tissue engineering scaffolds to enhance their blood compatibility and also to bind with heparin-binding growth factors *in vivo* to accelerate bone regeneration. The aim of the present study is to investigate the effects of different concentrations of heparin on the proliferation, differentiation, mineralized matrix formation and gene expression of MC3T3-E1 osteoblast-like cells.

Materials and Methods: MC3T3-E1 osteoblast-like cells (ATCC, Manassas, VA) were seeded onto 24-well tissue culture polystyrene (TCP) plates at a density of 5×10^4 cells/well. Cells were cultured in α-minimum essential medium supplemented with 10% FBS, 1% antibiotics, and different concentrations of heparin (Sigma, St. Louis, MO) at 0, 0.005, 0.05, 0.5, 5, 50 and 250 μg/ml. At day 7 and 14, cell proliferation was quantified using the MTS assay (Promega, Madison, WI). Alkaline phosphatase (ALP) activity of the cells was measured with an ALP substrate kit (Bio-Rad, Hercules, CA). Mineralized matrix formation was analyzed using Alizarin Red assay. In addition, total RNA was extracted from the cells using an RNeasy Mini Kit (Qiagen, Valencia, CA) and a real time RT-PCR was used to quantify the gene expression of ALP and osteocalcin (OCN). Data were normalized to the expression of housekeeping genes GAPDH and HRPT. All experiments were performed in triplicate, and statistical analysis was done using one way analysis of variance (ANOVA).

Results and Discussion: Figure 1 (a), (b), and (c) show the total cell number, ALP activity of the cells, and calcium deposition at both day 7 and day 14 respectively. MC3T3-E1 cells cultured with 250μg/ml heparin showed statistically significant decrease in proliferation after 7 days; however, after 14-day's culture, there is no significant difference in proliferation among the heparin concentrations studied (Fig. 1a), which is possibly because that cells reached confluency on the TCP. Comparing to cells with no heparin treatment, the ALP activity level peaked when the cells were treated at a lower heparin concentration (0.5μg/ml for day 7 and 5μg/ml for day 14), followed by a subsequent significant drop when treated with 50μg/ml and 250μg/ml heparin for respective time points (Fig. 1b). Alizarin Red assay demonstrated that there was no significant difference in calcium deposition at day 7 despite the heparin treatment, which may be because that cells were mostly still in the proliferation stage, and differentiation just started to take place. At day 14, calcium deposition was significantly increased at a heparin concentration of 0.05μg/ml, followed by significant decrease at higher heparin concentrations e.g. 5, 50, and 250μg/ml (Fig. 1c). RT-PCR data showed that 5μg/ml of heparin treatment up-regulated the gene expression of ALP and OCN at day 14, while further increase of heparin concentration down-regulated the gene expression of ALP.

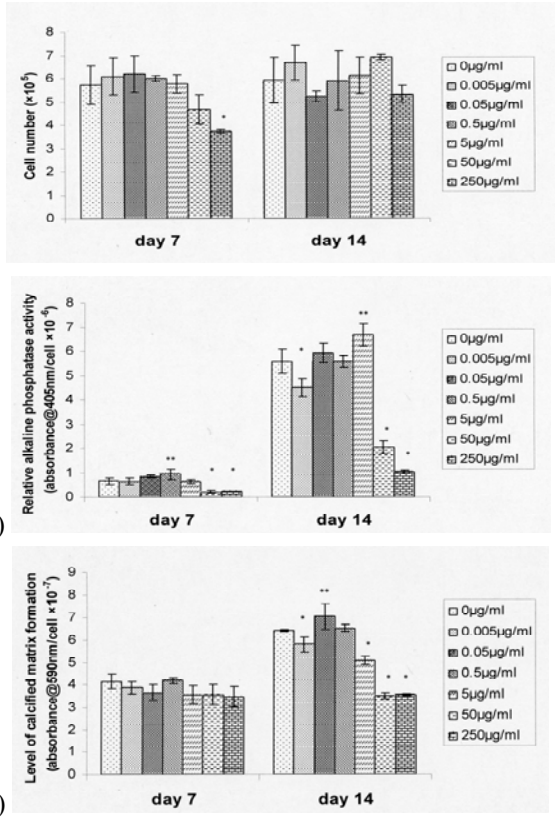

Fig.1 (a) MTS assay for cell proliferation; (b) Alkaline phosphatase activity for phenotype expression; (c) Alizarin Red assay for calcium deposition. Note: in (b) and (c), expression was normalized to each cell.; (*) and (**) indicates significant decrease and increase respectively comparing to heparin concentration of 0μg/ml. $p<0.05$.

Conclusion: Heparin concentrations higher than 50μg/ml tend to inhibit MC3T3-E1 osteoblast-like cell phenotype expression and mineralized matrix formation, while heparin concentrations between 0.05μg/ml and 5μg/ml have favorable effects on the differentiation of the cells.

References

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2. Capila I., et al. *Angew. Chem. Int. Ed.* 41: 390 (2002)