Chapter 5

Conclusion

This study demonstrated that cultivation of osteoblasts on a titanium disk was a useful study model for investigating behaviors of bone cells on different surfaces of biomaterials and for observing effects of various stimuli in culture medium on responding with bone cells grown on different titanium surfaces and biomaterials.

A study model for studying interaction between osteoblasts and titanium surface was developed. Fluorescence staining observed under a fluorescence microscope and SEM are effective methods for detecting morphology and attachment of cells on an opaque titanium surface. Progress of osteoblastic differentiation can be determined by detecting expression of osteoblastic phenotypes, such as ALP activity and osteocalcin when corresponding with the growth curve of cells.

The hypotheses of this study that (1) a reduction of growth and differentiation of osteoblasts by a specific COX-2 inhibitor directly associated with a reduction of prostaglandin synthesis and (2) therapeutic dose of specific COX-2 inhibitors NSAIDs reduced growth and differentiation of osteoblasts by inhibiting prostaglandin synthesis were unlikely from the findings. This was because the inhibitory effect of specific COX-2 had no effects on osteoblastic differentiation and its inhibitory effect on cell growth did not correlate to a reduction of PGE_2 synthesis. As it could be seen that specific COX-2 inhibitors, celecoxib and conventional NSAIDs, indomethacin, decreased PGE_2 synthesis of mouse osteoblast cell line, MC3T3-E1, on smooth surface titanium surface only in *static* and *plateau* phases not *log* phase, the inhibitory effect was associated with stages of growth of osteoblast.

An administration of celecoxib in high therapeutic doses might inhibit growth of osteoblasts during implant healing. Effects of 0.1 μM indomethacin (conventional NSAIDs) and 1.5 – 3 μM celecoxib on growth and differentiation of cells were not significantly different. The adverse effect on cell growth in a time dependent manner was found only in high concentrations, 9 μM celecoxib, which is equivalent to a therapeutic dose of 800 mg/day. The results did not

show that a specific COX-2 inhibitor NSAID, celecoxib, in low therapeutic doses (1.5 – 3 μM) had greater adverse effects on growth and differentiation of osteoblasts than conventional NSAID, indomethacin, in a therapeutic dose (0.1 μM). It might be postulated that effects of celecoxib upon osteoblasts on titanium implants possibly depend on doses and administration time of celecoxib and stages of implant healing.

Limitation of this study design was cell cultivation in serum free culture medium during medication treatment. It was unavoidable to employ serum free cell cultures because growth factors including PGE_2 in a supplemented bovine serum could interfere with inhibitory effects of the treated medications on the function of cyclooxygenase and thus PGE_2 synthesis from osteoblasts. A deprived condition of serum free culture medium limited duration of treatment to be not more than 5 days, which was much shorter than therapeutic periods in clinical cases.

Further study should be conducted in an animal study model to maximize a mimicking of a clinical application, to avoid adverse effects of serum free culture medium on cell viability and to study the effects of specific COX-2 inhibitors on angiogenesis in early stages of bone healing. A range of therapeutic doses of celecoxib should be administered for a longer period of time between 1 – 6 months in pre- and post-implantation periods. The effects should be compared with other NSAIDs that are commonly used for analgesic after implant surgery in therapeutic and high doses.
