Chapter 5

CONCLUSION

Part I: Osteogenic induction of bone marrow of fully mature rats

It is clearly demonstrated that bone marrow of fully mature rats contained pluripotential mesenchymal stem cells, which could be expanded *in vitro* and differented to osteoblasts and chondroblasts *in vivo*. In this cell culture condition, bone marrow cells expressed markers of osteoblastic differentiation and adipocytes. Expressions of ALP and collagen type I were markers of early osteoblastic differentiation. Continuous exposure to BMP-2 or VD3 was required to promote expression of markers of mature osteoblasts, which were osteocalcin and *in vitro* mineralization.

Implanted cultivated bone marrow cells had higher osteogenic potential than fresh bone marrow. Differentiated bone marrow cells were able to form bone in a larger amount and higher rate than fresh bone marrow, which were contributed to the intramembranous bone formation process induced by differentiated cells. Autogenous BMP-2, TGF- β1 and IGF-I secreted by differentiated osteogenic cells might enhance bone formation process and induce osteogenic differentiation of mesenchymal stem cells in subcutaneous tissue. The amount and rate of new bone formation should be able to be enhanced by supplementing BMP-2 in differentiated bone marrow cells. ICBM scaffold was suitable as a carrier for cell transplantation. It is possible to expand mesenchymal stem cells on structure of ICBM *in vitro* and transfer the reconstruction into the implantation site. This approach will enhance the osteogenic potential of cultivated cells and osteogenesis of the construct *in vivo*, as numbers and the differentiation state of cells are increased.

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It is clearly demonstrated that bone marrow cells of fully mature rats can be a source of osteoprogenitor cells for cell transplantation. The results confirm a possibility to use autogenous bone marrow cells of adults and aged patients to repair skeletal defects. Implantation of osteoprogenitor cells is another option to accelerate the bone regeneration process and a decrease in using allogenic materials. A synthetic scaffold should be reconstructed simulating structure and porosity of ICBM.

Part II: Platelet-rich plasma and osteogenic differentiation

When osteogenic cell culture of bone marrow cells was well established and osteoblastic differentiation markers could be identified, the techniques were applied in a three-dimensional cell culture to investigate the effects of PRP on osteogenic differentiation of osteoblast precursor cells and mesenchymal stem cells in bone marrow.

It is clearly demonstrated that PRP delivers high concentration of growth factors. The released growth factors inhibit osteogenic differentiation and promote proliferation of osteoprogenitor cells in a dose dependent manner. It is postulated that synergistic mitogenic effects of PDGF, TGF-β1, IGF and EGF promote cell proliferation and inhibit osteogenic differentiation. An impairment of an IGF-I stimulatory effect on osteoblastic differentiation reciprocates an inhibitory effect of PRP on osteogenic differentiation. There is no evidence to demonstrate an osteoinductive property of PRP. BMP-2 is a potent osteogenic inducer.

In order to verify effects of PDGF, TGF-β1 and IGF-I released from PRP, cells should be cultivated parallelly to the experimental groups of PRP in known concentrations of these growth factors in culture medium. Growth and differentiation of cells in the experimental group can be compared with cells in those control groups. The results will clarify effects of growth factors released from PRP and dose dependent effects of growth factors can be clearly explained.

General conclusion

Bone tissue engineering aims to reconstruct skeletal defects morphologically and functionally. The natural process of osteogenesis is modified and regulated by interactions of osteogenic proteins, osteogenic growth factors and osteogenic cells.

Three dimension scaffolds are mostly used as delivery vehicles of cells and growth factors into the implantation sites. Attempts have been made to develop a suitable framework which is biocompatible, resorbable, malleable and favourable to cell growth and differentiation. Controlling growth factors released from the scaffold is challenging, for the growth factors to be delivered in a suitable dose and time.

This study focused on osteoprogenitor cell transplantation and utilization of autologous products as repair materials. Osteogenic cell culture of bone marrow cells was developed to establish a study model of their growth and differentiation. Osteogenic differentiation of these cells can be modified by supplementation of osteogenic proteins and growth factors. It is believed that transplanted osteoprogenitor cells will enhance bone regeneration of skeletal defects. This study found out that there is a high potential for repairing skeletal defects with transplanted autologous osteoprogenitor cells.

It is the first time that the effects of PRP on osteoprogenitor cells were investigated by basic research. This study demonstrates that PRP offers no advantage on osteogenic differentiation of mesenchymal stem cells and pre-osteoblasts in contrast to results of clinical reports. The advantage of using PRP in bone grafting is questionable. PRP has no osteoinductive effects and is not an alternative growth factor to replace BMP-2.