Cytokines and Osteogenic Differentiation of Mesenchymal Stem Cells

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Implications for Bone Tissue Engineering

General Summary

GENERAL SUMMARY

The treatment of large bone defects is still a significant challenge for orthopaedic and maxillofacial surgeons. Large bone defects in the skull occur as a consequence of traumatic injuries and pathological conditions, and these defects represent a significant socioeconomic burden. Autologous bone graft is still considered as the “gold standard” for the treatment of large bone defects. However, its use has disadvantages such as limited availability and quality as well as donor-site morbidity. Allografts are another alternative for the treatment of bone defects, but infection risk concerns and donor availability issues limits their use.

Polymethyl methacrylate (PMMA) due to its high mechanical stability and low costs, is the most frequently used bone substitute in order to treat large cranial bone defects. Despite the positive clinical outcomes, the use of PMMA is still a matter of debate due to cytotoxic concerns. In a skull defect, the implanted PMMA will be surrounded by osteoblasts present within the bone tissue. Moreover, mesenchymal stem cells (MSCs) will be attracted to the implant site following PMMA implantation. Thus, MSCs and osteoblasts represent suitable cell types for the *in vitro* evaluation of possible cytotoxic effects of PMMA. Therefore, in **Chapter 2** we evaluated: 1) the potential adverse and cytotoxic effects of PMMA on human adipose stem cells (hASCs) and human osteoblasts (hOBs), and 2) Whether PMMA enhances the production of osteoclast factors by hASCs and/or hOBs. We found that PMMA is not cytotoxic, and does not interfere with the osteogenic differentiation potential of hASCs and hOBs, and that PMMA does not enhance production of osteoclast regulatory markers by hASCs and hOBs *in vitro.* We demonstrated that PMMA does not inhibit bone formation and that activation of osteoclasts by production of osteoclast-related factors due to PMMA is unlikely.

Despite recent advances in bone tissue repair techniques, biomaterials and stem cell technology, the treatment of large bone defects remains a difficult clinical problem due to the complexity of the damaged bone tissue that needs to be repaired. Bone tissue engineering approaches have the potential to overcome the limitations of using conventional bone grafts, and therefore they are a strategy for the treatment of large bone defects including large cranial defects. Bone tissue engineering techniques use the combination of stem cells including MSCs, scaffolds, and inductive factors to create a bio-active bone construct. A bone tissue engineered construct once implanted into a bone defect may enhance and contribute to bone repair.

Bone repair is a highly complex process. Bone repair involves hematoma formation and hypoxia, followed by an inflammatory response where different immune cells are attracted. The

inflammatory response triggers and initiates the repair process where cytokines such as TNF-α, IL-4, IL-6, IL-8, and IL-17F are released to the injury site. In **Chapter 3** the effects of the pro-inflammatory cytokines TNF-α, IL-6, IL-8, and IL-17F, and the anti-inflammatory cytokine IL-4 on proliferation and osteogenic differentiation of hASCs were studied. The cytokines were added to hASCs during 72 h mimicking the early stage of the physiological process of bone repair. We found that TNF-α, IL-4, IL-6, IL-8, and IL-17F have both enhancing and reducing effects on osteogenic differentiation of hASCs. Specifically IL-6 may be suitable to induce osteogenic differentiation of MSCs as a strategy for enhancing bone repair.

An hypoxic microenvironment is another important event occurring after bone fracture. Cytokines and hypoxia are also likely to occur in an implanted tissue engineered construct *in vivo*, where an inflammatory response takes place. Thus, cytokines as well as hypoxia are hallmarks of the early stages of fracture healing, where their activated pathways may interact at the level of signal transduction. Therefore, the effect of cytokines and hypoxia were investigated in **chapter 4,** to better predict cytokine modulation of MSC-aided bone healing for bone tissue engineering purposes to treat large bone defects. We found that IL-4 reduced osteogenic differentiation of hASCs, indicating that this cytokine might inhibit bone healing and regeneration. In addition, we demonstrated that the inhibitory effects of IL-4 were mitigated in the presence of IL-6 which are likely to occur through an mTOR independent pathway. Thus, for a better understanding of bone healing, specifically for tissue engineering purposes, it is important to study the effect of oxygen tension and the combination of cytokines.

A bone tissue engineered construct, once implanted into a bone defect, will be exposed to the effect of cytokines and hypoxia, and MSCs incorporated into a bone tissue engineered construct will interact with the scaffold substrate. The substrate may play a role by modulating the response of MSCs to cytokines and hypoxia. *In vitro* studies designed to evaluate the interaction between MSCs and biomaterials in an hypoxic environment and in the presence of cytokines, mimicking the *in vivo* inflammatory environment, are essential to successfully develop a tissue engineering construct for the treatment of large bone defects. In **Chapter 5** we investigated the direct effect of a 3 day-stimulation period with a cocktail of cytokines, i.e. TNF-α, IL-4, IL-6, and IL-17F, on osteogenic differentiation and VEGF expression by hASC cultured on biphasic calcium phosphate (BCP) scaffolds and on tissue culture plastic under hypoxia. We demonstrated that a combination of cytokines and hypoxia enhanced VEGF expression in hASCs cultured on BCP under hypoxia, but did not affect their osteogenic differentiation. Thus, cytokines and hypoxia affect MCS behavior, and the effect is substrate-dependent. This indicates that

principles of the *in vivo* process of bone repair (i.e. addition of cytokines, hypoxic culture conditions) must be incorporated in the evaluation of bone tissue engineered constructs.

Finally, in **Chapter 6** the major findings and conclusions of the studies described in this thesis are discussed as well as their implications for bone tissue engineering strategies in the field of orthopedics and maxillofacial surgery. The authors conclude that complex *in vitro* studies that incorporate principles of the *in vivo* process of bone repair as well as the principles of tissue engineering, are crucial for the successful development of a bone tissue engineered construct to treat patients suffering from large bone defects. The results described in this thesis contribute to an increased understanding in the mechanism of bone repair involving MSCs, and it may lead to the implementation of new bone tissue engineering research strategies.