

Challenges in bone tissue engineering

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The application of tissue engineering principles is commonly described by using a combination of cells, matrix and growth factors in order to regenerate or to replace tissue. Bone tissue engineering currently uses either growth factors on a variety of carriers or osteogenic cells seeded onto an even larger number of different materials. The former approach has been very successful in preclinical animal studies using bone morphogenic proteins for the healing of critical size skeletal defects in various locations.

Osteogenic growth factors

Clinical applications in the head and neck area, however, have shown ambiguous results or required extremely high dosages when compared to the natural content of BMPs in bone. Besides other reasons, the unfavourable release characteristics of carriers such as collagen or inorganic materials have been considered responsible for these results. Loading with BMP has been commonly performed by simply soaking the growth factor solution into the carrier. However, this type of loading is associated with rapid delivery of growth factors after implantation within the first 48 hours. As many polypeptide growth factors provide a heparin binding domain, modification of collagen carriers by covalent binding of heparin to the respective sites in the collagen molecule can substantially retard the delivery of loaded growth factors.

Alternatively, degradable polymers that give way to bone regeneration during resorption can be used as slow release systems for bone morphogenic proteins. Incorporation of growth factors into the polymer has been accomplished using organic solvents to liquefy the solid polymer and by gas foaming. As the former approach may be associated with residual amounts of solvents the later appears to be preferable. Gas foaming of amorphous poly-DL-lactic acid with incorporation of BMPs has shown controlled release from the porous implants that induced alkaline phosphatase *in vitro*. *In vivo*, bone formation was induced in heterotopic sites in the gluteus muscle of rats and bone regeneration was found in critical size defects in rat mandibles. This approach could help to provide a more controlled manner of growth factor delivery and thereby reduce the amount of growth factor that is required to induce bone regeneration.

Osteogenic cells

An alternative way to enhance bone regeneration is the use of osteogenic cells seeded onto biomaterials and implanted into skeletal defects. In adult human individuals these cells are most frequently derived from iliac crest bone marrow aspirates. Cytofacs analysis and magnetic bead sorting has shown that only a minor portion of aspirated cells are compatible with the surface markers of undifferentiated mesenchymal cells such as STRO-1. Extensive *ex vivo* expansion is, thus, required to obtain adequate numbers of cells for the repair of clinically relevant defects.

Despite a decade of experimental evaluation a number of unresolved questions remain. i) The expansion protocol has to prevent premature aging and differentiation during expansion. ii) Cell viability and proliferation is difficult to control during cultivation in three-dimensional scaffolds. iii) There is no agreement so far whether differentiated osteogenic cells or rather undifferentiated mesenchymal cells are preferable for implantation and subsequent bone formation *in vivo*. iv) It is largely unknown which parameters control the behaviour of cultivated human bone marrow stroma cells (hMSCs) after implantation in the *in vivo* environment.

Experimental evaluation of expansion protocols have shown that media using FCS or human serum tended to result in premature differentiation and growth arrest. Media using PDGF-BB and other growth factors have shown reliable expansion through a high number of passages. Short term vs. long term culturing as well as dynamic vs. static culturing of hMSCs both in organic and inorganic carriers porous carriers have not shown to result in significant differences in bone formation in critical size defects in athymic rats. Poor cell survival and inferior bone specific cellular activity after transplantation have to be considered as reasons which may result from premature differentiation and subsequent growth cessation and/or poor revascularization *in vivo*.

Future research has to focus on the combination of slow release scaffolds with growth factors and osteogenic cells that can improve vascularization and provide stabilization and/or stimulation of the seeded cells after implantation thereby enhancing the specific function of all three components of the engineered construct.