

STEM CELLS FOR MUSCULOSKELETAL REGENERATION

Stoddart M, Grad S, Gantenbein B, Verrier S and Alini M.

[AO Research Institute](#), AO Foundation, Davos, CH

The use of human stem cells to regenerate damaged tissue within the musculoskeletal system has been already applied to treat several of them, such as bone and intervertebral disc. Mesenchymal stem cell based approaches for treatment of other tissues, like cartilage, meniscus, ligament and tendon are at the later stages of development.

Although, some encouraging results have been observed, the clinical application of mesenchymal stem cells is far from being a routine approach in current medical treatments. Several biological and clinical issues have still to be improved. Presently, we are tackling two major problems, one related to the heterogeneity of mesenchymal stem cell isolation procedures and the second, which is more clinically relevant, concern the use of mesenchymal stem cells for repairing large bone defect. Mesenchymal stem cells are normally isolated by adherence to plastic or by using antibodies that recognize specific cell surface molecules (i.e. CD 90). With the first approach, other cells will also adhere to the well of the plate and indeed only few percent of all the adherent cells could be considered mesenchymal stem cells. The use of a CD antibody is also confronted with problems related to the specificity of the antibody, when used to fish out a homogeneous cell population from a heterogeneous mixture of phenotypically different cells. In order to improve the isolation of committed (osteogenic or chondrogenic) mesenchymal stem cells, we have developed a GFP-vector that once transduced into the cells (Adenovirus) will produce green fluorescence upon differentiation towards a specific phenotype (osteogenic or chondrogenic). So, we will be able to isolate those committed cells and further study their behavior. We could test if a more committed and homogeneous cells population would be more efficient (to form bone or cartilage) than those presently used, which are dispersed and fill the presence of different type of cells. Autologous bone grafting is the current golden standard for the repair of large bone defects, despite drawbacks such as limited availability of grafting material and donor site morbidity. Possible alternatives like allografts or xenografts also have serious limitations; the risk of infections, possible immune reactions and ethical issues. Due to these problems, researchers in the area of bone repair have explored alternative solutions. Calcium and

phosphate based materials as well as polymer scaffolds have shown some interesting osteoconductive properties. Nevertheless, the lack of osteoinductive potential prevents the healing of large bone defects treated only with such alloplastic materials. Many studies have shown that the lack of osteoinductive potential of such scaffolds can be partly overcome by seeding mesenchymal stem cells (MSC) onto the scaffold prior to implantation. However, a major problem still remains, namely the insufficient vascularization of the central part of these large grafts (>4cm). Thus, one of the most limiting aspects in obtaining tissue-engineered bone suitable for repairing large bone defects is the inadequate bone vascularisation. We have therefore addressed the enhancement of endothelial progenitor cells (EPC) as one of the key mechanisms in autologous bone grafting. The means by which these progenitors for neovascularisation can be isolated and characterized have recently been described. However, one of the major obstacles preventing the clinical application is the time needed to expand the EPC in vitro in order to obtain the required cell numbers. Out of the hundreds of common culture media compositions specifically designed to effectively culture cells of endothelial lineage none seems to be powerful enough for the desired purpose. Furthermore, these media are not autologous, which would be ideal for clinical use. We have therefore investigated the "Platelet-released growth factors" (PRGF) cocktail as a possible autologous source for EPC expansion. Our results show that PRGF is a highly efficient growth medium for EPC in vitro expansion. Moreover PRGF maintains the endothelial differentiation capacity of EPC. Immunostaining and PCR analysis showed persistence of angiogenic markers on CD34+ and CD133+ cells up to 21 days of culture. In addition, the capacity to form a cellular network after expansion in PRGF medium indicates that the EPC/PRGF association could have a positive influence on the formation of a vascular network within bone tissue engineered constructs.