OP010 Clinically optimized cell culture conditions in combination with a new 3D scaffold for human mesenchymal stem cell bone tissue engineering

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Objectives

Skeletal tissue loss due to congenital defects, disease and injury is normally treated by autologous tissue grafting. However, this method is limited by the availability of the host tissue, harvesting difficulties, donor site morbidity and the clinician's ability to manipulate delicate 3D shapes.

Therefore, the generation of autologous bone grafts *in vitro* avoiding the harvesting of autologous tissue at a second anatomic location is the ultimate goal in bone tissue engineering.

We have developed a protocol for the isolation and culture of adipose tissue-derived mesenchymal stem cells (AT-MSCs), which fulfils the strict European regulations concerning Advanced Therapy Medicinal Products. AT-MSCs were grown inside a new 3D scaffold developed by Industrie Biomediche Insubri (Switzerland). This matrix is a composite material based on bovine bone grafts, biodegradable polymers and bioactive agents. The bone grafts allow an adequate 3D structure to be maintained, the biopolymers permit good mechanical characteristics to be achieved and bioactive agents promote cell adhesion and proliferation. The differentiation of the AT-MSCs into osteogenic cells was triggered by a serum-free induction medium without the use of growth factors.

Materials & methods

Adipose tissue-derived mesenchymal stem cells were obtained from liposuction aspirates, cultured and expanded. After seeding the AT-MSCs into the scaffold, the cells were cultured for 2 weeks in the presence of a defined osteogenic induction medium which was optimized in our laboratory. The fixed scaffolds were cut into slices and examined by ESEM imaging in order to evaluate the morphology, spreading and adhesion properties of the cells. The ability of the cells to properly differentiate was explored by immunohistochemical techniques with typical markers of osteogenic lineage. A reverse-transcription PCR assay was performed to search for the expression profile of genes specifically involved in osteogenic differentiation, stem cell genes and genes involved in the cell cycle.

Results

Our composite scaffold, in combination with our defined culture conditions, is very suitable for the growth and differentiation of AT-MSCs into osteogenic cells.

Conclusions

This strategy involving cellular and biopolymeric components generating an *in vitro* bone graft appears to be very promising for the development of clinical protocols for the treatment of bone loss or injury.

Acknowledgements

The authors wish to thank the Department of Chemistry, Materials and Chemical Engineering, Politecnico Milano, Italy, for environmental scanning electron microscope imaging.