ISOLATION AND CULTURE OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN GMP QUALITY CONDITIONS FOR ITS FURTHER APPLICATION IN TISSUE ENGINEERING

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Introduction

In recent years, the clinical application of stem cells in tissue engineering and regeneration including the treatment of bone and cartilage defects is becoming more significant. Adipose tissue (AT) represents an abundant and accessible source of stem cells including mesenchymal stem / stromal cells (MSCs) and may be a starting material for isolation of AT-derived MSCs (AT-MSCs). MSCs can differentiate into multiple tissues including bone, cartilage etc. It has been demonstrated that upon intra-articular administration into injured joint, MSCs induce cartilage replacement and were detected in the newly formed tissues. However, growing evidence has recently indicated that the paracrine activity of the transplanted MSCs might be predominantly responsible for stimulating pro-regenerative effects in a place of transplantation including the cartilage repair. For clinical applications, AT-MSCs must be manufactured in accordance with Good Manufacturing Practice (GMP). Directly prior to intra-articular administration, AT-MSCs can be mixed with some biopolymers which can serve as a carrier creating 3D environment mimicking stem cell niches.

Materials and Methods

The human adipose tissue were collected during liposuction procedure from subcutaneous area of human donors. The procedure of AT-MSCs isolation and culture were optimized during a series of conducted experiments. All materials and reagents used for manufacturing of AT-MSCs were sterile and GMP quality or of equivalent standard. Morphology of adherent cells were analyzed by phase-contrast microscopy. Identification of these cells were carry out in accordance with the International Society for Cellular Therapy position statement paper. To evaluate potential application of AT-MSCs in regeneration of damaged cartilage, cells were differentiated into chondrocyte under high pressure (2 PSI, 5 PSI) and low oxygen (5%) conditions using Avatar Cell Control System. In the last step, we designed the composition of a carrier solution for AT-MSCs enhancing viability and functionality of these cells before and after application into site of injury.

Results and Discussion

Based on our translational research we selected the optimal condition for AT-MSCs isolation and their propagation *in vitro* using materials and reagents compliant with GMP. The cultured cells were confirmed as MSCs based on appropriate identity and phenotype assessed accordingly to specific criteria provided by the International Society for Cellular Therapy. We have shown that isolated and expanded adherent fraction of AT-MSCs maintained in standard culture conditions, express following antigens: CD44, CD73, CD90, CD105 (characterising MSCs) and do not possess CD45, CD34, CD14, CD19, HLA-DR antigens on their surface. Moreover, we have also demonstrated their capacity for trilineage mesenchymal differentiation (to osteoblasts, chondroblasts/ chondrocytes and adipocytes) *in vitro*. Moreover, the potential to differentiate into chondroblasts/chondrocytes was also confirmed in microenvironment resembling conditions in joints including appropriate oxygen concentration and pressure by employing a novel platform for cell culture such as *Avatar System* allowing for tuneable control of oxygen level and hydrostatic pressure (1-21% O_2 and 1-5 PSI, respectively). Our data suggest that AT-MSCs differentiate into chondrocyte-like cells in standard culture conditions (21% O_2; 1 PSI) and in the microenvironment resembling joint niche (5% O_2, 2 PSI). In the next step, we developed the optimal composition of medium for preparing of cell suspension for injection, which allows maintaining high viability of AT-MSCs before and after application into site of injury.

Conclusions

We successfully optimized efficient protocol for isolation and culture of AT-MSCs using GMP- grade reagents and materials. The optimized procedures for validation of the manufacturing process in GMP facility have been also established. During the validation of the manufacturing process accordingly to these procedures, the safety profile of the product will be assessed by analyses of genotypic stability, tumorigenicity, phenotypic profile and multipotent differentiation potential.
AT-MSCs due to their ability to differentiate into chondrocytes and secretion of wide range of bioactive factors can be effectively use in tissue engineering. AT-MSCs exhibit properties for “regenerating, repairing or replacing a human tissue” and can be classifies as “tissue engineered product” (According to Reg. 1394/2007).

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