GMP COMPLIANT ISOLATION AND CULTURE OF HUMAN ADIPOSE TISSUE- DERIVED MESENCHYMAL STEM CELLS FOR APPLICATIONS IN TISSUE ENGINEERING

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Introduction

Tissue engineering refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. For clinical applications, all the component must be manufactured in accordance with Good Manufacturing Practice (GMP) [1]. Subcutaneous human adipose tissue (AT) is an abundant source of mesenchymal stem/ stromal cells (MSCs). In comparison to bone marrow-derived MSCs, AT-MSCs occur at a 100–1000-fold higher frequency within adipose tissue on a volume basis [2]. MSCs can differentiate into multiple tissues including bone, cartilage, muscle etc. Moreover, it has been proposed that the functional benefits observed after MSCs transplantation in experimental models of tissue injury might be also related to the secretion of soluble factors acting in a paracrine fashion *e.g.* exhibiting immunosuppressive properties [3]. Thus, the goal of this study was to optimize isolation and culture methods of MSCs derived from adipose tissue in accordance with GMP rules for further applications in tissue engineering.

Materials and methods

Adipose tissue were obtained during liposuction from adult young male and female donors. To get rid of erythrocytes and other decontaminations, adipose tissue were washed several times in PBS (w/o Ca^{2+}, Mg^{2+}) supplemented with antibiotics and antimycotic. Then, after enzymatic digestion using GMP- grade collagenase stromal vascular fraction (SVF) were isolated and seeded onto cell culture flasks in three commercially available GMP-grade cell culture media (serum- free, animal component free). After removal of non-adherent cells, adherent fraction (AT-MSCs) were expanded until 5 passage. Morphology of adherent cells were monitored by phase- contrast microscopy. Identification of these cells were carry out in accordance with the International Society for Cellular Therapy (ISCT) position statement paper on antigenic profile and multipotency differentiation potential [3]. During optimization process, different concentrations of enzyme, culture media as well as culture conditions were examined. 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 *in vivo* study, selected dose of AT-MSCs were suspended into hyaluronic acid and injected to the injured pigs' knee to evaluate regenerative potential of these cells.

Results and Discussion

SVF with viability greater than 95% were obtained after isolation step. The yield of isolation were high and comparable between isolations (2,1 x 10^5 cells/ 1g of lipoaspirate). We selected the optimal enzyme concentration as well as the best culture medium. The kinetics of growth and proliferation of AT-MSCs were also comparable between isolations. The isolated cells: 1) were plastic- adherent when maintained in standard culture conditions; 2) possess phenotype characteristic for MSC cells ($CD45^-$, $CD34^-$, $CD14^-$, $CD11b^-$, $CD19^-$, HLA-DR-, $CD105^+$, $CD44^+$, $CD73^+$, $CD90^+$); 3) exhibit ability to differentiate into adipocytes, chondrocytes and osteoblasts; confirming their identity according to ISCT recommendations. Moreover, AT-MSCs can effectively differentiate into chondrocytes when culture under high pressure and low oxygen conditions mimicking the condition observed in the knee. AT-MSCs suspended into transport medium improving their viability, can be then suspended into fluid carrier such as hyaluronic acid and use in tissue engineering.

Conclusions

We successfully optimized efficient protocol for isolation and culture of AT-MSCs using GMP- grade reagents and materials as well as define interoperation control and release criteria. 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.

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