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Research Paper: Comparing the Effects of Human and Fetal Bovine Serum on Mesenchymal Stem Cells Under Oxidative Stress

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ABSTRACT

Objectives: Stem cells are undifferentiated cells capable of creating different types of cell in the body. Stem cell proliferation often is performed in the culture medium supplemented with Fetal Bovine Serum (FBS). Unknown compounds in the FBS, risk of contamination and disease transmission encourages the researches toward finding an alternative to FBS. Several factors are involved in the Mesenchymal Stem Cells (MSCs) precocious death in the transplanted tissue environment. Oxidative Stress (OS) is one of the main causes of stem cell apoptosis in the initial days after transplantation. The aim of this study was to evaluate the effect of Human Serum (HS) on the viability and oxidative related enzymes in human Adipose tissue-Derived Stem Cells (ADSCs) under oxidative stress in comparison with FBS.

Materials & Methods: Human serum were obtained from blood of a healthy donor persons, in respective intervals during few days. The ADSCs were isolated from lipolysis operation samples and their cuture media were supplemented with FBS or HS and different concentrations of H_2O_2 as the oxidative agent.

Results: The results showed that cell proliferation and viability of ADSCs under oxidative stress condition was significantly higher in the culture medium supplemented with HS in comparison with FBS supplemented medium ($P<0.05$).

Conclusion: This study showed that FBS could be replaced by HS in MSC culture medium with improved effects on cell proliferation and oxidative related enzyme activity under oxidative Stress condition.

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1. Introduction

Stem cells are undifferentiated cells that do not committed for any tissue specific function and under physiologic or controlled in vitro conditions can differentiate to the other cell types [1]. Mesenchymal Stem Cells (MSCs) are under intense studies for clinical cell therapy because of their availability, immunosuppressive properties and ability to differentiate into many cell types [2-5]. MSCs, based on International Society for Cellular Therapy (ISCT) definition, should have three characteristics. They are adherent cells, have adipogenesis, osteogenesis and chondrogenesis ability, expressing CD73, CD90 and CD105 surface marker while are negative for CD34 and CD45 [6, 7]. MSCs derived from adipose tissue can provide an accessible source for autologous transplantation and overcome the rejection of transplanted stem cells [8].

Culture media are often supplemented with Fetal Bovine Serum (FBS) as an essential component which mainly provides growth and adherence factors for stem cells [9-12]. However, variations in each FBS preparation, available unknown compounds and risk of virus and prion transmission strongly limited research and clinical use of proliferated MSCs [13, 14]. FBS also contains non-human antigens, such as N-glycolylneuraminic acid, which is an obstacle for its application in clinical cell therapy [15]. Human Serum (HS) has been introduced as an alternative for FBS that provide the required proteins stem cells with no risk of disease transmission and rejection [8, 14, 16].

After transplantations cells usually encountered an inappropriate environment, such as Oxidative Stress (OS), which leads to extensive cell deaths [17, 18]. Oxidative stress is also known as a cause for cell senescence and chronic age-dependent diseases [19] as well as a main mechanism in cell and tissue injury [9]. Considering the importance of cell viability after transplantation, the present investigation aimed to study the effect of HS and FBS on adipose-derived MSCs proliferation and osteogenic differentiation in the presence of oxidative stress.

2. Materials and Methods

ADSC isolation and characterization

After obtaining written informed consent, liposuction samples were collected from patient in a sterile condition and transfer to the laboratory. Blood and debris were washed off from the samples by rinsing in sterile PBS containing peniciline/ streptomycine (shelmax) and tissues sectioned in 1 mm pieces and then digested for around 30 minutes in 37°C water bath with 0.075% collagenase type I solution (GIBCO). The digesting tissues were shake each 5 minutes during incubation period, the final digested tissues were devided into 15 ml tubes and collagenase was neutralized by addition peniciline/ streptomycine, Dulbecco's Modified Eagle Medium, DMEM (Gibco) supplemented with 10% HS or FBS. Cultured cells were incubated in humidified incubated and 5% CO_2 atmosphere.

Cultured cells were inspected under the invert microscope every day and at 70-80% confluency, ADSCs were trypsinized by 0.25% Trypsine/EDTA solution and passaged into new cell culture flasks in 2:1splitting ratio. Isolated cells at passage 3-4 were tripsinized and suspended in freezing medium (1:10 DMSO:FBS) and then stored at -80°C until use. ADSCs were characterized by evaluating the expression of cell surface markers CD34, CD90 and CD105 using flowcytometry (BD Co.).

To assess the multipotency, osteogenic differentiation was induced in isolated ADSCs. Osteogenesis was induced by differentiation medium containing 1 µM Dexamethasone and 50 µg/ml Ascorbic acid-2 phosphate. Histochemistry by Alizarin Red was used to stain the calcium deposition in differentiated cells.

Serum preparation

In this study, around 90 ml blood sample were collected periodically from each healthy volunteer after obtaining informed consent. Each blood sample was centrifuged for 10 minutes in 2500 RPM, serum layer collected and incubated in 56°C for 30 minutes to inactivate the complement system. Serum samples were stored at -20°C until use. FBS were purchased (life technologies) and inactivated as mentioned earlier.

ADSCs proliferation under oxidative stress

The number of viable MSCs was determined by staining with Trypan blue and 5×10^4 cells/well cultured in 4 well plates with DMEM supplemented by 10% HS or FBS. After 24 hours media were replaced and 0, 50, 150 and 300 micromolar H_2O_2 added to each well. After one week, cells in 2 independent wells were counted for each HS or FBS treatment by Haemocytometer method to evaluate their proliferation.

Lipid oxidation assay

The amount of Malondialdehyde (MDA) was measured in cell supernatant as recommended by the kit (Zellbio) which

provides a standard method for assessment of lipid peroxidation in biological samples, such as serum, plasma, urin, homogenized tissues, cell lysates and supernatants. Briefly, in this colorimetric method, MDA (at 90-100°C) is combined with Thiobarbituric Acid (TBA) and produce a pink color which then were quantified by reading the optical densities at 530-540 nm using microplate reader (Stat Fax).

Antioxidant activity assay

Superoxide Dismutase (SOD) activity were measured by Zellbio kit in cell supernatants. This method is a colorimetric assay which measures the optical density of produced color at 420 nm at time 0 and 120 seconds after SOD interaction on its substrate, anion superoxide $2O^{2-}$. To measure the enzyme activity the following formula was used as recommended by the kit:

SOD activity (U/mL)=(VP–VC)/(VP)×60

VP=OD sample 120s–OD blank 120s

VC=OD sample 0s–OD blank 0s

Statistical analysis

Triple repeats were performed for each experiment, other than mentioned in the text. ANOVA and t-test were used for statistical analysis.

3. Results

ADSCs isolation, characterization and proliferation

Replacing FBS with HS led to the isolation of ADSCs with the same spindle shape morphology (Figure 1). Flowcytometry analysis showed that isolated cells were positive for CD90, CD105 and negative for CD34 which corresponded with the mesenchymal stem cell surface markers (Figure 2). Alizarin Red staining revealed the calcium deposition in ADSC culture after osteocyte differentiation (Figure 3). At day 7 after isolation in the first passage of ADSCs, HS resulted in higher number of cells compared to FBS, P<0.001 (Figure 4).

Figure 1. Spindle shape morphology of isolated ADSCs

Proliferation under oxidative stress

After 24 hours under different H_2O_2 concentration, ADSCs were counted. The results showed the concentration dependent adverse effect H_2O_2 on cell viability in both HS and FBS groups (Figure 5). HS showed more protective effect on ADSCs in oxidative stress conditions.

Lipid peroxidative assessment

MDA was measured in cell supernatant as an index for lipid peroxidation after addition of H_2O_2 and the results were shown as nM/ml (Figure 6). In both groups MDA measurements were increased by increasing the H_2O_2 concentration. No significant differences were observed between HS and FBS supplemented culture media.

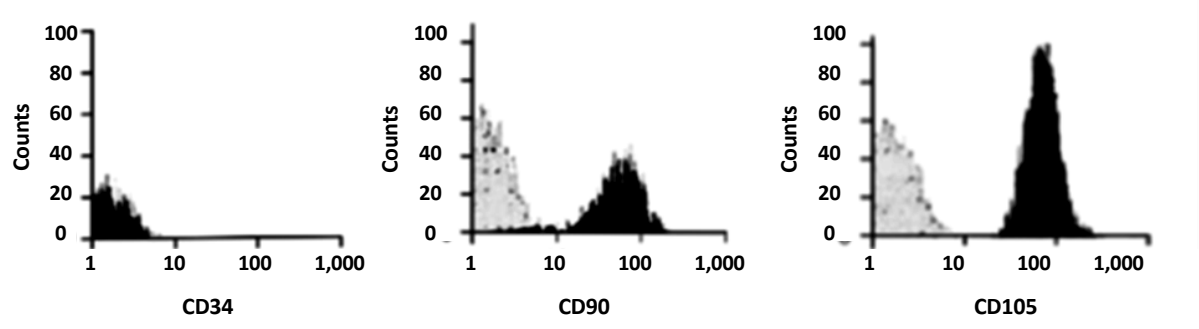

Figure 2. Cell surface marker expression pattern of isolated ADSCs

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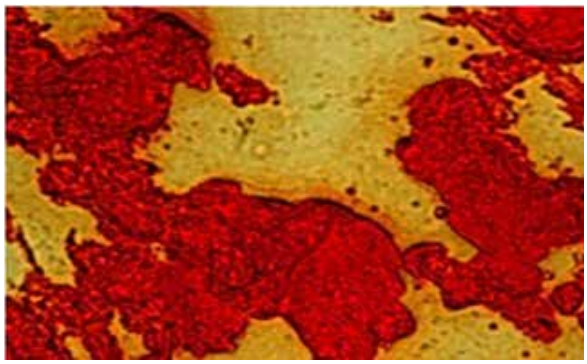

Figure 3. Alizarin red staining for osteogenesis differentiation

Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was measured as an index of antioxidant effects of HS and FBS. Optical densities showed a direct correlation between H_2O_2 concentration and SOD activity in both groups (Figure 7).

4. Discussion

MSCs, as one of the most studied type of stem cells, have proven their therapeutic effects in researches and clinical trials [3, 20-23]. Stem cell therapy often encounters reduced viability after implantation in the injured tissues. Microenvironment of injured tissues is far from the optimized conditions for cell survival, depending on the site and type of injuries [2, 9]. Among disturbing factors, oxidative stresses have a major role in cell apoptosis and any method that promote cell viability and proliferation could be valuable [17, 18, 24]. H_2O_2 has been usually used to stimulate oxidative stress in cell cultures in vitro [9]. In the current study, using H_2O_2, the oxidative condition was mimicked and HS hypothesized to be able to diminish the adverse effects of H_2O_2.

Based on our results, replacing FBS with HS were shown no harmful effect on lipid peroxidative and superoxide dismutase activities. Moreover, HS were more effective for preventing the dose dependent adverse effects of H_2O_2 on cell viability and proliferation compared to FBS. HS potential of replacing FBS in isolation of ADSCs, collectively increased the cell yield, as well as high cell doses is needed for clinical uses and cell therapies [11]. Although more in vivo in investigation is needed but it can be suggested that FBS could be replaced successfully with HS in vitro as the growth supplement in ADSCs media which help the cells to tolerate harsh oxidative stress.

It is also notable that any xeno product, such as FBS, is a point of debate for using in cell therapies because of the batch to batch variation and the risk of protein and

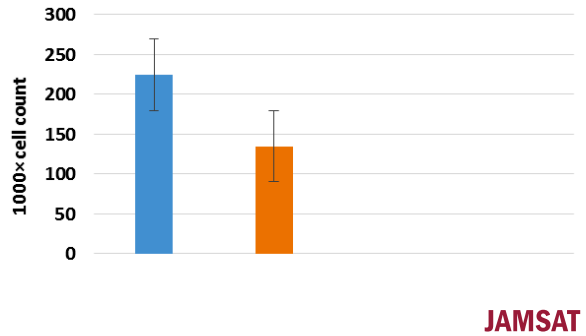

Figure 4. ADSCs isolation yield

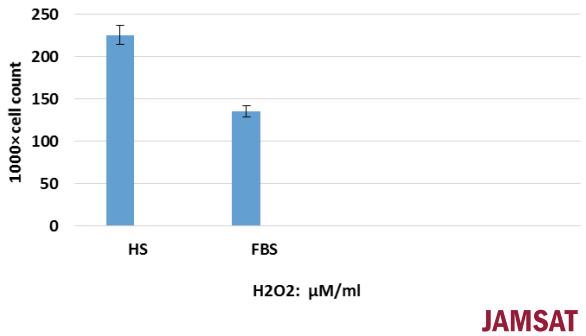

Figure 5. ADSCs proliferation under different concentrations of H_2O_2

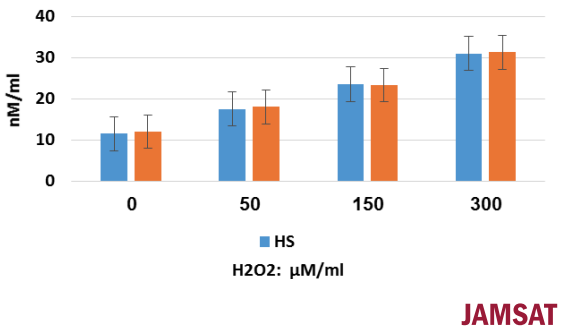

Figure 6. Malondialdehyde activity different concentrations of H_2O_2 in the presence of FBS and HS

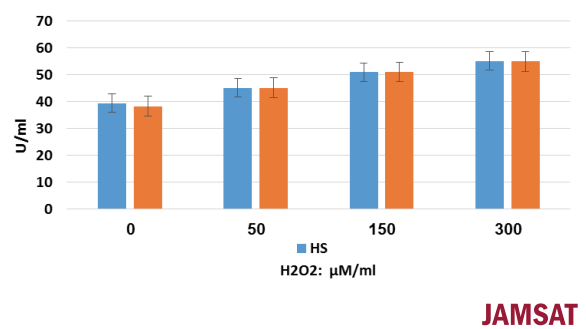

Figure 7. Superoxide dismutase activity at different concentrations of H_2O_2 in the presence of FBS and HS

diseases transmission [10, 13, 14]. Moreover, serum free media may burden high expenditures on in vitro and in vivo researches.

5. Conclusion

Considering above concerns, HS worth to survey more intensively and can also be recommended an exchangeable supplement with FBS in future studies with more beneficial effects on stem cell yield, especially in the presence of oxidative agents.

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Conflict of Interest

The authors declared no conflicts of interest.

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