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Comparison between human cord blood serum and platelet-rich plasma supplementation for Human Wharton's Jelly Stem Cells and dermal fibroblasts culture

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ABSTRACT

We carried out a side-by-side comparison of the effects of Human cord blood serum (HcbS) versus embryonic PRP on Human Wharton's Jelly Stem Cells(hWMSC)and dermal fibroblasts proliferation. Human umbilical cord blood was collected to prepare activated serum (HCS) and platelet-rich plasma (CPRP).Wharton's Jelly Stem Cells and dermal fibroblasts were cultured in complete medium with10% CPRP, 10%HCSor 10% fetal bovine serumand control (serum-free media).The efficiency of the protocols was evaluated in terms of the number of adherent cells and their expansion and Cell proliferation. We showed that proliferation of fibroblasts and mesenchymal stem cells in the presence of cord blood serum and platelet-rich plasma significantly more than the control group ($p \leq 0/05$). As an alternative to FBS, cord blood serum has been proved as an effective component in cell tissue culture applications and embraced a vast future in clinical applications of regenerative medicine. However, there is still a need to explore the potential of HCS and its safe applications in humanized cell therapy or tissue engineering.

Keywords: Human fibroblasts, Wharton's Jelly Stem Cells, Human cord blood serum, Human umbilical cord PRP, proliferation.

INTRODUCTION

The standard procedure for in vitro and ex vivo cell, tissue and organ cultures is based on supplementing cell culture media with fetal bovine serum (FBS). FBS contains essential components such as a large number of growth factors, hormones, vitamins, minerals, and extracellular matrix molecules that enhance cell attachment to plastic surfaces as well as cell proliferation and differentiation[1].

Theuse of FBS in cell cultures and tissue engineering involves both ethical and scientific concerns. FBS cultures are associated with possible allergic reactions caused by FBS proteins internalized in the stem cells and risks of transmitting bovine-viral and bacterial contamination, notably the mycoplasma infections [2-4]. Thus alternatives for FBS are needed.

Several investigators have explored the possibility of cell culturing in media containing alternative supplements to FBS. Among these, there are human blood derived alternatives such as autologous human serum (auto HS), allogeneic Human Serum (alloHS), platelet rich plasma, plasma from umbilical cord blood, umbilical cord blood serum and autologous plasma derived from bone marrow (AP)[5-9].

During pregnancy, cord blood as a lifeline of nourishment is delivered from mother to fetus. With the nutrition in cord blood, the embryo evolves from the fertilized egg into a mature individual form. In HCS, the content of growth factors is higher than adult blood serum, as some researchers have reported. For example, the levels of erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), and colony stimulating factor 1 (CSF-1) in HCS were higher than that in adult serum[10-12]. The content of stem cell factor (SCF), interleukin-3 (IL-3), and IL-6 in HCS was also higher[13, 14]. These factors can promote stem cells to grow. In cord blood, T and natural killer (NK) cells are naive and not primed for activation growth factor absent in cord blood, which is present in adult serum[15].

Platelet-rich plasma (PRP) contains increased levels of growth factors (GF) in their biologically determined ratios, including platelet-derived growth factor (PDGF), transforming growth factor-beta(TGF-b), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF), as well as the plasma components fibrin, fibronectin, and vitronectin[16-18].PRP plays an important role in the repair process types of cells, such as osteoblasts, fibroblasts, epithelial cells, endothelial cells and adult mesenchymal stem cells, on a large number of patients [17, 19, 20].

Fibroblasts are critical in supporting normal wound healing, involved in key processes such as breaking down the fibrin clot, creating new extra cellular matrix (ECM) and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound[21]. Also, Human Wharton's jelly MSCs compared to other original MSCs, have many advantages including availability of plentiful and inexpensive source of cells, short doubling time, high capacity of proliferation, lower immunogenicity and safety[22-24].The effect of umbilical cord MSCs on wound healing in severe burns has been studied in animal models[23, 25, 26].

Therefore, in this study, HCS and embryonic PRP were selected as the supplement in cell culturing ex vivo, compared FBS, because of no direct side-by-side comparisons between FBS and them on hWMSCs and human dermal fibroblasts have been formally performed.

MATERIALS AND METHODS

Human sera preparation

Human Umbilical Cord blood was collected from 8 informed healthy mothers undergoing cesarean section and donors, respectively. The blood samples were kept for 3-4 h without anti-coagulants and allowed to clot and then centrifuged at 3500 r.p.m. for 10min, and pure sera were aliquot and stored at -20°C until use.

PRP Preparation

Platelet-rich plasma is synthesized from umbilical cord blood and concentration using a series of centrifugations. Cord blood were collected in lithium heparin–coated collection tubes and initially centrifuged at 350 g for 10 min to separate the red blood cell (RBC) portion from the platelet-rich plasma. Next, the platelets are pelleted by a hard centrifugation of buffy coat plasma at 1600 g for 10 min.

The upper layer of the RBC portion was included as the platelets containing the largest amount of growth factors, and hence having the greatest potential biological activity. The inclusion of this small RBC layer imparted a red tinge to the PRP, as previously reported [27].

Cell Culture

Human dermal fibroblast

Human fibroblast cells were isolated from human foreskin and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), antibiotics such as: streptomycin, penicillin incubated in 5% Co2 incubator at 37 ° C. Culture media was replaced every 3 days. When the cells' density sticking to the bottom of the flask reached to 70 - 80 percent, cells' passage was done using 0.25% Trypsin-EDTA solution. Cells from passages 3 and 4 were used.

HWMSCs from Wharton's jelly of umbilical cord

The obtained umbilical cords were washed with phosphate buffered saline (PBS, pH = 7.2) to remove the blood, minced into 2-mm^2 pieces and transferred to 10-cm^2 culture plates containing DMEM/ F12 supplemented with 10% FBS, penicillin (100µg/mL) and streptomycin (100µg/mL). The plates containing Wharton's gel were incubated at

5% CO_2, 37 ˚C and 95% of humidity. After reaching 70% to 80% confluence, adherent cells were harvested by 0.25% trypsin-EDTA (Gibco, Germany); a single cell suspension was used for subsequent experiments.

Cell Proliferation

The viability of cells is determined by MTT assay that this method is based on the succinate dehydrogenase enzyme activity in mitochondria of living cells that turns the yellow MTT solution into the insoluble purple formazan crystals, which can be then dissolved in DMSO and measured with ELISA plate reader. Approximately (1×10^5) cells were transferred to 96-well plate and incubated for 24 h at 37 ° C, then treated with different concentration of PRP. The volume of the well was 100 micro liters. Micro plates containing cell extract for 24 were incubated in the same conditions. 10ml solution of MTT (5mg/ml) was added to each well and were incubated for 3 hours. 100 ml DMSO was replaced with incubated MTT medium. Then the optical absorbance was measured at a wavelength of 570 nm with ELISA reader. Viability percentage of cells that is affected by the umbilical cord serum and PRP was calculated by dividing the absorbance of treated wells to the absorbance of control well and then multiplied by 100.The results (mean ± SEM) are expressed using SPSS software[28].

Results

All hWMSCs and fibroblasts cultures retained normal morphology. After only 4 days of incubation in the presence of 10% CPRP and HCS, fibroblasts and hWMSCs completely covered the surface of the wells, forming a dense layer of cells (Figure1).

The effect of FBS (10%), HCS (10%), CPRP (10%) and control (serum free medium)in Proliferation on hWMSCs is shown in fig. 2.Both HuS and PRP supported hWMSCs growth. Growth cells in 10% HuS and CPRP was better than of 10% FBS, But this difference was not significant. Cells cultured in control were growth arrested. The effect of FBS (10%), HuS (10%), CPRP (10%) and control in Proliferation on human dermal fibroblasts is shown in fig.3. As shown in Figure, Fibroblast cell proliferation in presence of 10% HuS and CPRP was better than of 10% FBS.

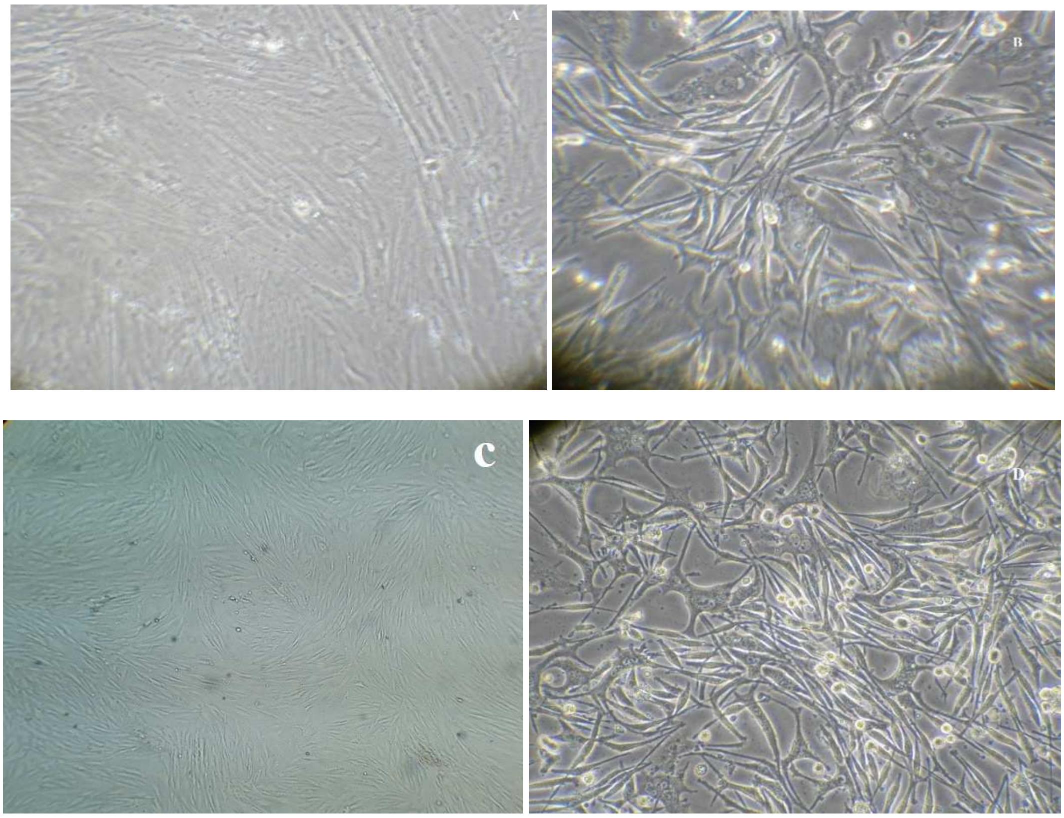

Figure 1. Human Wharton's jelly MSCs after 4 days of incubation with 10% FBS (A) and 10% HCS(C) and Fibroblast cells after 4 days of incubation with10% FBS (B) 10% HCS(D)

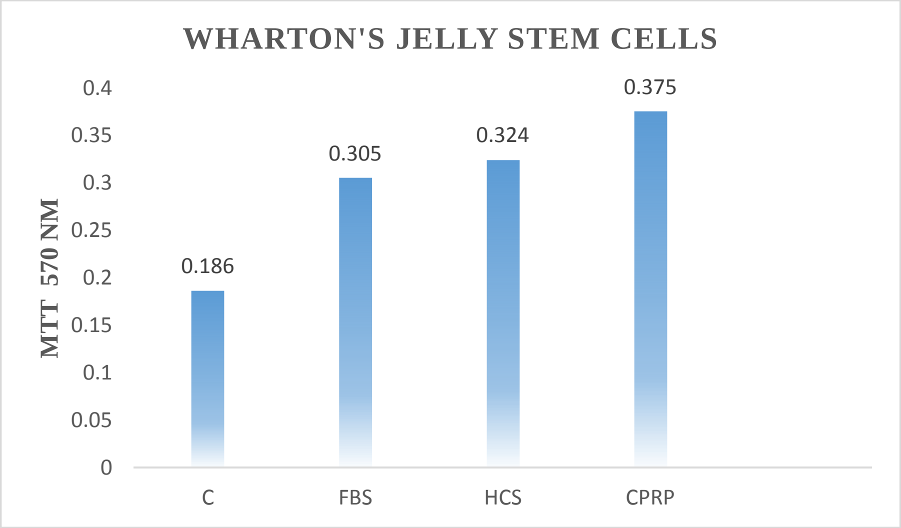

Figure 2.Comparison of the proliferation rate of Human Wharton's jelly MSCs (n = 4) cultured in the presence of fetal bovine serum orhuman umbilical cord serum and PRP.
C= Control; FBS = Fetal Calf Serum; HCS = Human Umbilical Cord Serum; CPRP= Umbilical Cord Blood PRP.

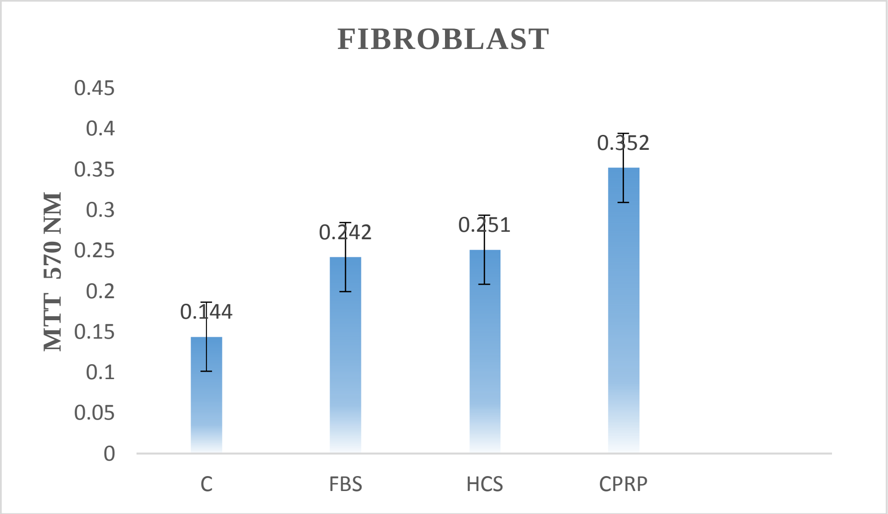

Figure 3.Comparison of the proliferation rate of humanfibroblasts (n = 4) cultured in the presence of fetal bovine serum or human umbilical cord serum and PRP.
C= Control; FBS = Fetal Calf Serum; HCS = Human Umbilical Cord Serum; CPRP= Umbilical Cord Blood PRP.

DISCUSSION

In our study we observed different morphological features of cells in CPRP and various supplemented sera. Primary dermal fibroblasts and human Wharton's jelly MSCs cultured in FBS, HCS and CPRP supplemented media dominantly were triangular in P0,whereas in late passages (P3) flat cells were more dominant. Flat cells attached to the dish more strongly in HCScultures than in FBS.

In this study we showed that HCS stimulates the proliferation of primary dermal fibroblasts and human Wharton's jelly MSCs in vitro. The MTT assay, which we used to measure the proliferation, is based on the reduction of MTT catalyzed mainly by mitochondrial enzymes (but also by a number of other non-mitochondrial enzymes). Also, the effects of CPRP on MSC and fibroblasts proliferation were evaluated in primary and secondary cultures. In primary culture, medium containing 10% CPRP, 10% HCS and 10% FBS significantly stimulated MSC and fibroblasts proliferation compared with that of control.

This study showed that HCS and CPRP are very similar to FBS which may be potentially used in cell therapy management, especially in the case of aged transplant patients, whose sera have typically insufficient levels of growth factors. Because the source of autologous a PRP was limited and the necessary number of CPRP and HCS for clinical application is high, we evaluated human embryonic serum and PRP incomplete medium, serum free medium as a control.

Human cord blood is a biological waste that is generally discarded after parturition. The placenta and the cord blood present therein nourish the developing fetus and are enriched with several growth promoting factors required for the cell/tissue culturing[29].The more interesting fact about the HCS is its striking natural resemblance of nutrients availability required for the cell/tissue culturing. Human HCS is much easier to isolate as compared to FBS and it is relatively free of animal based bacterial and viral pathogens. There are no strict ethical concerns on the isolation of HCS as this procedure is non-invasive and also does not pose any threat to the life of mother or fetus. Cord blood is collected immediately after the delivery of the baby and several routine tests are performed for screening of the blood, followed by room incubation for three to four hours to allow for the clotting. The serum is then was separated after centrifugation which was followed by heat inactivation and preservation at -20^{o}C for subsequent use [30, 31].

On the basis of detailed description of FBS being replaced with HCS, it can be concluded that HCS can be a better alternative of FBS as it has ample advantages over FBS in the form of ethical issues, surplus supply, non-xenogeneic and free from bovine induced pathogens[32]. HCS use in cell and tissue culture can cost efficiently and humanize the regenerative medicines and clinical applications of cell and tissue engineering[32].

The overview provided above highlights the liking of HCS by many investigators in in vitro culturing of stem/progenitor cells, epithelial cells and mature cells. Several lots of serum may be pooled for the purpose of general nutritional homogenization.

Our study contributes to this kind of knowledge because it shows the positive effect of HCS and CPRP similar FBS on the growth of human dermal fibroblasts and MSCs in vitro, therefore could be replaced it.

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