# RESEARCH ARTICLE



# Human Umbilical Cord Blood Serum Has Higher Potential in Inducing Proliferation of Fibroblast than Fetal Bovine Serum

Ferry Sandra<sup>1,2</sup>, Rita Lahirin<sup>3</sup>

**Background:** Cytokines and growth factors were reported to play an important role in stimulating fibroblast proliferation. *In vitro* culture, fibroblast is mostly culture in medium containing fetal bovine serum (FBS). Human umbilical cord blood (hUCB) has been reported to have low immunogenic property and potential in wound healing, so therefore hUCB serum (hUCBS) could be potential and were investigated in current study.

**Materials and Methods:** Five hUCBs were collected from healthy volunteers with normal delivering procedure. hUCB was *ex utero* immediately collected from umbilical vein in vacutainers and processed. NIH3T3 cells were cultured in DMEM with 10% FBS or 5-20% hUCBS for 48 hours. Cells were then quantified using MTT assay. Protein concentration of FBS and hUCBS were quantified using Bradford assay.

**Results:** NIH3T3 cells density grown in DMEM with 10% FBS was the lowest. NIH3T3 cells densities were increased along with the increment of hUCBS concentrations. MTT results showed that average number of NIH3T3 cells grown in DMEM with 10% FBS was  $6,185\pm1,243$ . Meanwhile average numbers of NIH3T3 cells grown in DMEM with 5%, 10% and 20% hUCBS were  $8,126\pm628$ ,  $9,685\pm313$  and  $12,200\pm304$ , respectively. Average numbers of NIH3T3 cells grown in DMEM with 5% hUCBS were significantly higher than the ones with 10% FBS (p=0.000). Bradford results showed that concentration of hUCBS was significantly higher than the one of FBS (p=0.000).

**Conclusion:** hUCBS could induce higher proliferation rate of NIH3T3 cells than FBS. Hence hUCBS could be suggested as an alternate of FBS in inducing fibroblast.

Keywords: NIH3T3, fibroblast, UCB, serum, FBS, proliferation

### Introduction

Fibroblasts are the cell type that can synthesize extracellular matrix and collagen, which plays an important role in wound healing.<sup>1,2</sup> Fibroblasts are cells were most

abundant in connective tissue.<sup>1,2</sup> Fibroblasts make collagen, glycosaminoglycan, reticular, elastin fibers and glycoproteins that are part of the extracellular matrix so that the increase of fibroblasts affect skin elasticity, muscle tone, bone strength and others where it is extremely

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important in preventing premature aging.<sup>1</sup> Cytokines and growth factors were reported to play an important role in stimulating fibroblast proliferation.<sup>3,4</sup> Meanwhile in *in vitro* culture, fibroblast is mostly culture in medium containing fetal bovine serum (FBS), which has plenty of cytokines and growth factors. Nevertheless, in searching for better supplement, FBS has been compared to various products, including human platelet lysate and serum.<sup>5</sup>

It has been confirmed that stem cell can induce and activate fibroblast to participate in wound healing by producing and expressing TGF- $\beta$  and other cytokines.<sup>6</sup> Therefore, stem cell were reported to be effective to promote re-epithelialization in burns.<sup>7</sup> Adult stem cells, which could be isolated from bone marrow<sup>8-10</sup>, umbilical cord blood<sup>10-12</sup>, adipose tissue<sup>9,13</sup>, peripheral blood<sup>10,14,15</sup>, skin and hair follicles, are being explored extensively to facilitate the healing of both acute and chronic wounds.<sup>7,16</sup>

Human umbilical cord blood (hUCB) has been reported to have low immunogenic property. <sup>16,17</sup> Therefore hUCB could be suggested as an alternative source of stem cells for allogeneic transplantation. <sup>16,17</sup> Beside in wound healing, secretome of hUCB stem cell was shown to induce apoptosis and inhibit growth of cancer cells. <sup>18</sup> Since hUCB was shown as a potential source, hUCB serum (hUCBS) were investigated in current study.

#### Materials and methods

# hUCB collection and hUCBS production

Five hUCBs were collected from healthy volunteers underwent normal delivery procedure at Mohammad Ridwan Meuraksa Military Hospital in Jakarta. Briefly, after volunteer mother signed informed consent and delivered baby, 50 mL hUCB was collected immediately *ex utero* from umbilical vein in 5 10mL-vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). Vacutainers were seated for 10 minutes and centrifuged. After complete separation resulted, hUCBS was collected and stored in a -20°C fridge. The study protocol was approved by The Ethical Committee of Faculty of Medicine Udayana University/Sanglah Central General Hospital (No. 570/Skrt/VIII/2010) and permitted by The Medical Committee of Mohammad Ridwan Meuraksa Military Hospital (No. SK/53-6/iV/2010).

# NIH3T3 cell culture

NIH3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA)

containing 10% FBS (Gibco), penicillin, streptomycin and amphotericin B (Sigma-Aldrich, St. Louis, MO, USA) in a humidified, 37°C, 5% CO2 incubator. Upon reaching 80% confluency, cells were detached with trypsin (Gibco), split and propagated.

# 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay

The MTT assay provides a quantitative measurement of viable cells by determining the amount of formazan crystals produced by metabolically active cells.  $^{19,20}$  Briefly,  $5x10^3$  cells were seeded into each well of 96-well plate in medium containing FBS, hUCBS or medium merely. Cells were incubated for 48 hours. Ten  $\mu$ l of 5 mg/mL MTT (Sigma-Aldrich) in phosphate buffer saline (PBS) was added to each well. The plate was then incubated for 4 hours, and then the medium was discarded and formazan crystals were dissolved in  $100~\mu$ l of 0.1~N~HCl. The absorbance was measured at 570 nm by a microplate reader (Bio-Rad, Richmond, CA, USA). Untreated cells were counted with a hemacytometer and used for interpolating the absorbance.

# Bradford assay

Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 dye to proteins. For this research, Quick Start™ Bradford Protein Assay (Bio-Rad) was applied. All procedures were conducted according to instruction manual. Briefly, bovine serum albumin (BSA) as the standard, FBS and hUCBS were serial diluted in a 96-well plate. Then, 1x dye reagent was added to each well and seated for 15 minutes at room temperature. The absorbance was measured at 595 nm by a microplate reader.

#### Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Macintosh version 21 (SPSS IBM, Armonk, NY, USA). Mann-Whitney test was used to compare two population means. Statistically significance is expressed as p<0.05.

### Results

# hUCBS induced higher proliferation rate of NIH3T3 cells than FBS

In Figure 1, NIH3T3 cells densities grown in DMEM with 10% FBS and various concentration of hUCBS could be

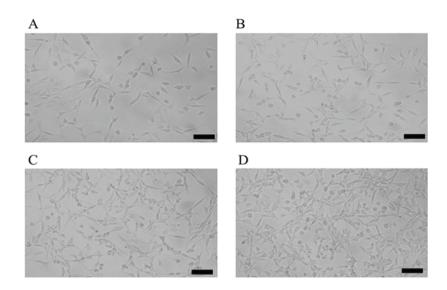
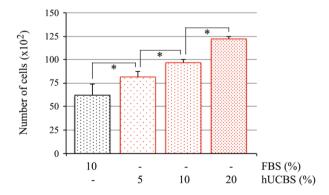


Figure 1. Growth of NIH3T3 cells in medium containing FBS or hUCBS. Five thousand NiH3T3 cells were seeded and cultured in 96-well plate with medium containing 10% FBS, 5% hUCBS, 10% hUCBS or 20% hUCBS for 48 hours. Cells were then documented using an inverted light microscope. Detailed procedures are described in "Materials and Methods". Each picture shows the typical result of 3 independent results. Black bar: 100 μm.

observed. NIH3T3 cells density grown in DMEM with 10% FBS was the lowest (Figure 1A). NIH3T3 cells densities were increased along with the increment of hUCBS concentrations (Figure 1B-D).

MTT results confirmed the densities results (Figure 2). Average number of NIH3T3 cells grown in DMEM with 10% FBS was  $6,185\pm1,243$ . Meanwhile average numbers of NIH3T3 cells grown in DMEM with 5%, 10% and 20% hUCBS were  $8,126\pm628$ ,  $9,685\pm313$  and  $12,200\pm304$ , respectively. Average numbers of NIH3T3 cells grown in DMEM with 5% hUCBS were significantly higher than the ones with 10% FBS (p=0.000). Numbers of NIH3T3 cells grown in DMEM with 10% hUCBS were significantly



**Figure 2. hUCBS induced proliferation of NIH3T3 cells.** Five thousand NiH3T3 cells were seeded and cultured in 96-well plate with medium containing 10% FBS, 5% hUCBS, 10% hUCBS or 20% hUCBS for 48 hours. Cells were then subjected to NIH3T3 assay. Detailed procedures are described in "Materials and Methods". This experiment was performed in triplicate and repeated three times. \*Mann Whitney test, *p*<0.05.

higher than the ones with 5% hUCBS (p=0.000). Numbers of NIH3T3 cells grown in DMEM with 20% hUCBS were significantly higher than the ones with 10% hUCBS (p=0.000).

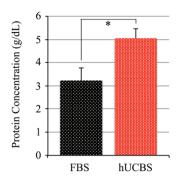
# Protein concentration of hUCBS was higher than FBS

As shown in Figure 3, protein concentration of FBS was  $3.23\pm0.54$  g/dL, while protein concentration of hUCBS was  $5.04\pm0.41$  g/dL. The concentration of hUCBS was significantly higher than the one of FBS (p=0.000).

### **Discussion**

Our current results showed that hUCBS induced proliferation of NIH3T3 cells in a concentration dependent manner. Although protein concentration of hUCBS was almost twice higher than the one of FBS, 5% hUCBS resulted significant higher average number of NIH3T3 cells than 10% FBS did. These results showed that the cell numbers resulted were not in a protein concentration dependent manner. hUCBS and FBS should have different protein components and hUCBS contained more potential components in inducing proliferation of NIH3T3 cells.

hUCBS has been reported superior in inducing proliferation of mesenchymal stem cell (MSC),<sup>21-24</sup> human bone marrow-derived progenitor cells,<sup>25</sup> human derived Panc-1 cell lines<sup>26</sup> and human foreskin fibroblasts<sup>27</sup> than FBS or fetal calf serum (FCS). Not only for cell growth, human pluripotent stem cell lines could maintain their pluripotencies, differentiation capacities, and karyotypic stabilities after being co-cultured for extended period with



**Figure 3.** Amount of protein in FBS or hUCBS. FBS and hUCBS used in this research were serial diluted and quantified with Bradford assay. Detailed procedures are described in "Materials and Methods". This experiment was performed in triplicate. \*Mann Whitney test, *p*<0.05.

hUCBS.<sup>24,27</sup> In addition, hUCBS cryopreservation has been performed extensively with modified methods.<sup>24,28</sup> Nevertheless, hUCBS is also useful when cells were induced for differentiation.<sup>22,25,26</sup> Regulatory effects of hUCBS on MSC included selective activation of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) signals in MSC.<sup>22</sup>

Proliferation of NIH3T3 cells could be regulated by several growth factors. EGF increased NIH3T3 cell proliferation along with the increment of EGF concentration until 25 ng/mL, which was the optimal EGF concentration to induce the maximum NIH3T3 cell proliferation.<sup>29</sup> EGF induced the NIH3T3 cell proliferation through Mitogen-Activated Protein Kinase (MAPK) / Extracellular signal-Regulated Kinase (ERK) signaling pathway,<sup>30</sup> which is a major signaling pathway involving cellular proliferation though Rat sarcoma (Ras) / Rapidly accelerated fibrosarcoma (Raf) / ERK cascade.<sup>31</sup> Another growth factor, PDGF, that was also contained in hUCBS,<sup>32</sup> were reported to induce phosphatidylinositol 3-kinase (PI3K) / Akt<sup>32,33</sup> and Ras/ ERK signaling pathways as well.<sup>32-34</sup>

In conclusion, our current results suggested that hUCBS might play an important role in proliferation of NIH3T3 cells, since hUCBS could induce higher proliferation rate of NIH3T3 cells than FBS. Hence hUCBS could be suggested as an alternate of FBS in inducing fibroblast. However, further research should be explored to test hUCBS for other fibroblast cell lines.

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