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Comparison of FBS and FBS substitute containing media in umbilical cord blood stem cell cultures: A preliminary study

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ABSTRACT

Aim: This study aimed to compare FBS and FBS substitute containing media in umbilical cord blood (UCB) derived stem cell culture.

Experimental: Processing of UCB was done by Ficoll gradient or simple centrifugation, which was followed by lysis buffer treatment. The pellets were then cultured in FBS and FBS substitute containing media.

Results: Attached cells were heterogeneous. There were fibroblastic cells and rounded cells of the macrophage-monocyte lineage. Ficoll gradient centrifugation and FBS containing DMEM, and simple centrifugation and VEGF and human AB serum supplemented DMEM yielded confluent cultures. However, after passage-1, the cells differentiated predominantly into osteoclast-like cells, cell growth was arrested, and further passage was impossible.

Conclusion: UCB is a poor source of MSCs, therefore, Ficoll gradient and simple centrifugation processing combined with FBS or FBS substitute containing media are not suitable methods for the isolation and culture of UCB derived MSCs.

KEYWORDS

Umbilical cord blood;
Mesenchymal stem cell;
Platelet rich plasma;
Human AB serum;
Osteoclast-like cells;
Ficoll gradient centrifugation.

INTRODUCTION

Umbilical cord blood (UCB) contains various kinds of stem cells, including hematopoietic and mesenchymal stem cells (MSCs). The mesenchymal stem cells are similar to the previously characterized bone marrow derived MSCs in terms of their surface antigens. UCB derived MSCs can be differentiated *in vitro* into chondrogenic, osteogenic, neural and hepatic lineages, and have higher proliferation potential compared to bone marrow derived MSCs[1]. Umbilical cord blood can be obtained easily, thus UCB derived stem cells are very promising for regenerative medicine.

Attempts to isolate UCB derived MSCs without separating them from hematopoietic stem cells showed variable results due to their low number. Moreover, pub-

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lished studies used fetal bovine/calf serum (FBS/FCS) containing media to culture them[2-4]. Fetal bovine/calf serum contains xenoproteins that may be internalized by the MSCs, and therefore, can not be removed even after several washings[5]. Xenoproteins may pose rejection problems, when the MSCs are transplanted to patients.

Studies on lipoaspirate and bone marrow derived MSCs showed that platelet rich plasma (PRP) was a good substitute for FBS/FCS[6-8]. Therefore, to know whether it is suitable or not in UCB derived stem cell culture, we compared FBS and FBS substitute (PRP and human AB serum) containing media in UCB derived stem cell culture.

EXPERIMENTAL

This is an experimental descriptive study that was done in the Integrated Laboratory, Faculty of Medicine Universitas Indonesia, from July to October 2012, and has got an approval from the ethical committee of the Faculty of Medicine University of Indonesia.

Sample

Umbilical cord blood was obtained from the Department of Obstetrics and Gynecology, from full term deliveries. The UCB was taken after the mother signed the informed consent form.

Sample processing

To get the cells from the UCB, two approaches were done. The first used Ficoll Paque Plus (GE health care) in a gradient centrifugation at 3000 rpm for 30 minutes, to obtain the mononuclear cells. The second used simple centrifugation at 1650 rpm for 10 minutes to obtain the mononuclear and polymorphonuclear cells.

Both kinds of cells from the two approaches were subjected to lysis buffer until the pellet was white in color, and viable cells were counted by tryphan blue exclusion method. Further, UCB smear and spot specimens were made from gradient and simple centrifugation derived cell suspension, and mononuclear/polymorphonuclear cell proportions were calculated.

Pellets from the two processing procedures were cultured in four kinds of media, i.e. MesenCult® (STEMCELL Technologies),10% FBS containing Dulbecco's Modified Eagle Medium (DMEM, Lonza), 10% PRP (Indonesian Red Cross Blood Bank) supplemented DMEM, and vascular endothelial growth factor (VEGF, Invitrogen) and 10% human AB serum (Gibco) supplemented DMEM, in a 12 well plate. All cultures were done in duplicate.

The cultures were observed daily under an inverted microscope. When the cells were attached, the medium was changed, and photographs of different kinds of attached cells were taken. However, when at day-5 the cells were not attached, half of medium was changed. Further, the medium was changed every 3-4 days.

After the cells became confluent, they were detached by TrypLE Select (Invitrogen) and sub-cultured in duplicate in the same medium with a seeding number of 10,000-12,000 cells/well. The medium of the subcultures (passage-1) was changed every 3-4 days, and the cultures were observed daily under an inverted microscope. Photographs of the different kinds of cells were taken.

Data collection and presentation

Data of the two approaches of cell suspension preparation were collected before culturing, i.e. mononuclear/ polymorphonuclear cell proportions, viable cell counts after centrifugation, and lysis buffer treatment, and seeding number. Data collected from primary cultures were the day when the cells were attached, the types of attached cells, and the day when the cells became confluence. From passages, the types of cells were noted. Further, the data were grouped and presented in Tables.

RESULTS

Two samples have been processed. The proportions of mononuclear/polymorphonuclear cells in UCB, gradient and simple centrifugation derived cell suspension of sample-2 were 46/54, 88/12, and 86/14 respectively. The amount of processed UCB, viable cell yield, and seeding number can be seen in TABLE 1, and the day when the cells were attached and the types of attached cells for the various approaches can be seen in TABLE 2.

In most instances, cell attachment occurred earlier in Ficoll gradient compared to simple centrifugation, and only sample-1 that was cultured in either 10%

FBS supplemented DMEM, or VEGF + human AB serum supplemented DMEM yielded confluence (Figure 1), while other cultures only showed cell attachment that did not become confluent. The attached cells were initially fibroblastic and mononuclear rounded cells (Figure 2), which later turned into rounded cells and large cells with multiple nuclei (osteoclast-like cells) (Figure 3).

TABLE 1 : UCB amount, cell yield, and seeding number in Ficoll gradient and simple centrifugation.

Sample	Processing	UCB amount (mL)	Viable cell yield after Centrifugation	Viable cell yield after Lysis buffer	Viable cell Seeding
1	FGC	22.5	35,900,000	21,950,000	2,550,000
	SC	24	121,600,000	49,760,000	6,000,000
2	FGC	12.5	Not counted	9,730,000	973,000
	SC	12.5	Not counted	4,605,000	460,500

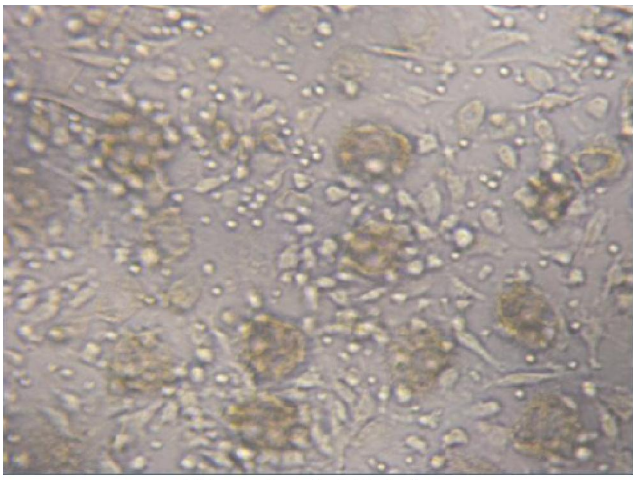
FGR= Ficoll gradient centrifugation, SG= simple centrifugation

TABLE 2 : The day of cell attachment and types of attached cells in the various approaches.

Proc	Medium	Sample	Day of cell attachment	Day of confluence	Types of attached cells
FGC	DMEM-10% FBS	S-1	Day -6	Day 10	Fibro, OLC
	MesenCult®	S-2	Day-5	N- conf	Fibro, OLC
	DMEM -10% PRP	S-2	Day-5	N-conf	Fibro, OLC
	DMEM-VEGF, huAB	S-2	Day-5	N-conf	Fibro, OLC
SC	MesenCult®	S-1	Day -3	Day-7 – CD	Fibro
	MesenCult®	S-2	Day-6	N Conf	Fibro, OLC
	DMEM-VEGF, huAB	S-1	Day-7	Day-11	Fibro, OLC
	DMEM-VEGF, huAB	S-2	Day-6	N conf	Fibro, OLC
	DMEM -10% PRP	S-1	Day-7	N-conf	Fibro, OLC
	DMEM -10% PRP	S-2	Day-6	N conf	Fibro, OLC
	DMEM-VEGF, huAB	Passage-1	Day-1	N-conf	Fibro, OLC

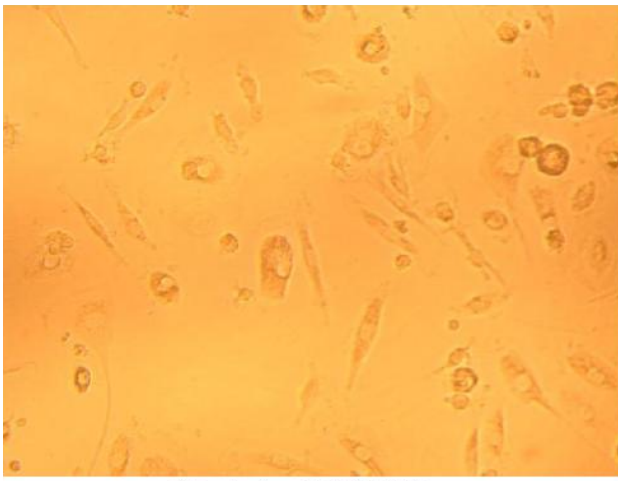
Proc= processing, FGR= Ficoll gradient centrifugation, SG= simple centrifugation, DMEM= Dulbecco's Modified Eagle Medium, FBS= fetal bovine serum, PRP= platelet rich plasma, VEGF= vascular endothelial growth factor, huAB= human AB serum, S-= sample-, N-conf= never confluent, CD= cells degenerated, fibro= fibroblastic cells, OLC= osteoclast-like cells

Compared to attached cells, the cells that were found in the supernatant and forming clones upon culture (hematopoietic stem cells) were much more abundant. For the attached cells, the culture that reach confluence (FGC – DMEM-10% FBS – sample 1 and SC- DMEM-VEGF, huAB–sample-1 (TABLE 2) showed heterogeneous types of cells, and upon passage yielded mostly osteoclast-like cells.



Unstained (400x)

Figure 1 : 80% confluent culture of sample-1 in VEGF and human AB serum supplemented DMEM.



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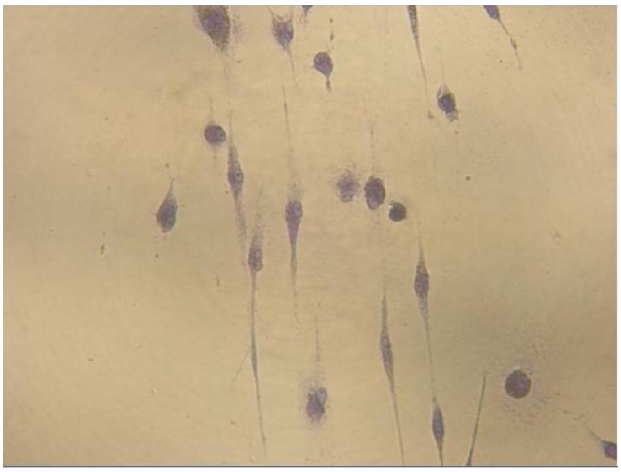

Giemsa Staining (100x)

Figure 2 : Initial cell attachment: fibroblastic and rounded cells.

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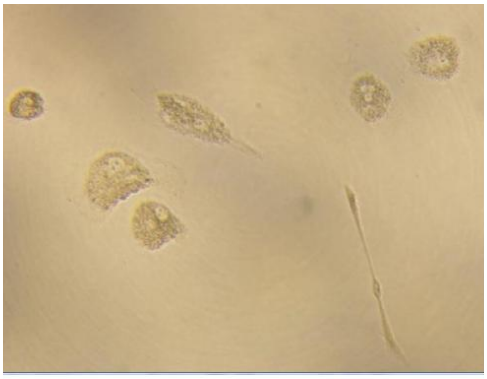

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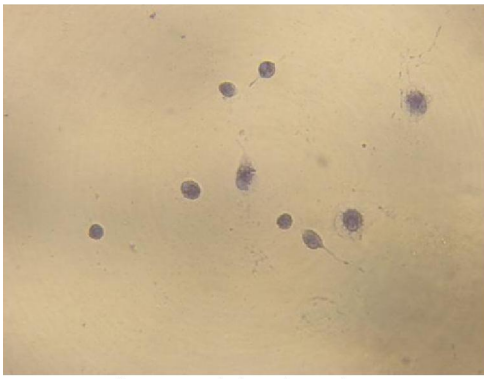

Giemsa staining (100x)

Figure 3 : Predominant round and osteoclast-like cells.

DISCUSSION

Umbilical cord blood is a rich source of hematopoietic stem cells, and very poor in mesenchymal stem cells. The frequency of MSCs in UCB ranges from 0-2.3 clones per 1x 10^8 mononucleated cells[2]. Therefore, to yield mesenchymal stem cells from UCB culture, special techniques to separate the mesenchymal stem cells from hematopoietic stem cells are required[9], which makes their isolation laborious and expensive[1]. Further, a study showed that the success rate of UCB derived MSC culture in FBS containing medium, which used ficoll gradient centrifugation as mononuclear cell isolation, was only 10%[10].

In our study, we used PRP containing media to culture the UCB derived MSCs, as there are no data about the use of PRP containing media for UCB derived MSCs. We used two UCB processing approaches before culturing. However, the results of both processing approaches were disappointing, and even the FBS containing media did not yield any MSCs. Our experience was shared by a study that used simple centrifugation as UCB processing before culturing, which yielded non confluent fibroblast-like cells with hematopoietic phenotype[11]. In sample-1, processing by simple centrifugation yielded more cells, compared to Ficoll gradient centrifugation. This result was in line with the result of Kawasaki-Oyama et al.[12]. However, sample-2 showed a different result. The result of sample-2 might be due to the lysis buffer treatment, which needed more treatment to get white pellet compared to sample-1.

In sample-2, simple and Ficoll gradient centrifugation yielded similar proportions of mononuclear/polymorphonuclear cells. This result suggests that simple centrifugation may yield high mononuclear cell proportion, after several lysis buffer treatments. We supposed that decantation of supernatant caused elimination of the polymorphonuclear cells. However, more samples are needed to get a reliable conclusion.

Another study that used Ficoll gradient and simple centrifugation before culturing the cells in FBS containing media showed that processing by simple centrifugation gave better results compared to Ficoll gradient centrifugation. Ficoll gradient centrifugation did not yield any confluent cultures from ten samples. On the other side, in simple centrifugation processing, three out of ten samples yielded confluent cultures, which can be passage several times during two to three months. Some of the attached cells were from macrophage-monocyte lineage, which appeared as big rounded cells with multiple nuclei (osteoclast-like cells)[12], as were found in our study.

ABBREVIATION

UCB	=	umbilical cord blood
MSCs	=	mesenchymal stem cells
FBS	=	fetal bovine serum
FCS	=	fetal calf serum
PRP	=	platelet rich plasma
DMEM	=	Dulbecco's Modified Eagle Medium
VEGF	=	vascular endothelial growth factor
FGC	=	Ficoll gradient centrifugation
SC	=	simple centrifugation

CONCLUSION

UCB is a poor source of MSCs, therefore, Ficoll gradient and simple centrifugation processing combined

with FBS substitute containing media are not suitable methods for the isolation and culture of UCB derived MSCs.

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