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Placental chorionic villus explant culture in xeno-free medium: A preliminary study

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ABSTRACT

Aim: to know whether placental chorionic villous-derived stem cells can grow in xenofree medium. In this study, we did explant culture of human chorionic villous in alpha minimal essential medium (MEM) supplemented with human AB serum. **Experimental:** The chorionic villous was washed briefly, and minced in complete alpha MEM medium, which contained penicillin/streptomycin, amphotericin B, 1% L-glutamine and 10% human AB serum. Two to three explants (diameter 2-3 mm) was placed in each well of a 12 well plate, and several drops of the complete medium were added. Further, the plate was incubated and when the explant attached to the base of the well, 0.5 mL fresh medium was added. Further, medium changes were done every 2-3 days. Observation was done daily to detect cell growth, and cell morphology. The day when fibroblastic cell growth and appearance of various morphologies of cells in each well was first detected was noted and tabulated. **Results:** Two types of cells were grown, i.e. fibroblastic and non-fibroblastic cells. The fibroblastic cells began to grow and attach on day-3 to day-5. The various morphologies of cells were apparent on day-5 to day-7. Long-term explant culture, more than four weeks, showed that hematopoietic stem cell derived osteoclast-like multinuclear non-fibroblastic cells were prominent and dominated the culture field. **Conclusion:** Placental chorionic villous explant culture can grow well in 10% human AB serum containing alpha MEM, but when washing is inadequate, the growing cells were dominated by osteoclast-like cells.

KEYWORDS

Placenta;
Chorionic villous;
Explant culture;
Human AB serum;
Osteoclast-like cells.

INTRODUCTION

Stem cells, which can be obtained from various tissues, are very promising for regenerative medicine. One of the various sources of stem cells is placenta[1]. As stem cell sources, placenta, along with umbilical cord tissue and blood have an advantage compared to other adult tissues. Moreover, as they are delivery wastes, their collection does not need invasive procedure, and their use does not pose ethical concern. Moreover, their supply is abundant in developing countries with high birth rate, such as Indonesia.

Various parts of placenta can be exploited, i.e. amniotic membrane that is the source of amniotic epithelial cells and amniotic mesenchymal stem cells (MSCs), and chorionic villous that is the source of chorionic trophoblastic cells and chorionic MSCs[1]. Various protocols have been developed to isolate and culture the MSCs from placenta, including explant[2,3] and enzymatic[4-7] methods. However, the various protocols used fetal bovine serum (FBS) supplemented medium.

Fetal bovine serum contains xenoproteins that can be incorporated into the cells, and may cause harmful immune rejection in recipient[8]. Therefore, this study used xeno-free medium combined with explant culture method to check whether placental chorionic villous explant derived stem cells could grow in xeno-free medium.

EXPERIMENTAL

This experimental descriptive study was done in Culture Laboratory of Stem Cell Medical Technology Integrated Service Unit, Cipto Mangunkusumo Central Hospital-Faculty of Medicine Universitas Indonesia, in June-July 2013. Ethical clearance for this study was obtained from the Ethical Committee, Faculty of Medicine, Universitas Indonesia. Full term normal placenta was obtained from Caesarean section, after the mother signed the informed consent.

Procedure

Two cotyledons of placenta were collected in 50 mL transport medium, which contained alpha minimal essential medium (alpha MEM [GIBCO 12000-022 1]), penicillin/streptomycin (final concentration 300U/mL [Gibco 15140-122]) and amphotericin B (final concentration 7500ng/mL [JR Scientific 50701]), and processed 3 hours after collection.

The chorionic villous was dissected and washed briefly in 0.5% betadine containing phosphate buffered saline pH 7.4 (PBS [Sigma P3813]), followed by washing in PBS to remove the blood. Further, the chorionic villous was minced in complete alpha MEM medium, which contained penicillin/ streptomycin (final concentration 100U/mL), amphotericin B (final concentration 2500ng/mL), 1% L-Glutamine (Lonza 17-605C), and 10% human AB serum (Gibco 34005-100). Two to three explants (diameter 2-3 mm) was placed in each well of a 12 well plate (growth area 3.8 cm2 [Biolite]), and several drops of the complete medium were added. Further, the plate was incubated in 37°C, 5% CO_2.

The cultures were observed daily to check whether additional medium was needed or not; and when needed, several drops of medium were added. When the explant attached to the base of the well, 0.5 mL fresh medium was added. Further, medium changes were done every 2-3 days.

Observation was done to detect cell growth, and the day when cell growth was first detected was noted for each well. Cell morphology was observed and photographed. The day when cell growth and appearance of various morphologies of cells in each well was first detected was noted and tabulated.

RESULTS

Observation of the chorionic villous explant showed red blood cell contamination. There were two major types of cells that moved out from the human chorionic

TABLE 1 : The appearance time of fibroblastic cells and various cell morphologies in each well

Well	Appearance of fibroblastic cells	Appearance of fibroblastic and non-fibroblastic cells
1	Day-3	Day-5
2	Day-4	Day-7
3	Day-4	Day-5
4	Day-5	Day-5
5	Day-4	Day-5
6	Day-3	Day-7
7	Day-4	Day-7

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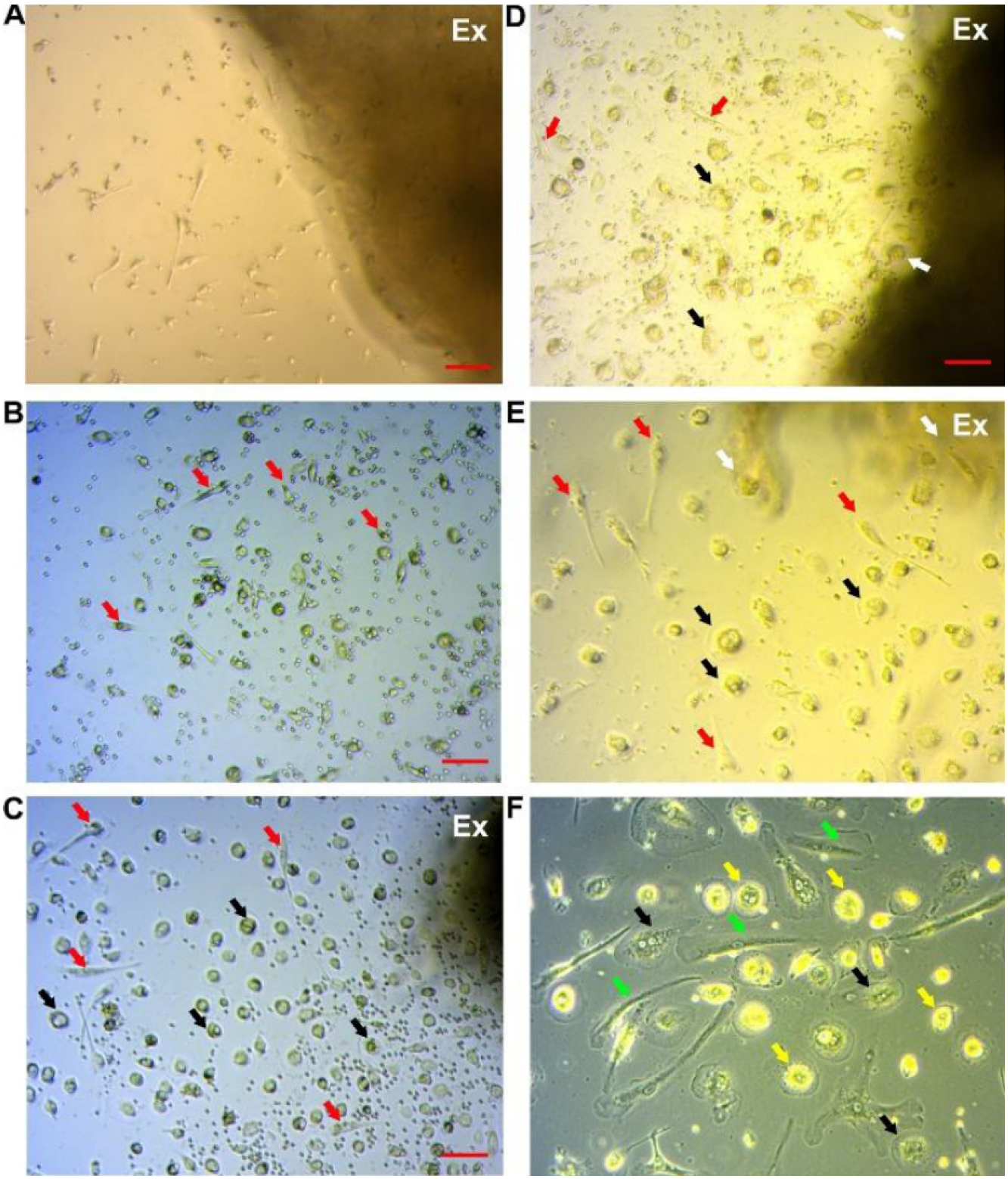

Figure 1 : Morphology of placental chorionic villous-derived cells cultured in 10% human AB serum-containing alpha MEM medium

villous explant, i.e. fibroblastic and non-fibroblastic cells. The first appearance of fibroblastic cells were observed in the first week, i.e. on day-3, 4 or 5 (TABLE 1; Figure 1A). Some of the cells were located near the explant, while some cells were located far from the explant. Many of these fibroblastic cells contained fine

granules in the cytoplasm (Figure 1B-C).

In the following days, mitosis was observed; however, the two daughter cells did not separate and became rounded cells with two or more nuclei (osteoclast-like cells; Figure 1B-C-D). After two to four weeks, colonies of heterogeneous morphology of cells could be seen prominently and some of the osteoclast-like cells started to die (Figure 1E-F). The non-fibroblastic cells seemed to be prominently sprouting from the explant after two weeks (Figure 1D-E and 2). These cells were mononuclear or multinuclear cells. In between these cells granule-devoid fibroblastic cells began to appear (Figure 2). The red blood cells were diminished after two weeks, and were not seen at the beginning of the third week (Figure 1F).

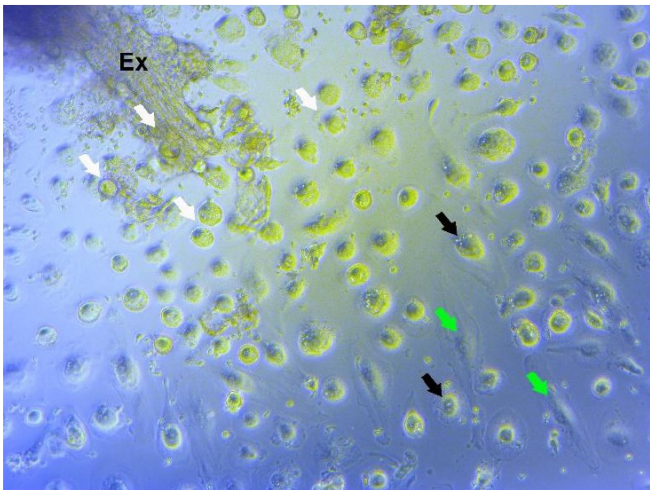

Figure 2 : Sprouting of non-fibroblastic cells from human placental chorionic villous explants

A) Day-5 culture: fibroblastic cells moved out from the explant (*Ex*). (B) Day-8 culture: there were fine granule-containing fibroblastic cells (*red arrows*) and rounded osteoclast-like cells. (C) Day-11, (D) Day-15, and (E) Day-16 culture: there were osteoclast-like cells (*black arrows*) that dominated the culture field, and fibroblastic cells (*red arrows*) were scattered among them. In (D) and (E), it is clear that sprouting cells are fibroblastic and also non-fibroblastic (rounded) cells (*white arrows*). (F) Day-16 culture from other explant shows heterogeneous morphology of adherent cells; mononuclear or multinuclear fibroblastic cells (*green arrows*) and multinuclear non-fibroblastic cells (*black arrows*), with some dying multinuclear cells scattered among them (*yellow arrows*). Many red blood cells are scattered among the growing cells in almost photographs (A-D). They are diminished in (E) and disappear in (F). *Red bars* 100µm.

This day-25 culture shows that rounded mononuclear or multinuclear cells (*white arrows*) move out from the explant (*Ex*). These cells would persist as non-fibroblastic (*black arrows*), and later, granule-devoid fibroblastic cells appeared (*green arrows*).

The results of cultures in term of the first appearance of fibroblastic cells, and various morphologies can be seen in TABLE 1.

DISCUSSION

Our attempt to culture the stem cells from placental chorionic villous by explant method in human AB serum containing alpha MEM showed that the stem cells began to attach and grow on day-3 to day-5. However, the cells that first attached were possibly hematopoietic stem cells (HSCs) from the contaminating blood rather than the mesenchymal stem cells, which later developed into non-fibroblastic multinuclear cells (osteoclast-like cells). This result was in line with our previous result on umbilical cord blood culture[9]. Moreover, a study on murine mid gestation placenta showed that placenta contains HSCs in the placenta vascular compartments[10]. In explant culture, the capillaries in chorionic villous can not be eliminated, and might be another source of HSCs, which might give rise to the macrophage precursor lineage that would differentiate into osteoclast-like cells that were observed in this study.

Another study by Igura et al (2004), which used explant culture method in 10% FBS containing low glucose Dulbeco's modified Eagle's medium (LG-DMEM), showed that twenty day culture yielded two types of cells, i.e. fibroblastic and large flat cells. The CD characteristics and differentiation capacity of the fibroblastic cells showed that they were MSCs. Further, the MSCs were contaminated by HSCs, endothelial and blood cells in early passages, but the contaminants decreases with passage, and disappeared after two to three passages[2]. Thus, this early result corroborates our result, though our results showed that the contaminant cells were more dominant. Moreover, Igura et al, washed the explant for five to ten times, until the supernatant was devoid of blood cells[2], while in our study, we only washed two to three

times. Therefore, to minimize the contaminants, washing should be done thoroughly, and should be ascertained that the blood cells were eliminated.

Besides MSCs, explant villous culture might release various kinds of cells, such as mesenchymal derived-macrophages (Hofbauer cells), fibroblasts, pericytes, endothelial cells, and also the syncytiotrophoblast and cytotrophoblast that cover the entire villous surface[11]. Study of Sima´n et al (2001) showed that cytotrophoblast growth occurred at culture of placental explant on a mesh. Even though in the first few days the trophoblast cells degenerated, after five days of culture, the trophoblast layer regenerated in the explant tissue[12]. In our study, in the second week of culture, degeneration of cells occurred followed by sprouting of non-fibroblastic cells that some of them might be trophoblast cells. These growing cells were remained viable until termination of the culture at day-32.

Our result showed that placental chorionic villous explant culture can grow well in 10% human AB serum containing alpha MEM. The growing cells were dominated by non-fibroblastic, rounded multinuclear cells or osteoclast-like cells rather than fibroblastic cells, when washing was inadequate.

CONCLUSION

Placental chorionic villous explant culture can grow well in 10% human AB serum containing alpha MEM, but when washing is inadequate, the growing cells were dominated by osteoclast-like cells.

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