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Evaluation of Two Cell Culture Media in Culturing Rat Full Term Amniotic Fluid Cells

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ABSTRACT

Introduction: Amniotic fluid (AF) consists of heterogenous population of cells with high diagnostic and therapeutic values. The study of rat amniotic fluid cells is very limited, despite the extensive use of this animal model in biomedical research. Primary culture of rat AF cells, especially from full term pregnancies has not been well established. Here we attempt to determine the suitable medium in culturing rat AF cells that would enhance the cell viability, growth rate and heterogeneity. **Methods:** The cell viability, growth rate and heterogeneity of rat AF cells were compared upon culturing the primary cells in two different media; Amniomax or RPMI. Cell viability study was carried out using trypan blue staining, while the growth rate was monitored based on the time required to passage the cells (population doubling time in hour). The heterogeneity of cells was examined based on the morphology of the cells. Statistical analysis was performed using t-test. **Results:** Amniomax was observed to provide a better culture condition in culturing rat AF cells as the cells are more viable, grow faster and more heterogenous as compared to the cells grown in RPMI. **Conclusion:** Amniomax is a more suitable medium for high quality and viability of full term rat AF cell culture, as compared to RPMI. Thus, warranting propagation of more rat AF cells for biomedical research.

Keywords: Full term rat amniotic fluid, cell culture, Amniomax, RPMI, amniotic fluid cells

INTRODUCTION

Amniotic fluid (AF) has been widely used in clinical setting for prenatal diagnosis, for more than 50 years, due to the presence of fetal cells in the fluid [2]. The fluid consists of a highly heterogenous population of differentiated and undifferentiated cells [1, 4, 16, 17]. The heterogeneity of AF cells might be explained by the direct contact of the fluid with the fetus [10] and the origin of the cells, which is from the three primary germ layers of amnion, fetal and embryonic tissues [1, 4, 7, 16, 17].

Recently, the use of AF has not been limited to only prenatal diagnosis, but they have been found to reside high potential stem cells, thus increases its therapeutic values. Exploring the potential values of these cells is indeed essential. AF can be retrieved from either mid- or full-term pregnancies, but most of stem cells findings from AF were established from mid-term pregnancies, even though the procedure to collect AF from this term is invasive (amniocentesis), with 1% risk of miscarriage and maternal/fetal complications [19, 21]. Minimizing these risks to the patient is important, thus full-term AF could be used as alternative, as they are usually discarded after birth and the procedure is much safer [6, 20, 21].

Despite the extensive use of rat animal model in biomedical research [5, 9, 10, 14], the culture of rat AF cells has not been well established, especially the AF cells harvested from full term pregnancies. Previous studies using human full term AF cells have demonstrated that the cells were difficult to grow and required long time for cell attachment [6]. Full term AF has been shown to contain less viable cells [4] as the fluid generally consists of excess cellular debris [12]. Therefore, establishment of appropriate conditions in culturing full term rat AF cells is indeed essential.

One of the important aspects in studies using rat AF is the culturing of the primary AF cells. Cell culture media is a factor that could affect AF cell culture [18] and even though various types of medium have been used in culturing AF cells [3-4], the best medium to culture AF cells from rat samples is still unknown.

Commercial and well-known medium to culture human AF cells, Amniomax has reported to be successful in

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propagating the bovine AF cells [3], hence providing an alternative option to be considered for culturing rat AF cells. Another common, cost-effective cell culture medium for various types of cell lines, RPMI could also be considered, as it has been used to culture murine AF cells in previous study [13]. Therefore, this study aims to evaluate the effects of these two different media on the growth rate, cell viability and heterogeneity of the rat AF cells as an effort to establish an optimal culture condition for the propagation and survival of rat full term AF cells. The established culture condition will certainly be useful in promoting future studies using AF cells. Thus, allowing the potential therapeutic values of AF cells to be explored.

MATERIALS AND METHODS

Sample collection

Time-mated pregnant Sprague Dawley rats from full-term pregnancies (day 20; n=8) were collected from animal center in University Malaya (UM) and sacrificed by excessive carbon dioxide (25-30L/min, 1000psi). Amnion sacs were removed from the uterus and washed with cold PBS (1x). Subsequently, amnion sacs were removed from the uterus by cesarean section and washed with PBS before placed in a petri dish. Then, AF from each sac were drawn and pooled under sterile condition. All rats received humane care and observed by a veterinarian in UM. Animal sampling procedures were approved by Animal Care and Use Committee (ACUC) of UPM.

Culturing and maintainence of AF cells

Prior to culturing, AF samples were centrifuged at 160xg for 10 minutes to pellet the cells. The cells were then resuspended in fresh medium by gentle pipetting and distributed into 6 well plates before incubated at 37°C in a humidified 5% CO2 incubator. The cells were subcultured after they reached 80-90% confluency by washing the cells with 1x PBS twice and trypsinized with 0.25% trypsin in 1mM EDTA (Gibco-Invitrogen). The trypsin was deactivated with medium containing serum and population doubling time (PDT) of the cells were recorded according to the formula: PDT = duration of culture x log2/log (inoculum cell number/cells harvested) [21].

Media preparation

Amniomax (Gibco-Invitrogen) complete medium was prepared by adding the supplements (containing serum and antibiotics) into the basal medium. RPMI 1640 with L-glutamine (Gibco-Invitrogen) was supplemented with 7.5% sodium bicarbonate (Sigma), 1% penicillin-streptomycin (Gibco- Invitrogen) and 20% fetal bovine serum (Gibco-Invitrogen).

Cell viability study & statistical analysis

Trypsinization and preparation of single cell suspension was performed prior to trypan blue staining. 10µl of the cell suspension was mixed with 10µl of trypan blue dye (Sigma) and the number of viable cells was determined using haemocytometer. The unstained cells were identified as viable cells whereas blue stained cells were identified as dead cells, as the dye was only absorbed by damaged cells. The experiments were performed in triplicate and for statistical analyses, t-test was conducted and P values <0.05 were considered statistically significant.

RESULTS

Two different media (Amniomax and RPMI) were used to evaluate the growth and maintenance of full term rat amniotic fluid (AF) primary cell culture based on cell viability, growth rate and heterogeneity. To compare the effects of Amniomax and RPMI, cells from the same batch of AF specimens were cultured in both medium upon collection for a culture period of 14 days, and in the same culture conditions.

VIABILITY OF AF CELLS

Both Amniomax and RPMI were observed to provide short attachment time of the primary rat AF cells to culture flask. The cells in both media were found to attach within 48 hours. However, the quality of the AF cells cultured in the respective medium was significantly different and this was assessed through cell viability study. From the cell viability study performed on the first and second passages of the cells, Amniomax was observed to significantly maintain more viable cells compared to RPMI. It was discovered that the number of viable cells grown in RMPI was reduced more than 70% (Figure 1), which may indicate that RPMI is not supporting the growth of AF cells or may suggest that RPMI is selective towards certain type of cells in AF.

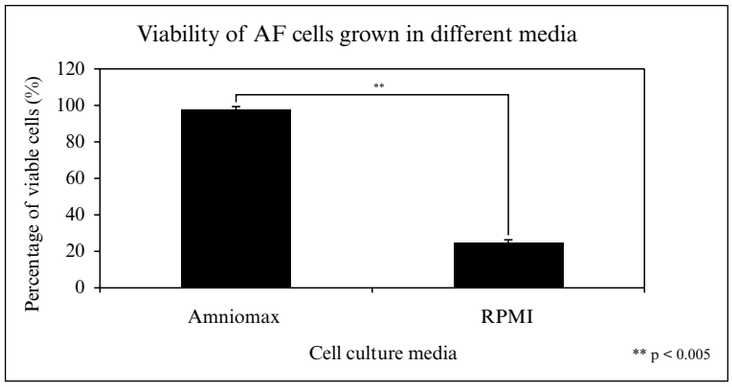

Figure 1. Comparison of AF cells viability grown in two different media, Amniomax and RPMI. Amniomax medium shows higher cell viability compared to RPMI medium (P2 & P3). Error bar is presented as mean ± S.E.M of 8 independent experiments conducted in triplicate, n=24

GROWTH RATE OF AF CELLS

The growth rate of AF cells in both media was also monitored. The cells were found to exhibit different growth behaviour. Cells grown in RPMI were observed to take longer time to reach confluency compared to cells cultured in Amniomax. The population doubling time for cells in RPMI was 125 hours compared to 40 hours for cells in Amniomax. These results highly suggest that Amniomax is providing a better culture condition in promoting the growth rate of AF cells compared to RPMI.

HETEROGENEITY OF AF CELLS

More heterogenous population of AF cells was observed when AF cells were grown in Amniomax compared to in RPMI (Figure 2). AF cells cultured in Amniomax exhibit at least four different morphology of cells; amniotic fluid cells (AF)-type (Figure 2A), epithelial–like (Figure 2B), fibroblast–like (Figure 2C), and neuronal-like cells (Figure 2D). Even though cells in RPMI took longer time, different types of cells was also observed in this medium. AF-type (Figure 2E), epithelial–like (Figure 2F) and fibroblast-like cells (Figure 2G) were observed being grown in RPMI. However, unlike in Amniomax, neuronal-like cells were not observed in RPMI. These observations suggest that Amniomax may be less selective compared to RPMI, thus promoting the propagation of more heterogeneous population of full term AF.

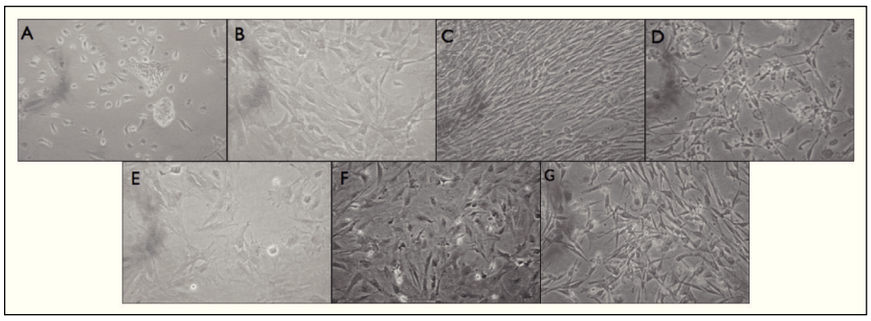

Figure 2. Heterogenous population of rat AF cells cultured in different media. More heterogeneous morphology of cells was observed when the cells were cultured in Amniomax; AF-type (A), epithelial–like (B), fibroblast–like (C), neuronal-like cells (D) compared to RPMI; AF-type (E), epithelial–like (F) and fibroblast-like cells (G). The magnification is 10X

DISCUSSION

The present study aims to establish a culture condition that would support the propagation of AF cells harvested from full term pregnant rats. AF cells harvested at this stage of pregnancy has been claimed to contain less number of viable cells [4], therefore culturing of the cells requires a good culture condition. One important aspect in establishing this

condition is to find the best suitable medium that would promote the cell attachment and enhance the viability, growth rate and heterogeneity of these cells. In this study, two types of media that have been used in culturing AF cells were evaluated. Amniomax, a specific medium to culture human AF cells, that has been previously reported to culture AF cells from bovine origin [3], and RPMI, a common medium that has been used to culture murine AF cells [13]. The ability of these two media in propagating and maintaining the rat AF cells was evaluated.

Both media managed to provide an environment that supported cell attachment. However, Amniomax showed better condition to culture rat AF cells as it was observed to support more viable cells than RPMI. The significance of the cell viability study was supported with the growth rate results, where AF cells cultured in Amniomax has lower PDT, indicating a faster growth rate.

The overall assessment of the cell culture success was observed by cell morphology, where more types of cells were observed in Amniomax, supporting the heterogeneous population of AF cells. At least four types of cells were observed in Amniomax compared to three in RPMI. Therefore, based on the cell viability, growth rate and heterogeneity, we discovered that rat AF cell culture quality is better with Amniomax medium as compared to RPMI (Figure 1, 2). Previous study on bovine AF cells supports our finding in which the AF cells grow very poorly in RPMI, but very well in Amniomax[3]. This may due to the variable contents of the medium, where Amniomax may have more additional supplements consisting growth factors and hormones specifically for the growth of rat AF cells [3], thus better in culturing AF cells, than RPMI [3, 15]. We suppose the basic basal components of RPMI may not be sufficient for rat AF cells, even though the difference of the constituents between these two media were unknown, as the composition of the Amniomax media is unavailable.

Overall, in our hands, Amniomax medium was found to be significantly better in culturing rat AF cells compared to RPMI. In conclusion, although Amniomax is a specific medium to culture human AF cells, it is also recommended for culturing the full term rat AF cells. This is the first finding to establish a culture condition that is not only managed to propagate the rat AF cells harvested from full-term pregnancies, but also enhanced the growth rate, viability and heterogeneity of the cells. The results will certainly be useful in promoting future studies using AF cells, especially full term AF cells. In that, it gives us the opportunity to have sufficient number of cells for isolation of stem cells residing in the fluid.

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