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Original Article

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2-3% fetal calf serum in cell media is sufficient for the development and growth of human mononuclear cell cultures

Abstract

Aim: It is a common practice that 10-20% fetal calf serum (FCS) is being used in the cell culture media for the development and maintenance of human mononuclear cells as well as other cell cultures. FCS is produced through a painful process involving animals and is one of the major cost factors. The reduction in the volume of FCS can make it less unethical and reduce the costs strongly.
Materials and Methods: Therefore 2-3 % FCS and 10% FCS were used in the development of different mononuclear cell cultures for making comparisons and different parameters like morphology, production of antibodies, isolation of DNA/RNA with mini-column and magnetic beads cell isolations along with the presence of human internal controls with real-time PCR were tested.
Results: It was found that there was no difference between 2-3 % FCS and 10 % FCS containing media in the above-said parameters. More than a hundred such cell cultures were developed and many of them were maintained for more than 3 years.
Conclusion: 2-3 % FCS is sufficient for the development and maintenance of human mononuclear cell cultures over a long period of time.

Keywords: Mononuclear cells, cell culture, fetal calf serum, ELISA

INTRODUCTION

In all laboratories around the world, it is common practice to use 10 % of fetal calf serum (FCS) in cell cultures like mononuclear cells, stem cells, etc. (1-7). This practice is being conducted for last many years (8,9). The production of FCS needs the processing of serum from the unborn fetus. This process involves steps, which may be called unethical according to animal rights because the heart of a fetus obtained through slaughter is being punctured to get the FCS, which causes a lot of discomfort to animals, and the serum is then filtered to ensure sterility (10,11). Hence there is a lot of research going on for the development of alternatives (11,12). The FCS is continuously used because its demand is increasing. As 10 to 20 % FCS is being used in processes involving the development and maintenance of the cell cultures, hence FCS is one of the major costs of cell cultures producing different proteins or during the conducting of cell culture research. These concepts of using 10-20% FCS can be traced bask toearly scientific literature; therefore, we decided to try and make studies to use the low concentration of 2-3 % in the development and maintenance of different human mononuclear cell cultures.

MATERIALS AND METHODS

The ethics committee of the institute has approved this research work. A human buffy coat (Red Cross blood collection center, Germany) was obtained, and the mononuclear cells were isolated with two different methods. One method was the density gradient method. In this method, the buffy coat was mixed with an equal volume of PBS (Biochrome) in a 50 ml sterile plastic tube. In another 50 ml sterile plastic tube, 30 ml of density gradient solution (Biochrome) was added. To this, 20 ml of diluted buffy coat was poured in such a way that the buffy coat makes a new layer on the density gradient solution, and it should not mix with the density gradient solution. This was centrifuged at 19000 rpm for 20 minutes to get a layer of white blood cells on the density gradient solution. These cells were removed and collected in another 50 ml sterile tube. The collected cells were washed 3 times with PBS and centrifuged as mentioned above. After that, the cells stained with trypan blue solution (Biochrome) were counted in the Neubauer chamber under the microscope.

These cells were cultured in different concentrations in 6 wells and 96 wells plates with different cell counts in media made of

2-3 % and 10 % FCS in DMEM or RMPI containing 1 % of Streptopenicillin (Biochrome, Germany) antibiotics as well as 1 % Glutamine (Biochrome, Germany).

DMEM and RMPI were used from 2 different manufacturers, which are Biochrome (Germany) and Lonza (USA). FCS was supplied by Biochrome.

Mononuclear cells were kept in a CO_2 incubator at 37 O^C as they were cultured starting from 5x106 cells per well. They were fed regularly with media used during the preparation. The Samples of the media were taken from these culture cells for testing purposes e.g., the presence of antibodies as well as cells were used to select the specific cells through the specific magnetic beads for further culturing. The isolated cells were observed under a fluorescent microscope (Zeiss, Germany). The cell cultures were observed under a microscope regularly. These cells were subcultured in cell culture flasks. The presence of antibodies was done with ELISA as well as magnetic beads ELISA kits (Genekam, Germany).

The presence of antibodies was done with ELISA (Genekam, Germany) as well as magnetic beads ELISA kit (Genekam, Germany). Magnetic beads ELISA kit is a new method; hence the manuscript is in preparation. ELISA kit is used as follows: coating the wells with antigen overnight in 96 well plates and blocking with the blocking buffer. The supernatant was added to each well overnight. Conjugate was added to each well and stopped with the stop solution. The results were read visually or with an ELISA reader.

DNA / RNA from these cell cultures were isolated successfully with mini-column isolation kits (Genekam, Germany) for testing the confirmation of successful isolation of RNA / DNA with real-time PCR kit as well as conventional PCR tests (Genekam, Germany) for internal control like β-actin and β-globin genes. This isolated RNA of mononuclear cells was used in PCR to find the presence of antibodies-specific genes in gel agarose.

A part of the mononuclear cells from each cell culture was taken out and centrifuged. They were stored in freezing media (Genekam, Germany) at -20 O^C and later shifted to a liquid nitrogen tank. Some of the cell cultures were thawed and cultured successfully again.

Alternatively, a new and simple method was used to isolate the mononuclear cells, where one mixes two different components and lets them stay till the erythrocytes are lysed. (Manuscript in preparation)

RESULTS

In the year 2013, we conducted the comparison between 2-3 % and 10 % FCS cell culture media and found no difference in the performance for different parameters, therefore our laboratory shifted to the use of 2-3% FCS cell culture media. We have made more than a different hundred mononuclear cell cultures with 2-3 % FCS cell cultures, we get always good performance. The cell cultures look healthy and good under the microscope in the Neubauer chamber (Figure 1 and 2).

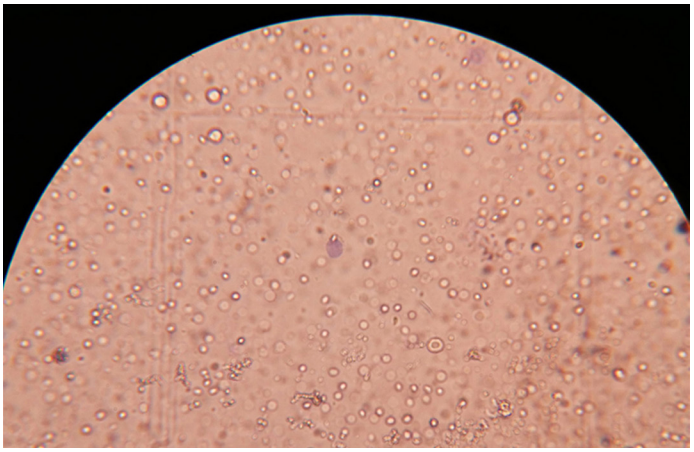


Figure 1. The human mononuclear cells under microscope in Neubauer chamber (100 X)

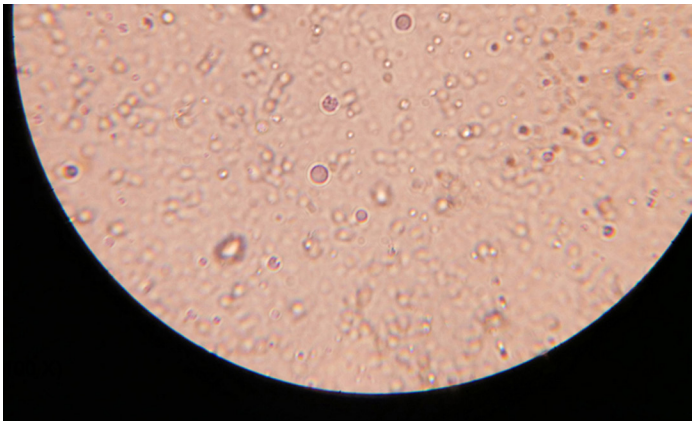


Figure 2. Mononuclear cells, which looks healthy and huge in number under microscope (100 X)

ELISA performed showed the presence of specific antibodies e.g., Chlamydia. The cells can be subcultured in flasks of many different volumes for the last many years (Figure 3).

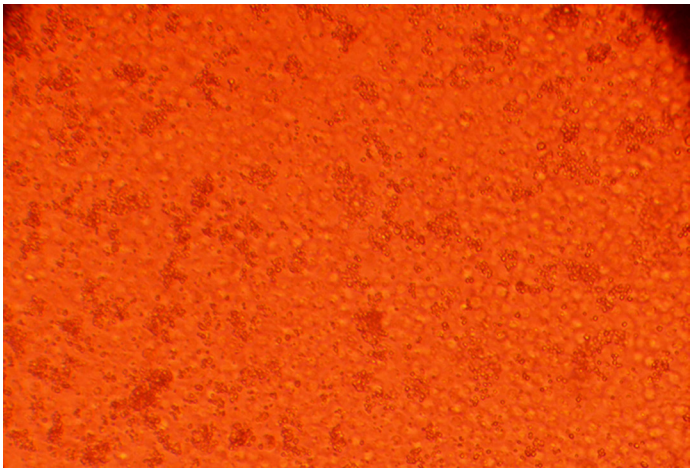


Figure 3. Human mononuclear cells in cell culture media after 2 months of culturing (100 X)

We isolated the specific cells e.g., plasma cells with anti-CD27 specific magnetic beads, they were observed under a microscope and cultured in flasks (Figure 4).

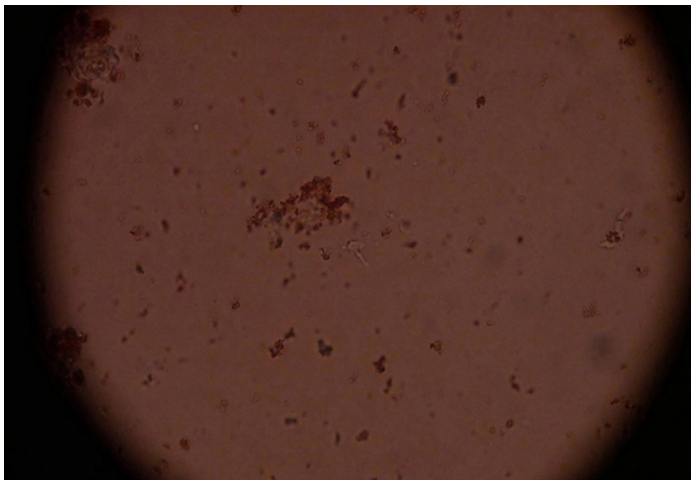
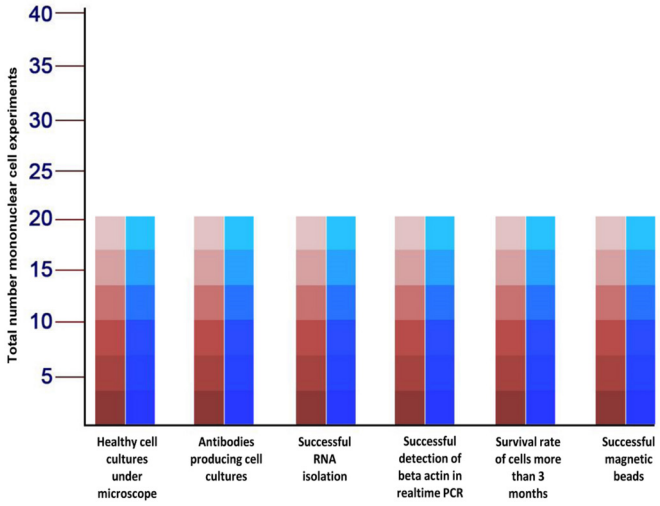


Figure 4. Mononuclear cells successfully isolated with magnetic beads under microscope (100 X)

The cells were cultured in different concentrations starting from 5 x 106 cells per well and they show always good performance in 2-3 % FCS-fed cell cultures. The results of 20 experiments as an example were presented here, where these cells were examined under a microscope for phenotype, and it was found that these cells are healthy. They were tested, whether they are producing the antibodies, and it was found that these cell cultures were producing human antibodies as tested with ELISA. Successful isolations of DNA / RNA were confirmed through β-actin and β-Globin internal control during real-time and conventional PCR (Graphic 1). Many cell cultures were kept for more than 2 years without loss of morphology except that some of the mononuclear cells stuck on the plastic floor of plates and flasks very strongly. The thawing of frozen cell cultures led to obtaining healthy mononuclear cell cultures



Graphic 1. The results of different parameters studied during 20 different experiments with 2-3% (red bars) an 10% (blue bars) FCS

DISCUSSION

We are showing here that 2-3 % FCS in DMEM and RPMI media are sufficient to culture the human mononuclear cells for different purposes e.g., production of antibodies, isolation of specific populations like CD27 as well as isolation of RNA / DNA for molecular analysis. Our laboratory is using this for the last 10 years and we have developed more than 100 different cell cultures. It was found that some authors have used also 2 % as well as 4 % FCS, but these are very rare reports in the literature (13). Cultured mononuclear cells were used to conduct the presence of tumor markers also after the specific markers' specific magnetic beads isolation (Data not shown as more research work is being done in the field of tumor markers in order to publish in future).

2-3 % FCS is sufficient to maintain the human mononuclear cell cultures, this helps to cut costs of doing research. In the case of industrial production, this work should help to reduce the costs of cell cultures, which should lead to a reduction in the production costs and development of cost-effective therapies, vaccines, and diagnostics. This work can contribute to reducing the pain caused to animals. We have used this media to isolate and maintain mesenchymal stem cells successfully. This research work will lead to saving money in many institutes.

CONCLUSION

In this work, there is successful use of 2 to 3% FCS to develop and maintain the human mononuclear cell cultures against 10-20% FCS is being used in most of institutes today. This research should lead to reduction in the monetary burden on the institutes and in commercial applications, it can reduce the cost of development and productions of many products like human antibodies, assays etc. One of biggest advantage of this work is that there should be improvement in animal rights through reduction in animal cruelty.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

This is not applicable here. Moreover Genekam Biotechnology AG is private company, hence there is no committee for this.

REFERENCES

1. Leshem B, Yogev D, Fiorentini D. Heat inactivation of fetal calf serum is not required for in vitro measurement of lymphocyte functions. J Immunol Methods. 1999;223:249-54.

2. Mannello F, Tonti GA. Concise review: no breakthroughs for human mesenchymal and embryonic stem cell culture: conditioned medium, feeder layer, or feeder-free; medium with fetal calf serum, human serum, or enriched plasma; serum-free, serum replacement nonconditioned medium, or ad hoc formula? all that glitters is not gold! Stem Cells.

2007;25:1603-9.

3. Witzeneder K, Lindenmair A, Gabriel C, et al. Human-derived alternatives to fetal bovine serum in cell culture. Transfus Med Hemother. 2013;40:417-23.
4. Messias CV, Lemos JP, Cunha DP, et al. Zika virus infects human blood mononuclear cells. BMC Infect Dis. 2019;19:986.
5. Alipour R, Fatemi A, Alsahebfosul F, et al. Autologous plasma versus fetal calf serum as a supplement for the culture of neutrophils. BMC Res Notes. 2020;13:39.
6. Bhatia, S. A simple method for isolation of rest of trypsinized stem cells with magnetic beads. Jurnal Teknologi Laboratorium. 2021;10:1–2.
7. Juhl M, Christensen JP, Pedersen AE, et al. Cryopreservation of peripheral blood mononuclear cells for use in proliferation assays: first step towards potency assays, J Immun Methods. 2021;488:112897.
8. Enders JF. Bovine amniotic fluid as tissue culture medium in cultivation of poliomyelitis and other viruses. Proc Soc Exp Biol Med. 1953;82:100-5.
9. Nowell PC. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. Cancer Res. 1960;20:462–6.
10. Jochems CE, van der Valk JB, Stafleu FR, et al. The use of fetal bovine serum: ethical or scientific problem? Altern Lab Anim. 2002;30:219-27.
11. van der Valk J, Bieback K, Buta C, et al. Fetal bovine serum (fbs): past - present - future. ALTEX. 2018;35:99-118.
12. Venkatesan M, Semper C, Skrivergaard S, et al. Recombinant production of growth factors for application in cell culture. iScience. 2022;25:105054.
13. Suchánek J, Kleplová TS, Kapitán M, et al. The effect of fetal calf serum on human dental pulp stem cells. Acta Medica (Hradec Kralove). 2013;56:142-9.
14. Bhatia S. A simple and quick isolation method to obtain a huge number of fibroblasts like mesenchymal stem cells from human umbilical cord. Jurnal Teknologi Laboratorium. 2022;11:60-4.