METHOD OF ISOLATION OF CELL CULTURES

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ABSTRACT:

In this paper, we report on the development of a method that allows a faster, cheaper and safer isolation of the desired cell type by the explant. The advantages of the method are in preventing the contamination of the vessel for cell culture, lack no need to use additional expensive reagents, good adhesion of the tissue to cell culture surface without the risk of detachment and stable isolation of a large number of cells in a short period of time from a small amount of tissue in a procedure.

KEYWORDS:

explants, cell culture, fibroblast

1. Introduction

One of the methods for better adherence of an explant to the cell culture surface during fibroblasts isolation is fetal bovine serum utilisation. According to this method in a period of about 30 days the fibroblasts reach to a 70-80 % confluence in a 75 cm^2 cell culture flask. The disadvantages of this method are the utilisation of an additional substance for cell culture surface coating in order to increase the adhesion of the explants, additional period of time for cell culture surface preparation, which presents a potential risk of contamination, the cell over-confluence around the explant with an uneven distribution of cells through cell culture flask, but also the long period of time needed for cell culture accompanied by the high risk of

cellular culture contamination and infection. There are also described methods of using type I collagen, gelatin or fibronectin to process cell culture surface to increase the adhesion of the explant to it. Also is known the method of primary bronchial epithelial cells isolation through explants, wherein the complete cell culture medium is used to make the cell culture surface more adherent. The disadvantages of this method in addition to those listed above is the weak attachment of the explant to the cell culture surface with lack of attachment and frecvent detachments.

2. Literature review, conceptual framework, hypotheses, etc.

The importance of our invention compared to the existing methods of isolating cells by explant is the lack of additional costs for the acquisition of various substances necessary to increase the adhesion of the explant to the cell culture surface, preserving of resources as time and reagents, but also the possibility to isolatie a wide and numerous range of animal cells in a short period of time from different tissues in a single procedure.

3. Material and methods

In the experiment was used the cellular isolation method proposed by Jian et al. (2021). For fibroblasts isolation, in sterile conditions, under general anesthesia from a rabbit, a piece of dermis was taken and introduced into the preheated fibroblast-specific culture medium. The tube was taken to the laboratory and placed in the hood with laminar air flow. The dermal tissue was washed 3 times with HBSS and culture medium, then placed in a Petri dish and cut into 2 pieces of approximately 2 x 4 x 5 mm each. The explant pieces were then placed in a 60 mm diameter plastic Petri dish, in which 6 ml of fibroblast culture medium was poured. The Petri dish with the tissue pieces was incubated at 37 °C, 5 % CO_2 and humid environment for 3 days, then the culture medium was changed and the volume of media poured back was reduced to 2 ml, a sufficient amount of media to maintain the explant moist but fixed to the cell culture surface. Than the Petri dish was placed in the incubator under the same conditions. Each day the culture medium was changed, if the explants

were fixed to the cell culture surface by the cells which migrated from the explant, in to the Petri dish were added 5 ml of complete media. When many colony-forming cells were determined near the explants, they were moved to the opposite poles of the Petri dish and incubated again in a small amount of medium to form another cell colonies

4. Results and Discussion

The attachment of the explants to the cell culture surface took place in the first 2 days of culture in a small volume of environment, without the risk of detachment of the explants at addition of a higher volume of cell culture media. Fibroblasts confluence of 70-80 % was obtained at 4 days after the first movement of the explants. The fibroblasts were then subcultured in a 75 cm^2 flask for 3 days, obtaining 3.32 x 10^6 cells, which were spread through another 4 flasks of 75 cm^2 and cultured for another 3 days. Thus, obtaining within 19 days from the beginning of the isolation of fibroblasts in the 3rd passage 1.28 x 10^7 cells. Which is much faster comparing with fetal bovine serum utilisation, where in 1st passage a to obtain a confluence in 75 cm^2 cell culture flask are needed 30 days and the number of isolated cells is much higher if comparing with methods of cell culture surface processing with other substances.

5. Conclusions

The used method of cell isolation by volumetric regulation from the explants is more efficient from the point of view of money, reactives, time and ensure an efficient isolation of a large number of cells.

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