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Improvement of protocol for detachment assays in B16F10 cells

Eric Almeida Xavier *, Isabela Canavezzi Matias and Anelise Freitas Sandei

Department of biochemistry institute of chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, Sao Paulo, 05508-900, Brazil.

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Abstract

B16F10 (B16) murine melanoma cells have the characteristic of strong adhesion to culture surfaces. The use of trypsin solutions in high doses to release this type of cell is not suitable. When B16F10 cells are released with high concentrations of trypsin they do not form a precipitate after centrifugation and consequently the cells will be lost when discarding the supernatant. To solve this problem, we established a solution with phosphate buffered saline (PBS) and ethylenediaminetetraacetic acid (EDTA) at low trypsin concentrations. Finally, we verified the efficiency of this solution on the quantity of cells recovered after centrifugation.

Keywords: Improvement of protocol; B16F10 melanoma cells; Detachment assays; Trypsin enzyme; PBS/EDTA assays

1. Introduction

Trypsin, a proteolytic enzyme, is the standard way to detach adherent cell cultures and monolayers [1]. This globular, pancreatic protease cleaves the C-terminal side of lysine and arginine, breaking down vessel-adhering proteins and allowing easy resuspension during cell harvesting [1,2].

The release of B16 cells into culture plates or bottles is generally done through the use of trypsin or PBS/EDTA solutions [1,2,3]. However, the use of trypsin in high concentrations is not recommended for certain strains such as B16F10.

B16 cell adhesion proteins are very sensitive to trypsin [4,5]. Therefore, this strain, when exposed to high concentrations of trypsin, acquires a morphological conformation similar to jelly or gelatin. This gelatin will remain floating in the supernatant after centrifugation and the cells are lost in the discard of the supernatant.

The use of solution only with PBS/EDTA takes too long for release of B16 cells. Thus, we elaborate a compound solution of both PBS/(EDTA 0.5 Mm) and PBS/EDTA + (trypsin 0.025%).

The B16 strain has a strong adhesion to the culture surface [5], however when the detached cells are centrifuged, they do not precipitate adequately. In this way, the cells are lost because they remain in the supernatant. If you use aspiration, the cells will be aspirated and therefore lost, or if the tube is inverted, the cells will also be lost as they are floating in the supernatant.

Therefore, to improve the method of releasing B16 cells and not lose the detached cells, we established a solution of PBS with EDTA with a small amount of trypsin.

* Corresponding author: Eric Almeida Xavier

2. Material and methods

2.1. Cell lines and culture conditions

The murine melanoma cell line B16F10-Nex2 was originally obtained from the Ludwig Institute for Cancer Research (LICR), São Paulo branch. The cell line grew in RPMI-1640 (Gibco, Grand Island, NY) medium supplemented with 10 mM of 2-(4-(2-hydroxyethyl) piperazin-1-yl) ethane sulfonic acid (HEPES; Sigma-Aldrich, St. Louis, MO), 24 mM sodium bicarbonate, 40 mg/L gentamicin (Hipolabor, Minas Gerais, Brazil), pH 7.2, and 10% fetal bovine serum (Gibco, Grand Island, NY). Cells were cultured at 37 °C and 5% CO2 and 95% humidity in the atmosphere.

2.2. Cell detachment assay

Viable cells were seeded (1 x 10^6) in a 6-well plate. And group 1 (Fig 1 A) detached with trypsin (0.25%, sterile-filtered, BioReagent, suitable for cell culture, 2.5 g porcine trypsin and 0.2 g EDTA, 4Na per liter of Hanks' Balanced Salt Solution with phenol red), group 2 (Fig 1 B) detached with PBS-EDTA (0.5 mM), and group 3 (Fig 1 C) detached with trypsin (0.025%) in PBS-EDTA (0.5 mM), for fifteen minutes at 37 °C, thus obtaining loose cells without mechanical action. The number of cells in 10 µl transferred to a Neubauer chamber was counted with the aid of a manual counter. Cell number was determined by the following formula: mean of four fields, multiplied by 1 x 10^4 as a correction factor, equivalent to the number of cells per mL. Cell number was compared to shedding cells versus the respective control system. The graphs were performed using the GraphPad Prism program.

3. Results and discussion

We can see in (figure 1 A) when the cells are released and centrifuged with pure trypsin they form a gelatinous supernatant. We found that B16 cells, despite remaining in the PBS/EDTA solution for fifteen minutes, few cells uncouple (figure 1B). On the other hand, there was a greater recovery of cells that were centrifuged with PBS/EDTA + trypsin (figure 1C).

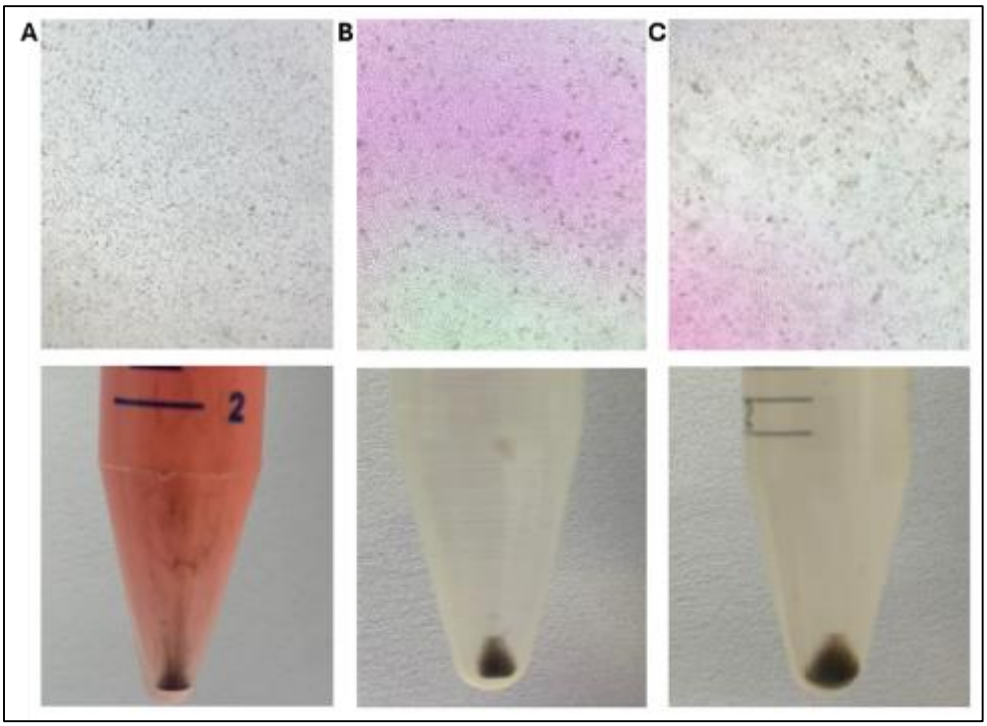

Figure 1 (A) shows a floating element with a gelatinous shape, which makes it difficult to remove the supernatant without removing the cells together. (B) PBS/EDTA we can see that the size of the precipitate is smaller in relation to A and C. (C) PBS/EDTA + trypsin we noticed a large and cohesive precipitate

In (figure 2 A) we can see in the numbers of released cells, and we can see through the cell number graphs a loss in cell recovery after discarding the supernatant (figure 2 B). Therefore, when we resuspended the trypsin precipitate, we had

a loss of 86.25%. In the same way there was a 50% loss in resuspension of PBS/EDTA. On the other hand, the loss of PBS/EDTA + trypsin was only 17.8% (figure 2 A-B).

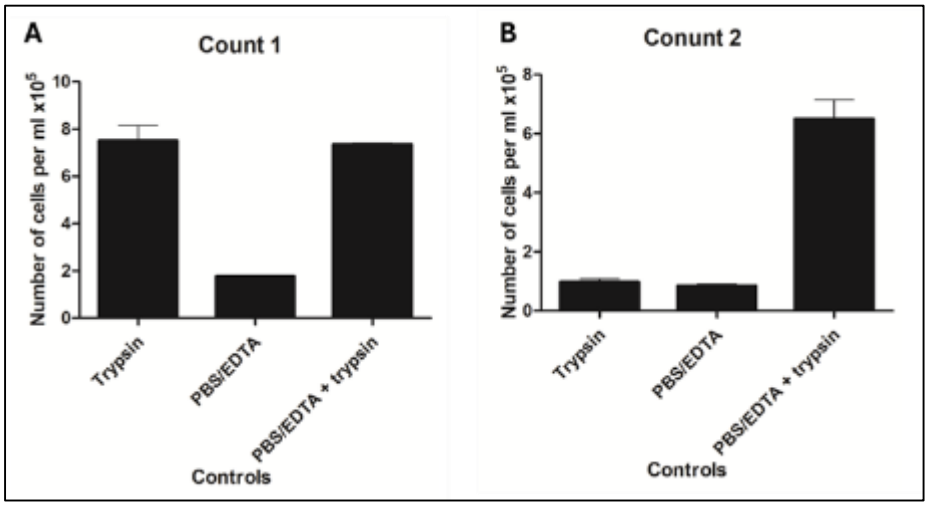

Figure 2 (A-B) numbers of released cells. (B) recovery cell safter after discarding the centrifuged supernatant. In trypsin precipitate, we had a loss of 86.25%. In PBS/EDTA we had 50% loss. And in PBS/EDTA + trypsin we had only 17.8% loss

4. Conclusion

This study contributes to the scientific community showing that when cells B16 are centifugated with PBS/EDTA with low trypsin concentrations, the precipitate is totally improved for use. In addition, we have a gain not only in the number of cells but also in the time when cells need to be in the solution to release.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest in constructing this manuscript.

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