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Efficient isolation and high yield of epidermal cells from foreskin biopsies by dynamic trypsinization

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ABSTRACT

Cultured keratinocytes play important roles in burn wound healing and scientific research studies. We aimed to modify the isolation method to avoid over-digestion, maximize the number of isolated epidermal cells and establish a more efficient and innocuous way of cell isolation. Compared to the conventional method, the modified method combines the more dynamic process of enzymatic digestion with multiple harvestings of dissociated cells *via* digestion. The cells from each harvesting were immediately re-suspended in culture medium with serum to avoid extended trypsinization and then pooled for further analysis. The number of viable cells isolated per gram of adult foreskin epidermis was $(18.88 \pm 13.22) \times 10^6$ cells in the control group and $(67.34 \pm 30.66) \times 10^6$ cells in the modified group ($p < 0.001$). No significant differences were observed in the proportion of CD49f-positive cells between the two groups ($p > 0.05$). The modified method was significantly more efficient in dissociating keratinocytes from each unit of skin biopsy, which is particularly important for treating severe burns when donor skin is limited.

1. Introduction

Skin is an essential barrier that protects organisms from the environment and is composed of epidermis, dermis and subcutaneous tissue [1]. Most cells in the epidermis are keratinocytes, which constitute 90% of the cell population in this layer [2]. Keratinocyte progenitor cells at the epidermal-dermal junction are essential for regenerating and maintaining the normal structure and function of the epidermis. Frequently, epidermal cells must be isolated and cultivated for scientific studies or clinical applications for wound care. Currently, human keratinocytes can be isolated from skin by direct trypsinization of minces, split-thickness skin following the separation of the epidermis from the dermis, or the outgrowth from tissue explanted in liquid medium [3]. Much progress has been achieved in the field of keratinocyte culturing. For instance, long-term keratinocyte

Abbreviations: Cnt-Pr, CnT-Prime, epithelial culture medium; CCK, cell counting kit; DMEM, Dulbecco's modified eagle medium; DPBS, Dulbecco's phosphate-buffered saline; EDTA, ethylene diamine tetraacetic acid; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PI, propidium iodide; PS, phosphatidylserine; SA-β-gal, senescence-associated β-galactosidase; SD, standard deviation.

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subcultures can be successfully achieved without using fetal bovine serum (FBS), feeder layers, fibroblast-conditioned medium or bovine pituitary extract [4]. Meanwhile, various growth factors are involved in the regulation of human keratinocyte function [5,6]. Nevertheless, isolating the maximum number of viable and proliferative epidermal cells from a skin biopsy is critical for initiating the cell cultivation process. Although several digestive reagents are used in the process of keratinocyte isolation, trypsin is the most commonly used reagent. The conventional keratinocyte isolation method is usually performed by trypsin digestion at 37°C for a fixed time period during which the cells are gradually released into the enzymatic solution [7]. The cells are then harvested by centrifugation after the digestion is completed. The enzymatic activity of the trypsin preparation could vary from lot to lot among different skin biopsies [8,9]. Additionally, over trypsinization could injure the cells [10,11], and a small time-window exists between successful detachment and irreversible cell damage. Because the cells released during the early phase of the conventional method remain in the trypsin solution until the completion of the digestive process, these cells could be subjected to enzymatic damage due to extended exposure. In this study, we aimed to modify the isolation method to maximize the isolation of epidermal cells from skin biopsies while avoiding cellular injury due to over trypsinization. Our modified method combines the more dynamic digestion process that enhances the number of cells released with multiple harvestings of the freshly released cells during the digestive process to immediately quench the trypsin digestion of already released cells. Several techniques and parameters were used to evaluate the modified method in comparison with the conventional method.

2. Materials and methods

2.1. Foreskin sample collection

The foreskin samples used in this study were donated by healthy adults aged 20–30 years who underwent surgical circumcision, and all donors were of the Asian race. Fifteen samples were used in the overall study. Fresh foreskins were obtained with the donors' consent and the approval of the Ethics Committee of the First Affiliated Hospital of People's Liberation Army General Hospital, Beijing, China. The foreskin tissue was surgically removed using aseptic techniques. The collected samples were kept in sterile physiological saline and transported at 2–8°C to the laboratory for processing.

Upon receipt, the circular foreskin samples were transferred to a sterile 60-mm tissue culture dish (Corning Incorporated, NY, USA) and rinsed thoroughly using a pair of forceps in two repeated washes with Dulbecco's phosphate-buffered saline (DPBS, MACGENE Biotech, Beijing, China). The skin specimens were then turned dermal side up, and the subcutaneous fat and reticular dermis were dissected using curved scissors. The skin was cut into strips of approximately 0.5 cm × 1 cm and washed again in a new sterile dish containing 6–7 ml of DPBS. The tissue strips were transferred to a 15-ml conical centrifugation tube (Corning Incorporated, NY, USA) containing 10 ml of a 2.3 U/ml dispase II solution (Bacillus polymyxa, Gibco, NY, USA), 200 IU/ml penicillin, 200 μg/ml streptomycin and 0.5 μg/ml amphotericin B (MACGENE Biotech, Beijing, China). After ensuring that the tissue was submerged in the dispase II solution, the tube was securely capped and laid horizontally in a refrigerator for 12–18 h at 2–6°C. The dispase-treated skin tissues were retrieved from the refrigerator and gently transferred to a 60-mm tissue culture dish in a Vertical Flow Clean Bench and rinsed with DPBS. The strips were placed into a clean sterile dish, and the epidermis was separated from the dermis by holding the dermal side of the skin with a pair of forceps and the edge of the epidermis with a second pair of forceps. All epidermal pieces were rinsed again with DPBS, quickly dried using sterile gauze to absorb the DPBS and divided into two equal parts as soon as possible using scissors. Each part of the epidermal tissue was weighed in a sterile dish using an electronic balance, and the weight was recorded; then, each part was randomly allocated to either the conventional digestion control group or the modified digestion group.

2.2. Isolation of epidermal cells

Digestion with 0.25% trypsin+0.02% EDTA for 10 min at 37°C is widely performed; thus, this method was performed in the control group in this study.

In the control group, the conventional digestion method was performed for the cell isolation. Briefly, the epidermal tissue was rapidly transferred to a new sterile 60-mm dish, and 200 μl of DPBS were immediately added to wet the samples. The epidermal tissue was finely minced to less than 1 mm in diameter using curved scissors. Then, 3 ml of 0.25% trypsin-EDTA preheated to 37°C were added to the dish to digest the tissue at 37°C for 10 min. The dish was gently shaken every 3 min during the digestion. Subsequently, 3 ml of complete medium (DMEM containing 10% FBS) were immediately added, and the solution was pipetted up and down vigorously using a 5-ml pipette to further release the epidermal cells. The cell suspension was passed through a 70-μm cell strainer (Biologix Group Limited, Jinan, China.), which was positioned on top of a 50-ml conical tube, to remove the remaining undigested tissue debris. The strainer was subsequently washed with 10 ml of DPBS to collect all released cells.

In the modified group, a new method combining the more dynamic digestion with agitation and five quick harvestings of the freshly released cells during a 10-min digestion period was adopted. First, a 50-ml conical tube with 15 ml of complete medium was prepared. The epidermal pieces were rapidly transferred to a new sterile 60-mm dish in the same manner as that performed in the control group. Then, 3 ml of 0.25% trypsin-EDTA preheated at 37°C were added to the dish with the unminced epidermal pieces. Then, the solution was continuously stirred with a 1-ml pipette tip for 2 min, and the solution became cloudy as the individual epidermal cells were released. The solution was immediately transferred to a 50-ml conical tube to stop the enzymatic digestion. Another 3 ml of 0.25% trypsin-EDTA were added to the dish, and the mixture was stirred again for 2 min and transferred to the same conical tube. These steps were repeated 5 times during the 10-min digestion period. Finally, the cell suspension was passed through a 70-μm cell strainer in the same manner as that performed in the control group (Fig. 1).

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