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Primary Culture by Enzymatic Disaggregation

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ABSTRACT

Primary cells are essential in cellular experiments because of their features, such as high biological relevance, original genome, and the best experimental models for *in vivo* studies. The primary culture originated with Wilhelm Roux's efforts, but it began in 1907 with Ross Harrison's experiments on frogs and the growing of neuron fibers. Enzymatic disaggregation is a helpful tool to separate cells from organs or tissues. This method is one of the three main disaggregation methods (*fine dissection, enzymatic disaggregation, and mechanical disaggregation*) that are used in primary culture. This study established a new enzymatic disaggregation method to harvest murine prostate cells by collagenase enzyme.

Keywords: Primary Culture, Methodologies, Enzymatic Disaggregation

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1. Introduction

Cell culture's development significantly modified the biology science area and contributed to remarkable medical advancements. Wilhelm Roux used saline solution to maintain chicken embryos alive outside the egg for a few days in the 1800s; it was the first observation leading to cell culture development. Therefore, the basic principles of cell culture were raised **(1-3)**. However, primary cell culture began in 1907, when Ross Harrison successfully used the hanging drop method to culture frog neurons **(4)**. For this experiment, he applied small fragments of frog embryonic tissue immersed in a solution of lymph droplets on the cover slide. Then, he turned the plate, maintained the primary cell culture, and watched the growing neuron fibers **(4, 5)**. Primary cells have advantages compared to cell lines; for example, they have high biological relevance, original genome, and the best experimental models for *in vivo* studies **(6)**. Primary cells are taken directly from organs and tissues through *fine dissection* (Chopping down to explant size), *enzymatic disaggregation, or mechanical disaggregation* (e.g., sieving, syringing, or vigorous pipetting) and are considered primary until subculture (the first passage) **(7, 8)**. Enzymatic method has three disaggregation types: cold trypsin, warm trypsin, and collagenase **(8)**. This study established a new enzymatic disaggregation method to harvest murine prostate cells by collagenase enzyme.

2. Protocol

1. Mouse prostate tissues were transferred to the cell culture lab in collection medium: Hanks buffer (Biowest, Nuaillé, France) or phosphate-buffered saline (PBS) supplemented with 10% Penicillin-Streptomycin (Biosera, East Sussex, UK) and 70 µl amphotericin B (2.5 mg/ml; Sigma, Germany).

2. Tissues were transferred to a Petri dish. After dissecting fat and necrotic tissues, samples were washed twice with PBS. The tissues were then chopped into 1 mm^3 pieces with a sharp scalpel.

3. The chopped cubes were incubated in Hanks buffer with collagenase type I (100 U; Invitrogen, Massachusetts, USA) for two hours at 37 °C. The cells were dissociated by sampling up and down slowly every 15 min or incubation in a shaker incubator at 65 rpm.

4. Following incubation, the disaggregated tissue was passed through a 40 µm filter mesh with the medium. The suspension was centrifuged at 1700 rpm for 6 min to obtain single cells.

5. Cell pellet was resuspended in DMEM-F12 (Biowest) supplemented with 15% heat-inactivated Fetal Bovine Serum (FBS; Biowest) and 1% penicillin-streptomycin and propagated in a 25 cm^2 tissue culture flask.

3. Results and Discussion

Two days post-seeding, the medium was changed. Cells isolated from prostate tissue by enzymatic and mechanical digestion methods were grown in 5% CO_2 and 37 °C in DMEM-F12 medium containing 15% FBS and 1% Penicillin-Streptomycin. After 5 days, the cells were observed with spindle-shaped morphology (Figure 1).

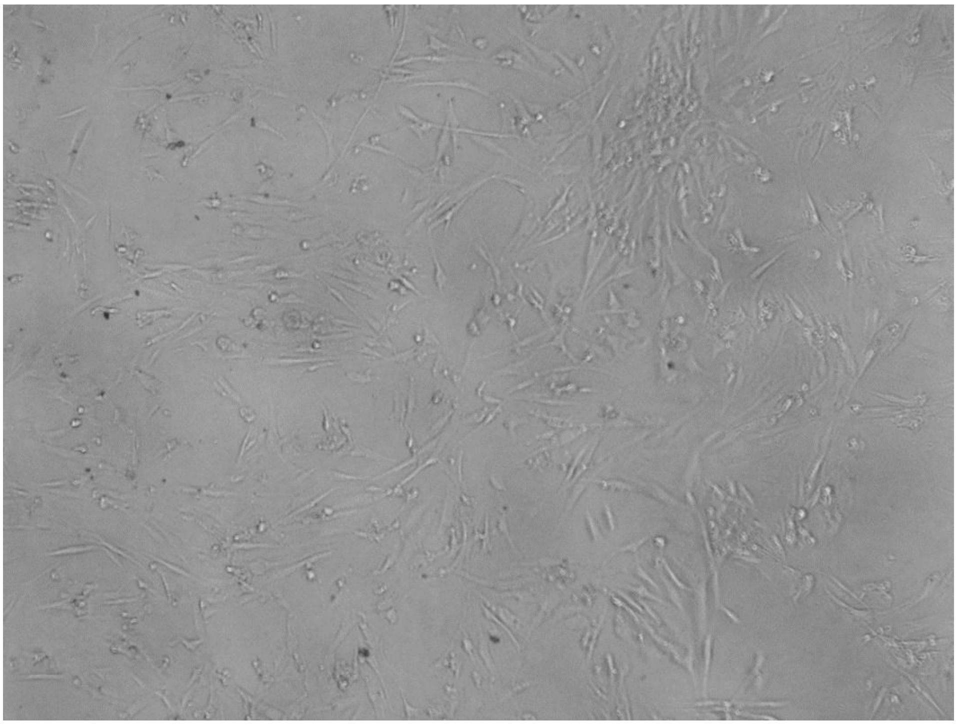

Figure 1. The spindle-shaped morphology of isolated primary cells after 5 days, magnification 10 X (Design by Authors, 2024)

Our study has some advantages and limitations; Enzymatic disaggregation is highly efficient when applying a suitable cell dissociation enzyme. This process provides a better yield than other methods when more tissue is available and yields a higher number of cells.

However, it is more time-consuming and can also unintentionally change cell surface marker **(9, 10)**.

For further investigation, the cells should be characterized e.g. immunophenotyping of selective biomarkers to validate the type of isolated cells **(11-13)**.

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Ethical Considerations

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

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