

Primary Culture by Enzymatic Disaggregation

Shima Aliebrahimi¹ , Ahmad Habibian Sezavar² , Babak Sabet³ , Seyed Nasser Ostad^{2,4*} ,
Vahideh Montazeri^{1*} 

1. Department of Artificial Intelligence, Smart University of Medical Sciences, Tehran, Iran
2. Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
3. Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Toxicology and Poisoning Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

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

Received: 17 August 2023;

Accepted: 12 December 2023;

Published Online: 21 January 2024;

Corresponding Information:

Vahideh Montazeri, Department of Artificial Intelligence, Smart University of Medical Sciences, Tehran, Iran & Email: vmontazeri2012@gmail.com

Seyed Nasser Ostad, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran & Email: ostadnas@tums.ac.ir



Copyright © 2024. This is an original open-access article distributed under the terms of the [Creative Commons Attribution-noncommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/) International License which permits copy and redistribution of the material just in noncommercial usages with proper citation.

Use DOI and watch the video article online



Highlights:

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. In this study, we established a new enzymatic disaggregation method for prostate cells from the tissue by collagenase enzyme.

Use a device to scan and read the article online



Aliebrahimi Sh, Habibian Sezavar A, Sabet B, Ostad SN, Montazeri V. Primary Culture by Enzymatic Disaggregation. J Vis Med. 2024;1:e0101.

Download Citation:

[RIS](#) | [EndNote](#) | [Mendeley](#) | [BibTeX](#) |

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 mm³ 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 cm² 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% CO₂ and 37 °C in DMEM-F12 medium containing 15% FBS and 1% Pen/Strep. After 5 days, the cells were observed with spindle-shaped morphology (Figure 1).

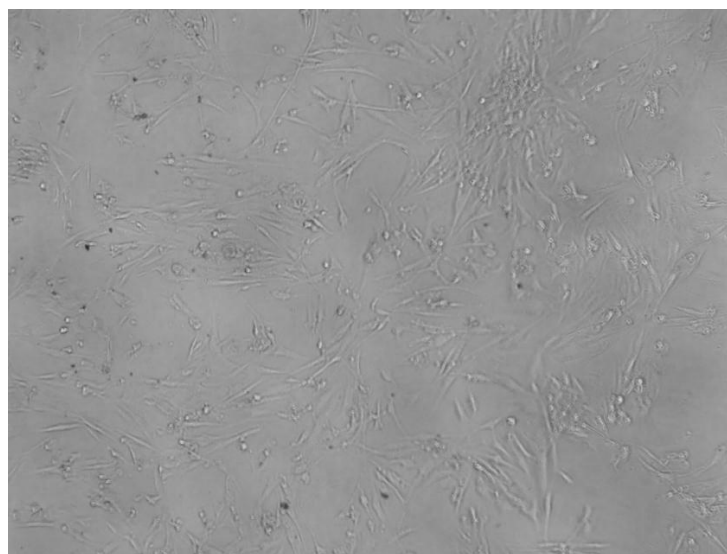


Figure 1. The spindle-shaped morphology of isolated primary cells after 5 days, magnification 10 X (Design by Authors, 2024).

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 (10-13).

4. Declarations

Acknowledgment

The authors are grateful to the Faculty of Pharmacy, Tehran University of Medical Sciences for technical support.

Ethical Considerations

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

Financial Support and Sponsorship

This research was supported under grant number 544 of Smart University of Medical Sciences.

References

1. Eitan E, Zhang S, Witwer KW, Mattson MP. Extracellular vesicle-depleted fetal bovine and human sera have reduced capacity to support cell growth. *J Extracell Vesicles*. 2015;4(1):26373. [DOI:10.3402/jev.v4.26373] [PMID] [PMCID]
2. Fischer R, Stubenrauch M, Straube A, Wedrich K, Goj B, Bartsch H, et al. System for automated cell cultivation and analysis: Universitätsbibliothek Ilmenau; 2017. Accessed from: [https://www.tu-ilmenau.de/universitaet/quicklinks/universitaet-sbibliothek]
3. Merten O-W. Introduction to animal cell culture technology-past, present and future. *Cytotechnology*. 2006;50(1-3):1. [PMID] [PMCID] [DOI:10.1007/s10616-006-9009-4]
4. Abercrombie M. Ross Granville Harrison, 1870-1959. The Royal Society London; 1961.
5. Maienschein J. Ross Granville Harrison (1870-1959) and perspectives on regeneration. *J Exp Zool Part B: Mol Dev Evol*. 2010;314(8):607-15. [DOI:10.1002/jez.b.21368] [PMID]
6. Richter M, Piwocka O, Musielak M, Piotrowski I, Suchorska WM, Trzeciak T. From donor to the lab: A fascinating journey of primary cell lines. *Front Cell Dev Biol*. 2021;9:711381. [PMCID] [DOI:10.3389/fcell.2021.711381] [PMID]
7. Magdalena J-S. History of Cell Culture. In: Sivakumar Joghi Thatha G, editor. *New Insights into Cell Culture Technology*. Rijeka: IntechOpen; 2017. p. Ch. 1.
8. Freshney RI. *Culture of Animal Cells*: Wiley; 1993.
9. Freshney RI. *Culture of animal cells: a manual of basic technique and specialized applications*: John Wiley & Sons; 2015.
10. Masters J. *Animal cell culture: a practical approach*: OUP Oxford; 2000.
11. Taylor JA, Richter CA, Suzuki A, Watanabe H, Iguchi T, Coser KR, Shioda T, vom Saal FS. Dose-related estrogen effects on gene expression in fetal mouse prostate mesenchymal cells. *PLoS One*. 2012;7(10):e48311. [PMID] [PMCID] [DOI:10.1371/journal.pone.0048311]
12. Richter CA, Taylor JA, Ruhlen RL, Welshons WV, Vom Saal FS. Estradiol and bisphenol A stimulate androgen receptor and estrogen receptor gene expression in fetal mouse prostate mesenchyme cells. *Environ Health Perspect*. 2007;115(6):902-8. [DOI:10.1289/ehp.9804] [PMID] [PMCID]
13. Bhandari RK, Taylor JA, Sommerfeld-Sager J, Tillitt DE, Ricke WA, Vom Saal FS. Estrogen receptor 1 expression and methylation of Esr1 promoter in mouse fetal prostate mesenchymal cells induced by gestational exposure to bisphenol A or ethinylestradiol. *Environ Epigenetics*. 2019;5(3):dvz012. [DOI:10.1093/eep/dvz012] [PMID] [PMCID]