# Establishment of simpler and effective primary cultures from ovarian cancer specimens of Eastern India Population

Shuvojit Moulik, Sayantani Karmakar, Siddikuzzman, Chandan Mandal, Asama Mukherjee, Arup K Pattanayak, Mousumi Som, Dona Chakraborty, Manisha Karar, Barnali Ghosh, Ratnaprabha Majhi and **Asima Mukhopadhyay**.

## Introduction

### Comparison between Primary Cells and Continuous cell lines

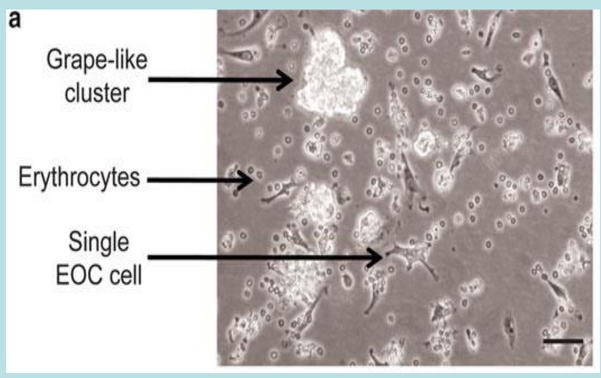
| Properties | Primary cells | Continuous cell lines |
|---|---|---|
| Life span and cell proliferation | It is finite (i.e. limited to a less number of cell divisions) | It is infinite when handled properly (i.e. for a long period, approx. 30 cell divisions) |
| Consistency | Variability exists between donors and preparations | Minimal variability |
| Genetic Integrity | Retains in vivo tissue genetic makeup through cell doublings | Subject to genetic drift as cells divide (undefined set of mutations) |
| Biological relevance | More closely mimics the physiology of cells in vivo | Relevance can drift over time as cells divide (minimal biological relevance) |
| Ease of use (freeze-thaw & use) | Needs optimized culture conditions and careful handling | Well established conditions and robust protocols exist |
| Time & expense to use | Needs more time and less abundance of cells | Needs less time and more abundance of cells |

Epithelial ovarian cancer (EOC) remains the most lethal of the gynecological malignancies, due to lack of distinct symptoms during the early course of the disease and insidious peritoneal spread at later stages. Established cell lines (secondary cultures) are easy to culture and maintain for prolonged periods possess but with genetic and biochemical abnormalities driven by immortalized growth. On the contrary short time cultures known as primary cultures mimic better the clinical setting.

### Advantages and Drawbacks of Primary Cells

| Advantages | Drawbacks |
|---|---|
| Use of primary culture avoids many ethical objections raised against animal experiments Allows experiments on human tissues which otherwise could not have been done in vivo. | Primary cells takes more time to grow than other cell lines, it possess limited growth potential even under optimal growth conditions and eventually senesce and die. |
| The use of primary cells provides more relevant results than cell lines. Pre-screened primary cells are good models to represent the signaling in vivo very closely. | The cells taken from different donors behave differently in response to pro-inflammatory cytokines (unless they are pre-screened).2 The growth of metabolic regulatory mechanism that exist under in vivo conditions are absent in culture condition. |
| Primary cells are cost-effective as they help reduce the expenditure on animal models required for in vivo studies | The cost of isolation and culture is often high and prohibitive though cheaper that animal models. The tissue culture may not be always possible. The characteristics of primary cells may change with each subsequent passage if optimum culture conditions are not maintained. |

For the isolation and culturing of solid tumor EOC cells, we have employed three different methods: (1) enzymatic dissociation with collagenase, (2) mechanical tissue dis-ruption via a cell scraper, and (3) derivation of EOC cells via explant cultures. In these the first two methods are used generally for setting up primary ovarian cell cultures.




## Aim

1. To check the feasibility of primary culture by mechanical shearing in clinical specimen from eastern India
2. To estimate the cost effectiveness of the procedure
3. To see preliminary differences between two techniques at molecular level

## Method

**Mechanical Shearing**

1. The ovarian tumor specimens must be collected aseptically and transported to the laboratory on ice
2. Pre-warm the OSE complete medium in a 37 °C water bath
3. Working in a tissue culture biosafety cabinet and with sterile forceps transfer the tumor tissue (0.5–1 $cm^2$) into 100 mm culture dish with 8mL of pre-warmed complete (20% FBS )RPMI medium
4. While holding the piece of tumor tissue with the forceps, gently dissociate cells from the tissue with a sterile cell scraper
5. Incubate the dissociated cells overnight in a tissue culture incubator (37ºC, 5% $CO_2$, 95% air).
6. The next day, remove the medium with a sterile Pasteur pipette fixed to a vacuum and waste receptacle system.
7. With a sterile disposable serological pipette, add 8mL of pre-warmed, fresh OSE complete medium
8. Tumor-derived EOC cells can be characterized for the presence of epithelial and other contaminating cell types via immunocytochemistry

**Disclaimer :**
The following issues are fully considered while dealing with human tissues:
1. The consent of the patient and/or relatives for using tissues for research purposes.
2. Ownership of the cell lines developed and their derivatives.
3. Consent for genetic modification of the cell lines.

**Enzymatic dissociation**

1. Collect the tumor from ovarian cancer patient
2. Wash with PBS and transfer to 15 ml centrifuge tube in Dispase II solution (1.6U /ml, for 2-3 ml)
3. Keep at $4^0$C for 16hr followed by 1 hr room temperature
4. Collect the Dispase II solution and wash with PBS
5. Transfer the tissue in 0.025% trypsin- EDTA solution, dissected into, ~3mm pieces
6. Keep 12 min at $37^O$C with gentle shaking
7. Neutralized the solution with equal volume DMEM (10% FCS)
8. The cell suspension will be transferred to a 15ml tube, centrifuged at 400xG for 5 minutes,
9. PBS wash, re-suspended in RPMI medium (20% FCS) and will be placed in a T25 flask.

## Result and Discussion

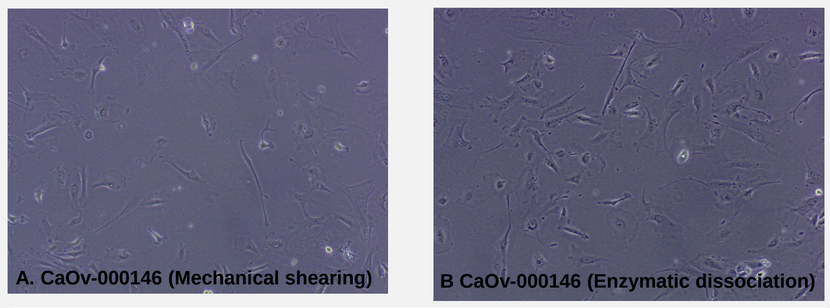



Fig: Representative pictures of Primary Culture from mechanical shearing (A) and enzymatic dissociation (B) techniques.

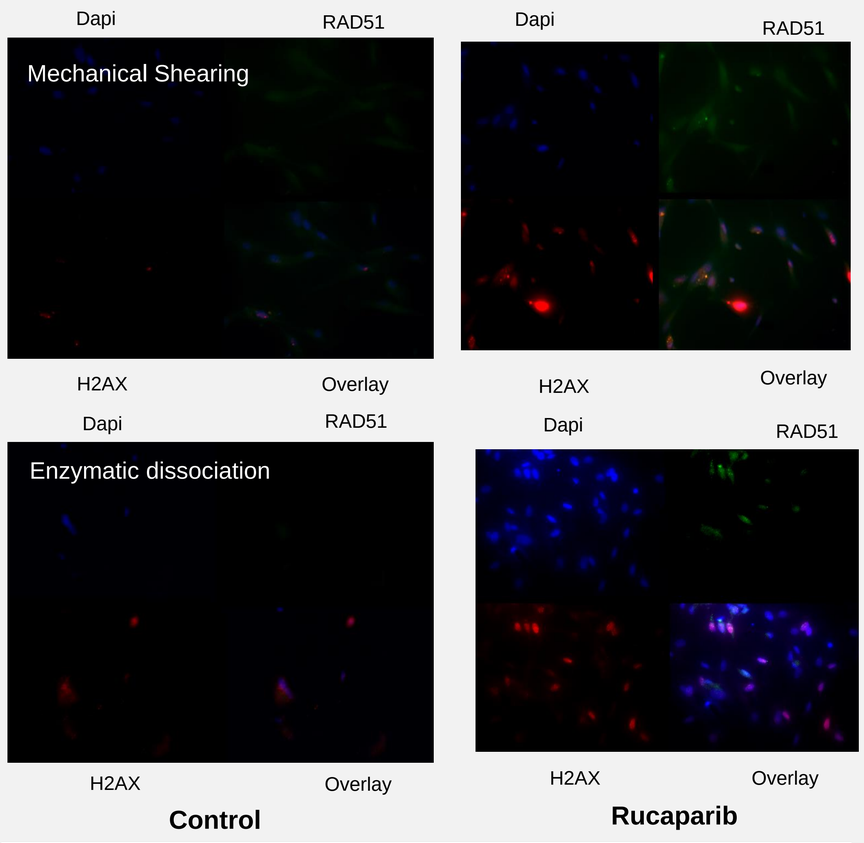



Fig: Representative pictures of co-localization of γ-H2AX and RAD51 foci on Rucaparib (PARP-i) treated at dose of 10uM for 24hrs to investigate activity of homologous recombination.

Primary culture by mechanical shearing after initial 2 days yields less cells than cells by enzymatic dissociation. This is primarily because the cell scrapping technique is based on only loosely adhered cells.

Co-localisation of gammaH2AX and RAD51 foci on Rucaparib , a PARP inhibitor shows a two fold increase with respect to control by both the techniques.

The cost analysis for both the techniques showed that Enzymatic dissociation is almost 4 times costlier than mechanical shearing

Human EOC consists of several different histological subtypes, and we receive samples that reflect the expected prevalence observed in patients. Serous, mucinous, clear cell, and poorly differentiated EOC cells have been successfully cultured and archived.

| Mechanical Shearing | Enzymatic dissociation |
|---|---|
| Only a scraper is used to dissociate the tissue. No other forms of mechanical disruption like scalpel is being used. **Less harsh techniuque** | Apart from mechanical disruption like using of scalpel , enzymes like collagenase disapase , trypsin are being used which **causes cell damage** due to breakage of covalent bonds |
| Rapid technique, effective time being 2-3 hours | Slow technique, effective time being 24-36 hours |
| Cell yield is low | Cell yield is high |
| **Approximate total cost per sample is 866/-** | **Approximate total cost per sample is Rs 3048/-** |

## Conclusion

1. Less harsh technique for primary cell culture
2. Cost effective than other techniques
3. Simpler way of mimicking clinical settings

## Future Direction

1. To standardise further the technique and characterize it in molecular level
2. To make 3 D ovarian cultures to check drug efficacy

**Acknowledgements**

- Prof. Vaskar Saha. Prof. Usha Menon
- TCS (TTCRC), Medgenome, DST-UKIERI

TATA MEDICAL CENTER

**TATA MEDICAL CENTRE, Kolkata**
**14 MAR (E-W), New Town,**
**Rajarhat,**
**Kolkata 700 160**
**E-mail: asima7@yahoo.co.in**
**Phone: +91 33 6605 7000**