Experiment time: 2025-03-06, 18:00 - 2025-03-06, 20:00

Cell Subculture and Medium Change

I. Objectives of the experiment

1.1 The T24 cell line and the moderately resistant and highly resistant T24-RC48 cell lines were passaged to maintain continuous cell proliferation and ensure an adequate supply of nutrients.

1.2 The low-dose resistant T24-RC48 cell line was was performed to remove metabolic waste products and replenish fresh culture medium.

II. Materials and reagents

2.1 Materials

Pipette tips and 15ml centrifuge tube were purchased from Axygen

Serological pipettes were purchased from a domestic company

Micropipettes: Eppendorf

T-25 cell culture flasks were purchased from Corning

Human bladder transitional cell carcinoma cell line (T24)

Low, medium, and high drug-resistant cell lines T24-RC48

2.2 Reagents

Phosphate-Buffered Saline (PBS) and trypsin were purchased from Gibco, USA

McCoy's 5A medium from Shanghai Yuanpei Biotechnology Co., Ltd., China

Fetal bovine serum (FBS, Gibco)

III. Experimental instruments

Clean bench: Suzhou purification equipment factory

Benchtop low-speed centrifuge at room temperature: Eppendorf

IV. Experimental steps

4.1 Wipe the surface of the ultra-clean workbench with alcohol cotton. Sort out the experimental tools. Put the required reagents and consumables into the ultra-clean workbench. Turn on the ultraviolet lamp, and disinfect for 30 minutes.

4.2 Disinfection: Hands were disinfected with alcohol. An alcohol lamp was ignited. Using

forceps, a graduated serological pipette was taken. The caps and openings of all reagent bottles were briefly passed through the outer flame of the alcohol lamp. PBS and McCoy's 5A medium bottles were opened. The complete culture medium, supplemented with FBS and antibiotics, was placed in an accessible position.

4.3 Take the cell culture bottle containing T24 and T24-RC48 cell lines out from the 37℃ constant temperature box, and observe the cell culture under microscope: a large number of cells grow on the wall.

4.4 The neck of the culture flask was briefly passed through the flame of the alcohol lamp to sterilize it before the medium was decanted into a waste container.

4.5 Transfer about 4ml of PBS with a graduated pipette into the culture bottle. Place the culture bottle horizontally. Gently shake the culture bottle front and back. Rinse off cell fragments and residual culture medium, and pour the cleaning liquid into the waste tank. Repeat this step twice.

4.6 Transfer 5ml of complete medium with a graduated pipette into the culture bottle containing low drug resistance T24-RC48 cell line.

4.7 1 mL of trypsin solution was added to each flask using a new sterile pipette. The flasks were incubated at 37°C for 2 minutes. Upon microscopic examination, cells appeared shrunk and rounded but not fully detached. Incubation was continued for an additional 30 seconds. Subsequent observation revealed that the cells detached and assumed a 'sandy' appearance under the microscope. Intercellular connections were lost, and rounded cells were suspended in the medium without significant clumping.

4.8 Add 2 mL of complete culture medium to the culture flask using a calibrated pipette to terminate the trypsin digestion. After gently pipetting the medium up and down several times to dissociate the cells, transfer the remaining liquid to a 15 mL centrifuge tube. Mark the tube and place it in the centrifuge. After equilibration, centrifuge at 800rpm for 5 minutes.

4.9 During centrifugation, add two tubes of complete culture medium (3ml/bottle) to each of the three cell culture bottles with a new pipette.

4.10 Take out the centrifuge tube and pour out the supernatant. Add about 6ml of full culture liquid into the centrifuge tube with a graduated pipette, and the cell pellet was resuspended by gently pipetting up and down. Then divide the culture liquid into three cell culture bottles with a

graduated pipette. The flasks were gently rocked back and forth and side-to-side to ensure even cell distribution.

4.11 Label the date and cell type on the cell culture bottle.

4.12 Set up the ultra-clean table and wipe it with alcohol cotton balls.

V. Photos of experimental operation:

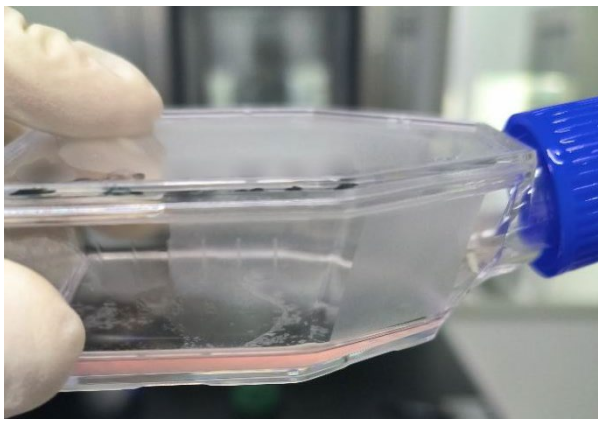

Figure 1. Visual observation of digested cells

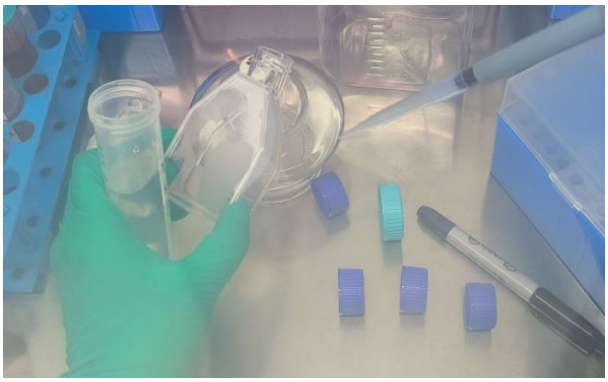

Figure 2. Wash off the residual medium with PBS

VI. Experimental results

6.1 For each of the three cell lines (T24, moderately resistant, and highly resistant T24-RC48), three new culture flasks were successfully established through passaging. The next day, under the microscope, it was observed that all the cells were attached to the wall, but their morphology was slightly distorted morphology with mild elongation.

6.2 The morphology of the low-resistant T24-RC48 cell line remained good after fluid replacement.

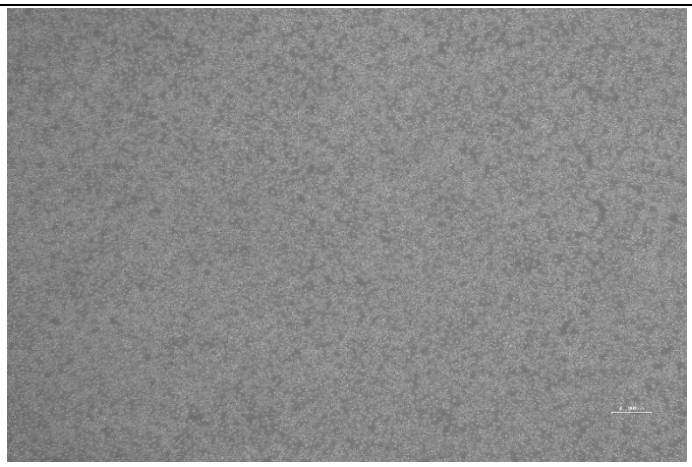

Figure 3. Cells after digestion observed under a phase-contrast microscope

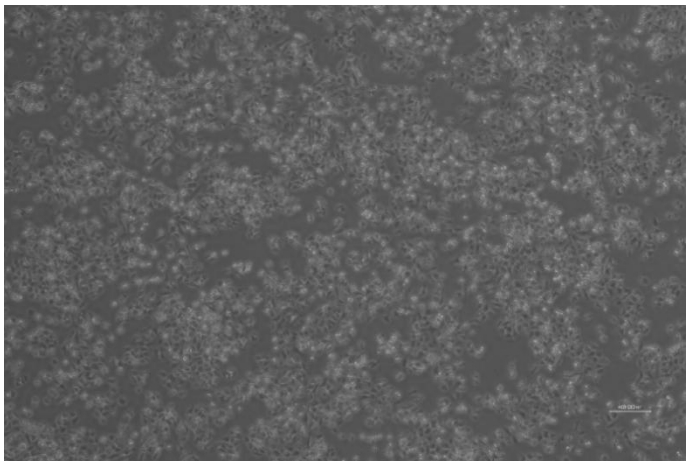

Figure 4 Status of low drug resistance T24-RC48 cell line after fluid replacement

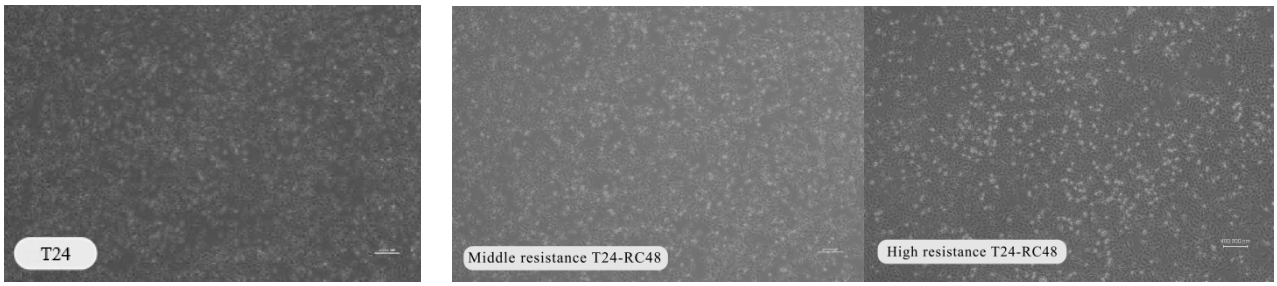

Figure 5. Growth of passaged cells overnight under an inverted phase microscope

VII. Results analysis

The T24 cell line and moderately to highly resistant T24-RC48 cell lines exhibited slightly distorted morphology with mild stringing on the second day, while the low-resistance T24-RC48 cell line maintained better morphology after culture medium replacement. This morphological discrepancy is likely attributable to the compromised state of the T24 parental line and the moderately/highly resistant T24-RC48 sublines. These cells were subjected to a suboptimal, forced

passaging due to high cell density shortly after recovery, before they had fully stabilized. In contrast, the low-resistance T24-RC48 subline experienced a longer period of stable growth after recovery, allowing it to maintain a fuller morphology and healthier status.