Cell Adhesion Assay

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[Abstract] Cell adhesion, the binding of a cell to the extracellular matrix (ECM), other cells, or a specific surface, is essential for the growth and survival of the cell and also its communication with other cells. The process of cell adhesion involves a range of biological events such as three-dimensional re-organization of the cytoskeleton, biochemical reactions in the cell, and changes in molecules on the surface of the cell. Cancer cells, especially the highly metastatic types, are believed to have enhanced adhesion ability that often facilitates the migration of the cells to a new site to establish new tumors in the body. Cell adhesion assay is therefore often used to evaluate the metastatic ability of cancer cells. In addition, the assay can also be used to assess the effect of certain treatment (*e.g.*, exposure to chemicals) on the ability of cells to adhere. A modified cell adhesion assay protocol is described here for studying the interactions between cells and extracellular materials.

Materials and Reagents

1. Hela cells (ATCC, catalog number: CCL-2™)
2. MTT cell proliferation assay kit (ATCC, catalog number: 30-1010K™)
3. Collagen I (Sigma-Aldrich, catalog number: C7661)
4. Dulbecco's modified eagle medium (DMEM) (Life Technologies, Invitrogen™, catalog number: 10313-021)
5. Fetal bovine serum (FBS) (ATCC, catalog number: 30-2020™)
6. 0.5 M EDTA solution (pH 8.0) (Life Technologies, Invitrogen™/Ambion®, catalog number: AM9260G)
7. Bovine serum albumin (BSA) (Life Technologies, Invitrogen™, catalog number: 15561-020)
8. Phosphate buffered saline (PBS) (Life Technologies, Invitrogen™, catalog number: 14190-144)

Equipment

1. Corning 96-well polystyrene plate (Fisher Scientific, catalog number: 07-200-91; Corning Incorporated, catalog number: 3598)
2. Cell culture incubator: 37 °C and 5% CO_2
3. Spectrophotometer that can measure absorption at 570 nm with 96-well format

Procedure

1. Grow the Hela cells in DMEM supplemented with 10% FBS.
2. Prepare 40 μg/ml Collagen I solution in PBS, store at 4 °C; prepare 0.1% BSA solution in DMEM.
3. Coat the 96-well plate (30 μl/well) with the Collagen I solution at 4 °C.
4. After 12 h of coating, remove the Collagen I solution and air-dry the plate at room temperature in the tissue-culture hood.
5. Deprive cells of serum for 8 h before the adhesion assay. To do so, wash cells three times with serum-free DMEM and grow them in DMEM.
6. Use 10 mM EDTA in DMEM to detach the cells and then observe them under a microscope to confirm complete dissociation of the cells, which would take ~10 min.
7. Wash cells twice with DMEM to remove EDTA, resuspend cells at 2 x 10^5 cells ml^{-1} in DMEM with 0.1% BSA.
8. For cell-substratum adhesion assay, add 100 μl cell suspension (from step 7) to each of the Collagen I-coated wells. Incubate the plate at 37 °C for 20 min to allow the cells to adhere to the surface.
9. Add 100 μl DMEM to each well to wash off any non-adherent cells, wash four times.
   Note: To achieve consistency, always add/remove DMEM gently with multi-channel pipetter for multiple wells.
10. After washing, add DMEM with 10% FBS and incubate the cells at 37 °C for 4 h for recovery.
11. Add 10 μl of MTT substrate to each well and continue incubation for an additional 2 h at 30 °C.
12. Next, lyse the MTT-treated cells in 100 μl DMSO (or other lysis buffer of choice) and measure absorbance at 570 nm on a spectrophotometer (see Note 1).

Notes

1. Consider including the following reference group for monitoring each step of the procedure: Wells not coated with Collagen I; wells not washed with DMEM; wells not added with cells; wells not added with MTT (background for MTT assay).

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References

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