

***In vivo* Matrigel Plug Angiogenesis Assay**

Hong Lok Lung and Maria Li Lung*

Department of Clinical Oncology and Center for Cancer Research, The University of Hong Kong,
Hong Kong, Hong Kong SAR

*For correspondence: mlilung@hku.hk

[Abstract] The matrigel plug angiogenesis assay is a simple *in vivo* technique to detect the newly formed blood vessels in the transplanted gel plugs in nude mice. The matrigel matrix is derived from the engelbroth-holm-swarm (EHS) mouse sarcoma, and its composition is comparable to the basement membrane proteins. The matrigel can induce differentiation of a variety of cell types such as hepatocytes, mammary epithelial cells, and endothelial cells. In our case, tumor cells are mixed with the matrigel gel and are injected into the mice. The later immunohistochemistry (IHC) staining with the endothelial marker indicates the presence of the newly formed capillaries in the sectioned gel plugs.

Materials and Reagents

1. BALB/cAnN-nu (*Nude*) female mouse, 6 to 8 week-old
2. Tumor cells
3. Matrigel matrix (BD Biosciences, Falcon[®], catalog number: 354234)
4. 10% formalin solution-neutral buffered (Sigma-Aldrich, catalog number: HT501128-4L)
5. Anti-rat CD34 (Santa Cruz, catalog number: sc-18917)
6. Biotin goat Anti-Rat IgG (BD Biosciences, catalog number: 559286)
7. Streptavidin-peroxidase conjugate (Dako, catalog number: P0397)
8. Diaminobenzidine (DAB) (Dako, catalog number: K3467)

9. Paraffin
10. Potassium chloride
11. Potassium phosphate monobasic
12. Sodium chloride (NaCl)
13. Sodium phosphate dibasic anhydrous
14. Hematoxylin and eosin (H&E)
15. Trypsin
16. Complete medium
17. Plain medium
18. Phosphate buffered saline (PBS) (see Recipes)

Software

1. Aperio ScanScope System
2. ImageScope v10 software (Aperio, Vista)

Equipment

1. Aperio Scanscope CS-S microscopic slide scanning system
2. Tumor cell culture set up
3. Hemocytometer
4. Centrifuges
5. 24G syringe
6. T175 flask

Procedure

1. Remove the medium from a T175 flask containing an 80-90% confluent monolayer of tumor cells (e.g. the nasopharyngeal carcinoma HONE1 cells) and wash the cells with PBS.
2. Detach the tumor cells by incubating with 3 ml trypsin for 5-10 min; stop trypsinization with 10 ml complete medium.
3. Count the number of cells with a hemocytometer, spin down the cells at 1,200 rpm for 5 min and wash once with PBS.
4. Mix a total of 5×10^6 to 1×10^7 cells (cell type-dependent) with 50 μ l plain medium and 250 μ l ice-cold matrigel (the matrigel maintains as liquid form at 2-8 °C and solidifies rapidly at 22 -37 °C).
5. Subcutaneously inject the 300 μ l cell matrigel mixture into a flank of five female athymic nude mice (one injection site per mouse) with an ice-cold syringe with a 24G one inch needle.
6. Polymerize the matrigel mixed with the cells to form a solid gel plug, which allows cell growth and blood vessel formation.
7. After inoculation for 7 days, excise the matrigel, fix with formalin overnight, embed in paraffin, and section onto slides.
8. Stain the slides with hematoxylin and eosin (H&E) for histological observation.
9. Stain the blood vessels formed with 150 μ l endothelial cell marker CD34 monoclonal antibody (1:40) overnight at 4 °C and 150 μ l secondary biotin-conjugated goat anti-Rat IgG antibody (1:100) for 1 h at room temperature (RT).
10. Incubate the antibody with 150 μ l streptavidin-peroxidase conjugate (1:200) for 1 h at RT.
11. Add the substrate DAB for signal detection.
12. Scan the slides by the Aperio ScanScope System and quantify the signal by ImageScope v10 software.

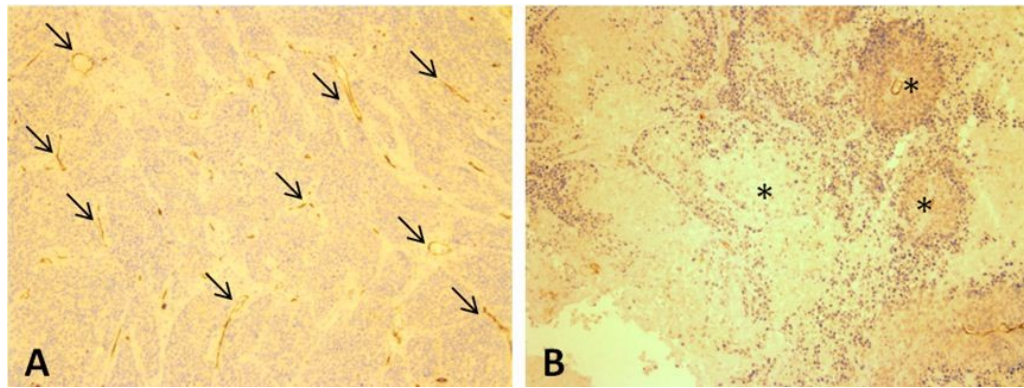


Figure 1. Representative results from matrigel plug assay. The endothelial cells were stained with anti-CD34 antibody, as indicated by the brown stain (indicated by arrows). A. Plenty of blood capillaries are formed in the gel plug section with growing tumor cells. B. Representative image of cell necrosis observed in center region of tumor nodules formed with lack of blood capillaries (indicated by asterisks).

Recipes

1. PBS (pH 7.4)
 - 2.67 mM potassium chloride
 - 1.47 mM potassium phosphate monobasic
 - 137.93 mM sodium chloride (NaCl)
 - 8.1 mM sodium phosphate dibasic anhydrous

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