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Methods for Implantation of Corning® Matrigel® Matrix into Mice and Tissue Fixation

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Introduction

Corning Matrigel Matrix is a solubilized basement membrane preparation extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in ECM proteins. Its major component is laminin, followed by collagen IV, heparan sulfate proteo-glycan, and entactin. Corning Matrigel Matrix is effective for the attachment and differentiation of both normal and transformed anchorage-dependent epithelial and other cell types.

Corning Matrigel Matrix is highly useful in various studies including 3D cell culture, cell invasion and migration assays, drug metabolism/toxicology, *in vitro* and *in vivo* angiogenesis assays. This report describes the use of Corning Matrigel Matrix for *in vivo* applications such as angiogenesis and human tumor cell implantation in mice.[1-8]

1. Corning Matrigel Matrix (Cat. Nos. 354234 and 356234) is suitable as a scaffold for supporting the implantation of various tumor cells. Growth Factor Reduced (GFR) Corning Matrigel Matrix (Cat. Nos. 354230 and 356230) is also available for studies in which a reduced growth factor composition is required.
2. Corning Matrigel Matrix phenol red-free (Cat. No. 356237) and GFR Corning Matrigel Matrix, phenol red-free (Cat. No. 356231) are typically used for the cyanmetrohemoglobin method that measures hemoglobin content (measurement of reddish-brown absorption) in angiogenesis studies. Corning Matrigel Matrix has been shown to enhance the process of angiogenesis *in vivo*.
3. Corning Matrigel Matrix High Concentration (HC) (Cat No. 354248) is suited for *in vivo* applications where a high protein concentration augments growth of tumors. The high protein concentration (18-22 mg/mL) also allows the Corning Matrigel Matrix plug to maintain its integrity after subcutaneous injection into mice. This keeps the injected tumor cells and/or angiogenic compounds localized for *in situ* analysis and/or future excision.

Procedures

Subcutaneous injection of Corning Matrigel Matrix into a mouse

1. Since Corning Matrigel Matrix forms a gel above 10°C, Corning Matrigel Matrix solution should be kept at low temperatures, and thus all equipment and reagents (syringes, needles, Corning Matrigel Matrix solution, etc.) should be chilled on ice prior to injection.
2. After mixing Corning Matrigel Matrix with a cell suspension [Note 1], the Corning Matrigel mixture is injected into a mouse subcutaneously [***Note*** 2] (Figure 1). An appropriate needle size (21-25G) should be selected to prevent the destruction of cells. To increase the contact area of the injected Corning Matrigel mixture into subcutaneous tissues, a wide subcu-taneous pocket should be formed by swaying the needlepoint right and left after a routine subcutaneous insertion. The Corning Matrigel mixture is then injected into the pocket. When the Corning Matrigel mixture is injected into a particular area without swaying the needlepoint, the mixture will form a large cell clump and a subsequent growth defect may result due to inefficient perfusion of nutrients to the cells within the core of the clump.

Note 1*: In this experiment, undiluted Corning Matrigel Matrix alone was injected into the mouse. For tumor implantation applications, approximately 2x10^7 cells/mL of cell suspension should be mixed with Corning Matrigel Matrix, resulting in a final cell concentration of ~10^6 cells/mL. To prevent incomplete gel formation in mice, do not dilute Corning Matrigel Matrix to a final concentration below 4 mg/mL.*

Note 2*: In this experiment, 0.7 mL of the Corning Matrigel was injected. The injection volume of Corning Matrigel takes into account the absorption of Corning Matrigel into the tissue and allows for easy removal of the resultant tissue 'plug'. The optimal injection volume should be determined according to the requirements of your experiment.*

While the injection of ~0.1 mL of a Corning Matrigel mixture into mice may be sufficient for the augmentation of tumor growth, the injection of at least 0.5 mL is recommended for in vivo angiogenesis studies.

Figure 1*. Subcutaneous injection site.*

Removal of the Corning Matrigel plug from the mouse

3. After an appropriate incubation period [Note 3], the mouse is anaesthetized and a square segment of tissue is excised with scissors. To ensure complete excision of the plug, cut ~5 mm wider than the implantion site on all sides.To maintain the shape of the Corning Matrigel plug, excise the subcutaneous tissue, peritoneum, as well as skin. These tissues are then fixed with formalin. Figure 3 shows the implanted Corning Matrigel viewed from the peritoneal side following excision. The volume of the implanted Corning Matrigel is reduced from the injected volume due to absorption and partial degradation of Corning Matrigel *in vivo*. The excised Corning Matrigel plug is usually clear yellowish in color. If blood vessels are formed within the Corning Matrigel plug, the color of the Corning Matrigel will appear red (***Figure 4***).

Note 3*: In this experiment, the Corning Matrigel plug was removed after one week. When the quantity of hemoglobin is used to assess angiogenesis, Corning Matrigel containing VEGF and heparin should be injected to promote angiogenesis. After about three days, the Corning Matrigel plug containing newly formed blood vessels can be easily removed.*

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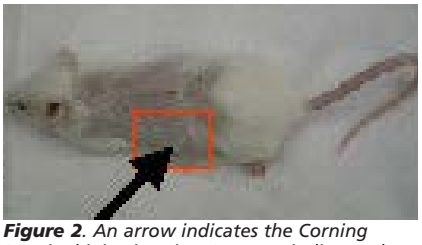

***Figure 2**. An arrow indicates the Corning Matrigel injection site. A square indicates the excised region for the sample.*

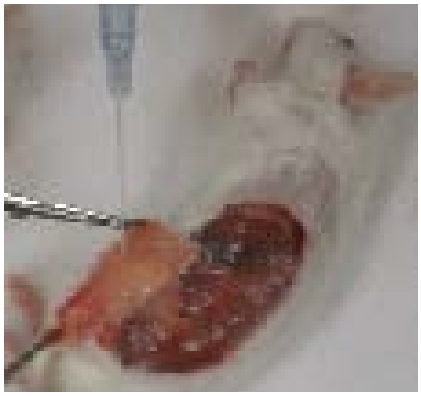

***Figure 3**. The implanted Corning Matrigel plug viewed from the peritoneal side (needlepoint).*

***Figure 4**. The removed Corning Matrigel plug from the subcutaneous tissue.*

Fixation of tissues including Corning Matrigel Matrix

4. The excised tissue should be stretched and put on a sheet of thick paper (e.g., poster board) to avoid the formation of wrinkles. The tissue is then placed in a nylon bag for protection. Fix the tissue in 10% neutralized formalin solution for at least one day at room temperature [Note 4]. This treatment will harden the tissue in preparation for slicing the sample (Figure 6). Care should be taken to ensure that the thickness of the slice is adequate to retain the implanted Corning Matrigel plug.

Note 4: Fixation of Corning® Matrigel® under 8°C may cause depolymerization of Corning Matrigel. Therefore, Corning Matrigel should be fixed at room temperature.

5. The fixed Corning Matrigel plug can be embedded in paraffin to prepare sections for histochemical staining. Figure 7 shows a section of the Corning Matrigel plug stained with hematoxylin-eosin (HE). Corning Matrigel appears pink to light reddish in color with HE staining.

***Figure 5.** The removed tissue was fixed in a nylon bag. The skin side is facing upward.*

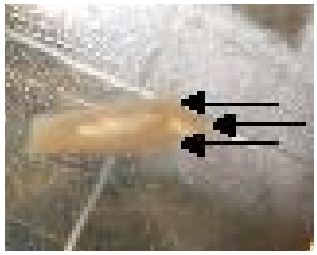

***Figure 6**. Arrows indicate the cut surface of the fixed Corning Matrigel plug. (Upper arrow = Skin; Middle arrow = Corning Matrigel; Lower arrow = muscle layer).*

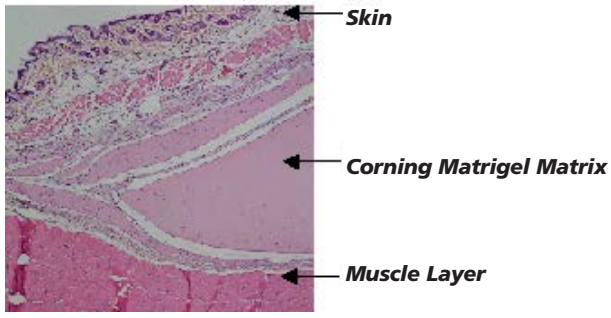

***Figure 7**. HE stain image.*

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Data provided by Dr. Kazuo Ohashi and Dr. Takashi Yokoyama at Nara Medical University.

Corning acquired the Discovery Labware Business including the Matrigel® brand. For information, visit www.corning.com/discoverylabware.

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