

1. Equipment, reagents and consumables

1.1 Equipment: Biosafety cabinet, pipette, carbon dioxide incubator, inverted microscope, centrifuge(Low-speed)

1.2 Reagent: DMEM, ECM complete medium, Trypsin Solution, 1×PBS solution

1.3 Consumables: sterile pipette tips; 96-well cell culture plate; Sterile EP tube and other consumables. (Or be adjusted according to the experimental design).

2. Experimental contents and methods

2.1 Preparation before Experiment

2.1.1 Put the Matrigel in the ice box and put it in the refrigerator at 4°C so that the Matrigel can slowly melt overnight; (Do not allow this product to warm up above 4°C during manipulation. Keep the product on ice and dilute using ice-cold solutions or cell suspensions.)

2.1.2 Consumables or reagents that come into contact with Matrigel, such as sterile centrifuge tube, sterile pipette tips and DMEM, were pre-cooled at 4°C in advance;

2.2 Plate coating procedure

2.2.1 Prepare the EP tube for placing on ice. Add each component according to the following table.

Ratio (Matrigel: DMEM)	Stock	2:1
DMEM(μL)	0	40
Matrigel(μL)	100	80

2.2.2 After mixing the above components in sequence, add 50μL/ well to the 96-well plate, (two repeated wells are recommended), mark the information and the experiment date, and solidified in a 37°C incubator for at least 1 hour.

2.3 Incubate the HUVEC

2.3.1 HUVEC were selected for pancreatic enzyme digestion. Cell density was adjusted to 4×10^5 cells /mL by using ECM medium.



Protocol

2.3.2 Take the “2.2.2” Coated 96-well plate and add the cell suspension in a volume of 50 μL per well (It means that the final number of HUVEC is 2×10^4).

2.3.3 The results of vascular structures can be observed after after 4 h and 24 h incubation in a carbon dioxide incubator at 37 °C.

