Vascular Tissue Blank™ Cell Seeding Protocol

Prellis Biologics Vascular Blanks can be stored at 4°C for up to 6 months.

Materials:

- Prellis Biologics Vascular Blank Structures (https://www.prellisbio.com/products)
- Sterile 1X PBS
- Transfer Pipets
- Cell Culture medium
- Tissue culture plate
- Optional: Poly-L-Lysine: (Sigma Aldrich #P8920), Collagen Type I (Advanced Biomatrix #5153), Geltrex (Thermo Fisher #A1569601)

Instructions

All steps should be carried out in a cell culture hood using proper sterile techniques.

Preparation of cells:

Remove cells of interest from culture and resuspend in culture medium at an appropriate concentration. Refer to Table I for the recommended cell count and required seeding volume for each structure.

Note: Optimize seeding density per structure according to cell type and application.

Note: Collagen Type I (1.0 - 2.0 mg/mL) in 1X PBS, neutralized to a pH between 6 - 7, can also be used to suspend cells in place of culture medium.

Table I: Recommended Cell Seeding Conditions for Vascular Blanks

Vascular Blank Structure	Minimum, Maximum Total Cell Count	Seeding Volume (µL)	Tissue Culture Plate Used for Seeding	Cell Adherence Time (mins)
Baskets	2.5×10^3, 2×10^6	10	12 well	20
Tissue Chips	5×10^5, 2×10^6	20	12 well	20
Vascular Bundles	2.5×10^3, 2×10^6	200	96 u-bottom well	120

Note: The number of cells seeded should be optimized according to desired culture duration and cell type.

Preparation of structures:

1. Wash the structure with sterile 1X PBS (3 x 1 minute rest). Make sure not to pipette directly on and off the structure.

2. Prepare coating solution, if desired. Refer to Table II for recommended conditions for coating solutions.

Table II: Recommended Coatings for Vascular Blanks

Coating	Recommended Concentration (w/v)	Incubation Time (mins)	Incubation Temp (°C)
Poly-L-lysine	0.1%	20	21.5 (R.T.)
Geltrex	N/A	60	37

Note: Alternative ECM components may be used to coat the structures.
Note: ECM component concentration and temperature/duration of coating should be optimized for each cell type used.

3. Remove all the PBS from the structure and add coating solution to well. Make sure the structure is entirely covered with coating solution.

4. Incubate at desired conditions, recommended 20 minute maximum.

5. Remove coating solution and wash the structure with 1X PBS (3 x 1 minute rest).

6. Transfer the structure to a new well. Avoid using the well used for coating the structure for cell seeding.

Cell Seeding of Baskets & Tissue Chips:

1. Add enough PBS to the well to just cover the top of the structure.
   Note: It is best to see a surface tension ring around the structure.

2. Additional: If using baskets, check that the structure is positioned upright with the flat side facing up and the curved side resting on the bottom of the well. Adjust the position of the basket gently with a transfer pipette.
   Note: Having the right orientation is critical for a successful seeding. If you can clearly see the horizontal crossbars in the top plane, the basket is positioned correctly.

3. Add cells in the required amount of media (Table I) directly to the top of each structure.
   Note: It is normal to observe some diffusion of the cells from the structure.

4. Gently transfer the well plate to the incubator and leave at 37°C for 20 minutes.

5. Transfer the structure to a new well to avoid the adherence and growth of cells to the surface of the well. Add fresh media.

6. Check the structure every day; change media every 2 days or as needed.
   Note: Ensure that you do not directly pipette the structure during media changes. Never directly aspirate the media or PBS. For best results, use a wide tip such as a transfer pipet or 1000 µL pipette tip.

Cell Seeding of Vascular Bundles:

1. Add cells in the required amount of media/cell suspension solution (Table I) directly to the top of each structure.

2. Gently transfer the well plate to the incubator and leave at 37°C for the required amount of time (Table 1).

3. Transfer the structure to a new well to avoid the adherence and growth of cells to the surface of the well. Add fresh media.

4. Check the structure every day; change media every 2 days or as needed.
   Note: Ensure that you do not directly pipette the structure during media changes. Never directly aspirate the media or PBS. For best results, use a wide tip such as a transfer pipet or 1000 uL pipette tip.

Optional: If culturing more than one cell type per vascular blank structure, sequentially seed cell types 1-5 days apart.