ADVANCED BIOMATRIX
A BICO COMPANY

Hydrogel Cell Seeding/Encapsulation Protocol

Description

This protocol outlines procedures for culturing cells using collagen hydrogels. It presents different seeding methods, including: (1) encapsulating cells within the hydrogel; (2) seeding cells on top of the hydrogel; and (3) culturing cells between hydrogel layers.

Materials Needed

- PureCol® (Cat #5005) or other Type I Collagen
- 1M NaOH
- Cells/cell lines of interest
- Cell culture media
- Trypsin-EDTA (0.25%)
- Phenol red (Gibco, Cat # 25200056)
- Cell culture plates
- Cell culture flasks
- Centrifuge tubes
- Serological pipettes
- Micropipette and tips
- Ice bucket

Procedure

1. Culture cells following manufacturer's protocol and prepare the cell pellets. Place cell pellets on ice to maintain cell viability.

2. Prepare the PureCol® solution by slowly adding 1 part of chilled 10X culture media to 8 parts of chilled collagen solution and gently swirling the mixture until homogenous.

3. Carefully adjust pH of mixture to 7.0–7.5 using sterile 0.1 M NaOH (~1 part). Monitor pH adjustment using a pH meter, phenol red, or pH paper.

4. Adjust the final volume to a total of 10 parts with sterile water. Place the collagen solution on ice to prevent pre-gelation*.

***Note:** Atelocollagen and Telocollagen have different gelation properties. PureCol® is Atelocollagen. Learn more here.

Method 1: Encapsulating cells within the hydrogel

1) From your vial containing cells, gently remove the excess cell culture medium by aspiration and add chilled collagen solution to the cell pellet.

2) Gently mix the collagen solution with the cells by pipetting the collagen solution up and down using the serological pipette. The cells should be well suspended in the collagen before plating.

3) Add cell-laden collagen solution to cell culture plates. Recommended seeding volume per well is listed below:

Table 1. Cell seeding volume per well

Plate size	Volume of Cell-Laden Collagen
6 well	1.5 ml
12 well	1 ml
24 well	0.5 ml
48 well	0.3 ml
96 well	0.1 to 0.2 ml

4) Place the lid on the culture plate and place the plate in the incubator (37 °C, 5% CO_2) to allow collagen gelation for >60 minutes (Atelocollagen) or >30 minutes (Telocollagen).

ADVANCED BIOMATRIX
A BICO COMPANY

5) Add cell culture medium to the plate and culture cells for desired culturing time.

Method 2: Seeding cells on top of the hydrogel

1) Add chilled collagen solution to the wells according to table 1. The hydrogel should cover the entire bottom surface of the well.

2) Place the lid on the culture plate and place the plate in the incubator (37 °C, 5% CO_2) to gel for >60 minutes (Atelocollagen) or >30 minutes (Telocollagen).

3) Resuspend cell pellet in fresh cell culture medium to achieve desired seeding density/concentration, i.e. 50,000 cells per well in 1 mL medium for a 24-well plate.

4) Gently add the cell suspension on top of the hydrogel layer in the well.

5) Incubate the plate at 37 °C for cell culture.

Method 3: Culturing cells between hydrogel layers

1) Add chilled collagen solution to the wells to cover the entire surface of the well. This may be slightly less than the volumes in table 1, to account for a second hydrogel being added later.

2) Place the lid on the culture plate and place the plate in the incubator to gel for >60 minutes (Atelocollagen) or >30 minutes (Telocollagen).

3) Resuspend cell pellet in fresh cell culture medium to achieve desired seeding density/concentration, i.e. 50,000 cells per well in 100 µL medium for a 24-well plate.

4) Place the lid on the plate and incubate the plate in the incubator for 60 to 120 minutes to allow for cell attachment to the hydrogel layer.

5) Add the same volume of collagen solution from Step 1 on top of the first gelled collagen layer to encapsulate cells between two hydrogel layers.

6) Incubate the plate in the incubator for 60 minutes to allow the gelation of the second hydrogel layer.

7) Supply cell culture medium to the wells and return the plate to the incubator for cell culture.

