READY-TO-USE HYDROGELS

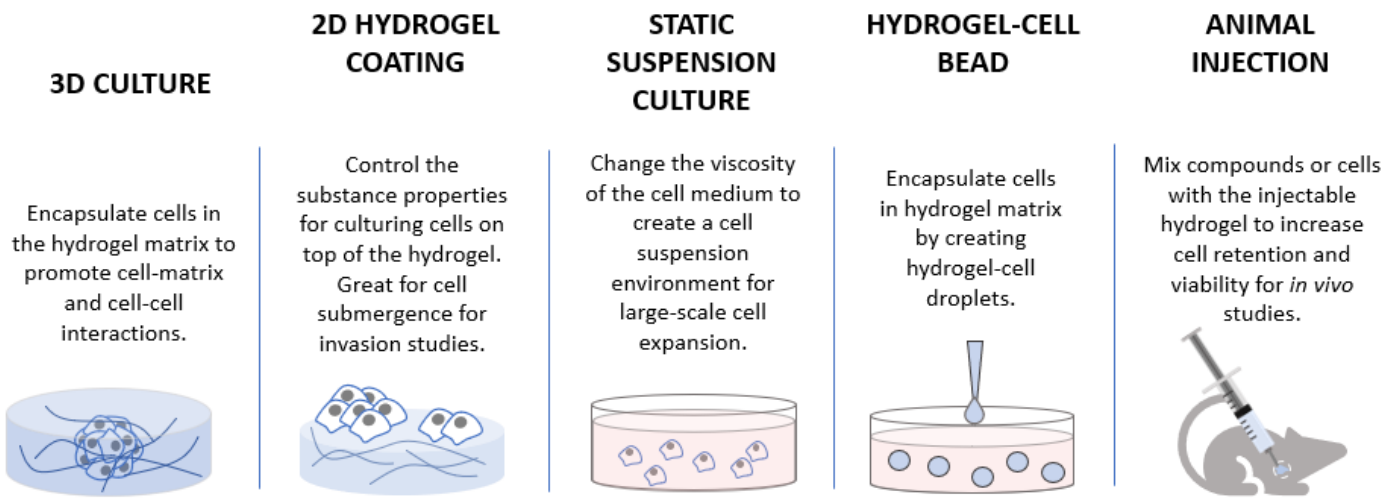
# FIVE CELL CULTURE METHODS WITH THE READY-TO-USE VITROGEL® HYDROGELS

## CULTURE METHODS & PROTOCOL EXAMPLES

### Applies to the Ready-to-Use VitroGel Hydrogels

The XENO-FREE bio-functional VitroGel is a versatile hydrogel system for many cell culture applications. With two different hydrogel systems, choose the **"Ready-to-Use"** VitroGel hydrogels for optimized formulation and ease of use for different cell culture applications or select the tunable **"High Concentration"** VitroGel hydrogels which has the ability for researchers to "mix & match" the hydrogels to create customized microenvironments. With either VitroGel hydrogel systems, there are many ways to fulfill your research needs.

To expand on the robustness of the VitroGel systems, we have listed below the five most popular cell culture methods to use with the hydrogels:

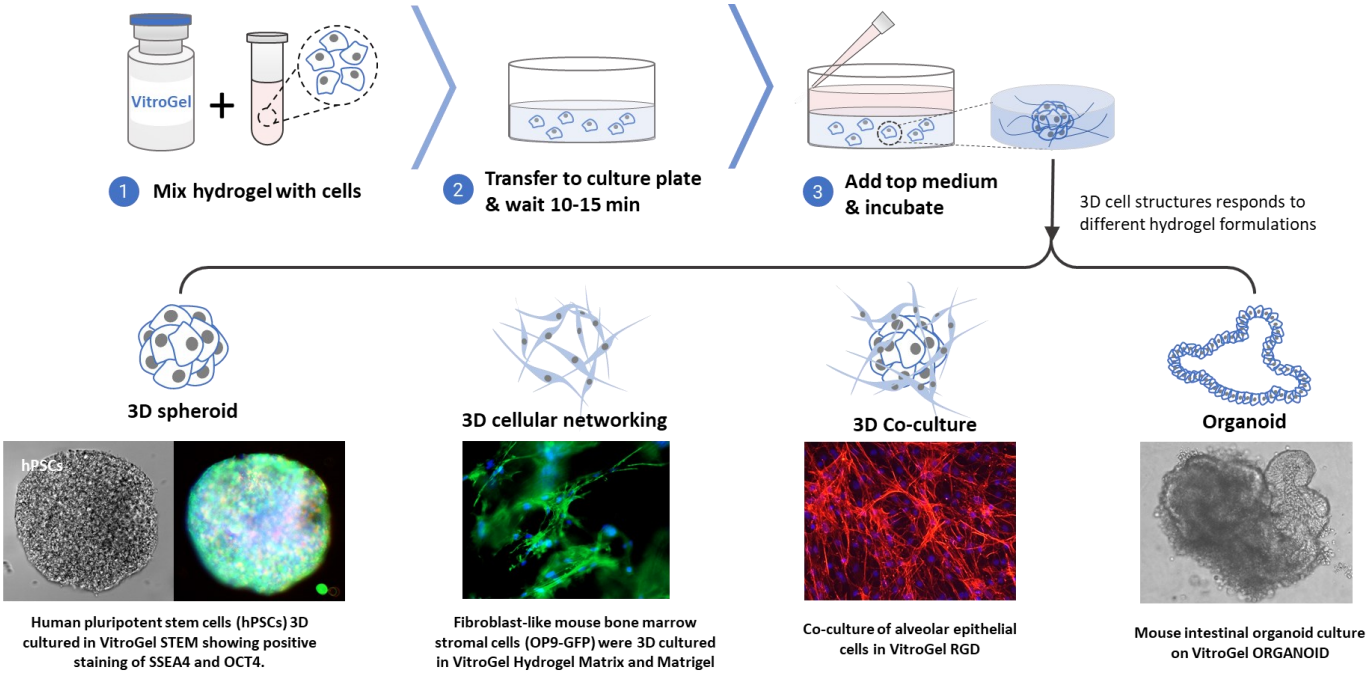



The following five culture methods and example protocols in this document can be applied to all of our "Ready-to-Use" VitroGel hydrogels. While the five culture method applies also to the "High Concentration" hydrogels, refer to the "VitroGel High Concentration Kit Handbook" for protocol details. Cells cultured in these methods can be easily harvested with the VitroGel® Cell Recovery Solution for downstream analysis or subculture.

**Learn more about all the VitroGel Cell Culture Methods:**
https://www.thewellbio.com/3d-cell-culture-hydrogel/3d-cell-culture-methods-with-vitrogel/

# 3D CELL CULTURE

The VitroGel system is ideal for 3D cell culture. By simply mixing VitroGel solution with cell suspension at room temperature, transferring to a culture plate and adding top medium, the cells are ready for incubation. This 3D culture method make a full cellular encapsulation, which enhance the cell-hydrogel matrix interactions. Many downstream analysis such as drug screening, immunofluorescence analysis, and cyto-toxicity assays can perform in hydrogel directly.




# 3D Cell Culture—EXAMPLE PROTOCOL

**VitroGel Hydrogel Matrix (Cat# VHM01) is used as an example below. The 3D Cell Culture method applies to all VitroGel hydrogels. For VitroGel High Concentration hydrogels, please check the "VitroGel High Concentration Kit Handbook " for protocol usage and details.**

## MATERIALS AND REAGENTS (AS AN EXAMPLE)

- VitroGel® Hydrogel Matrix (Cat# VHM01)
- Cells
- Cell culture medium
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture plate

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in the cell culture medium.
   - Recommended cell concentration 1-2 x $10^6$ cells/mL.
   - **Optional:** To control the critical growth factors/inhibitors/serum in hydrogel, add desired supplement in cell suspension at 3X concentration. The cell suspension then can mix with VitroGel hydrogel solution to get 1X final concentration in step 3.
3. Add 1 mL VitroGel hydrogel solution to 500 µL cell suspension from step 2 and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio).
   **If using cell culture medium with low salt concentration such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio. Example, 500 µL VitroGel hydrogel to 500 µL cell suspension.*

4. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture for specific well plate types are list below.

| | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate |
|---|---|---|---|---|---|
| **Volume per well** | 1200 µL | 600 µL | 300 µL | 150 µL | 50 µL |

5. Wait 10-15 min at room temperature for a soft gel formation.
   - **Note:** During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.

6. Carefully add additional medium to cover the hydrogel. The recommended volumes of cover medium for specific well plate types are list below.

| | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate |
|---|---|---|---|---|---|
| **Volume per well** | 1200 µL | 600 µL | 300 µL | 150 µL | 50 µL |

7. Place the well plate in an incubator. For long term culture, change the cover medium every 48 hours.
   - **Note:** We recommend to only change 50-80% of the top medium without disturbing the hydrogel.

## VIDEO PROTOCOLS

- **Ready-to-use VitroGel:** https://www.thewellbio.com/3d-cell-culture-with-vitrogel-ready-to-use-hydrogels/
- **VitroGel High Concentration:** https://www.thewellbio.com/3d-cell-culture-with-vitrogel-hydrogel-system-demonstration/
- **Cell recovery from 3D cell culture:** https://www.thewellbio.com/3d-2d-cell-recovery/

## DATA EXAMPLES

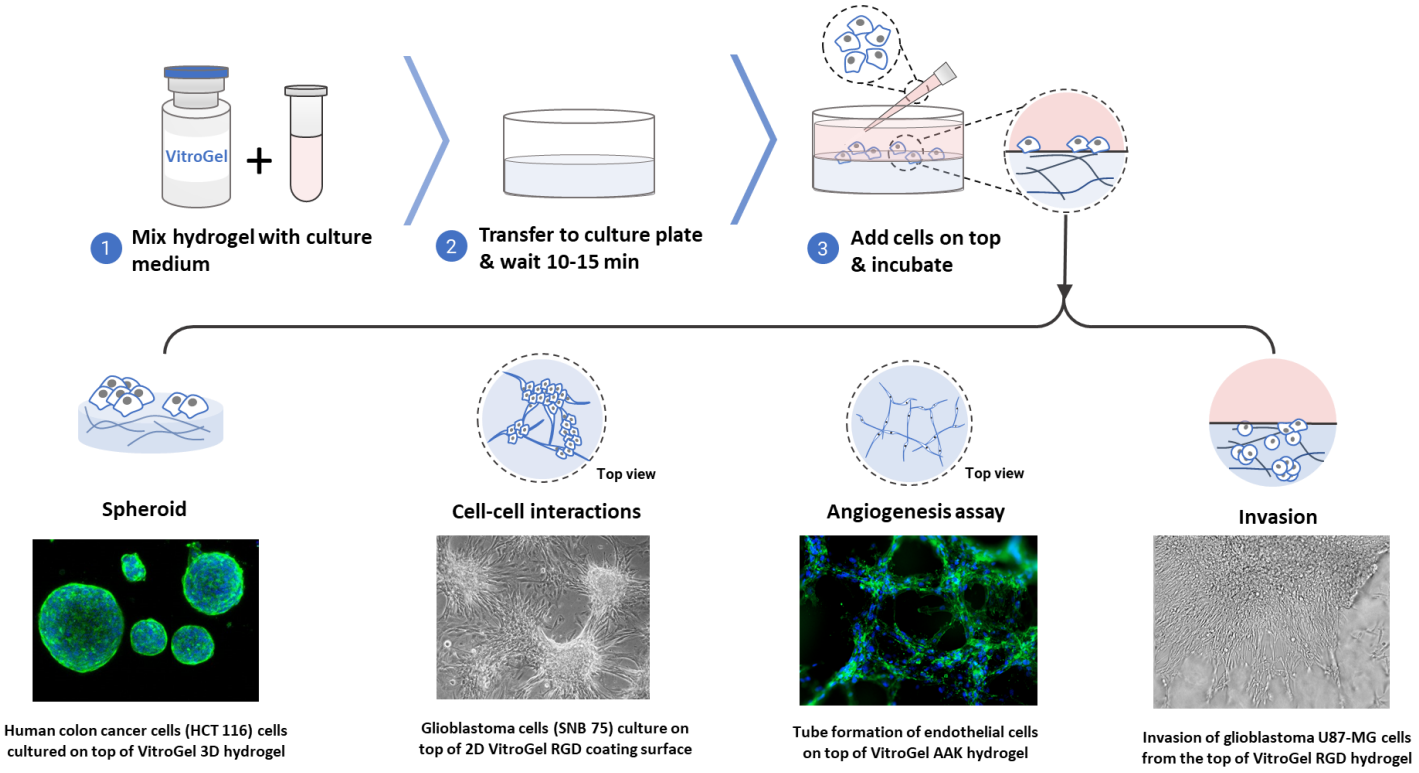
Check product page for more 3D culture data of each hydrogel. Please click the link below to learn more about how 3D cell structure responses to different hydrogel properties such as mechanical strength, functional ligands, degradation, and supplement

- https://www.thewellbio.com/applications/in-vitro-3d-cell-models/

# 2D HYDROGEL COATING

2D hydrogel coating method is great to generate the bridge between the traditional 2D culture and 3D cellular encapsulation culture. VitroGel can be coated at the bottom of the culture plate as a thick layer of hydrogel by mixing VitroGel solution with cell culture medium at room temperature and transfer to the culture plate. Cells can be added directly on the top of hydrogel.

The 2D hydrogel coating method allows cells to interact with/submerge into the functional hydrogel substance and maintain an excellence exposing surface to the top medium. The quick cell spheroid formation can happen when cells migrate/aggregate on the surface of the hydrogel. The 2D hydrogel coating method can be used as an alternative co-culture method in combine with the 3D cell culture method: encapsulate a cell type in the hydrogel for 3D culture and then add another type of cell on top of the hydrogel as 2D coating culture. The 2D coating method can be a powerful system to study cell invasion, angiogenesis assay and layer by layer co-culture. Besides 2D thick gel coating, VitroGel is also good to be diluted for thin gel coating method.




# 2D Hydrogel Coating—EXAMPLE PROTOCOL

**VitroGel Hydrogel Matrix (Cat# VHM01) is used as an example below. The 2D Hydrogel Coating method applies to all VitroGel hydrogels. For VitroGel High Concentration hydrogels, please check the "VitroGel High Concentration Kit Handbook " for protocol usage and details.**

## MATERIALS AND REAGENTS (AS AN EXAMPLE)

- VitroGel® Hydrogel Matrix (Cat# VHM01)
- Cells
- Cell culture medium
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture plate

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Add 1mL VitroGel hydrogel solution to 500 µL cell culture medium and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell medium at 2:1 v/v mixing ratio).
   * *If using cell culture medium with low salt concentration such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio. Example, 500 µL VitroGel hydrogel to 500 µL cell medium.*

   - **Optional:** To control the critical growth factors/inhibitors/serum in hydrogel, add desired supplement in cell culture medium at 3X concentration. The medium then can mix with VitroGel hydrogel solution to get 1X final concentration.

3. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture for specific well plate types are list below.

| | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate |
|---|---|---|---|---|---|
| **Volume per well** | 1200 µL | 600 µL | 300 µL | 150 µL | 50 µL |

4. Wait 10-15 min at room temperature for a soft gel formation.
   - **Note:** During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.

5. Carefully add medium with cells on top of hydrogel (Recommend cell concentration of 0.5-1 x $10^6$ cells/mL). The recommended volumes of cell medium for specific well plate types are listed below.

| | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate |
|---|---|---|---|---|---|
| **Volume per well** | 1200 µL | 600 µL | 300 µL | 150 µL | 50 µL |

   - **Optional Seeding Method:** To ensure cells are seeded on the surface of hydrogel, add 50% of the medium (without cells) on top of hydrogel first. Wait 5-10 min then add the rest 50% of medium with cells on top of the hydrogel.
     Example: For a 24 well plate, add 150 µl medium (without cells) first. Wait 10-15 min. Then, add 150 µl medium with 1-2 x $10^6$ cells/mL on top.

6. Place the well plate in an incubator. For long term culture, change the cover medium every 48 hours.
   - **Note:** We recommend to only change 50-80% of the top medium without disturbing the hydrogel.

## VIDEO PROTOCOLS

- **Ready-to-use VitroGel:** https://www.thewellbio.com/2d-coating-vitrogel-ready-to-use-hydrogels/
- **VitroGel High Concentration:** https://www.thewellbio.com/2d-cell-coating-culture-with-vitrogel-hydrogel-system/
- **Cell recovery from 2D hydrogel coating culture:** https://www.thewellbio.com/3d-2d-cell-recovery/

## DATA EXAMPLES

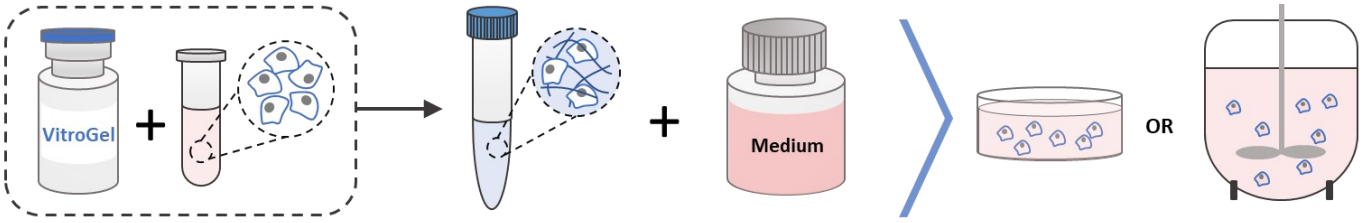
- **2D hydrogel coating applications:** https://www.thewellbio.com/applications/2d-cell-culture/
- **Invasion assay applications:** https://www.thewellbio.com/applications/invasion-assay/

# STATIC SUSPENSION CULTURE

3D static suspension culture protocol is a unique culture method of VitroGel hydrogel system. By simply mixing the VitroGel solution and cells for a soft hydrogel formation, the researchers can further directly mix the hydrogel-cell mixture with additional culture medium to make a hydrogel-cell suspension. The hydrogel matrix disperses within the cell culture medium can increase the viscosity of the whole mixture and help cells to maintain the suspension status without a strong agitation.

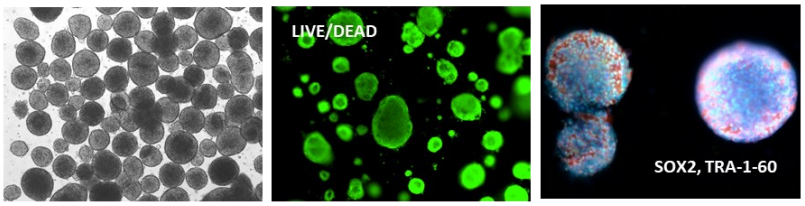
The 3D static suspension culture is easy to prepare and flexible to adjust the final viscosity for various cell types and seeding densities, by simply changing the mixing ratio of hydrogel and cell medium. For example, researcher can use a fixed 2:1 (hydrogel solution: cells, v/v) to prepare the hydrogel-cell mixture and then mix it with cell culture medium at 1:1 to 1:10 ratios to get different viscosity of final hydrogel-cell suspension.

This culture method can be easily used for lab size or large industry size cell culture scale-up. There is no fancy bioreactor or costly culture vessel needed for research lab. We use it for stem cell spheroids and HEK293 spheroids generation. The method is also applied to all our ready-to-use VitroGel and high concentration VitroGel system. The cells generated from VitroGel static suspension culture can be easily harvested out by centrifuging.

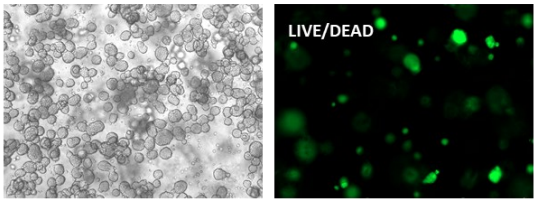



**Data examples**

Human pluripotent stem cells (hPSCs) 3D static suspension culture in VitroGel STEM




HEK293 cells 3D static suspension culture in VitroGel HEK293




**3D spheroid**

**Easy 3D cell scale-up and harvesting**

# Static Suspension Culture—EXAMPLE PROTOCOL

**VitroGel Hydrogel HEK293 (Cat# VHM05) is used as an example below. The Static Suspension Culture method applies to all VitroGel hydrogels. For VitroGel High Concentration hydrogels, please check the "VitroGel High Concentration Kit Handbook " for protocol usage and details.**

## MATERIALS AND REAGENTS (AS AN EXAMPLE)

- VitroGel® HEK293 (Cat# VHM05)
- Cells
- Cell culture medium
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture vessel (plate, flask, bioractor)

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in the cell culture medium.
   - Recommended cell concentration 0.5-2 x $10^6$ cells/mL.
   - **Optional:** To control the critical growth factors/inhibitors/serum in hydrogel, add desired supplement in cell suspension at 3X concentration. The cell suspension then can mix with VitroGel hydrogel solution to get 1X final concentration in step 3.
3. Add 1 mL VitroGel hydrogel solution to 500 µL cell suspension from step 2 and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio).
   * *If using cell culture medium with low salt concentration such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio. Example, 500 µL VitroGel hydrogel to 500 µL cell suspension.*
4. Disperse the hydrogel-cell mixture from step 3 in cell culture medium by mixing them at 1:1 to 1:10 v/v ratios for a hydrogel-cell suspension (e.g. mix 1.5 mL of hydrogel-cell mixture with 4.5 mL cell culture medium for 1:3 mixing ). Carefully pipette up and down to mix homogeneously.
   - **Note:** the final viscosity of the hydrogel-cell suspension can be adjusted by changing the mixing ratio between hydrogel-cell mixture and cell culture medium to fulfill the culture conditions of various cell types, cell seeding density and culture vessels. Usually the 1:1 to 1:5 mixing ratio can main good hydrogel-cell suspension without plate shaker or agitation. For mixing ratio over 1:5, the hydrogel can help to maintain a great cell suspension under low agitation speed (10-40 rpm) to reduce shearing force and promote cell growth.
5. Transfer the hydrogel-cell suspension to a culture vessel for incubation.

## VIDEO PROTOCOLS

- **Static suspension culture:** https://www.thewellbio.com/hek293-3d-suspension-culture/
- **Cell recovery from VitroGle static suspension culture:** https://www.thewellbio.com/hek293-3d-suspension-harvesting/

## DATA EXAMPLES

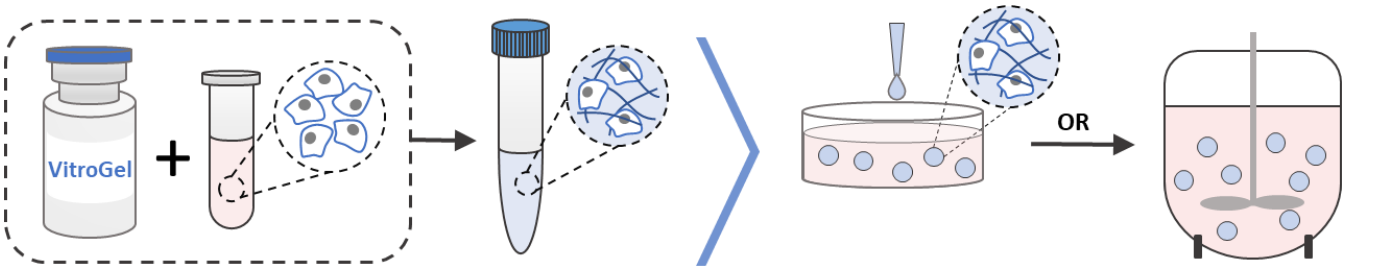
- **Static suspension culture of human pluripotent stem cells (hPSCs):** https://www.thewellbio.com/applications/stem-cells/

# HYDROGEL-CELL BEAD

As an injectable hydrogel system, VitroGel has a unique shear-thinning and rapid recovery rheological property. The hydrogel solution can mix with cells for a soft hydrogel, which then can add to the cell culture medium as droplets for hydrogel-cell bead formation.

This culture method not only encapsulates cells within the hydrogel matrix to enhance cell-matrix interactions, but also allow the whole hydrogel-cell bead to suspense in cell culture medium for optimal medium penetration. Researchers can adjust the size of the hydrogel beads by changing the volume of droplets added to the culture medium.

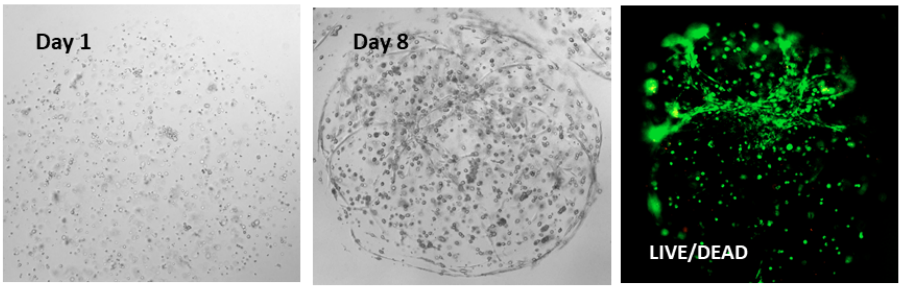
For cells that need strong attachment to grow such as mesenchymal stem cells (MSCs), this hydrogel-cell bead culture method is an excellent way to replace microcarriers for 3D cell scale-up. Since the excellent medium and oxygen penetration, cells cultured in hydrogel beads can maintain high cell viability for long term culture. The method is also applied to all our ready-to-use VitroGel and high concentration VitroGel system. The cells generated from VitroGel hydrogel-cell bead culture can be harvested by VitroGel Cell Recovery Solution.



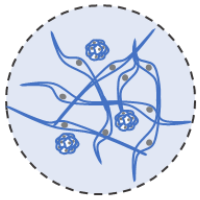

1 **Mix hydrogel with cells & wait 10-15 min**

2 **Add mixture as droplet into culture medium & incubate**

**Data examples**




**Mesenchymal stem cells (MSCs) 3D culture in VitroGel MSC hydrogel beads**



**3D Cell growth within the hydrogel bead**

# Hydrogel-Cell Bead—EXAMPLE PROTOCOL

**VitroGel MSC (Cat# VHM02) is used as an example below. The Hydrogel-Cell Bead method applies to all VitroGel hydrogels. For VitroGel High Concentration hydrogels, please check the "VitroGel High Concentration Kit Handbook " for protocol usage and details.**

## MATERIALS AND REAGENTS (AS AN EXAMPLE)

- VitroGel® MSC (Cat# VHM03)
- Cells
- Cell culture medium
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture vessel (plate, flask, bioractor)

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in the cell culture medium.
   - Recommended cell concentration 0.5-2 x $10^6$ cells/mL.
   - **Optional:** To control the critical growth factors/inhibitors/serum in hydrogel, add desired supplement in cell suspension at 3X concentration. The cell suspension then can mix with VitroGel hydrogel solution to get 1X final concentration in step 3.
3. Add 1 mL VitroGel hydrogel solution to 500 µL cell suspension from step 2 and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel and cell suspension at 2:1 v/v mixing ratio). Incubate the hydrogel-cell mixture at room temperature for 10-15 minutes to use in step 5.
   * *If using cell culture medium with low salt concentration such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio. Example, 500 µL VitroGel hydrogel to 500 µL cell suspension.*
4. Add cell culture medium to a well plate. The recommended volumes of cell medium for specific well plate types are listed below.

| | 6 well plate | 12 well plate | 24 well plate | 48 well plate | 96 well plate |
|---|---|---|---|---|---|
| **Volume per well** | 3000 µL | 1500 µL | 750 µL | 300 µL | 10 µL |

5. Using a pipettor with a 100 µL tip, carefully pipette the hydrogel-cell mixture from step 3 into the well plate as droplets (roughly 5-10 droplets per 100 µL of hydrogel-cell mixture). The ratio between hydrogel-cell mixture and cell culture medium in a well plate is about 1:5 (v/v) (e.g. 600 µL hydrogel-cell mixture for 3 mL cell culture medium in each well of a 6-well plate).
   - **Optional:** Control the final size of the hydrogel-cell beads by adjusting the volume of the droplets. For small beads, 1-5 µL per droplet and for large beads, 20-50 µL per droplet).
     **Tip:** Press the pipette plunger to create a droplet on the pipette tip, lower the pipette tip to release the droplet by contacting the surface of culture medium.
6. Place the well plate in an incubator and change the medium every 48-72 hours.
   - **Note:** we recommend to only change 50-80% of the top medium without disturbing the hydrogel beads

## VIDEO PROTOCOLS

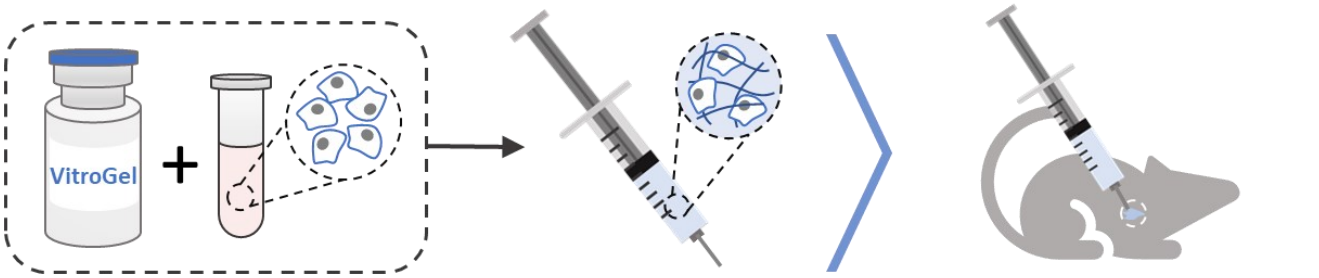
- **3D hydrogel-cell bead culture:** https://www.thewellbio.com/mesenchymal-stem-cell-3d-culture-bead-droplet-protocol/
- **Cell recovery from hydrogel-cell bead culture:** https://www.thewellbio.com/msc-mesenchymal-stem-cell-3d-culture-scale-up-cell-harvesting/

# ANIMAL INJECTION

VitroGel is an outstanding injectable carrier for animal injections. Under the mechanical shearing force such as injection through a syringe, the hydrogel performs a gel-sol transition and becomes free-flowing status. However, once the shearing force ceased, the mechanical strength of the hydrogel can rapidly recover with a sol-gel transition and become a hydrogel status again. With this injectable property, VitroGel can be used for in vivo cells/drug delivery for cell therapy or controlled release.

Our xeno-free functional hydrogel system is designed based on the application needs and biochemical/biophysical properties. Scientists can choose the hydrogels that fit their research objectives from either the "Ready-to-Use" VitroGel hydrogels or the "High Concentration" Vitro-Gel hydrogels where the injectable hydrogel can be controlled at different hydrogel strengths.

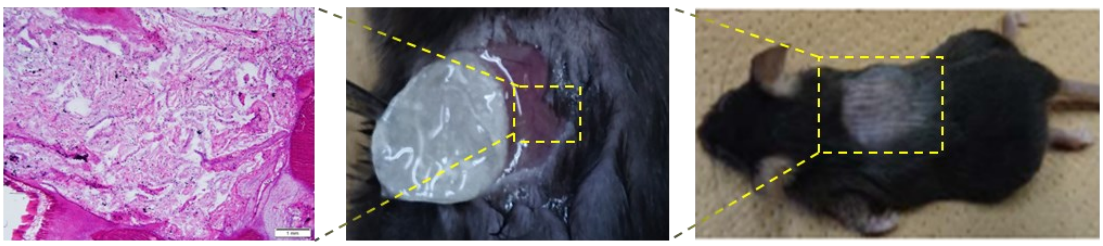
Simply mix the VitroGel hydrogel solution with cells/compounds at room temperature, the hydrogel is ready for injection in 20 minutes. VitroGel has a unique rheological property that can maintain an injectable status for hours after mixing with cells.




1 **Mix hydrogel with cells/compounds & wait 10-15 min**

2 **Ready for animal injection**

**Data examples**



**Hydrogel is stable after injection. The material is biocompatible without toxicity**

# Animal Injection—EXAMPLE PROTOCOL

All VitroGel hydrogels are injectable and excellent for xenografts. Researchers have full control of the supplement/growth factors in the hydrogel-cell mixture. **Simply adding 3-5X of FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with hydrogel can boost the cell growth after injection.**

**VitroGel Hydrogel Matrix (Cat# VHM01) is used as an example below. The Animal Injection method applies to all VitroGel hydrogels. For VitroGel High Concentration hydrogels, please check the "VitroGel High Concentration Kit Handbook " for protocol usage and details.**

## MATERIALS AND REAGENTS (AS AN EXAMPLE)

- VitroGel® Hydrogel Matrix (Cat# VHM01)
- Cells or molecular compounds
- Cell culture medium or PBS
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Syringe

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in PBS.
   - Adjust the cell/molecular concentration accordingly to experiment (prepare cell suspension at 2X desired concentration for later mixed with VitroGel for 1X final concentration.
3. Mix VitroGel hydrogel with cell suspension at 1:1 (v/v) ratio and gently pipette up and down 5-10 times to mix thoroughly. Example: 1 mL VitroGel hydrogel solution to 1 mL cell suspension in PBS.
   (The recommended mixing ratios with other solutions are listed in the table below.)

| Medium used to prepare cell suspension/drug solution | VitroGel | Cell suspension/drug solution |
|---|---|---|
| 1X PBS at 1:1 gel/cell ratio (v/v) | 1 mL | 1 mL |
| Cell culture medium at 2:1 gel/cell ratio (v/v) | 2 mL | 1 mL |

4. Transfer the hydrogel mixture to a syringe. Stabilize the hydrogel mixture either by putting on ice or at 4°C for 5-10 min. Alternatively, stabilize at room temperature for 15 min.
5. After stabilization, the hydrogel mixture is ready for injection. The hydrogel mixture can be kept at room temperature during injections. VitroGel has a unique rheological property that can maintain an injectable status for hours after mixing with cells without issues of needle clogging.

   ***Optional for step 2**: Adding 30-50% FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with the hydrogel can boost the cell growth after injection.*

## VIDEO PROTOCOLS

- **Injectable hydrogel: thewellbio.com/xenograft-injection-video**