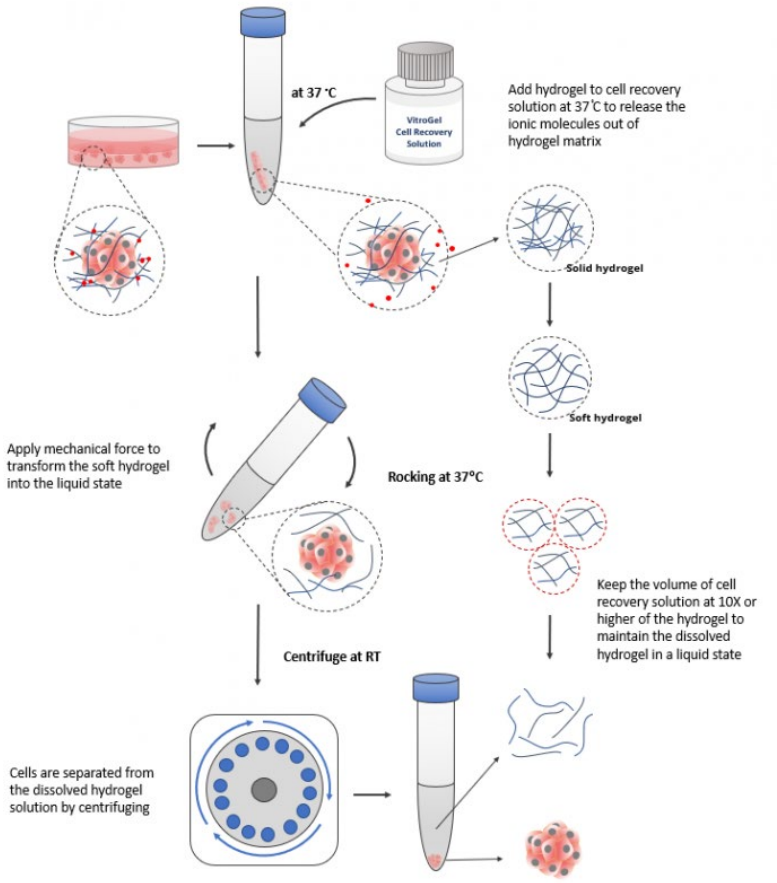
PROTOCOL

CELL RECOVERY FROM GEL USING VITROGEL® CELL RECOVERY SOLUTION

HOW DOES CELL RECOVERY WORK IN VITROGEL?

Harvesting cells from a hydrogel matrix is not an easy task. Using harsh chemical solutions, strong enzyme, or changing the temperature may damage your cells. The yield rate of cell recovery is low with these methods. With the VitroGel system, harvesting cells from the hydrogel matrix is as easy as it is to perform 3D cell culture. Using our enzyme-free VitroGel® Cell Recovery Solution (Cat# MS03-100), scientists can recover cells at neutral pH and 37 °C for the operating temperature. The solution can maintain high cell viability during the recovery process. Cells can be sub-cultured in both 2D and 3D culture after recovery. The VitroGel Cell Recovery Solution helps to release the ionic molecules from the hydrogel matrix, which converts the solid hydrogel back to the soft hydrogel state. At this state, the hydrogel maintains the unique shear-thinning properties; it can further transform into a liquid state with a little mechanical disruption (such as rocking or gentle pipetting) and dilution. Once the hydrogel dissolves as a liquid form, cells can harvest by centrifuging.



PROTOCOL-1

CELL RECOVERY FROM 3D CULTURE AND 2D HYDROGEL COATING OF VITROGEL

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured with VitroGel system
- VitroGel Cell Recovery Solution (Cat# MS03-100)
- DPBS (Wash Buffer, no calcium, no magnesium)
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Micropipette; Low retention pipette tips
- Dry bath or water bath set to 37 °C
- Centrifuge
- Lab spatula

PROTOCOLS: (using 24 well-plate, 300 µL gel/well as an example)

The selection of Method 1 and Method 2 below is depended on the conditions of cells and hydrogel: if the sizes of cells in hydrogel are bigger than 500 µm in diameter, Method 1 is recommended; if using VitroGel at a high gel concentration (1-0 or 1-1 dilution) or the sizes of cells in hydrogel are smaller than 500 µm in diameter, Method 2 is recommended.

ONLINE VIDEO PROTOCOL: https://www.thewellbio.com/3d-2d-cell-recovery/

Method 1 (using a serological pipette to break the hydrogel into small pieces)

1. Warm the VitroGel Cell Recovery Solution to 37 °C.
2. Take the cells out of the incubator and remove the medium covering the top of the hydrogel. Wash the hydrogel two times with DPBS.
3. Add 1 mL warm VitroGel Cell Recovery Solution to the well and use a 10 mL serological pipette to gently break the hydrogel into small pieces by gently pipetting up and down. This step can accelerate the hydrogel dissolving process.
4. Add 5 mL warm VitroGel Cell Recovery Solution to a 15 mL conical tube and transfer the hydrogel to the tube.

   Optional:
   Rinse the well with 1 mL warm VitroGel Cell Recovery Solution and combine the solution to the centrifuge tube.
5. Use a 10 mL serological pipette to gently pipette the mixture up and down 3-5 times and put the tube back to the water bath for 2-3 minutes. Repeat this cycle 2-3 times. (Optimize the pipetting times and repeats according to the gel strength and cell type).
6. Centrifuge at 100 x *g* for 3-5 minutes at room temperature to collect the cell pellet. (Optimize the speed and time of centrifuge according to different cell types).

   Optional:
   If there is still some hydrogel on top of the cell pellet, resuspend the cell with 5 mL warm cell recovery solution and repeat steps 4 to 6 one more time.

PROTOCOL-1

CELL RECOVERY FROM 3D CULTURE AND 2D HYDROGEL COATING OF VITROGEL

Method 2 (using a lab spatula)

1. Warm the VitroGel Cell Recovery Solution to 37 °C.
2. Take the cells out of the incubator and remove the medium covering the top of the hydrogel. Wash the hydrogel two times with DPBS.
3. Add 1 mL warm VitroGel Cell Recovery Solution to the well and use a spatula to detach the hydrogel from the well plate.
4. Add 5 mL warm VitroGel Cell Recovery Solution to a 15 mL conical tube and transfer the hydrogel to the tube.

   Optional:
   Rinse the well with 1 mL warm VitroGel Cell Recovery Solution and combine the solution to the centrifuge tube.

5. Rock the conical tube for 20 times and then put the tube back to the water bath for 2-3 minutes. Repeat this cycle for 3-5 times. (Optimize the rocking time and repeats according to the gel strength and cell type).
6. Centrifuge at 100 x *g* for 3-5 minutes at room temperature to collect the cell pellet. (Optimize the speed and time of centrifuge according to different cell types).

   Optional:
   If there is still some hydrogel on top of the cell pellet, resuspend the cell with 5 mL warm cell recovery solution and repeat steps 5 and 6 one more time.

IMPORTANT NOTES:

- KEEP THE SOLUTION WARM: It is important to keep the cell recovery solution and the mixture warm at 37 °C during the whole process. The warm temperature is essential to accelerate molecular exchanges to release the ionic molecules from the solid hydrogel, which can transform into a soft hydrogel.
- APPLY MECHANICAL FORCE: The mechanical force such as rocking the tube or using a serological pipette to mix the hydrogel with the cell recovery solution helps to transform the hydrogel into the liquid state.
- DILUTION: Adding the cell recovery solution at the volume of 10X or higher than the hydrogel maintains the dissolved hydrogel in a liquid state.
- CENTRIFUGE AT ROOM TEMPERATURE

PROTOCOL-2

CELL RECOVERY FROM HYDROGEL-CELL BEADS

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured in VitroGel-cell beads
- VitroGel Cell Recovery Solution (Cat# MS03-100)
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Micropipette; Low retention pipette tips
- 37 °C water bath or dry bath
- Centrifuge

PROTOCOLS:

The ratio between the hydrogel-cell beads (no medium) and VitroGel Cell Recovery Solution is appoximately 1:10 v/v. Example below**:** Using a 6 well-plate with 600 µL hydrogel-cell beads in 3 mL medium per well, 5 mL VitroGel Cell Recovery Solution is used.

ONLINE VIDEO PROTOCOL: https://www.thewellbio.com/msc-hydrogel-bead-cell-harvesting

1. Warm the VitroGel Cell Recovery Solution to 37 °C.
2. Take the cells out of the incubator and carefully remove the cell culture medium from the well without disrupting the hydrogel-cell beads.
3. Add 5 mL warm VitroGel Cell Recovery Solution to the well and transfer the hydrogel-cell beads to a 15 mL conical tube by using a 10 mL serological pipette. (The ratio between hydrogel-cell beads and cell recovery solution is appoximately 1:10 v/v.)
4. Using the serological pipette, gently pipette the mixture up and down 3-5 times and put the tube to a dry bath or water bath and incubate at 37°C for 3-5 minutes.

   Optional*:* Pipette the mixture up and down 3-5 times again before centrifuge.

5. Centrifuge at 100 x *g* for 3-5 minutes at room temperature to collect the cell pellet. (Optimize the speed and time of centrifuge according to different cell types).

   Optional*:* If there is still some hydrogel on top of the cell pellet, resuspend the cell with 5 mL warm cell recovery solution and repeat steps 4 and 5 one more time.

IMPORTANT NOTES:

- KEEP THE SOLUTION WARM: It is important to keep the cell recovery solution and the mixture warm at 37 °C during the whole process. The warm temperature is essential to accelerate molecular exchanges to release the ionic molecules from the solid hydrogel, which can transform into a soft hydrogel.
- APPLY MECHANICAL FORCE: The mechanical force such as rocking the tube or using a serological pipette to mix the hydrogel with the cell recovery solution helps to transform the hydrogel into the liquid state.
- DILUTION: Adding the cell recovery solution at the volume of 10X or higher than the hydrogel maintains the dissolved hydrogel in a liquid state.
- CENTRIFUGE AT ROOM TEMPERATURE

PROTOCOL-3

CELL RECOVERY FROM CELL STATIC SUSPENSION CUTLURE IN VITROGEL

RECOMMENDED MATERIALS AND REAGENTS

- Cells static suspensional cultured in VitroGel system
- VitroGel Cell Recovery Solution (Cat# MS03-100)
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Micropipette; Low retention pipette tips
- 37 °C water bath or dry bath
- Centrifuge

PROTOCOLS: (using 6 well-plate, 3 mL cell suspension per well as an example)

1. Warm the VitroGel Cell Recovery Solution to 37 °C.
2. Take the cells out of the incubator and transfer the cell suspension to a 15 mL conical tube by using a 10 mL serological pipette.
3. Add additional 5 mL warm VitroGel Cell Recovery Solution to a 15 mL conical tube.
4. Use a 10 mL serological pipette to gently pipette the mixture up and down 3-5 times and put the tube to a dry bath or water bath and incubate at 37°C for 3-5 minutes.

   Optional:
   Pipette the mixture up and down 3-5 times again before centrifuge.

5. Centrifuge at 100 x *g* for 3-5 minutes at room temperature to collect the cell pellet. (Optimize the speed and time of centrifuge according to different cell types).

   Optional:
   If there is still some hydrogel on top of the cell pellet, resuspend the cell with 5 mL warm cell recovery solution and repeat steps 4 and 5 one more time.

IMPORTANT NOTES:

- KEEP THE SOLUTION WARM: It is important to keep the cell recovery solution and the mixture warm at 37 °C during the whole process. The warm temperature is essential to accelerate molecular exchanges to release the ionic molecules from the solid hydrogel, which can transform into a soft hydrogel.
- APPLY MECHANICAL FORCE: The mechanical force such as rocking the tube or using a serological pipette to mix the hydrogel with the cell recovery solution helps to transform the hydrogel into the liquid state.
- DILUTION: Adding the cell recovery solution at the volume of 10X or higher than the hydrogel maintains the dissolved hydrogel in a liquid state.
- CENTRIFUGE AT ROOM TEMPERATURE