Home for Cells and Beyond

PGmatrix^{TM} System Using Guides

The **PepGel PGmatrix^{TM} system** is a powerful tool for 3D cell culture, high content biologically formed spheroids, 3D bioprinting, and beyond with more accurate in vivo predictions for life science research and development. A typical PepGel PGmatrix kit consists of a vial of ***PGmatrix*** patented peptides nanofiber solution, a vial of ***PGworks*** trigger solution. The PGmatrix nanofibrils are formulated into a basic or a customer desired cell culture medium in neutral pH. A 3D microenvironment can be formed accordingly for cell/spheroid growth. With PGmatrix, cells no longer suffer acidic or chill conditions; cultured cells/spheroids are easily harvested from the matrix; all operating procedures can be completed at room temperature or 37°C in neutral pH.

PRODUCT:	PepGel PGmatrix^{TM} Research Kits
CONTENT:	PGmatrix solution and PGworks solution
QUANTITY:	20 mL of PGmatrix and 1 mL of PGworks
	10 mL of PGmatrix and 0.5 mL of PGworks
	6 mL of PGmatrix and 0.3 mL of PGworks
	2 mL of PGmatrix and 0.3 mL of PGworks
STORAGE:	Stored at 4°C
LOT NUMBER:	See product label

FOR IN VITRO RESEARCH USE ONLY. PLEASE READ MATERIAL USING AGREEMENT FOR MORE DETAILS. FOR IN VIVO TEST, PLEASE ASK FOR PG IN VIVO PRODUCTS.

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A. Protocols for 3D cell culture in PGmatrix System

I. FOR FIRST TIME USER, PLEASE READ THE FOLLOWING THREE MESSAGES

MESSAGE I: Mixing Ratio Notice

The PGmatrix solution contains 1% W/V standard peptides. Most cells from soft tissue grow well in the peptide concentration from 0.1% to 0.5%. If you are first time user, we recommend using a few mixing ratios in the range of 0.1-0.6% W/V final peptide concentration for 3D cell encapsulation to identify the best mixing ratio for your cells. The following **Table 1** presents two mixing ratios at 0.2% and 0.5% concentration as example, respectively. Please use the following table as reference to mix PGmatrix solution and cell suspension.

Remember: add the PGworks to your cell suspension FIRST before you mix PGmatrix solution with cell suspension. If you still have questions, please contact technical support by email to customerservice@pepgel.com

Table 1: Examples of mixing ratios of PGmatrix solution, cell suspension and PGworks* solution and maximum plating volume per well for different well plates.**

Well Plate Size	0.2% W/V			0.5% W/V			*Maximum plating volume per well (µL)*
	Cell suspension (µL)	*PGworks solution (µL)*	*PGmatrix solution (µL)*	*Cell suspension (µL)*	*PGworks solution (µL)*	*PGmatrix solution (µL)*	
6	1560	40	400	960	40	1000	2000
12	780	20	200	480	20	500	1000
24	390	10	100	240	10	250	500
48	195	5	50	120	5	125	250
96	78	2	20	48	2	50	100

NOTE: *PGworks is always 2% of the total volume of PGmatrix + cell suspension + PGworks together.
**** Nontreated culture plate is recommended for 3D cell culturing in PGmatrix system.**

MESSAGE II: **Add medium on the top of hydrogel to prevent drying and to feed the cells for long term culture**

After hydrogel formation (30-60 min at 37 °C after mixing), cell medium needs to be added on the top of the gel to provide fresh nutrition and prevent drying for long term culture. The following **Table 2** presents the recommended volume of medium to add to each well based on different well sizes.

Table 2: The recommended volume of medium to be added on the top of hydrogel

	6-well	12-well	24-well	48-well	96-well
Volume to add (µL)	4000	2000	1000	500	200

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MESSAGE III: Examples of Cell Density and Gel Concentration

***Table 3: Cell density and gel concentration recommendation for 3D cell cultures in PGmatrix System**

Cells	PGmatrix System£	Gel concentration (%)	Gelation time (min)	Cell seeding density (Cell/mL)	Proliferation (times)	Cell viability (%)
Jurkat Clone E6-1 (ATCC)	PGR	0.2	60	1×10^5 - 2×10^5	40-45	90-95
Jurkat Cell (ASC)	PGR	0.2	60	1×10^5 - 2×10^5	40-45	90-95
CHO-S (ASC)	PGP/PGD	0.2-0.4	60	1×10^5 - 2×10^5	40-45	90-95
MCF-7 (ATCC)	PGD	0.2-0.3	60	2.8×10^5	4	90
Hela cell	PGD	0.2	60	8×10^4	5-6	90-95
Head Neck cell	PGD	0.5	30	4×10^4	6-7	90-95
A549	PGD	0.5	30	5×10^4- 1.5×10^5	6-7	90
LET 1	PGD	0.5	30	5×10^4- 1.5×10^5	6-7	90
HepG2 (ATCC)	PGD	0.5	30	4×10^4 - 1×10^5	10	90-95
SW480 (ATCC)	PGP/PGL	0.5	30	4×10^4 - 1×10^5	10	90-95
PANC 1 (ATCC)	PGD	0.5	30	2×10^4 - 5×10^4	10-15	90-95
4t1 (ATCC)	PGD	0.5	30	4×10^4 - 1×10^5	10-20	90-95
FHC (ATCC)	PGP	0.3-0.5	30-60	1×10^6 - 3×10^6	**§**	90-95
Human Adipose-Derived Mesenchymal Stem Cells (ATCC)	PGD	0.5	30	6×10^6	**§**	90

£**PGD**=PGmatrix DMEM; **PGR=**PGmatrix RPMI; **PGL**=PGmatrix L15; **PGP**=PGmatrix PURE, **PGmatrix with other basal medium would be also available upon request**.

******Note:*** Results reported here are from 48-12 well plate under culturing condition at 37°C and 5% CO_2 and can only be used as reference. It is users' responsibility to use appropriate cell medium and growth supplement or consult with PepGel.

§ No proliferation for cells, while adjacent cells form spheroids spontaneously with the size of 20 μm to 100 μm.

II. PGmatrix™ System QUICK START* for 3D cell culture

For Example: 3D Cell Culture Procedure for a 24 well plate at the ratio of 1:1 (PGmatrix:Cell suspension) that will yield 0.5% (w/v) PGmatrix hydrogel concentration.
(Preparation: Bring the PGmatrix system kit and cell solution to room temperature or 37 °C)

- **Step 1** Prepare cell suspension (960 µL), add serum or other growth factors as needed to your cell suspension (pipet well without introducing air bubbles**)
- **Step 2** Add PGworks (40µl) to the cell suspension from Step 1 (pipet well without introducing air bubbles) (Graphic illustration Step 2)
- **Step 3** Mix PGmatrix solution (1000µL) with the cell suspension from Step 2 at 1:1 ratio (pipet well without introducing air bubbles) (Graphic illustration Step 3)
- **Step 4** Transfer the mixture of step 3 to a 24 well plate (500 µL/well, 4 wells total)
- **Step 5** Place the 24-well plate in a 37 °C incubator for 30 min or up to 60 min for gelation
- **Step 6** After gelation, add 1000 µL of the cell medium gently on top of the gel to prevent the matrix from drying and to feed the cells, (**HINT:** add the medium along the well wall to avoid disturbing the gel, change top medium every other day or as needed to provide cells with fresh nutrition)
- **Step 7** Place the 24-well plate back to the incubator for cell growth

*The mixing ratio given above is 1:1 at 0.5% PGmatrix concentration. **Most** cells grow well in gel concentration of 0.1-0.5%, in those cases, ratio of PGmatrix vs cell suspension can be 1:2, 1:3, 1:4, 1:5 or 1:9. PGworks is always 2% of the total volume of PGmatrix + cell suspension together. For more detailed information, such as other ratios, cell feeding, cell isolation or immuno-staining, please read the complete User Guides.

****NOTE**: To avoid introducing air bubbles, keep pipetting within the solution or mixture)

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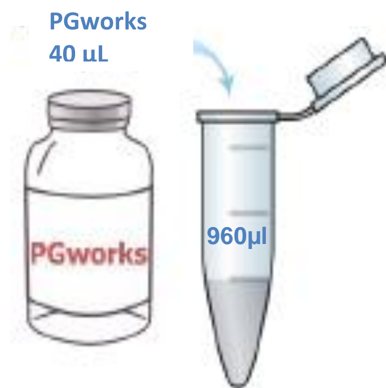
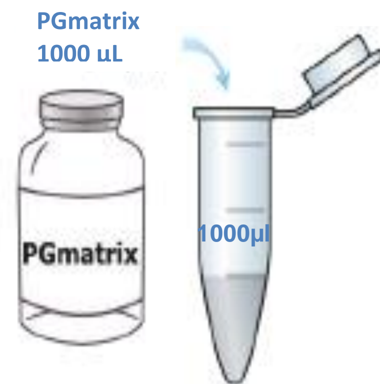
Cell Encapsulation illustration of the Example given in the QUICK START
(Pipet without introducing air bubble)

STEP 2: Add PGworks to cell suspension



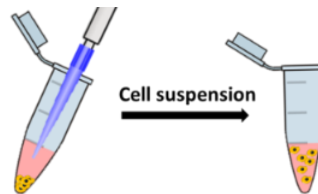
STEP 3: Add PGmatrix solution to mixture



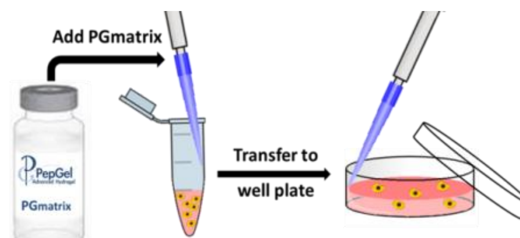
0.5% gel concentration for a 2000 µl volume: 960 µL Cell suspension +40 µL PGworks + 1000 µL PGmatrix solution

III. ENCAPSULATE CELLS FROM SUBCULTURE (2D or 3D) or CRYOPRESERVED CELLS

1. Bring the PGmatrix solution and PGworks solution to room temperature (15 - 25 °C) or 37 °C (37 °C water bath).
2. Suspend cells in desired cell culture medium then add PGworks solution to the cell suspension according to the Mixing Ratio in **Table 1** on page 3, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).



3. Mix the PGmatrix solution carefully into the cell suspension of step 2 at the Mixing Ratio indicated in **Table 1** on page 3 (pipet well without introducing air bubbles). Transfer the mixture into the center of each well, then swirl the plate to uniformly cover the entire well bottom surface. (***Note:*** Please select the PGmatrix solution accordingly that matches your cell culture medium or contact PepGel for special medium requirement by email to customerservice@pepgel.com). For cell seeding density and gel concentration, please see examples in **Table 3** on page 5.
   ***** Hand-shaking the 6-well plate front to rear and side to side is necessary for gel to uniformly cover the entire well bottom.



4. Incubate the plate at 37°C (5% CO_2) for 30-60 min to complete the gelation.
5. After gelation, place cell medium on top of the gel of each well to prevent the matrix from drying (recommended volume of each well is listed in **Table 2** on page 4). (**Tips**: carefully add cell culture medium along the wall of each well on the top surface of the hydrogel without disturbing the hydrogel).
6. To feed the cells, change the medium very gently by replacing 1/3 - 2/3 of medium above

the gel with fresh medium every day or every other day, depending on how often the cells need to be fed or how soon the cell medium color change to yellowish.

IV. THAWING CELLS DIRECTLY IN 3D PGMATRIX SYSTEM*

1. Bring the PGmatrix solution and PGworks solution to room temperature (15 - 25 °C) or 37 °C (37 °C water bath).
2. Thaw the vial with frozen cells by gently agitating on in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
3. Remove the vial from the water bath as soon as the contents start to thaw. Add 1 mL of pre-warmed complete culture medium and pipet until the cell suspension is totally thawed.
4. Transfer the cell suspension to a 15 mL conical tube, and use another 1 mL of complete culture medium to rinse the vial for the remaining cells, and combine the solution to the conical tube.
5. Dilute the mixture to 10 mL by using complete culture medium. And centrifuge at 100 g 200 g for 5 min (depending on cell types).
6. Remove the supernatant and resuspend the cell pellet gently in complete culture medium.
7. Follow the steps 2-6 in Section I "**ENCAPSULATE CELLS FROM SUBCULTURE (2D or 3D) or CRYOPRESERVED CELLS"** for the cells encapsulation in 3D PGmatrix.

*** The recommended cell seeding density for initial thawing cells in 3D PGmatrix is one to two times higher than encapsulation cells from subculture, in order to maintain good cell viability and proliferation.**

V. CELL RECOVERY FOR PASSAGING, COUNTING AND DOWNSTREAM APPLICATIONS

1. **Gel disruption:** Without removing the upper layer cell medium, mechanically disrupt the gel **THOROUGHLY** (**gel disruption thoroughly is very important for the cell isolation from the gel) by pipetting the gel and medium mixture. Transfer the mixture to a 2 mL-50 mL conical centrifuge tube (recommended conical centrifuge tube size vs well plate size are listed in **Table 4** on Page 12).
2. **Rinse:** Use PBS or DPBS (without Mg^{2+}/Ca^{2+}) to rinse the well and combine the solution to the centrifuge tube. Recommend using the volume as double the maximum plating volume for each well listed in **Table 1** on page 3 (i.e., 200 µL for 96-well plate or 1000 µL for 24-well plate).
3. **Gel dilution:** Pipette the mixture **THOROUGHLY** and add additional PBS or DPBS to further dilute the mixture by 10-20 folds of the original plating volume (See examples in **Table 4** on Page 12), and mix well.
4. **Centrifuge:** Centrifuge at 200 g-700 g for 5-6 min by using swing bucket centrifuge, depending on cell. Discard supernatant and collect the pellet.

Note: 1. Centrifuge force for cells harvesting from 3D PGmatrix should be higher than conventional 2D cell harvesting. 500 g-700 g is highly recommended for harvesting cancer cells spheroid colonies from PGmatrix, and 250g - 400 g for stem cells (i.e. hiPSC) spheroid colonies.
2. Always check if there are many cells or cell spheroid colonies remaining in the supernatant after centrifuge, then you may increase the centrifuge force or time accordingly for further cell isolation.
3. If you still have cell isolation issues, you may reduce your gel concentration for cell culture (i.e., if you used 0.5% PGmatrix for cell culture, you may reduce gel concentration to 0.3% or 0.2%), or consult with PepGel for technical support.

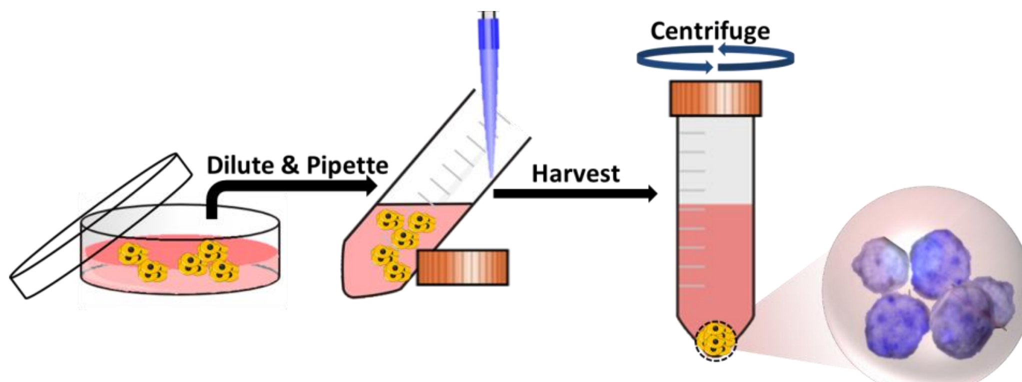


Table 4*: **The recommended conical centrifuge tube size vs well plate size for gel dilution**

	6-well	12-well	24-well	48-well	96-well
Gel plating volume	2000 µL	1000 µL	500 µL	250 µL	100 µL
Final volume of diluted gel and cell mixture from one well (10-20 folds)	40-45 mL	10-20 mL	5-10 mL	2.5-5 mL	1-2 mL
Suggested conical tube size	50 mL	50 mL	15 mL	15 mL	2 mL

*** The conical tube size suggested here is only good for one well cultured cells harvesting. If more than one well cultured cells are harvested at the same time, the tube size need to be larger accordingly. For example, 50 mL tube can be used if 3 wells of 24-well plate cultured cells are harvested at the same time (3 x 10 mL).**

5. **Spheroid colonies breakup:** To break the colonies, follow your conventional culture protocol, add enzyme solution or other dissociation agents, or follow procedures described below.

   – To break the spheroid colonies, add Trypsin-EDTA solution (i.e., 0.25%Trypsin+0.02%EDTA) to the cell pellet, the recommended Typsin-EDTA solution amount can be equal to the gel plating volume listed in **Table 1** on page 3 (i.e., 100 µL for 96-well plate or 500 µL for 24-well plate).
   – Mix gently to disrupt the cell pellet, then incubate the conical tubes at 37°C (5% CO_2) for 5 min.
   – After incubation, pipet the cell solution up and down to mechanically break up the cell spheroid colonies. If there are still some colonies left, continue to incubate the cell solution at 37°C (5% CO_2) for another 5 min or longer as needed and pipet, until majority of spheroid colonies become single cells observed under microscopy.
   – Then add culture medium or trypsin neutralization solution equal to the volume of Trypsin-EDTA solution to the conical tube, centrifuge at 100 g-200 g for 5 min (depending on cell types).
   – Discard supernatant and re-suspend the pellet in complete culture medium for further cell counting or cell passage.

   Note: The Enzyme concentration and incubation time for spheroid colonies disruption depend on the cell types, colonies size and number: certain cell lines need longer trypsinization time up to 20 min - 30 min to break spheroid colonies (50-100 um) into single cells, like 4t1, PANC 1 and HepG2 etc. Therefore, the condition listed above can only be used as reference. It is the users' responsibility to choose the appropriate condition for their cell spheroids breakup.

VI. CELL CRYOPRESERVATION

1. Re-suspend cell pellet in freezing medium or complete growth medium with 5%-10% DMSO to a concentration of 1×10^6 to 1×10^7 cells/mL depending on cell types. Allow the cells to sit at room temperature 15 min, so the cryoprotectant can diffuse into the cell.
2. Cells should be frozen slowly at 1 °C/min. This can be achieved by using a programmable cooler or using Mr. Frosty Freezing container with isopropanol placed in a -80 °C freezer for at least 24 hours.
3. Quickly transfer the vial to liquid nitrogen or -130 °C freezer for long term storage.

VII IMMUNO-STAINING

1. Gently remove the "free" medium on top of the gel in the culture well
2. Gently rinse the gel surface once with DPBS (without Ca^{2+} and Mg^{2+} ions)
3. Fix cells in gels with 10% neutral buffered formalin for 30-40 min
4. Wash the formalin out twice with DPBS (without Ca^{2+} and Mg^{2+} ions), 15 min for each rinsing.
5. Prepare washing buffer and blocking solution

   A): Washing buffer: DPBS (with Ca^{2+} and Mg^{2+} ions) + 0.2% triton X-100 + 0.1% cold water fish gelatin

   B): Blocking solution: washing buffer + 10% serum from the same source animal as the secondary antibody. Mix blocking solution thoroughly by pipetting

   C): Filter through syringe filter (Millex GP .22 micrometer PES membrane). Part of the solution will be lost due to filter clogging. Mix at least one extra mL more than you need.

Note: Blocking solution should be prepared just before applying it to the sample.

6. Remove the DPBS on top of the gel, add washing buffer and incubate at room temperature for 10 min. Wash twice.

Note: If the sample is not blocked and stained immediately, it can be stored after adding washing buffer. To store the sample, the plate should be sealed with parafilm and stored at 4°C.

7. Blocking (Day 1):

   Remove the washing buffer, add blocking solution to the fixed samples (i.e., 500-800 µL per well blocking solution, for 24-well plate). Let set overnight (or > 12 hrs).
8. Primary Antibody (1AB) (Day 2)

   A): Prepare primary antibody solution by adding the primary antibody solution to washing buffer. The primary antibody concentration for 3D staining can be similar or higher than that used for 2D culture staining (i.e., Goat Oct 3/4 antibody (N-19) with final concentration of 3 µg/mL was used for hiPSC staining directly in 3D PGmatrix-hiPSC)[9].

   B): Add primary antibody solution to the gel, incubate at room temperature overnight

Note: Use sufficient volume of antibody solution to completely soak the gel (i.e., 1 mL for gel in 24-well plate, 500 µL for gel in 48-well plate).

   C): Process one sample without 1AB as a negative control to assess background staining

9. Rinse (Day 3): Rinse with washing buffer 4 times (2 hrs per wash)
10. Secondary antibody (2AB) (Day 3)

    A) Prepare secondary antibody solution by adding the secondary antibody solution to washing buffer. The secondary antibody concentration for 3D staining can be similar or higher than that used for 2D culture staining (i.e., Rabbit anti-goat lgG (H+L) 2nd antibody alexa fluor 488 with final concentration of 5 µg/mL was used for hiPSC staining directly in 3D PGmatrix-hiPSC).

B) Wrap the plate with aluminum foil, incubate at room temperature, overnight (or > 12 hrs).

11. Rinse and Imaging (Day 4)

    A): Rinse at least 6 times (1 hr per wash) with washing buffer

    B): Replace washing buffer with Glycerin for imaging

    C): Proceed with imaging

Note: 1. to achieve a strong and specific signal, it is necessary to thoroughly block non-specific binding of antibodies to the gel matrix (PGmatrix) as well as allowing diffusion of antibodies through the gel matrix (PGmatrix). Therefore, extended blocking and incubation times and multiple washes are required.

2. Since it is a 3D system, the background is influenced by other cells at different planate positions. Therefore, post-processing of images may be needed to reduce background brightness.

B. Protocols for cell invasion (Thin gel method) in PepGel PGmatrix System

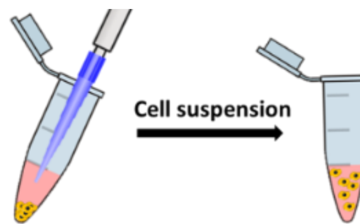
1. Bring the PGmatrix solution, for example, and PGworks solution to room temperature (15 - 25 °C) or 37 °C (37 °C water bath)
2. Mix PGworks first with cell medium with or without growth factors as users' need, and then mix with PGmatrix solution according to the Mixing Ratio in **Table 1** on page 3 (pipet well without introducing air bubbles)

   ***Note**:* Please select the PGmatrix solution accordingly that matches your cell culture medium or contact PepGel for special medium requirement by email to customerservice@pepgel.com
3. When properly mixed, plate the mixture into the center of each well (i.e., volume per well is recommended as 250 µl - 500 µl for 24-well plate), then swirl the plate to coat the entire well bottom surface, or different amount of mixture to get a desirable gel thickness as user wishes.
4. Place plates at 37°C for 30-60 min for gelation, then plate cell suspension on top of the thin gel for cell growth.

Note: Please follow the detailed procedures described in the user guides for desirable ratio of each mixing step, or conduct preliminary experiment to find out optimum mixing ratio for your experiment.

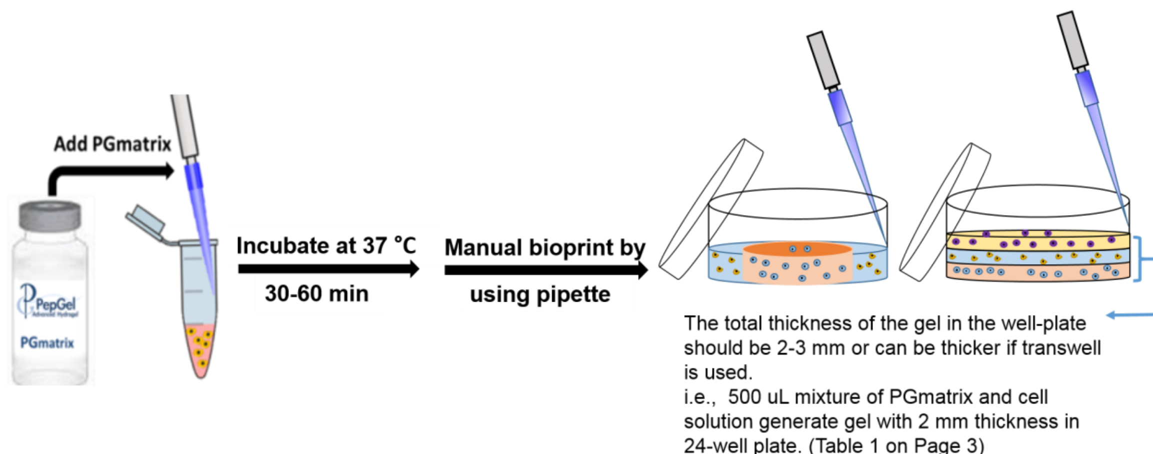
C. Protocols for 3D Manual Bioprinting of PepGel PGmatrix System using pipet or syringe

1. Bring the PGmatrix solution and PGworks solution to room temperature (15 - 25 °C) or 37 °C (37 °C water bath)
2. Suspend cells in desired cell culture medium with appropriate growth factors then add PGworks solution to the cell suspension according to the Mixing Ratio in **Table 1** on page 3, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).



3. Mix the PGmatrix solution carefully into the cell suspension of step 2 at the Mixing Ratio indicated in **Table 1** on page 3 (Please select the PGmatrix solution accordingly that matches your cell culture medium or contact PepGel for special medium requirement by email to customerservice@pepgel.com). For cell seeding density and gel concentration, please see examples in **Table 3** on page 5*****.
4. Incubate the vial with mixture of cell and PGmatrix (PGmatrix-Cell) at 37°C (5% CO_2) for 30-60 min to complete the gelation. (***Note:*** 30 min gelation time is recommended for 0.5% PGmatrix concentration, and 60 min gelation time for PGmatrix concentration lower than 0.5%). Then PGmatrix-Cell gel should be ready for manual bioprinting by using the pipet (i.e., the following diagram shows the potential for co-culture of two or three different cells by manual bioprinting, or users can bioprint other patterns for cell co-culture based on their needs).

   ***** **Note:** To avoid bubbles, the PGmatrix-cell solution can be uploaded into **syringe** at Step 3, and incubate, then bioprint with the syringe.



REFERENCE

1. Soltantabar P, Calubaquib EL, Mostafavi E, Ghazavi A, Stefan MC. Heart/liver-on-a-chip as a model for the evaluation of cardiotoxicity induced by chemotherapies. Organs-on-a-Chip. 2021 Nov 1;3:100008.
2. Li Q, Qi G, Liu X, Bai J, Zhao J, Tang G, Zhang YS, Chen-Tsai R, Zhang M, Wang D, Zhang Y. Universal Peptide Hydrogel for Scalable Physiological Formation and Bioprinting of 3D Spheroids from Human Induced Pluripotent Stem Cells. Advanced Functional Materials. 2021:2104046.
3. Carter T, Qi G, Wang W, Nguyen A, Cheng N, Ju YM, Lee SJ, Yoo J, Atala A, Sun XS. Self-Assembling Peptide Solution Accelerates Hemostasis. Advances in Wound Care. 2020. (https://www.liebertpub.com/doi/abs/10.1089/wound.2019.1109)
4. Harrison R, Luckett J, Marsh S, Lugo Leija HA, Salih S, Alkharji R, Sottile V. Magnetically Assisted Control of Stem Cells Applied in 2D, 3D and In Situ Models of Cell Migration. Molecules. 2019 Jan;24(8):1563.
5. Zhu Q, Hamilton M, Vasquez B, He M. 3D-printing enabled micro-assembly of a microfluidic electroporation system for 3D tissue engineering. Lab on a Chip. 2019;19(14):2362-72.
6. Thippabhotla S, Zhong C, He M. 3D cell culture stimulates the secretion of in vivo like extracellular vesicles. Scientific reports. 2019 Sep 10;9(1):1-4.
7. Xu J, Qi G, Sui C, Wang W, Sun X. 3D h9e peptide hydrogel: An advanced three-dimensional cell culture system for anticancer prescreening of chemopreventive phenolic agents. Toxicology in Vitro. 2019 Dec 1;61:104599.
8. Li L, Liu G, Timashev P, Sun XS, Criswell T, Atala A, Zhang Y. Biofabrication of tissue-specific extracellular matrix proteins to enhance the expansion and differentiation of skeletal muscle progenitor cells. Applied Physics Reviews. 2019 Jun 26;6(2):021309.
9. Nethercott HE, Brick DJ, Schwartz PH. Immunocytochemical analysis of human pluripotent stem cells. Human Pluripotent Stem Cells: Methods and Protocols. 2011:201-20.
10. Liang J, Liu G, Wang J, Sun XS. Controlled release of BSA-linked cisplatin through a PepGel self-assembling peptide nanofiber hydrogel scaffold. Amino acids. 2017 Dec 1;49(12):2015-21.
11. Liang J, Sun XS, Yang Z, Cao S. Anticancer drug camptothecin test in 3D hydrogel networks with HeLa cells. Scientific reports. 2017 Feb 1;7(1):1-9.
12. Miller PG, Shuler ML. Design and demonstration of a pumpless 14 compartment microphysiological system. Biotechnology and bioengineering. 2016 Oct;113(10):2213-27.
13. Kumar D, Kandl C, Hamilton CD, Shnayder Y, Tsue TT, Kakarala K, Ledgerwood L, Sun X, Huang H, Girod D, Thomas SM. Anti-HGF antibody ficlatuzumab mitigates tumor-associated fibroblast-facilitated head and neck cancer progression. JAMA Otolaryngology.

2015;141(12):1133-9.

14. Kumar D, Kandl C, Hamilton CD, Shnayder Y, Tsue TT, Kakarala K, Ledgerwood L, Sun XS, Huang HJ, Girod D, Thomas SM. Mitigation of tumor-associated fibroblast-facilitated head and neck cancer progression with anti–hepatocyte growth factor antibody ficlatuzumab. JAMA Otolaryngology–Head & Neck Surgery. 2015 Dec 1;141(12):1133-9.
15. Li X, Galliher-Beckley A, Huang H, Sun X, Shi J. Peptide nanofiber hydrogel adjuvanted live virus vaccine enhances cross-protective immunity to porcine reproductive and respiratory syndrome virus. Vaccine. 2013 Sep 23;31(41):4508-15.
16. Huang H, Ding Y, Sun XS, Nguyen TA. Peptide hydrogelation and cell encapsulation for 3D culture of MCF-7 breast cancer cells. PloS one. 2013 Mar 20;8(3):e59482.
17. Huang H, Shi J, Laskin J, Liu Z, McVey DS, Sun XS. Design of a shear-thinning recoverable peptide hydrogel from native sequences and application for influenza H1N1 vaccine adjuvant. Soft Matter. 2011;7(19):8905-12.