PepGel

Home for Cells and Beyond

PGmatrix-Spheroid Bioprinting Using Guide

The PepGel™ PGmatrix-Spheroid (PG-S) kit consists of a vial of ***PGmatrix-spheroid*** peptides nanofiber solution and a vial of ***PGworks*** trigger solution. PG-S kit creates a microenvironment mimicking in vivo ECM accordingly for cells growing and migrating in 3D manner. All operating procedures can be completed at room temperature or 37°C in neutral pH condition. PG-S can be used as bioink alone for bioprinting into large scale sheet or small-scale constructs (< 64 mm^3) without needing UV or chemical crosslinkers. PG-S can also combine with acrylated hydrogels [i.e., Poly (ethylene glycol) diacrylate (PEGDA) or Gelatin Methacryloyl (GelMA)] for bioprinting into large scale constructs with the aid of UV light crosslinking or any other bioinks. PG-S promotes cell biocompatibility when it is used in combination with other bioinks.

PRODUCT:	PepGel™ PGmatrix-Spheroid Research Kit
CONTENT:	PGmatrix-spheroid solution and PGworks solution
QUANTITY:	20 mL of 4% PGmatrix-spheroid and 4 mL of PGworks
	10 mL of 4% PGmatrix-spheroid and 2 mL of PGworks
	6 mL of 4% PGmatrix-spheroid and 1 mL of PGworks
	2 mL of 4% PGmatrix-spheroid and 1 mL of PGworks
	20 mL of 2% PGmatrix-spheroid and 2 mL of PGworks
	10 mL of 2% PGmatrix-spheroid and 1 mL of PGworks
	6 mL of 2% PGmatrix-spheroid and 1 mL of PGworks
	2 mL of 2% PGmatrix-spheroid and 1 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.

CONTACT: PepGel LLC

101 BIVAP Bldg.
1980 Kimball Ave
Manhattan, KS 66506
E-mail: info@pepgel.com
Phone: email to info@pepgel.com for call schedule
Fax: 785-532-7193
Web: www.pepgel.com

To Order: customerservice@pepgel.com, or online www.pepgel.com

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FOR FIRST TIME USER, PLEASE READ THE FOLLOWING THREE MESSAGES

MESSAGE: Mixing Ratio Notice

1. **PG-S kit alone as bioink:** The PGmatrix-spheroid (PG-S) solution contains 2-4% W/V standard peptides. Most cells from soft tissue grow well in PG-S peptide concentration from 0.5% to 3%. If you are first time user, we recommend using a few mixing ratios in the range of 0.5%-3% W/V final peptide concentration for 3D cell encapsulation to identify the best mixing ratio for your cells. The following **Tables 1 and 2** present mixing ratios at 0.5%, 1%, 2%, and 3% concentration as reference examples, respectively. Then follow Protocol A for bioink fabrication.

   Remember: add the PGworks to your cell suspension **FIRST** before you mix PG-S 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 4% PG-S solution, PGworks and cell suspension**
(PGworks is always 16% of the total volume of PG-S solution.)

Final concentration	PG-S solution (µL)	PGworks (µL)	Cell suspension	Total volume (µL)
3%	750	120	130	1000
2%	500	80	420	1000
1%**	250	40	710	1000

Table 2*: **Examples of Mixing ratios of 2% PG-S solution, PGworks and cell suspension**
(PGworks is always 8% of the total volume of PG-S solution.)

Final concentration	PG-S solution (µL)	PGworks (µL)	Cell suspension	Total volume (µL)
1%**	500	40	460	1000
0.5%**	250	20	730	1000

NOTES: *Cells will not perform well without appropriate growth factors, it is users' preference what growth factors are needed for their cells, or contact customerservice@pepgel.com for suggestion.

**Bioink from 0.5 – 1% peptide concentration is only suitable for ink-jet printing or manually pipetting for small construct desirable for Lab-on-Chip research.

2. **PG-S in combination with acrylated hydrogels as bioink:** Please use **Tables 3 and 4** as reference to mix PG-S solution, cell suspension and PEGDA hydrogel for example. Then follow Protocol B for UV light curable bioink fabrication.

Table 3: Examples of Mixing ratios of 4% PG-S solution, cell suspension and PEGDA solution

Final concentration	PG-S solution (µL)	Cell suspension + PEGDA solution (µL)	Photo initiator (mg)	Total volume (µL)
3%	750	250	1	1000
2%	500	500	1	1000
1%	250	750	1	1000

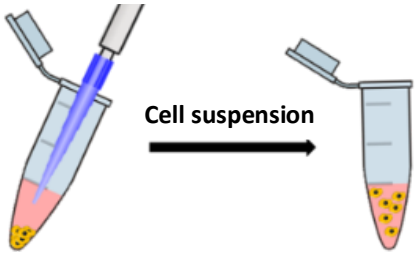
Table 4: Examples of Mixing ratios of 2% PG-S solution, cell suspension and PEGDA solution

Final concentration	PG-S solution (µL)	Cell suspension + PEGDA solution (µL)	Photo initiator (mg)	Total volume (µL)
1%	500	460	1	1000
0.5%	250	730	1	1000

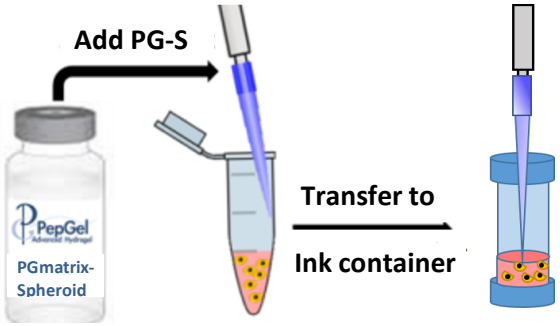
NOTE: Photo initiator is 0.1% (W/V) of total hydrogel volume or as customers' desire, PEGDA concentration and molecular weight is depending on users' interests.

A. Protocols for PG-S Bioink Preparation

1. Bring the PG-S 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 **Tables 1** and **2** on page 2, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).



3. Mix the PG-S solution carefully into the cell suspension of step 2 at the Mixing Ratio indicated in **Tables 1** and **2** on page 2 (pipet well without introducing air bubbles). Transfer the mixture into the center of the bioink container (i.e., syringe or pipet).
4. Incubate the bioink container at 37°C (5% CO_2) for 30 min to complete the gelation, then ready for bioprinting either by printer or manual pipetting.



B. Protocols for UV Curable Bioink Preparation

1. Bring the PG-S 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 according to the Mixing Ratio in **Tables 3 or 4** on page 3, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).
3. Dissolve photo initiator to the solution from Step 2, pipet well and avoid light.
4. Mix PEGDA solution to the mixture from Step 3, pipet well and avoid light. (PEGDA concentration can be used as customers' desire, depending on cell type, Mw of PEGDA etc.)
5. Add PG-S solution with the mixture from Step 4, pipet well, then transfer the mixture to ink container and avoid light, then ready for bioprinting and UV polymerization. (Customer needs to follow the procedure from Manufacturer to select appropriate UV curing system including type of light and time of exposure to get desired PEGDA hydrogel properties)

NOTE: PG-S kit is sterilized before shipping, it is users' responsibility to assure all other components (i.e., PEDGDA or photo initiator) be sterilized.

REFERENCE

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. Miller PG, Shuler ML. Design and demonstration of a pumpless 14 compartment microphysiological system. Biotechnology and bioengineering. 2016 Oct;113(10):2213-27.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. 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.
13. 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.

14. 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)