PepGel

Home for Cells and Beyond

PGmatrix-BioInk Using Guide

PGmatrix-BioInk (PGbioInk) takes the advantage of its unique features including shear-thinning, rapid gel-recovery (self-healing), and tunable gel strength capability, offering the best cyto-compatible hydrogel to meet advanced tissue engineering and organ biofabricaton via 3D bioprinting techniques without using light or chemical crosslinking agent. PGbioInk Kit consists of a vial of PGmatrix-BioInk nanofiber solution and a vial of PGworks trigger solution. PGbioInk 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.

NOTE 1: PGmatrix-Spheroid (PG-S) kit with similar mechanical properties and cyto-compatibility as the PGbioink kit can also be used for 3D bioprinting following the PGbioInk protocol.

NOTE 2: PGbioink kit can also be co-printed with light sensitive polymers such as Poly (ethylene glycol) diacrylate (PEGDA) or Gelatin Methacryloyl (GelMA)] for bioprinting into large scale constructs with the aid of UV or visible light crosslinking or any other bioinks. For co-printing with PEGDA or GelMA, PGworks is not necessary. or **NOTE 3:** choose PGmatrix-Bioink-LightC for light sensitive bioprinting**]**

PRODUCT:	PepGel™ PGmatrix-BioInk Research Kit
CONTENT:	PGmatrix-BioInk solution and PGworks solution
QUANTITY:	20 mL of 4% PGmatrix-BioInk and 4 mL of PGworks
	10 mL of 4% PGmatrix-BioInk and 2 mL of PGworks
	6 mL of 4% PGmatrix-BioInk and 1 mL of PGworks
STORAGE:	Stored at 4°C

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: 785-226-6917
Fax: 785-532-7193
Web: www.pepgel.com

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

PGmatrix-BioInk Using Protocol

1. Mixing ratios: The PGmatrix-BioInk (PGbioInk) solution contains 4% W/V standard peptides. Most cells from soft tissue grow well in PGbioInk peptide concentration from 0.5% to 3%. **Tables 1** presents mixing ratios of PGbioink solution and PGworks to obtain final gel concentration of 0.5%, 1%, 2%, and 3% as reference, respectively. Then follow procedure **#2** below for bioink preparation. Recommended final cell seeding density in PGbioInk hydrogel for printing can be 2 x10^5-4 x10^5 cell/mL.

Table 1: Examples of Mixing ratios of 4% PGbioInk solution, PGworks* and cell suspension (PGworks is always 16% of the total volume of PGbioInk solution.)

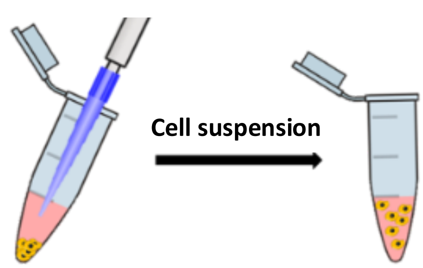
Final concentration**	PGbioInk solution (µL)	PGworks (µL)	Cell suspension	Total volume (µL)
3%	750	120	130	1000
2%	500	80	420	1000
1%	250	40	710	1000
0.5%	125	20	855	1000

NOTES: * add PGworks to your cell suspension **FIRST** before you mix PGbioInk solution with cell suspension.

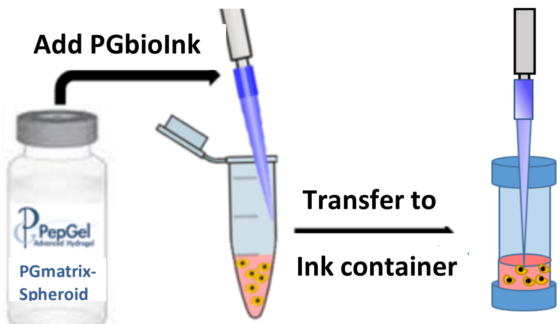
**Bioink from 0.5 – 1% peptide concentration is suitable for ink-jet printing or manually pipetting for small construct desirable for Lab-on-Chip research, and bioink from 1.5% above is for extrusion printer or other printing devices.

2. Protocol for PGbioInk Preparation

1. Bring the PGbioInk 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**, pipet well without introducing air bubbles (always immersing pipet tip in cell solution during pipetting).



3. Mix the PGbioInk solution carefully into the cell suspension from step 2 at the Mixing Ratio following **Table** 1 (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 it is ready for bioprinting. (**NOTE:** for hydrogel infusion purpose, the mixture from step 3 can be used, and then perform step 4 for gelation after infusion).
5. Culture the bioprinted construct at 37°C (5% CO_2) with desirable culture medium and perform downstream characterization and analysis as needed.



REFERENCE

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

14. 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.
15. 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)