GENProtocols

Protocol

Preparing a Brain-Mimetic 3D Matrix for Culturing Human Pluripotent Stem Cells

ANALYTICAL TESTING TRANSLATIONAL MEDICINE

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Abstract

This is a protocol for culturing human pluripotent stem cells (hPSCs) using a 3D matrix. It provides instructions for preparing a brain-mimetic 3D scaffold that can support neuronal growth and for culturing hPSCs. This injectable scaffold can be potentially employed in the field of regenerative medicine. The hydrogels can be prepared in approximately 45 mins.
This protocol does not require sophisticated experimental skills or expensive laboratory equipment, uses inexpensive water-based chemistry to synthesize the hydrogel precursors, and employs cell-friendly extracellular matrix-mimetic polymers. The hydrogel can be fabricated into any shape depending on the shape of the mold.

Introduction

Culturing and studying hPSC-derived neurons are a challenge due to the lack of a 3D matrix that mimics brain tissue composition physiologically and supports neuronal growth and network formation.

Hyaluronic acid (HA) and HA-chondroitin sulfate (HA-CS) composite gels are used for culturing hPSC-derived neurons. The covalent addition of dopamine (DA) moieties to the HA-CS (HADA-CS) gel enhances the scaffold stability. Additionally, HADA-CS gel can entrap cell-secreted ECM. Neurons cultured in HADA-CS gel express all the markers essential for cell adhesion and cell-ECM signaling, thus promoting neuronal network formation.

The protocol is simple to execute and inexpensive. However, prior gel handling experience will come in handy to avoid introducing bubbles in the hydrogels, especially important in presence of encapsulated cells as it interferes with imaging experiments.

This protocol can be used for studying neurobiology, disease pathology, and disease modeling.

Reagents and Equipment

1. Hyaluronic acid (MW 100-150 kDa; Cat # HA100K-5 LifeCore Biomedical (Chaska, USA))
2. Dopamine (2-(3,4-Dihydroxyphenyl) ethylamine hydrochloride) (Cat # H8502-10G Sigma Aldrich)
3. 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide hydrochloride (EDC) (Cat # E7750-5G Sigma Aldrich)
4. 1-hydroxy benzotriazole hydrate (HOBt) (Cat # 54802-10G-F Sigma Aldrich)
5. N-hydroxysuccinimide (NHS) (Cat # 130672-100G Sigma Aldrich)

6. Carbohydrazide (CDH) (Cat # C11006-25G Sigma Aldrich)
7. 3-amino 1,2-propanediol (Cat # A76001-25G Sigma Aldrich)
8. Sodium periodate (Cat # 311448-5G Sigma Aldrich)
9. Chondroitin sulfate (Cat # C9819-25G Sigma Aldrich)
10. Short laminin peptide with 12 amino acid sequence TFA salt (no modifications, 99% purity) (CCRRIKVAVWLC) (GenScript, USA)
11. Dialysis membranes (MWCO 3500 Spectra Por-6; Cat # 10142634 Fisher Scientific)
12. Sucrose (Cat # S0389-500G Sigma Aldrich)
13. Phosphate buffered saline (Cat # P3813-10PAK Sigma Aldrich)
14. Laminin (LN521, Cat # LN521-05 Biolamina, Sweden)
15. LDN193189 hydrochloride (Cat # SML0559-5MG Sigma Aldrich)
16. SB431542 hydrate (Cat # S4317-5MG Sigma Aldrich)
17. Fibroblast growth factor-2 (FGF2; Cat # 234-FSE-025/CF R&D Systems)
18. StemPro Accutase (Cat # 11599686 Thermo Fisher Scientific)
19. Brain-derived neurotrophic factor (BDNF; Cat # 248-BDB-01M R&D Systems)
20. Glial-derived neurotrophic factor (GDNF; Cat # 212-GD-01M R&D Systems)
21. Dibutyryl-cyclicAMP (db-cAMP; Cat # D0627-25MG Sigma Aldrich)
22. Ascorbic acid (AA; Cat # A4544-25G Sigma)
23. Regea08/023 (hESCs) and UTA04511.WTs (hiPSCs)
24. Tissue culture 24-well plates with glass bottom Cat # P24G-1.5-13-F (MatTek Corporation, Ashland, MA, USA).

Procedure

Preparation of transparent gels

1. Dissolve HA-CDH or HADA-CDH in 10% sucrose solution to reach a concentration of 14 mg/mL.
2. Dissolve CS-Ald or HA-Ald in PBS (pH 7.4) to reach a concentration of 14 mg/mL.
3. Prepare transparent gels by mixing equal volumes of aldehyde (HA-Ald or CS-Ald) and hydrazide (HADA-CDH or HA-CDH) derivatives to have four different gel compositions:

I. HA-HA: HA-CDH + HA-Ald
II. HADA-HA: HADA-CDH + HA-Ald
III. HA-CS: HA-CDH + CS-Ald
IV. HADA-CS: HADA-CDH + CS-Ald

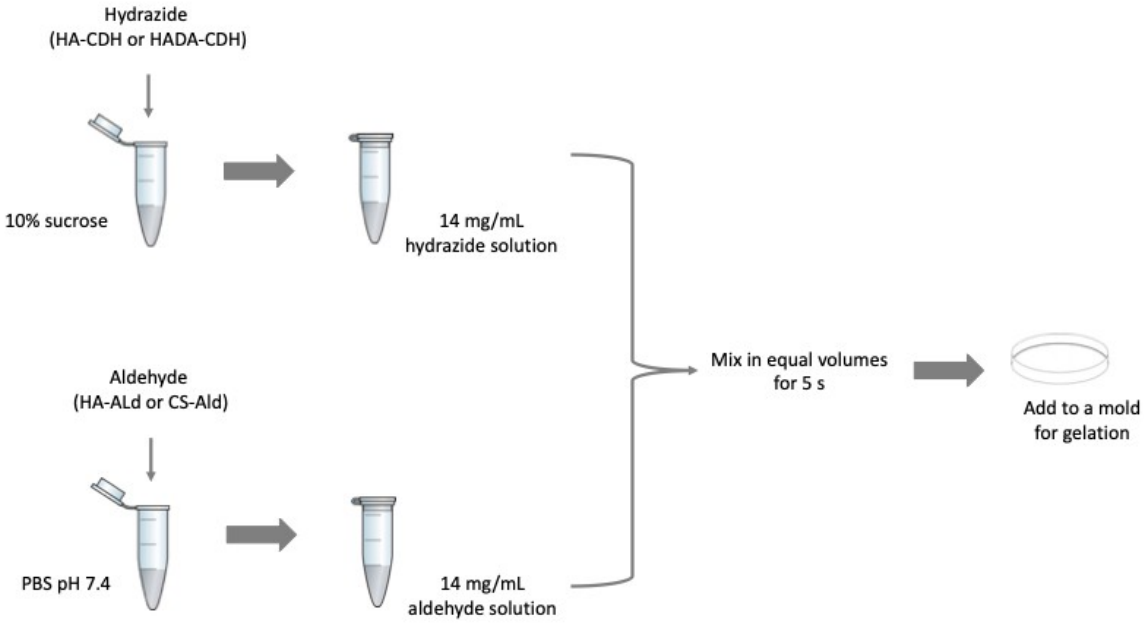

Figure 1. Preparation of transparent gels

Cell culture

1. Plate stem cells onto the laminin-coated surface in the presence of 100 nM LDN193189 and 10 µM SB431542 for neural induction in the culture medium.
2. Add 20 ng/mL FGF2 as a supplement.
3. Detach cells using StemPro Accutase and count them.
4. Pellet the required number of cells for each gel in a microfuge tube removing the supernatant in the process and add the appropriate volume of the HADA-CDH or HA-CDH required for one gel. Mix them thoroughly.
5. Add HA-Ald or CS-Ald to the well plates. Then add equal volumes of the cells along with the hydrazide mixture to the well plate. Mix well but quickly and allow the gel to cure for 20 min inside the incubation chamber at 37°C.
6. Add medium to the well plates. Supplement medium with 20 ng/mL BDNF, 10 ng/mL GDNF, 500 µM db-cAMP, and 200 µM AA.
7. The medium is replaced every two days for a period of 14 days after which the gels are taken, and the cells are analyzed.

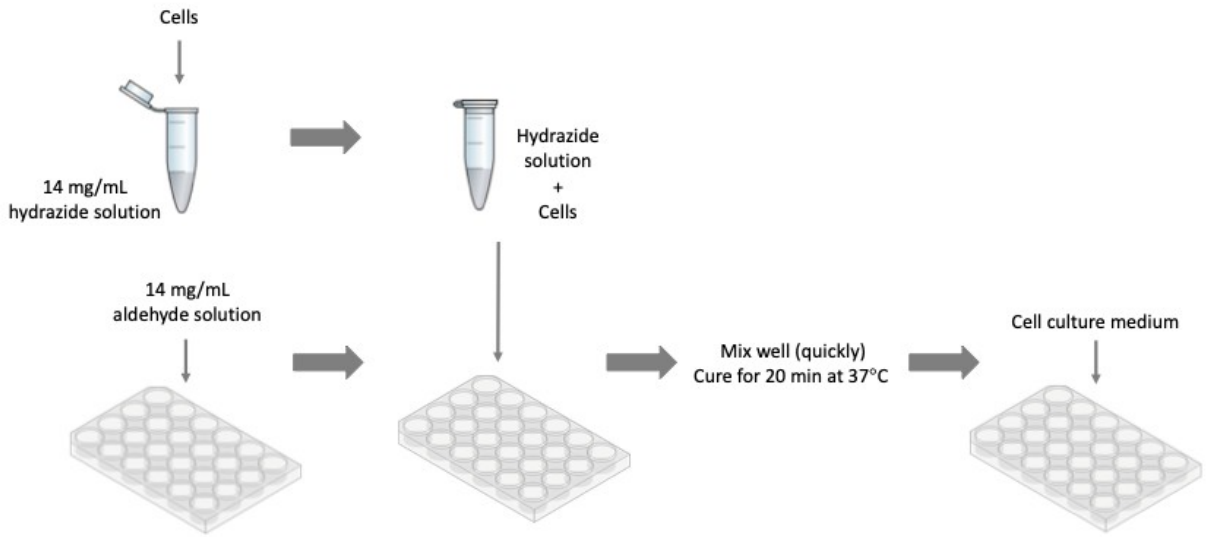

Figure 2. Cell culture

Time Taken

Hydrogel fabrications: 45 to 50 min. Encapsulated (3D) or on top (2D) cell culture: Depending on the research objective, up to 2 weeks with a similar percentage of functional modifications of the polymers as described in the original article.

Notes and Comments

Preparation of transparent gels

1. Let the polymer be in a fluffy stage while you add the respective solvents to dissolve them. Do not firmly press the solid polymer into the microfuge tube and add solvents to it. It will take a longer time to dissolve.
2. Incubation at 37°C along with gentle shaking will help faster dissolution.
3. Invert the microfuge tube and make sure the solution flows like liquid to ensure complete and homogenous dissolution.
4. Avoid introducing bubbles during the handling phases, especially with the pipettes.
5. The gelation is relatively fast. Prepare the mold where you want to cast your gels beforehand, place the hydrazide derivative first. Then add the aldehyde derivative quickly and mix both the solutions immediately manually. Be careful while dispensing the second component into the first component to avoid introducing any bubbles in the gel. Make sure to punch the air bubble with a sharp needle while the mixture is still in the liquid state to avoid any bubbles in the solidified gel. For this chemistry (or gelation), do not mix the two components for more than 5 s to avoid gel disruption.

Cell culture

1. To have homogenous cell density across the replicates of the same gel group, count the total number of cells required and mix them with the total volume of hydrazide derivative.
2. Always take into account the volume loss during pipetting, therefore prepare the mixture for at least one extra gel.
3. Avoid bubble formation while mixing aldehyde, hydrazide, and cells.

References

Samanta, S., Ylä-Outinen, L., Rangasami, V. K., Narkilahti, S., Oommen, O. P. (2022). Bidirectional cell-matrix interaction dictates

neuronal network formation in a brain-mimetic 3D scaffold. Acta Biomaterialia. 140:314-23. doi:10.1016/j.actbio.2021.12.010

Acknowledgments

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This protocol was written and submitted by Shital Sarah Ahaley.

Associated Publications

1. Oommen, O. P., Wang, S., Kisiel, M., Sloff, M., Hilborn, J., Varghese, O. P. (2013). Smart design of stable extracellular matrix mimetic hydrogel: synthesis, characterization, in vitro and in vivo evaluation for tissue engineering. Advanced Functional Materials. 23 (10): 1273-80. doi:10.1002/adfm.201201698
2. Samanta, S., Rangasami, V. K., Sarlus, H., Samal, J. R. K., Evans, A. D., Parihar, V. S., Varghese, O. P., Harris, R. A., Oommen, O. P. (2022). Interpenetrating gallol functionalized tissue adhesive hyaluronic acid hydrogel polarizes macrophages to an immunosuppressive phenotype, Acta Biomaterialia. 142: 36-48. doi:10.1016/j.actbio.2022.01.048