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A Protocol for Colorectal Tumor Spheroid Culture in Tunable Stiffness Alginate-Based Hydrogels and Subsequent Immunohistochemical Analysis

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Abstract

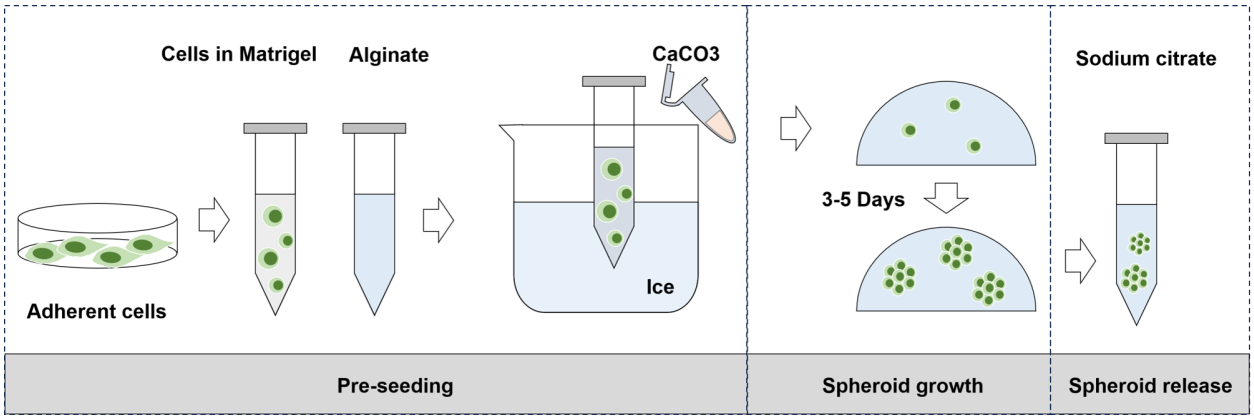
Tumor mechanical microenvironment, particularly extracellular matrix stiffness, plays a critical role in regulating cancer cell behavior, including proliferation, quiescence, and drug resistance. Conventional 2D culture or stiff 3D scaffolds fail to recapitulate the physiological soft (normal) or pathologically stiff (tumoral) mechanical niches. Here, we present a detailed protocol for establishing a tunable 3D tumor spheroid culture system using sodium alginate–based hydrogels crosslinked with calcium ions at different concentrations to achieve soft or stiff conditions that mimic normal colon and colorectal cancer tissues, respectively. We describe the step-by-step procedures for fabricating stiffness-tunable hydrogels, culturing colorectal cancer spheroids, releasing spheroids for downstream analysis, and performing immunohistochemical staining on intact spheroids. This protocol enables the reproducible investigation of mechanosensitive pathways and drug resistance mechanisms in a physiologically relevant 3D context.

Key features

- Tunable stiffness hydrogel system based on sodium alginate and calcium carbonate crosslinking.
- 3D tumor spheroid culture that recapitulates normal and tumor mechanical microenvironments.
- Gentle spheroid release using sodium citrate chelation, preserving morphology and viability.
- Compatible with immunohistochemistry.

Keywords: Tumor spheroid, Alginate hydrogel, Matrix stiffness, 3D culture, Immunohistochemistry

Graphical overview



Tumor spheroid culture and release based on sodium alginate hydrogel

Background

Colorectal cancer (CRC) is the third most common cancer worldwide, with metastasis and recurrence remaining the primary causes of mortality [1,2]. The tumor microenvironment (TME) plays a crucial role in inducing poor prognosis in CRC [3]. Among various TME factors, increased matrix stiffness resulting from excessive collagen deposition and crosslinking is a fundamental physical hallmark of solid tumors [4–6]. Normal colon tissue has a stiffness of <0.5 kPa, whereas CRC tissue typically exhibits a stiffness of 1–4 kPa [7]. Elevated stiffness levels are associated with poor prognosis and drug resistance. However, most existing in vitro models fail to recapitulate this stiffness range, often using supraphysiological stiffness that does not reflect in vivo conditions. Moreover, most studies use cells seeded on the surface of hydrogels with different stiffnesses to simulate the impact of this mechanical environment on tumor behavior [8]. Yet, this two-dimensional mechanical cue differs greatly from the true in vivo environment, potentially introducing bias into the conclusions.
Sodium alginate is widely used for three-dimensional cell culture due to its excellent biocompatibility and crosslinkability [9]. To address the gap mentioned above, we employed a tunable alginate-based 3D hydrogel system that independently mimics soft (normal) and stiff (tumor) mechanical niches. Here, we provide a detailed optimized protocol for preparing stiffness-tunable hydrogels, culturing tumor spheroids, releasing them without disrupting their morphology, and performing immunohistochemical (IHC) analysis on paraffin-embedded sections. In this protocol, we use the human colorectal cancer HCT-116 and SW-620 cell lines for tumor spheroid culture and compare, via IHC, the expression of the ANXA2 protein—an annexin closely associated with the extracellular microenvironment—in tumor spheroids grown in hydrogels of different stiffnesses. This protocol offers a robust platform for investigating mechanobiology in a physiologically relevant context.

Materials and reagents

Biological materials

1. Colorectal cancer cell lines HCT-116 (ATCC, catalog number: CCL-247) and SW-620 (ATCC, catalog number: CCL-227)

Reagents

1. Sodium alginate (Sigma, catalog number: A0682)
2. Calcium carbonate ($CaCO_3$) (Aladdin, catalog number: C100633)
3. Growth factor reduced Matrigel (Corning, catalog number: 356231)
4. Dulbecco's modified Eagle medium (DMEM) (Gibco, catalog number: 11965092)
5. Fetal bovine serum (FBS) (Gibco, catalog number: 10099141)

6. Penicillin-streptomycin (PS), 100× (Gibco, catalog number: 15140122)
7. Phosphate-buffered saline (PBS) (Cytiva, catalog number: SH30028.FS)
8. Sodium citrate (Sigma, catalog number: S1804)
9. Paraformaldehyde (PFA), 16% EM grade (Electron Microscopy Sciences, catalog number: 15710-S)
10. Low-melting-point agarose (Sigma, catalog number: A4018)
11. Ethanol, absolute (Sinopharm, catalog number: 10009218)
12. Xylene (Sinopharm, catalog number: 10023418)
13. Citrate antigen retrieval buffer (Beyotime, catalog number: P0083)
14. Hydrogen peroxide (H_2O_2), 30% (Sigma, catalog number: H1009)
15. Normal goat serum (Thermo Fisher, catalog number: 16210064)
16. ANXA2 primary antibody (Cell Signaling Technology, catalog number: 8235)
17. HRP-conjugated secondary antibody (Cell Signaling Technology, catalog number: 7074)
18. DAB Substrate kit (Vector Laboratories, catalog number: SK-4100)
19. Hematoxylin (Sigma, catalog number: MHS32-1L)
20. Cytoseal 60 (Fisher Scientific, catalog number: 23-244256)
21. Tween-20 (Sigma, catalog number: P1379)
22. HCl (Bolinda, catalog number: 7647-01-0)
23. Paraffin (CITOTEST, catalog number: 80200-0015)

Solutions

1. Sodium alginate stock solution (see Recipes)
2. $CaCO_3$ suspension (see Recipes)
3. Cell–alginate–Matrigel mixture (see Recipes)
4. Crosslinking $CaCO_3$ volumes for stiffness tuning (see Recipes)
5. Sodium citrate release solution (see Recipes)
6. 4% PFA fixative solution (see Recipes)
7. Low-melting-point agarose for spheroid embedding (see Recipes)
8. Citrate antigen retrieval buffer (see Recipes)
9. IHC blocking buffer (see Recipes)
10. DAB substrate working solution (see Recipes)
11. Graded ethanol series for dehydration (see Recipes)

Recipes

1. Sodium alginate stock solution (2% w/v, 50 mL)

Reagent	Final concentration	Quantity or volume
Sodium alginate powder	2% (w/v)	1 g
Ultrapure water	n/a	to 50 mL

Note: Dissolve by stirring overnight at 4 °C. Adjust pH to 7.4 with 1 M NaOH. Autoclave or filter sterilize through a 0.22 μm PES membrane. Store at 4 °C for up to 3 months.

2. $CaCO_3$ suspension (0.5 M, 10 mL)

Reagent	Final concentration	Quantity or volume
$CaCO_3$ powder	0.5 M	0.5 g
Ultrapure water	n/a	to 10 mL

Note: Autoclave the suspension. Vortex vigorously for 1 min immediately before each use to ensure a uniform particle suspension. Do not store for more than one week.

3. Cell–alginate–Matrigel mixture (for 2 mL gel)

Reagent	Volume per 2 mL gel
Cells in DMEM (2.5×10^5 cells/mL)	500 μL
Growth factor reduced Matrigel	500 μL
2% sodium alginate solution	1 mL

4. Crosslinking $CaCO_3$ volumes for stiffness tuning (per 2 mL gel)

Stiffness	Final Ca^{2+} concentration	Volume
Soft	10 mM	40 µL
Stiff	20 mM	80 µL

5. Sodium citrate release solution (100 mM, 50 mL)

Reagent	Final concentration	Quantity or volume
Sodium citrate	100 mM	1.47 g
1× PBS	n/a	to 50 mL

Note: Adjust pH to 7.4. Sterilize by filtration through a 0.22 µm PES membrane. Store at 4 °C for up to one month.

6. 4% PFA fixative solution (100 mL)

Reagent	Final concentration	Volume
16% PFA, EM grade	4%	25 mL
1× PBS	n/a	75 mL

Note: Prepare fresh or store at 4 °C for up to one week, protected from light.

7. Low-melting-point agarose for spheroid embedding (2%, 50 mL)

Reagent	Final concentration	Quantity or volume
Low-melting-point agarose	2% (w/v)	1 g
1× PBS	n/a	to 50 mL

Note: Heat in a microwave or on a hot plate until completely dissolved. Cool and maintain at 40–42 °C in a heat block before use.

8. Citrate antigen retrieval buffer (10 mM, pH 6.0, 1 L)

Reagent	Final concentration	Quantity or volume
Sodium citrate tribasic dihydrate	10 mM	2.94 g
Tween-20	0.05%	500 µL
Distilled water		to 1 L

Note: Adjust pH to 6.0 with 1 M HCl. Store at room temperature for up to 3 months.

9. IHC blocking buffer (for peroxidase and protein blocking, 50 mL)

Reagent	Final concentration	Volume
30% H_2O_2	3%	5 mL
Normal goat serum	5%	2.5 mL
1× PBS		to 50 mL

Note: Prepare fresh. The H_2O_2 blocks endogenous peroxidase, and the goat serum blocks nonspecific protein binding.

10. DAB substrate working solution (1 mL)

Reagent	Volume
DAB chromogen concentrate	1 drop (approximately 30 µL)
DAB buffer	1 mL

Note: Prepare fresh. Mix immediately before use. Protect from light. Discard after 30 min.

11. Graded ethanol series for dehydration (each 100 mL)

Solution	Volume
70% ethanol	70 mL absolute ethanol + 30 mL distilled water
80% ethanol	80 mL absolute ethanol + 20 mL distilled water
95% ethanol	95 mL absolute ethanol + 5 mL distilled water
100% ethanol	100 mL absolute ethanol

Laboratory supplies

1. 50 mL centrifuge tube, sterile (Celltreat, catalog number: 229106)
2. 15 mL centrifuge tube, sterile (Celltreat, catalog number: 229411)
3. 1.5 mL microcentrifuge tube, sterile (Eppendorf, catalog number: 0030120086)
4. 25 mL serological pipette, individually wrapped (Thermo Fisher, catalog number: 170357N)
5. 10 mL serological pipette, individually wrapped (Thermo Fisher, catalog number: 170356N)
6. 5 mL serological pipette, individually wrapped (Thermo Fisher, catalog number: 170355N)
7. 1,000 µL pipette tip, with filter (VWR, catalog number: 89082-350)
8. 200 µL pipette tip, with filter (VWR, catalog number: 89082-366)
9. 20 µL pipette tip, with filter (VWR, catalog number: 89082-338)
10. Wide-bore 200 µL pipette tip (VWR, catalog number: 89082-366, cut with sterile scissors)
11. Low-attachment 35 mm culture dish (NoninBio, catalog number: NBH2035)
12. Low-attachment 24-well plate (NoninBio, catalog number: NBH1124)
13. 0.22 µm PES syringe filter, 33 mm diameter (Millipore, catalog number: SLGP033RB)
14. 10 mL sterile syringe (Fisher Scientific, catalog number: 14-823-435)
15. Sterile cell scraper (Fisher Scientific, catalog number: 08-100-241)
16. Plastic embedding mold for histology (VWR, catalog number: 25608-922)
17. Tissue cassette for paraffin embedding (VWR, catalog number: 18000-134)
18. Superfrost Plus microscope slide (Thermo Fisher, catalog number: 12-550-15)
19. Coverslip, 24 × 60 mm (Fisher Scientific, catalog number: 12-543-5)
20. Microtome blade, disposable, high profile (Leica, catalog number: 819)
21. Pap pen or hydrophobic barrier pen (Vector Labs, catalog number: H-4000)
22. Humidified staining chamber (Thermo Fisher, catalog number: 50-195-9078)
23. Glass staining dish with slide rack (Thermo Fisher, catalog number: 900200)
24. Cytoseal 60 mounting medium (Fisher Scientific, catalog number: 23-244256)
25. Sterile spatula (Fisher Scientific, catalog number: 21-401-10)
26. Forceps, fine tip (Fisher Scientific, catalog number: 08-953G)

Equipment

1. Laminar flow hood (Baker, model: SterilGARD)
2. CO_2 incubator (Thermo, model: Forma SteriCycle i160) (for cell culture at 37 °C, 5% CO_2)
3. Benchtop centrifuge for 15/50 mL tubes (Thermo, model: Sorvall Legend X1)
4. Microcentrifuge for 1.5 mL tubes (Eppendorf, model: 5425)
5. Horizontal shaker with temperature control (Thermo Fisher, model: 88880022) (for gel dissolution and washes)
6. Heat block, capable of 40–42 °C and 65 °C (VWR, model: 13259-036)
7. Water bath, capable of 37 °C and 40 °C (Thermo Fisher, model: ISOTEMP 215)
8. Pressure cooker or electric pressure steamer for antigen retrieval (Instant Pot, model: Duo Plus) (achieves ~120 °C for 2 min)
9. Microtome (Leica, model: RM2255)
10. Tissue floatation water bath for section mounting (Leica, model: HI1210)
11. Slide drying oven (Thermo Fisher, model: 131481)
12. Light microscope for routine inspection (Olympus, model: CK2)
13. Digital light microscope for IHC imaging (Olympus, model: BX43)
14. Magnetic stirrer with heating function (Thermo Fisher, model: SP131830)
15. Analytical balance (Mettler Toledo, model: ML204T)
16. pH meter (Mettler Toledo, model: FE28)
17. Autoclave (Tuttnauer, model: 3870E)
18. Vacuum filtration system (Millipore, model: Stericup Quick Release)
19. Refrigerator (4 °C) and freezer (-20 and -80 °C) (for reagent storage)
20. Liquid nitrogen dewar for long-term storage (Taylor-Wharton, model: LS750)

Procedure

A. Fabrication of stiffness-tunable alginate hydrogels and tumor spheroid culture

1. Prepare 2% sodium alginate stock (see Recipes). Store at 4 °C.
2. Prepare a 0.5 M $CaCO_3$ suspension freshly (see Recipes). Vortex vigorously for 30 s immediately before use to ensure uniform particle distribution.
3. Harvest cells by trypsinization, count, and resuspend in DMEM at 2.5×10^5 cells/mL.
4. To prepare a final gel volume of 2 mL on ice, prepare cell–alginate–Matrigel mixture (see Recipes). Gently mix the mixture by pipetting up and down 5–6 times using a wide-bore tip.
5. Initiate gelation by adding the appropriate volume of 0.5 M $CaCO_3$ suspension based on the desired stiffness: 40 µL for soft gels (10 mM final Ca^{2+}) or 80 µL for stiff gels (20 mM final Ca^{2+}). After adding the $CaCO_3$ suspension, mix gently but thoroughly.
6. Dispense 40 µL of the mixture into a low-attachment culture dish or plate (Figure 1).

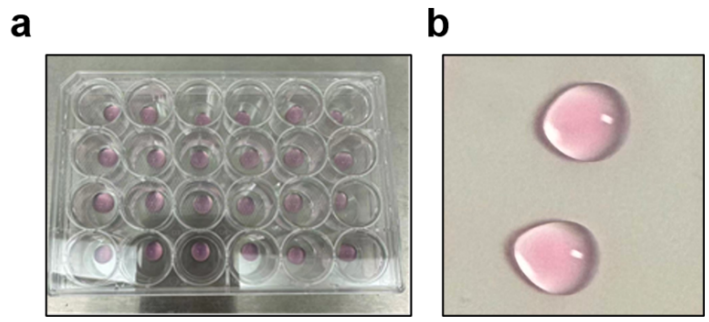


Figure 1. Crosslinking of sodium alginate solution into a hydrogel. (a) The uncrosslinked gel dispersion is shown in a 24-well low-adhesion culture plate. (b) After crosslinking, it becomes a transparent matrix, and the dome-shaped structure facilitates tumor spheroid growth.

7. Induce gelation by placing the dish in a 37 °C incubator for 30 min.
8. Overlay with 2 mL of prewarmed DMEM to promote complete ion chelation. Incubate for 1 h at 37 °C.
9. Replace the overlay DMEM with 2 mL of complete culture medium (DMEM + 10% FBS + 1% PS).
10. Culture for 3–5 days without disturbing the gel. Change medium every 2–3 days. Spheroid formation is typically observed within 3–5 days (Figure 2).

Critical: Keep all alginate–Matrigel–cell mixtures on ice before gelation to prevent premature crosslinking.

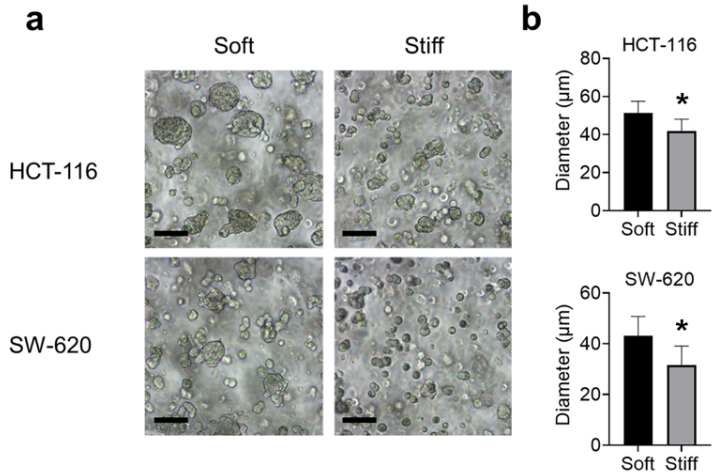


Figure 2. Tumor spheroid cultures in alginate gels with tunable stiffness. (a) HCT-116 and SW-620 spheroids grown in soft or stiff matrix after 5 days of embedded culture. Scale bars, 50 µm. (b) Quantification of tumor spheroid diameters. Data

represent mean ± SD of three biological replicates, each consisting of two technical replicates. Statistical significance was determined by Student's t-test. *p ≤ 0.05.

B. Gentle release of tumor spheroids from Matrigel–alginate hydrogel

Note: Perform all steps at room temperature unless specified.

1. Calculate the volume of sodium citrate required based on a molar ratio of sodium citrate to total $CaCO_3$ of 1.5:1. For a 2 mL gel, the calculation is as follows:

Gel stiffness	0.5 M $CaCO_3$	Total Ca^{2+} (μmol)	100 mM sodium citrate (μL)
Soft	40 μL	20 μmol	300 μL
Stiff	80 μL	40 μmol	600 μL

2. Transfer the gel into a 15 mL sterile centrifuge tube using a sterile spatula or wide-bore pipette.
3. Add the calculated sodium citrate release solution (see Recipes) and bring the total volume to 10 mL with PBS.
4. Incubate on a horizontal shaker at 50 rpm for 5–10 min until the gel is completely dissolved.
5. Centrifuge at 200× *g* for 5 min at room temperature.
6. Carefully remove the supernatant, leaving ~100 μL to avoid losing spheroids.
7. Add 10 mL of PBS and gently resuspend by rolling the tube horizontally between the palms. Do not pipette up and down.
8. Centrifuge again at 200× *g* for 5 min.
9. Fix spheroids in 4% PFA for 30 min at room temperature with gentle agitation.
10. Wash once with PBS and proceed to embedding (section C).

Note: Spheroids are now ready for agarose embedding and IHC.

C. Low-melting-point agarose embedding of tumor spheroids

1. Prepare 2% low-melting-point agarose in PBS. Heat to dissolve completely, then cool and maintain at 40–42 °C in a heat block.
2. Pellet fixed spheroids by centrifugation (200× *g*, 5 min). Carefully remove supernatant, leaving ~50–100 μL.
3. Resuspend spheroids in 100 μL of PBS using a wide-bore tip.
4. Add 100 μL of prewarmed (40–42 °C) 2% low-melting-point agarose to the spheroid suspension. Mix gently by stirring with the pipette tip; do not pipette up and down to avoid mechanical shearing.
5. Quickly transfer the mixture to a pre-chilled plastic embedding mold.
6. Solidify at 4 °C for 30 min.
7. Extract the agarose block from the mold. Trim excess agarose around the spheroids if needed.

D. Paraffin embedding and sectioning

1. Dehydrate the agarose block through a graded ethanol series as follows: 70% ethanol for 30 min, 80% ethanol for 30 min, 95% ethanol for 30 min, and 100% ethanol for two 30-min changes.
2. Clear with xylene twice for 30 min each time.
3. Embed in paraffin using a standard histology protocol. Orient the block to ensure spheroids are positioned for sectioning.
4. Section at 4 μm thickness using a microtome.
5. Float sections in a 40 °C water bath and mount onto Superfrost Plus slides.
6. Dry slides overnight at 37 °C.

E. IHC staining

1. Deparaffinize slides by incubating them sequentially as follows: xylene for two 10-min changes, 100% ethanol for two 5-min changes, 95% ethanol for 5 min, 80% ethanol for 5 min, 70% ethanol for 5 min, and distilled water for 5 min.
2. Perform antigen retrieval by filling a pressure cooker with citrate antigen retrieval buffer (pH 6.0), immersing the slides in the buffer, heating under pressure for 2 min at 120 °C, and then allowing to cool to room temperature (approximately 20 min).

3. Block endogenous peroxidase: Incubate slides in 3% H_2O_2 in PBS for 10 min at room temperature, then wash 3 × 5 min with PBS.
4. Block nonspecific binding: Incubate with 5% normal goat serum in PBS for 1 h at room temperature.
5. Incubate with primary antibody: Dilute primary antibody (anti-ANXA2 1:200) in IHC blocking buffer, apply to sections, and incubate overnight at 4 °C in a humidified chamber.
6. Wash 3 × 5 min with PBS.
7. Incubate with HRP-conjugated secondary antibody: Dilute secondary antibody 1:500 in IHC blocking buffer, then incubate sections for 1 h at room temperature.
8. Wash 3 × 5 min with PBS.
9. Develop with DAB: Prepare DAB substrate according to the manufacturer's instructions, apply to sections, monitor under the microscope (typically 1–5 min), and stop the reaction by rinsing with distilled water.
10. Counterstain with hematoxylin: Apply hematoxylin for 30 s to 1 min, then rinse with running tap water for 5 min.
11. Dehydrate and mount: Dehydrate sections through a graded ethanol series: 70% ethanol for 1 minute, 80% ethanol for 1 minute, 95% ethanol for 1 min, 100% ethanol for two 1-min changes, followed by xylene for two 1-min changes. Finally, mount with Cytoseal 60 and a coverslip.
12. Image using a light microscope (Figure 3).

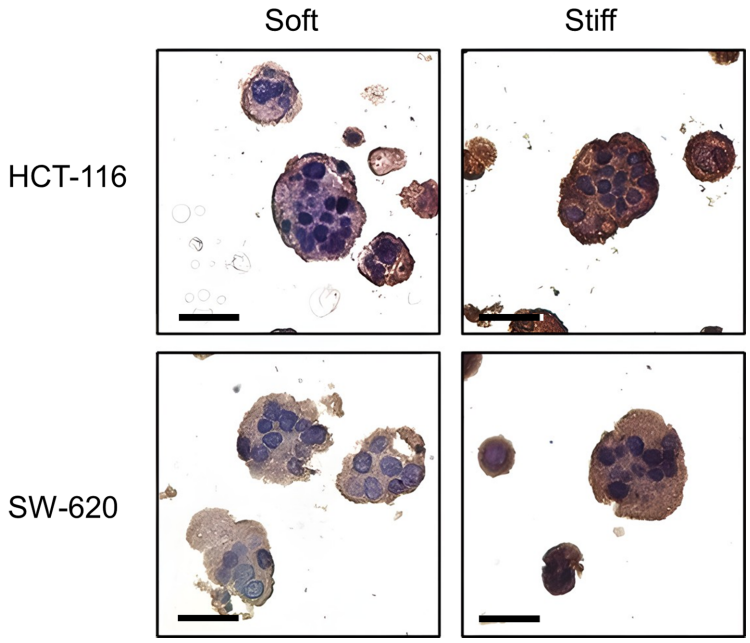


Figure 3. Immunohistochemical (IHC) staining of tumor spheroids after release from the gels. The ANXA2 protein expression was detected in HCT-116 and SW-620 spheroids grown in soft or stiff matrices. Scale bars, 50 μm.

Validation of protocol

The Matrigel–alginate matrix used in this protocol supported the growth of HCT-116 and SW-620 cells under both soft and stiff conditions. After 5 days of culture, tumor spheroids with a diameter of approximately 30–50 μm were obtained (Figure 2). These tumor spheroids were collected and sectioned, exhibiting intact morphology. Moreover, due to growth in matrices with different stiffnesses, these spheroids showed distinct ANXA2 expression levels (Figure 3). These results sufficiently validate this protocol.
This protocol is an optimized version based on a previous calcium alginate gel system and has been validated in the following research article:
Lemarie et al. [10]. Human Induced Pluripotent Spheroids' Growth Is Driven by Viscoelastic Properties and Macrostructure of 3D Hydrogel Environment. *Bioengineering (Basel)*. https://doi.org/10.3390/bioengineering10121418

General notes and troubleshooting

General notes

1. Always prepare the $CaCO_3$ suspension fresh and vortex immediately before use.
2. Matrigel must be thawed overnight at 4 °C and kept on ice at all times before use. Always use pre-chilled pipette tips when handling Matrigel to avoid premature gelation at room temperature.
3. Keep alginate–Matrigel–cell mixture on ice to prevent premature gelation.
4. Spheroid release using sodium citrate is gentle but must be monitored; over-incubation (>15 min) may cause spheroid dissociation.
5. Low-melting-point agarose must be maintained at 40–42 °C.
6. Due to the small size of the spheroids (approximately 50 μm), to avoid obtaining blank sections during microtomy, we recommend embedding at least 50 spheroids per agarose block and trimming the block to expose the spheroids before collecting ribbons.
7. For rare spheroids, minimize washes and use wide-bore tips throughout.

Troubleshooting

Problem 1: Gel does not form or is too soft.
Possible causes: Insufficient $CaCO_3$ or uneven mixing.
Solutions: Ensure $CaCO_3$ is vortexed thoroughly; increase $CaCO_3$ volume by 10%–20%.

Problem 2: Spheroids do not form or remain as single cells.
Possible causes: Low cell density or insufficient Matrigel.
Solutions: Use at least 2.5×10^5 cells/mL; ensure Matrigel is not frozen and thawed repeatedly.

Problem 3: Spheroids dissociate during release.
Possible cause: Over-incubation in sodium citrate.
Solution: Reduce incubation time to 5–7 min; check every 2 min.

Problem 4: Spheroids are lost during agarose embedding.
Possible causes: Centrifugation too fast or pipetting too vigorous.
Solutions: Use 200× *g* maximum; always use wide-bore tips; roll the tube instead of pipetting.

Problem 5: Weak or no IHC signal.
Possible causes: Incomplete antigen retrieval or antibody concentration too low.
Solutions: Extend pressure cooker time to 3 min; perform titration for primary antibody.

Problem 6: High background in IHC.
Possible causes: Endogenous peroxidase not fully blocked, or secondary antibody concentration too high.
Solutions: Ensure 3% H_2O_2 incubation for a full 10 min; reduce secondary antibody dilution to 1:1,000.

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Competing interests

The authors declare no competing interests.

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References

1. Biondo, S., Frago, R. and Kreisler, E. (2024). Primary tumor resection for asymptomatic colorectal cancer with unresectable metastasis: the end of the dilemma. *Ann Oncol.* 35(9): 757–759. https://doi.org/10.1016/j.annonc.2024.06.017
2. Siegel, R. L., Giaquinto, A. N. and Jemal, A. (2024). Cancer statistics, 2024. *CA Cancer J Clin.* 74(1): 12–49. https://doi.org/10.3322/caac.21820
3. Zhong, X., He, X., Wang, Y., Hu, Z., Huang, H., Zhao, S., Wei, P. and Li, D. (2022). Warburg effect in colorectal cancer: the emerging roles in tumor microenvironment and therapeutic implications. *J Hematol Oncol.* 15(1): 160. https://doi.org/10.1186/s13045-022-01358-5
4. Li, X., Pan, J., Liu, T., Yin, W., Miao, Q., Zhao, Z., Gao, Y., Zheng, W., Li, H., Deng, R., et al. (2023). Novel TCF21(high) pericyte subpopulation promotes colorectal cancer metastasis by remodelling perivascular matrix. *Gut.* 72(4): 710-721. https://doi.org/10.1136/gutjnl-2022-327913
5. Wang, K., Ning, S., Zhang, S., Jiang, M., Huang, Y., Pei, H., Li, M. and Tan, F. (2025). Extracellular matrix stiffness regulates colorectal cancer progression via HSF4. *J Exp Clin Cancer Res.* 44(1): 30. https://doi.org/10.1186/s13046-025-03297-8
6. Zheng, Y., Zhou, R., Cai, J., Yang, N., Wen, Z., Zhang, Z., Sun, H., Huang, G., Guan, Y., Huang, N., et al. (2023). Matrix Stiffness Triggers Lipid Metabolic Cross-talk between Tumor and Stromal Cells to Mediate Bevacizumab Resistance in Colorectal Cancer Liver Metastases. *Cancer Res.* 83(21): 3577–3592. https://doi.org/10.1158/0008-5472.CAN-23-0025
7. Liu, C., Pei, H. and Tan, F. (2020). Matrix Stiffness and Colorectal Cancer. *Onco Targets Ther.* 13: 2747–2755. https://doi.org/10.2147/OTT.S231010
8. Ye, Y., Jia, Y. and Li, W. (2025). TAGLN: a stiffness-driven prognostic biomarker for metastatic colorectal cancer. *Cancer Cell Int.* 26(1): 54. https://doi.org/10.1186/s12935-025-04157-3
9. Jiang, T., Munguia-Lopez, J., Flores-Torres, S., Grant, J., Vijayakumar, S., De Leon-Rodriguez, A. and Kinsella, J. M. (2018). Bioprintable Alginate/Gelatin Hydrogel 3D In Vitro Model Systems Induce Cell Spheroid Formation. *J Vis Exp.* (137). https://doi.org/10.3791/57826
10. Lemarie, L., Dargar, T., Grosjean, I., Gache, V., Courtial, E. J. and Sohier, J. (2023). Human Induced Pluripotent Spheroids' Growth Is Driven by Viscoelastic Properties and Macrostructure of 3D Hydrogel Environment. *Bioengineering (Basel).* 10(12). https://doi.org/10.3390/bioengineering10121418