Cell Organoid

Methodology

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A novel *in-situ* fixation and embedding method improves lung cancer organoid histopathology

Authors

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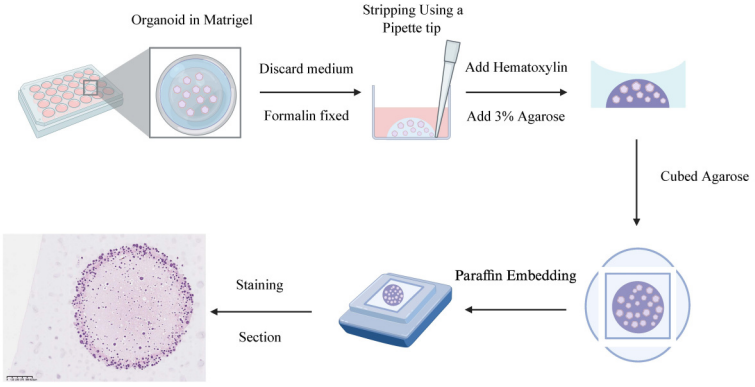
Graphical abstract



In Brief

This study introduces a simple method to prepare paraffin blocks from lung cancer organoids using a single Matrigel droplet. Organoids are fixed *in situ*, embedded in agarose, and processed without dissociation, minimizing cell loss. The approach maintains tumor marker consistency with original tissues and is efficient for histopathological analysis of early-stage organoids.

Highlights

- Only one single Matrigel droplet is required for organoids histopathology.
- *In-situ* fixation and pre-embedding simplify protocols by avoiding Matrigel dissociation.
- It effectively reduces organoids loss during fixation and paraffin embedding processes.
- It significantly reduces culture costs and time and is particularly useful for the histological examination of early-stage organoids.

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A novel *in-situ* fixation and embedding method improves lung cancer organoid histopathology

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ABSTRACT

Patient-derived lung cancer organoid provides a valuable platform for various applications. Histopathological examination of organoids is crucial to verify their consistency with the original tissue. Traditional methods require collecting multiple Matrigel domes and dissociating organoids from the matrix before histopathology. In this study, we report a fast and convenient method for making a paraffin block using only one Matrigel droplet. Organoids cultured in a 24-well plate were fixed with formalin *in situ* for 72 hours, pre-embedded in 3% agarose to block the Matrigel dome, and processed through routine dehydration, embedding, sectioning, and staining. We compared this method with others, including traditional small specimen paper wrapping and collecting dissociated organoids in EP tubes before embedding. Using only a single droplet, this approach yields a large number of organoids per section. Our method eliminates the need for Matrigel dissociation, effectively reduces organoids loss during fixation and paraffin embedding processes. In lung cancer organoids, tumor markers were consistent with the corresponding original tumor tissues. This novel protocol allows *in-situ* processing of patient-derived lung cancer organoids from a single Matrigel dome. It significantly reduces culture costs and time and is particularly useful for the histological examination of early-stage organoids.

KEYWORDS

lung cancer, organoids, histopathology, *in-situ* fixation and pre-embedding, agarose block

Introduction

Organoids, three-dimensional cell clusters mimicking *in vivo* tissue structures, bridge the gap between cell lines and tissues[1]. Since the pioneering studies conducted by Hans Clevers' and Kevin Kuo's laboratories in 2009[2,3], organoid culture technology has undergone remarkable advancements[4], profoundly influencing basic research, pre-clinical drug screening, and even personalized cancer therapy[5]. With lung cancer being a leading cause of cancer mortality worldwide[6], patient-derived lung cancer organoids hold great potential for patient-specific drug trials and proof-of-concept studies[7,8]. Patient-derived organoids faithfully maintain the histological and genetic characteristics of their respective parental tumor tissues[9].

Maintaining the histopathological consistency between organoids and parental tissue is critical for their applications. Histological analysis of organoids is essential for most laboratories conducting organoid research. Several methods have been developed. Fujii et al. reported pre-embedded organoids in agarose gel using a special polymer-coated conical tube[10]. To reduce sample volume requirements, Li et al. resuspended organoids in a 1.5 mL tube in phosphate-buffered saline (PBS), mixed it with 2% agarose, and pipetted it onto parafilm[11]. Zhang et al. developed an improved method using agarose to directly pre-embed organoids in tubes, which were centrifuged upside down to release blocks[12]. However, these methods require larger quantities of organoids, which lead to higher cultivation expenses and extended culture timelines. Furthermore, the necessity for Matrigel dissociation and meticulous manipulation renders them less suitable for early-passage organoids.

Here, we describe a single-droplet *in-situ* fixation and embedding technique, which simplifies histopathological examination of organoids without dissociating the Matrigel and has broad utility in organoid research settings.

Methods and materials

Tumor tissue processing and organoid culture

This study was approved by the Ethics Committee of Beijing

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Chest Hospital. Patient data and surgically resected primary tumors were collected from patients who provided informed consent. The patient information is listed in Table 1. The clinical diagnosis of lung cancer subtypes was validated by two pathologists. Additionally, a case of lung inflammatory pseudotumor which was firstly considered as adenocarcinoma was included as well. The protocol for establishing Non-Small Cell Lung Cancer (NSCLC) organoids has been described previously[7–9]. The organoid culture procedure in this study was modified according to these methods.

For organoid culture, tumor tissues were washed with ice-cold PBS and minced into small pieces (1–2 mm^3), then digested in collagenase I (Gibco, Cat. No. 17100-017, 2 mg/mL) and Dispase II (Sigma, cat. No. D4693, 2 mg/mL). The tissue was incubated at 37 °C with gentle shaking for 10–20 min. After digestion, the cell suspension was filtered through a 100 μm mesh, centrifuged at 300× *g* for 3 min, washed with PBS, and subsequently pelleted by centrifugation at 300× *g* for another 3 min. The cell suspension was prepared in PBS and subsequently combined with Matrigel (Biogenous, Cat. No. 354234) before being plated into 24-well culture plates. The plates were incubated at 37 °C for 5 min to allow Matrigel solidification, then inverted and incubated for an additional 15 min. Finally, 500 μL of organoid culture media was added to each well. The media was replaced every 3–4 days, and organoids were passaged every 1–2 weeks. During passaging, organoids were digested using mild enzyme TrypLE Express (Gibco, Cat. No. 12604-021), and Matrigel domes were broken up by mechanical dissociation (pipetting 20 times with a P1000 tip).

The basal media consisted of advanced DMEM/F12 (Gibco, Cat. No. 12634-010), B27 (Gibco, Cat. No. 17504-044), 10 mmol/L HEPES (Gibco, Cat. No. 15630-106), and GlutaMAX (Gibco, Cat. No. 35050-061). The basal media was supplemented with various growth factors and small molecules, including 100 ng/mL Noggin (Biogenous, Cat. No. 807-NOG), 50 ng/mL EGF (Biogenous, Cat. No. 568-EGF), 50 ng/mL FGF10 (Biogenous, Cat. No. 816-FGF), 500 nmol/L A83-01 (TargetMol, Cat. No. T3031), 10 μmol/L Y27632 (TargetMol, Cat. No. T1725), 250 nmol/L CHIR99021 (TargetMol, Cat. No. T2310), 100 nmol/L

Table 1. The list of patients donating samples for organoids culture.

Organoid ID	Gender	Age	Stage	Pathology diagnosis	Site of sampling	Driver mutation	Treatment
BCH_OG_2297	Male	51	IIB	LAC	Primary tumor	EGFR 19del	NA
BCH_OG_0264	Female	66	IV	LAC	Pleural effusion	EGFR 19del+T790M	Icotinib
BCH_OG_2285	Male	58	IB	LAC	Primary tumor	KRAS G12A/G12V/G12R/G12C/G13C	NA
BCH_OG_2339	Male	59	IB	LAC	Primary tumor	EGFR L858R, PDGFRA K646N	NA
BCH_OG_1454	Female	57	IB	LAC	Primary tumor	EGFR L858R	NA
BCH_OG_1791	Male	55	IB	LAC	Primary tumor	EGFR P772_H773dup, TP53 R267Tfs*5	NA
BCH_OG_1820	Male	35	IA	LAC	Primary tumor	NA	NA
BCH_OG_1864	Male	68	IIB	Lung spindle cell carcinoma	Primary tumor	NA	NA
BCH_OG_1543	Male	56	NA	Lung inflammatory pesudotumor	Primary leison	NA	NA
BCH_OG_4771	Female	68	IIB	LAC	Primary tumor	KRAS G12C, MET V1092I	NA
BCH_OG_2336	Female	70	IA	LAC	Primary tumor	EGFR L858R	NA
BCH_OG_5659	Male	75	IV	LAC	Pleural effusion	KRAS Q61K, TP53 H193R, ESR1 R548C	Pemetrexed+carboplatin+ camrelizumab
BCH_OG_8967	Female	64	IIB	LAC	Primary tumor	EGFR L858R	NA
BCH_OG_6397	Male	58	IV	SCLC	Pleural effusion	EGFR 19750del, ERBB2 A1039T, PIK3CA E545K, TP53 I232Kfs*4	Osimertinib, etoposide+carboplatin+ durvalumab, anlotinib
BCH_OG_3281	Male	46	IA	LAC	Primary tumor	EGFR L858R	NA
BCH_OG_3684	Male	64	IV	LAC	Pleural effusion	BRAF V600E	Dabrafenib+trametinib+ bevacizumab
BCH_OG_5861	Male	79	IV	SCLC	Pleural effusion	TP53 R273H	NA
BCH_OG_5153	Male	76	IIIB	LSC	Pleural effusion	TP53 S215R	Taxol+carboplatin+tislelizumab
BCH_OG_9411	Male	68	IV	LAC	Pleural effusion	EGFR L858R	Osimertinib+bevacizumab
BCH_OG_8941	Female	64	IB	LASC	Primary tumor	EGFR L858R, MET H837Y, PTEN D190N, STK11 G52E, PIK3CA H1047R	Almonertinib
BCH_OG_9474	Male	68	IB	LSC	Primary tumor	PIK3CA H1047R/E545K	NA
BCH_OG_3338	Male	69	IA	LAC	Primary tumor	EGFR L858R, TP53 R175H	NA

LAC: lung adenocarcinoma, SCLC: small cell lung carcinoma, LSC: lung squamous carcinoma, LASC: lung adenosquamous carcinoma.

SAG (TargetMol, Cat. No. T1779), 1 mmol/L NAC (Sigma, Cat. No. A9165), and antibiotic-antimycotic solution (Gibco, Cat. No. 15240-062).

Organoids fixation and pre-blocking by agarose *in situ*

Organoids were fixed *in situ* by replacing the culture medium with 1 mL of formalin at room temperature for 72 h (Fig. 1a). After fixation, formalin was removed, and 100–200 μL of hematoxylin was added to stain the Matrigel dome (Fig. 1b) for easier visualization during sectioning. Organoids were gently detached from the well bottom using a 200 μL pipette tip. 1 mL of 3% agarose (melted at 95 °C and cooled to ~50 °C) was gently added into the well to embed the Matrigel dome. A cut P1000 tip was used to pipette the agarose due to its viscosity. If the Matrigel dome is not properly positioned in the well, it can be lightly adjusted by gentle nudging with a 200 μL pipette tip.

The solidified agarose block was removed from the well using a microtome blade (to cut the circular block into a square) and a modified syringe needle (to remove the surrounding agarose) (Fig. 1c). The resulting square agarose block containing the organoids (Fig. 1d) was transferred to an embedding cassette for standard histological processing.

Histology staining and imaging

Formalin-fixed tissue and agarose-blocked organoids were processed by standard histology procedures, including dehydration (SAKURA, Tissue-TEK VIP 6 AI), paraffin embedding (Epredia, HistoStar), and sectioning (Leica, HistoCore Multicut). To obtain paraffin embedded organoid sections, the paraffin block was chilled on ice. Hematoxylin staining allows visualization of the Matrigel layer during block trimming. Organoid sectionsv(5 μm in thickness) were prepared using a rotary microtome (Leica, HistoCore Multicut). Paraffin sections were unfolded on water at 42 °C in a water bath.

Hematoxylin and eosin (H&E) staining was performed automatically using a Dako CoverStainer. The slides were mounted with mounting medium (DAKEWE, CS500). For immunohistochemistry (IHC) staining, antigen retrieval was performed automatically (Dako, PT Link). The samples were incubated with primary antibodies, including anti-TTF-1 (MXB, Cat. No. MAB-0599), anti-CK7 (MXB, Cat. No. MAB-0828), anti-p40 (MXB, Cat. No. RMA-1006), and anti-CK5/6 (MXB, Cat. No. MAB-0744). The sections were then incubated with secondary antibodies (Dako, Cat. No. EnVision FLEX/HRP SM802) and visualized using the DAB Detection kit (Dako, Cat. No. EnVision FLEX SM803). These procedures were performed automatically on a machine (MXB, LUMATAS). Nuclei were counterstained with hematoxylin. Images were acquired using a digital pathology slides scanner (KFBIO, Cat. No. KF-SCAN-ST).

Results

In-situ agarose embedding simplifies organoid processing

The Graphical Abstract outlines the procedure for organoids *in-situ* embedding in agarose and subsequent standard histopathology. The first step involves *in-situ* formalin fixation of organoids without removing them from the Matrigel (Fig. 1a). After 72 h of fixation, formalin is replaced with 100 μL of hematoxylin for visualization during sectioning (Figs. 1b and 1g). The Matrigel dome is gently detached from the well bottom using a pipette tip, as shown in the Graphical Abstract.

Melted 3% agarose (1000 μL) is added to the well plate to encapsulate the detached droplet (Fig. 1b). After agarose solidification, the gel is cut into a square by a microtome blade (short side), as shown in Figs. 1c and 1d. A hook made from a 1 mL syringe needle (Fig. 1c) is used to carefully remove the semicircular agarose surrounding the square, without damaging the square gel. The resulting square agarose gel, containing the organoids (Figs. 1d and 1e), can then be easily removed from the 24-well plate and placed into a dehydrating cassette (Fig. 1f) for standard formalin-fixed paraffin-embedded (FFPE) processing. Sectioning reveals clear boundaries between the embedded

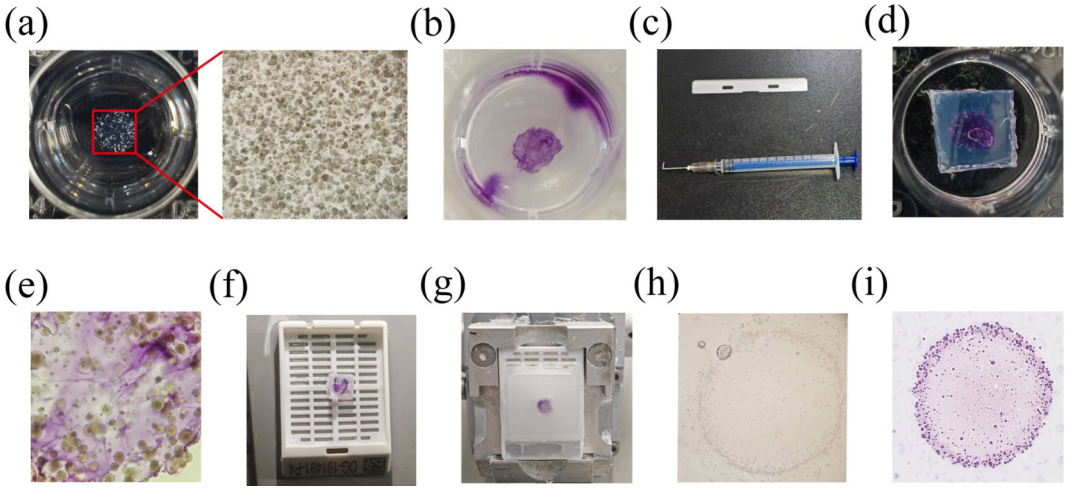

Figure 1. Key steps involved in the preparation of paraffin organoid blocks. (a) Photograph of organoids fixed with 10% neutral formalin in the well. (b) The Matrigel dome, pre-stained with hematoxylin, is completely blocked by 3% agarose in the well. (c) Tools used for processing and extracting the block from the well. (d) Image of the agarose block after processing, where the short edge of a blade is used to cut the round block into a square shape. A small hook, made from a modified syringe needle, is used to remove residual agarose from the edges. The square block in the center is then ready for further processing. (e) Photograph of *in-situ* embedded organoids. (f) The agarose block, ready for dehydration in an embedding cassette. (g) Photograph of paraffin-embedded organoids. (h) A bright-field image of a microscope slide containing lung cancer organoids. (i) A representative bright-field image of organoids after H&E staining.

Matrigel, the square agarose block, and the surrounding paraffin (Fig. 1g). Hematoxylin staining enables clear visualization of the Matrigel layer during block trimming (Fig. 1g). When the entire dome is cut, a uniform distribution of numerous organoids within the circular dome can be clearly seen under the microscope (Fig. 1h). An overview of the representative H&E staining results is presented in Fig. 1i.

H&E and IHC staining of paraffin-embedded organoid sections

Using our *in-situ* fixation and embedding method, we performed H&E and IHC staining on organoids derived from NSCLC tumor tissue. The method effectively stains both large and small organoids. Figure 2a shows representative images of lung squamous carcinoma (LSC) organoids (top row) with corresponding patient tumor tissue (bottom row). Consecutive sections show p40 and CK5/6 expression, consistent with lung squamous carcinoma. Similarly, lung adenocarcinoma (LAC) organoids expressed CK7 and TTF-1, consistent with the markers of the corresponding patient tumors (Fig. 2b). These results highlight the reliability of our method for histopathological evaluation.

Comparison with existing methods demonstrates the superiority of our approach

For small biopsy specimens, the traditional method is to wrap the tissue in filter paper for dehydration to prevent tissue loss[13,14]. Existing methods for organoid identification, mainly derived from routine histopathology techniques, require Matrigel dissociation followed by various collection methods (centrifugation in 50 mL[10], 2.0 mL[12], and 1.5 mL[11] tubes) and resuspension with gel. We compared our method to these methods (Fig. 3 and Table 2).

We first tested the traditional paper-wrapping method. Specifically, we scraped the entire Matrigel dome with a pipette tip, transferred it to a 1.5 mL tube using a Pasteur pipette, and fixed it in formalin for 72 h. After centrifugation, the organoid pellets were collected and wrapped in filter paper for dehydration[13,14]. We tried using 1, 2, or 4 gel droplets for making paraffin blocks. The number of gel droplets wrapped in paper does not scale predictably with the quantity of organoids

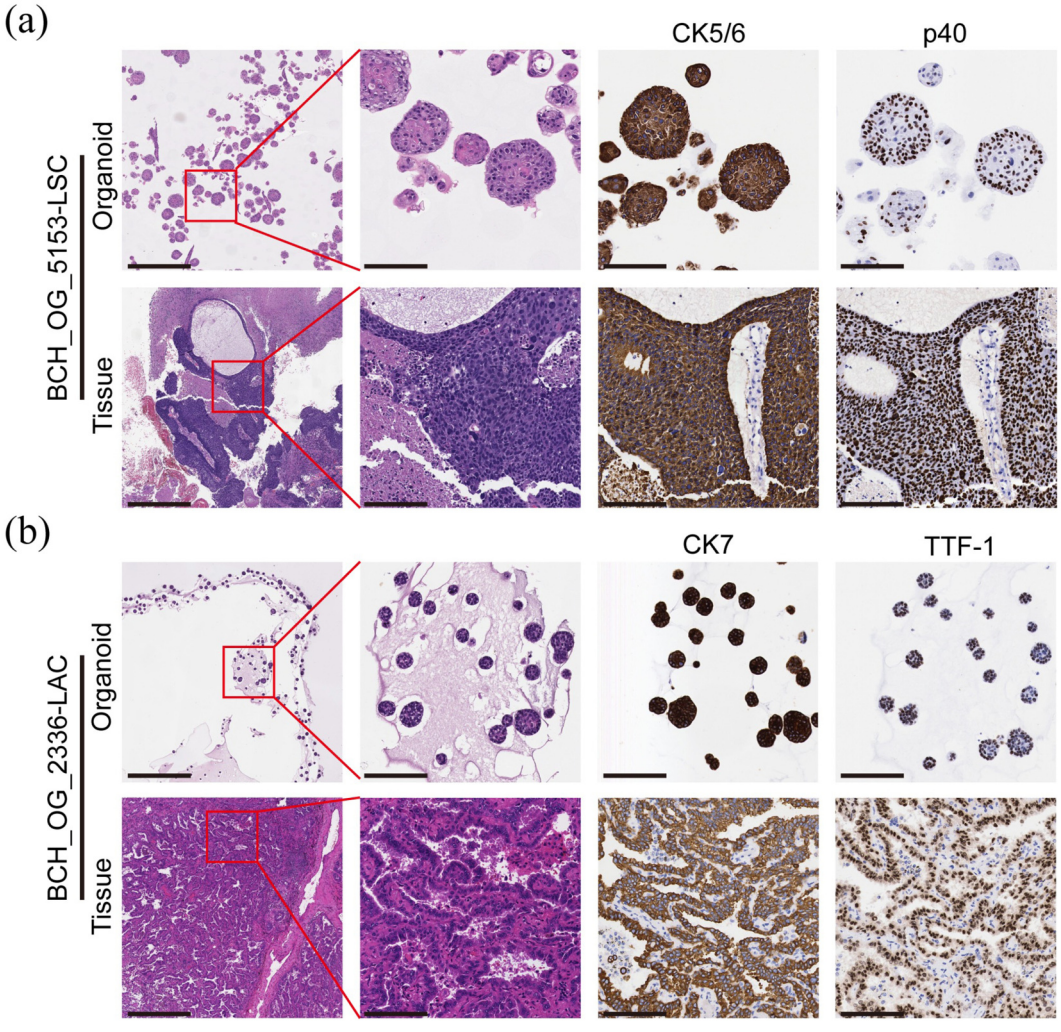

Figure 2. NSCLC organoids preserve the histopathological features of the original tumor tissues. (a) Histological H&E and IHC images of LSC organoids (upper panel) and the corresponding parental tumor (lower panel). Paired LSC organoids and tumor tissue were consecutively sectioned and stained for LSC markers CK5/6 and p40. Nuclei were stained with hematoxylin (blue). (b) Histological H&E and IHC images of LAC organoids (upper panel) and the corresponding parental tumor (lower panel). Paired LAC organoids and tumor tissue were consecutively sectioned and stained for LAC markers CK7 and TTF-1. Nuclei were stained with hematoxylin (blue). Scale bar: 250 μm for the left column; 50 μm for the last images

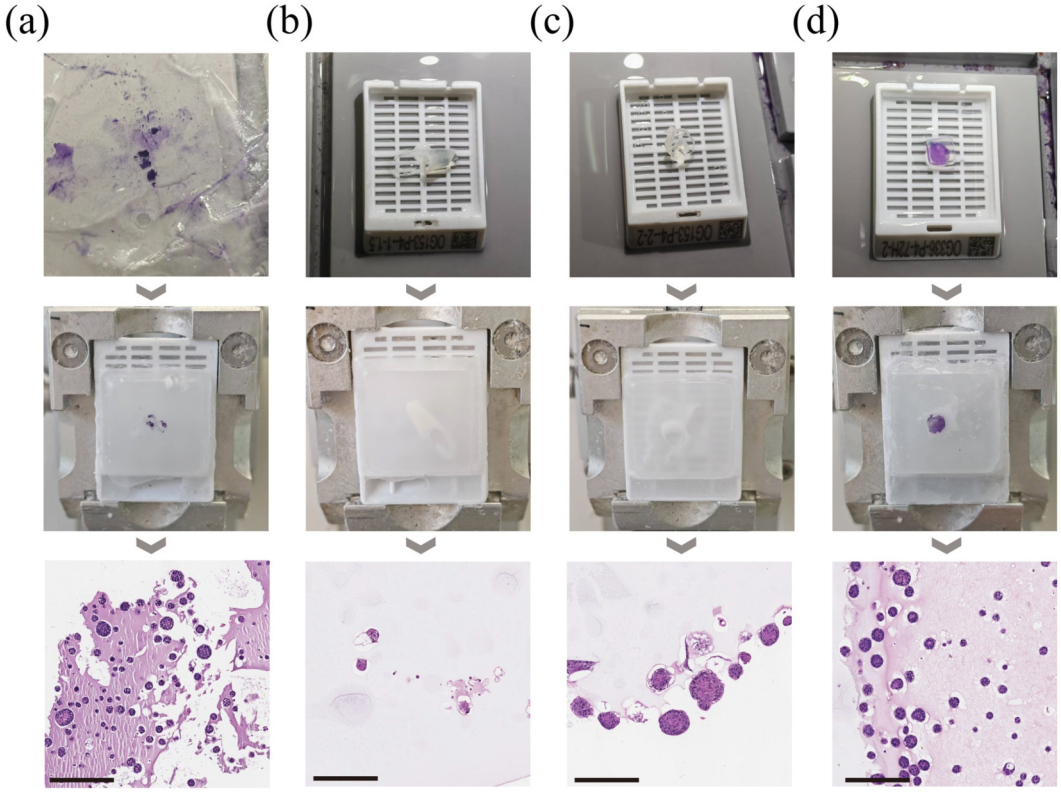

Figure 3. Comparison of traditional block and agar-based cell block methods. (a) The traditional paper-wrapping method, commonly used for pathological detection of small biopsy tissues, applied to organoids. (b) Organoids collected in a 1.5 mL EP tube using the agarose resuspension method. (c) Organoids collected in a 2 mL EP tube using the agarose resuspension method. (d) The method developed in this study for *in-situ* embedding of organoids with agarose in a well plate. The top row shows organoids after dehydration and ready for paraffin embedding. The middle row shows paraffin-embedded organoid cytology blocks. The bottom row displays the corresponding H&E staining results. Scale bar: 100 μm.

Table 2. Comparison of different sampling methods for organoid cytopathology.

Methods	Matrigel domes	Matrigel dissociation	Loss of organoids	Bubbling or Solidifying	Embedding operation	Sectioning location	Organoids Amount after H&E staining
Paper wrapping	≥2	Unnecessary	Partial	Unsusceptible	Difficult	Easy	Moderate
1.5 mL tube collection	≥2	Required	Partial	Susceptible	Inconvenient	Difficult	Limited
2.0 mL tube collection	≥2	Required	Partial	Susceptible	Inconvenient	Difficult	Limited
In-situ embedding	1	Unnecessary	None	Unsusceptible	Easy	Easy	Abundant

embedded in paraffin blocks. This may be because Matrigel is softer than agar, causing it to crumble during dehydration and leading to partial loss. This also makes it more difficult to manipulate during embedding, as liquid paraffin can easily disperse the aggregated Matrigel. Figure 3a shows typical results of the paper-wrapping method. The top image displays dehydrated organoid pellets on filter paper, the middle image shows an embedded organoid block with dispersed pellets, and the bottom image presents a representative H&E result with increased background staining.

Additionally, we attempted to resuspend organoids in liquid agarose within centrifuge tubes for embedding. Regardless of using 1.5 mL or 2.0 mL tubes, the resulting agarose blocks were irregular (Figs. 3b and 3c). These blocks with conical shape (1.5 mL tube) or convex-concave shape (2.0 mL tube) make embedding inconvenient. Both methods also require at least 100–200 μL of agarose for resuspension, which requires more organoids to meet the identification requirements. This results in higher time and economic costs, and lowers the likelihood of identifying early passage organoids due to limited numbers.

From a technical perspective, aspirating 100–200 μL of 2% agarose to resuspend and mix the organoids settled at the bottom of the tube is not easy. Bubbles can easily form and the gel will solidify quickly. Li et al. suggested resuspending organoids with a small amount of PBS first, then mixing with 2% agarose[11]. To make the gel block more regular, they aspirated the mixture onto a piece of parafilm and inverted it to form a dome shape. We also tried this method and found that bubble formation and gel solidification still occurred frequently, requiring quick and careful operations. Zhang et al. reported an improvement by inverting the 2.0 cryotube for centrifugation to facilitate gel block extraction[12]. Although the gel block is easy to extract, the key disadvantage compared to our method is that it requires more organoids, and manipulation needs to be more careful and faster to prevent bubbles and solidification. As summarized in Table 2, the advantages and disadvantages of these methods are compared. Representative images of the related techniques are shown in Fig. 3, with corresponding quantitative statistics provided in Fig. 4.

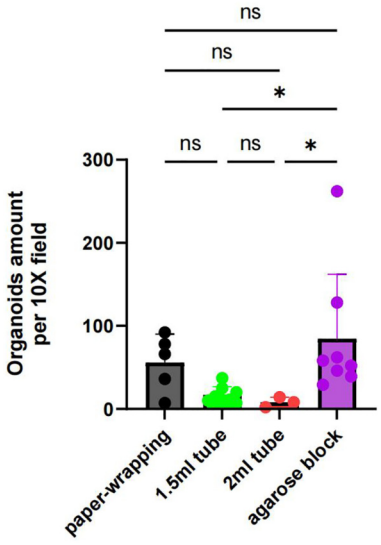

Figure 4. Quantitative analysis of organoid numbers across different methods. One section was cut from each paraffin block for H&E staining. The number of organoids in the H&E-stained sections was quantified. Due to the excessively high number of organoids in our method, making full-section counting difficult, we selected a representative 10× field of view for statistical analysis. ns: no significance; *$p < 0.05$.

Technical details impacting results

Using 3% agarose instead of 2% agarose. Although the literature on organoid pathological identification typically suggested 2% agarose[10,11], our own experience shows that 2% gel blocks sometimes crumble during embedding or dehydration (Fig. 5a). We switched to 3% agarose, which eliminated this issue. The 3% agarose is more viscous; to prevent solidification, we cut a small portion from the tip of a 1 mL pipette tip before aspiration.

Since both agarose and Matrigel are translucent and very close to paraffin, it is difficult to clearly determine whether the target layer has been cut during sectioning. To better determine the position of the dome during sectioning, we tried adding a small amount of hematoxylin or eosin (100–200 μL) to the well plate after formalin fixation and before agarose pre-embedding. Hematoxylin better delineates the position of the dome compared to eosin (Fig. 5b).

Small specimens from needle aspiration and bronchoscopy are generally fixed overnight or for 24 h. However, we found that fixing organoids in Matrigel for only 24 h is insufficient. Following 24-h fixation, H&E staining occasionally exhibits suboptimal cellular preservation, characterized by membrane disruption, cytoplasmic depletion, and a predominance of denuded nuclei. (Fig. 5c). Increasing the fixation time to 48 or 72 h improved

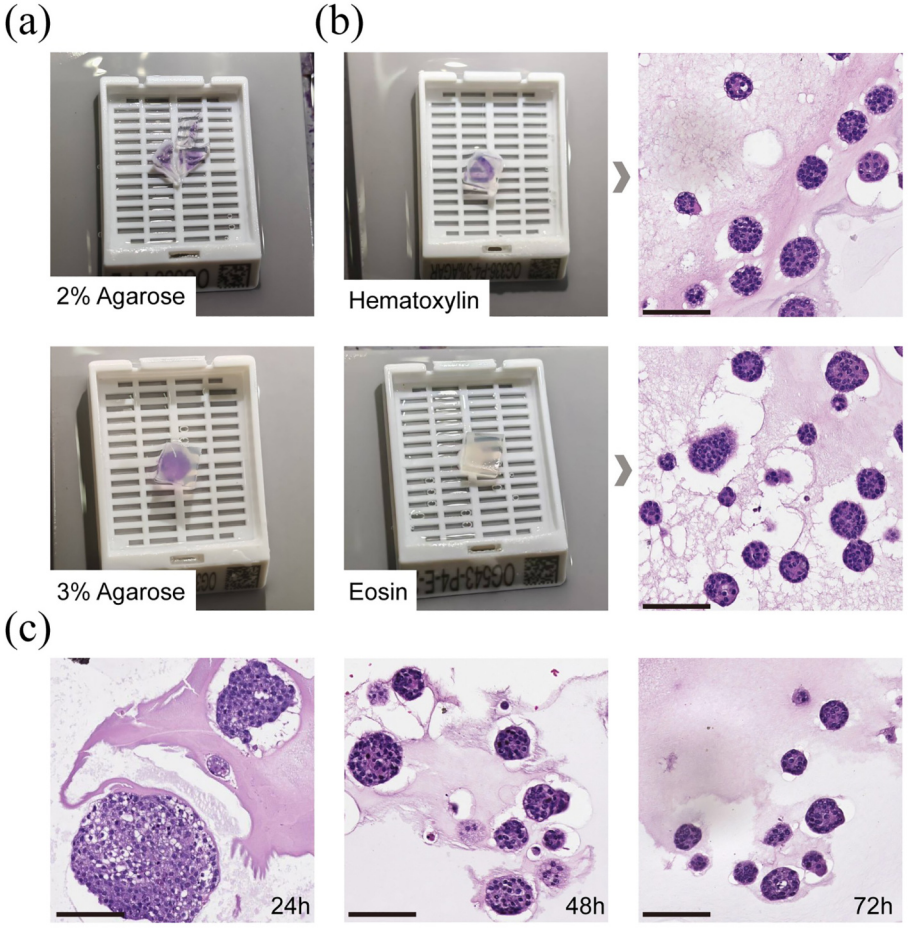

Figure 5. Tips for achieving optimal performance with our method. (a) Photograph of organoids embedded in 2% or 3% agarose in an embedding cassette. (b) Photograph of hematoxylin or eosin pre-stained agarose blocks and corresponding H&E staining results. Hematoxylin is more effective than eosin in helping to locate slides during sectioning, although both stains do not affect the H&E staining outcome. Scale bar: 100 μm. (c) H&E staining results of organoids fixed for different periods. Fixation for 24 h sometimes leads to poor staining, with loss of staining in the cell membrane and cytoplasm, leaving only the nuclei stained. Scale bar: 100 μm.

staining results (Fig. 5c).

Discussion

Numerous methods and procedures exist for the preparation of cell blocks (CBs) from cytological samples[15]. The most common CB methods are embedding cell pellets using plasma and thrombin (23.3%), agar (17.1%), Shandon/Epredia Cytoblock (11.4%), HistoGel (7.9%), and Cellient (3.5%)[16]. Each of these methods has advantages and disadvantages in terms of simplicity, versatility, and resource requirements[16]. As an inexpensive and simple method, plasma-thrombin cell blocks have the potential for DNA contamination[17], which may interfere with molecular testing results[18]. Due to their small size, organoids cannot be processed with conventional biopsy methods. Most organoids-related publications only briefly describe methods for histological analysis. Due to limited experimental details, several groups have systematically optimized organoid histopathology technology[10,12,19].

Fujii et al. reported a method for agarose embedding and histopathological analysis of organoids[10], where they pre-embedded organoids in agarose gel to accumulate organoids onto a single plane. However, this method requires a special polymer-coated 50 mL conical tube, which may not be readily available to other researchers, as well as the use of a large quantity of input organoids. This can be problematic when there are limited organoids, such as early passages (P0 or P1). In our method, *in-situ* fixation and embedding avoid any possible loss of organoids. which are constrained within a smaller volume (30–50 μL per Matrigel dome).

To reduce the requirement for organoid sample volume, Li et al. resuspended dissociated organoids in a 1.5 mL tube in PBS first and then mixed the solution with 2% agarose before pipetting onto a piece of parafilm to form a dome structure[11]. However, 2% garose is viscous, difficult to thoroughly mix, and prone to bubble formation. The small gel volume also leads to easy solidification during mixing. In our method, researchers simply add 3% agarose into the well, avoiding bubbles and premature solidification.

Zhang et al. reported a simple method for collecting the solidified agarose by inverting the cryotube for centrifugation[12]. To make the organoid block more complete, the authors tested various centrifuge tubes and found that 1.8 mL cryovials work best and determined that the most suitable volume of melted agarose gel is about 200 μL. Organoids are evenly distributed in such a large volume, necessitating the use of several matrix droplets. Moreover, this method also requires dissociation of Matrigel before embedding, which may lead to organoid loss and experiment failure[19]. Our method does not require Matrigel dissociation during the experiment.

For organoid culture, we also recommend avoiding excessive dilution of Matrigel. If the Matrigel is too soft after formalin fixation, the dome tends to flatten, and organoids will adhere to the bottom of the dish, making it difficult to scrape for embedding. In addition, hematoxylin pre-staining aids in sectioning and target visualization without interfering with downstream staining. Of note, this approach is optimized for histopathological assessment of organoids cultured in Matrigel within 24-well or larger plates. Its feasibility for 96-well plates is limited due to smaller droplet volumes and operational challenges.

In summary, we demonstrate that organoid sections maintain integrated cellular and tissue structures, suitable for standard hematoxylin and eosin staining and immunohistochemistry. It is a simple, cost-effective, and efficient method for the histopathological analysis of cancer organoids, promoting organoid-related research and applications.

Research ethics and patient consent

This study was approved by the Ethics Committee of Beijing Chest Hospital (2024-13). Patient data and surgically resected primary tumors were collected from patients who provided informed consent.

Availability of data and material

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of conflicting interests

The authors declare no conflict of interests.

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Author contributions

Hong Zhao conceived the project. Zuyu Sun and Hong Zhao performed most of the experiments. Li Zhang and Lijuan Zhou provided substantial support for H&E and IHC staining. Yuqing Huang and Jing Tan conducted extensive work on organoid culture. Zuyu Sun conducted data curation and figure preparation. Hong Zhao wrote the manuscript. Yi Liu provided substantial support for the preservation of tissue samples and organoids, as well as the provision of experimental facilities. Nanying Che supervised the project and revised the manuscript. All authors contributed to the article and approved the submitted version.

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