Cell Organoid

Review

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The pros and cons of mechanical dissociation and enzymatic digestion in patient-derived organoid cultures for solid tumor

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In Brief

This review compares mechanical dissociation and enzymatic digestion in deriving patient-derived organoids (PDOs) for cancer research. It examines their impact on organoid properties like stemness, heterogeneity, and long-term culture, and discusses their applications in drug screening and cancer modeling. The choice of method depends on tissue type and study requirements, with technological advances enhancing organoid production efficiency.

Graphical abstract

Highlights

- Mechanical dissociation is a compelling choice in solid tumor-derived organoid cultures for personalized medicine approaches because of its capacity to preserve more tumor microenvironment.
- Enzymatic digestion can generate a more homogenous population of cell, thus guaranteeing the reproducibility and controllability required by large-scale drug screening.
- The choice of tissue dissociation method and process depends on different tissues and the requirements of the following study.

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The pros and cons of mechanical dissociation and enzymatic digestion in patient-derived organoid cultures for solid tumor

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ABSTRACT

Patient-derived organoids (PDOs) are revolutionizing cancer research, serving as invaluable models for tumor biology and therapeutic screening. The fidelity and applicability of these organoids are fundamentally shaped by the tissue dissociation techniques employed, namely mechanical dissociation and enzymatic digestion. This comprehensive review delves into the nuances of these two methods, scrutinizing their effects on solid tumor organoid properties, including stemness, heterogeneity, long-term culturing. We discuss the advantages and limitations of each technique, with a focus on their impact on tumor microenvironment preservation, their application in drug screening and cancer modeling. Moreover, we examine how recent technological breakthroughs have bolstered the efficiency and scalability of organoid production through these methods. Our analysis is designed to assist researchers in choosing the optimal tissue dissociation strategy for their research objectives and to fuel the evolution of organoid-based cancer models.

KEYWORDS

organoids, tissue dissociation, tumor microenvironment, tumor heterogeneity

Introduction

Cancer is a multifaceted disease characterized by intricate molecular interactions and cellular heterogeneity that contribute to its complexity and therapeutic resistance[1]. One of the key aspects of cancer classification is the distinction between solid tumors and non-solid tumors, which are fundamentally different in their biological behavior and therapeutic approaches. Solid tumors, such as carcinomas, are characterized by the formation of solid tumors within tissues[2], while non-solid tumors, such as leukemias, involve the uncontrolled growth of abnormal cells in the blood or bone marrow[3].

The development of advanced models that accurately represent the tumor microenvironment is essential for understanding cancer biology and facilitating the discovery of novel therapeutics. Traditional two-dimensional (2D) cell cultures and animal models have provided valuable insights but often fall short in recapitulating the intricacies of human cancers, especially solid tumors, due to their complex three-dimensional (3D) architecture and the presence of a diverse tumor microenvironment[4]. There is, therefore, an urgent need for more sophisticated models that can better mimic the genetic and phenotypic landscapes of solid tumors[5].

Organoids, 3D cultures derived from patient tissues, have emerged as powerful tools in cancer research, offering a more physiologically relevant model for studying solid tumors[6,7]. These miniaturized, self-organizing structures maintain the architectural and functional properties of the original tissue, offering a more physiologically relevant model for studying solid tumor[8]. Patient-derived organoids (PDOs), in particular, replicate the architecture and cellular heterogeneity of the original tumor tissue, derived from a patient's biopsy, surgical specimen, or malignant effusion[7,9], and maintain the genetic and phenotypic characteristics of the tumor, offering a personalized model for cancer research and therapy development[10]. Compared to traditional 2D cell cultures and animal models, PDOs offer several advantages, including the preservation of the tumor's microenvironment and the 3D structure that allows for the study of cell–cell and cell-matrix interactions that are not possible in monolayer cultures[11]. PDOs can be established from a wide range of tumor types, providing a platform for high-throughput drug screening and enabling the rapid evaluation of multiple compounds for their efficacy and toxicity in a patient-specific context[12,13]. They are currently utilized in various applications within cancer research, such as models for studying tumor progression, metastasis, and the identification of

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potential biomarkers for early diagnosis and prognosis, as well as for chemosensitivity testing and the investigation of mechanisms of drug resistance[14].

The process of generating organoids begins with the dissociation of tissue into a small multi-cellular unit or a single-cell suspension, a critical step that can impact the subsequent culture's success and the organoids' fidelity to the original tissue. Mechanical dissociation and enzymatic digestion are two primary methods used for tissue dissociation. Mechanical methods involve physically disrupting the tissue, while enzymatic digestion uses enzymes to break down the extracellular matrix (ECM), allowing for the isolation of viable cells. Selecting the appropriate dissociation technique is crucial for preserving cell viability and maintaining the tissue's native characteristics, which are essential for the organoids' representativeness in cancer research.

In this review, we reveal the pros and cons of these two tissue-dissociation methods, highlight their applications in solid tumor research, and propose improvement strategies for leveraging them in PDOs-based cancer modeling.

Mechanical dissociation

Numerous procedures have been well documented for dissociating solid tumors. They are usually multistep procedures involving one or a combination of mechanical, enzymatic, or chemical manipulations. Generally, mechanical dissociation of tissue is the first step to make the tissue part or tumor biopsy smaller. Mechanical dissociation encompasses a variety of manual and semi-automated methods designed to physically separate tissue into smaller fragments or single cells[15]. Traditional techniques include mincing with scissors or sharp blades, scrapping the tissue surface, homogenization, filtration through a nylon or steel mesh, vortexing, repeated aspiration through pipettes or small gauge needles, abnormal osmolality stress, or any combination of these techniques[16–18]. These methods maintain cell viability and preserve partial three-dimensional architecture of tissues, which is crucial for organoid formation and function (Fig. 1a). Different with the fragmentation applied in organ explant or organotypic slice cultures, where a large piece of tissue is cultured as an intact unit, mechanical dissociation allows

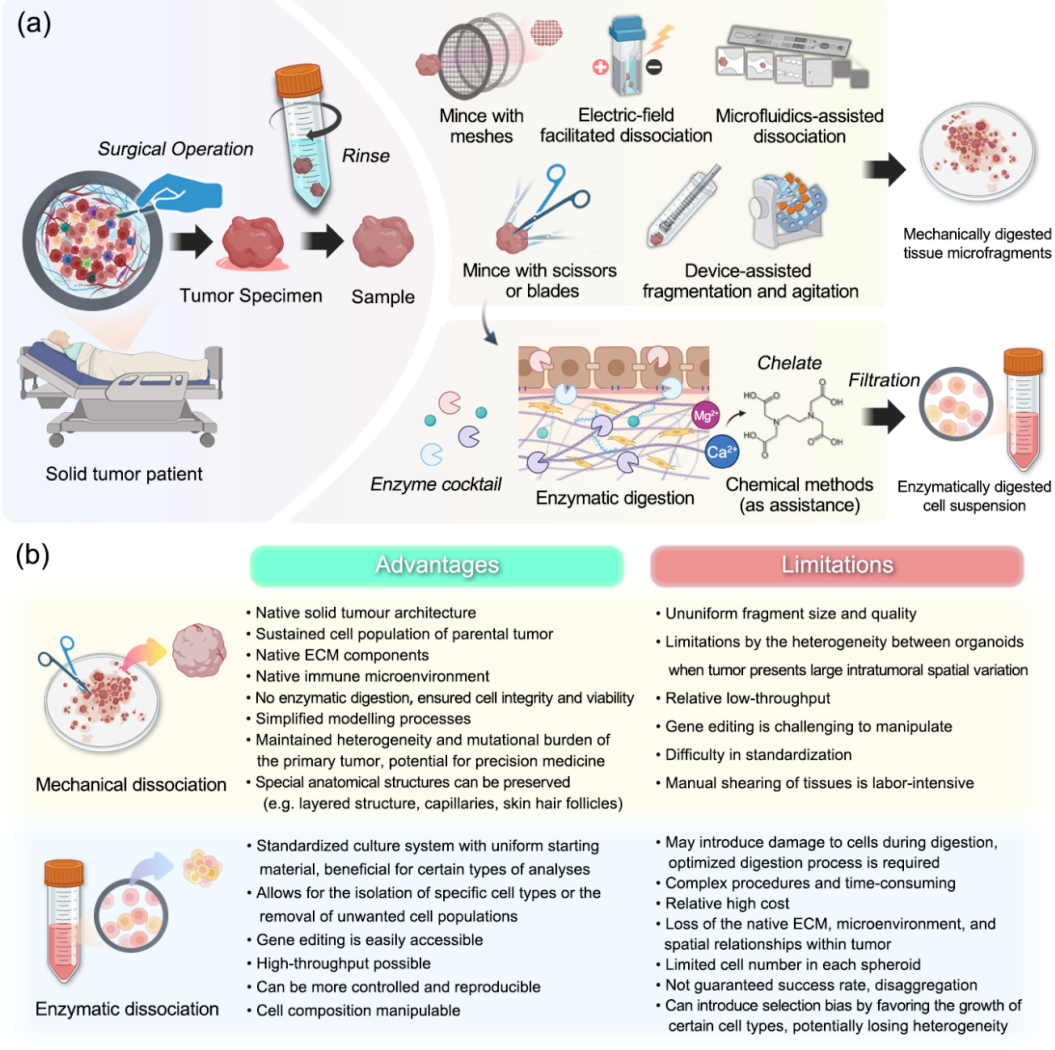

Figure 1. Mechanical vs. enzymatic digestion for tissue dissociation. (a) Schematic diagram of the processes for solid tumor samples obtained clinically treated with two different methods. Mechanical dissociation employs various techniques, such as shearing with scissors or blades, fragmenting with meshes, or utilizing electric fields[19], microfluidics[20, 21], and various fragmentation devices to dissociate the tissue[22, 23]. Enzymatic digestion often involves the use of an enzyme cocktail, supplemented with EDTA to chelate calcium and magnesium ions, thereby enhancing the efficiency of digestion[24, 25]. (b) A comparison of the advantages and disadvantages of each dissociation method when used individually[26–29].

organoids to initiate from small multicellular units generally 0.5–1 mm in diameter.

Enzymatic digestion

Various enzymes, such as trypsin, dispase, pepsin, papain, collagenase, elastase, hyaluronidase or trypsin (or its replacement enzyme TrypLE), pronase, chymotrypsin, and catalase, can be utilized individually or in combination such as Liberase (an off-the-shelf combination of collagenases I and II and thermolysin) to digest desmosomes, stromal elements, and both extracellular and intercellular adhesions (Fig. 2). Among these, collagenase and dispase are the most frequently employed due to their effectiveness. To further facilitate cell dissociation and prevent reaggregation, DNase is often used alongside these proteolytic enzymes to hydrolyze DNA–protein complexes that may trap cells. The distinct specificities of these enzymes enable the design of tailored dissociation protocols for specific tumors and purposes. It's important to note that many of these enzymes are crude extracts, which can contain varying levels of contaminating proteolytic enzymes.

The choice of enzyme is tailored to the tissue type and desired outcome, with protocols often requiring optimization to ensure its ability not only to release a large number of cells but also to preserve cellular integrity and viability.

In addition, chemical dissociation often complements enzymatic techniques and mechanical process, targeting the removal or sequestration of Ca^{2+} and Mg^{2+} ions, which are essential for preserving the intercellular matrix and cell surface integrity. Specifically, ethylene-diaminoacetate (EDTA) or citrate ions are frequently employed for this purpose. However, these chemical methods may not be sufficient for completely dissociating all tissue types.

Impact on cancer organoid characteristics

Influence on stemness and differentiation

Stemness refers to the ability of cells to self-renew and differentiate into various cell types, a property that is particularly important in the context of organoid cultures and cancer research[43,44]. The method of tissue dissociation can influence the stemness and differentiation potential of the resulting organoids.

Mechanical dissociation, by preserving the tissue architecture, may also maintain the niche interactions that are critical for stem cell behavior. This could result in organoids that more closely recapitulate the stem cell properties of the original tissue. However, the physical stress of mechanical dissociation could potentially impact the stem cell compartment, affecting the viability or functionality of stem cells[45,46]. Ma et al., reported that the distribution of liver cancer stem cells is related to the mechanical heterogeneity of ECM stiffness, and matrix stiffening could facilitate the stemness of cancer stem cells by promoting stemness-associated gene expression, reduce drug sensitivity, and enhance sphere-forming and clonogenic ability[47]. Li et al. also disclosed that ECM-derived mechanical force regulated tumor stemness and cell quiescence in breast cancer cells through integrin-DDR signaling[48]. Adding mechanical stretch to intestinal organoid cultures could increase the proliferation of intestinal stem cells and stimulate the expansion of SOX9+ progenitors by activating the Wnt/β-Catenin signaling, resulting in the boost of the stemness of intestinal stem cells and organoid growth[49]. Although mechanical dissociation is not studied in the above

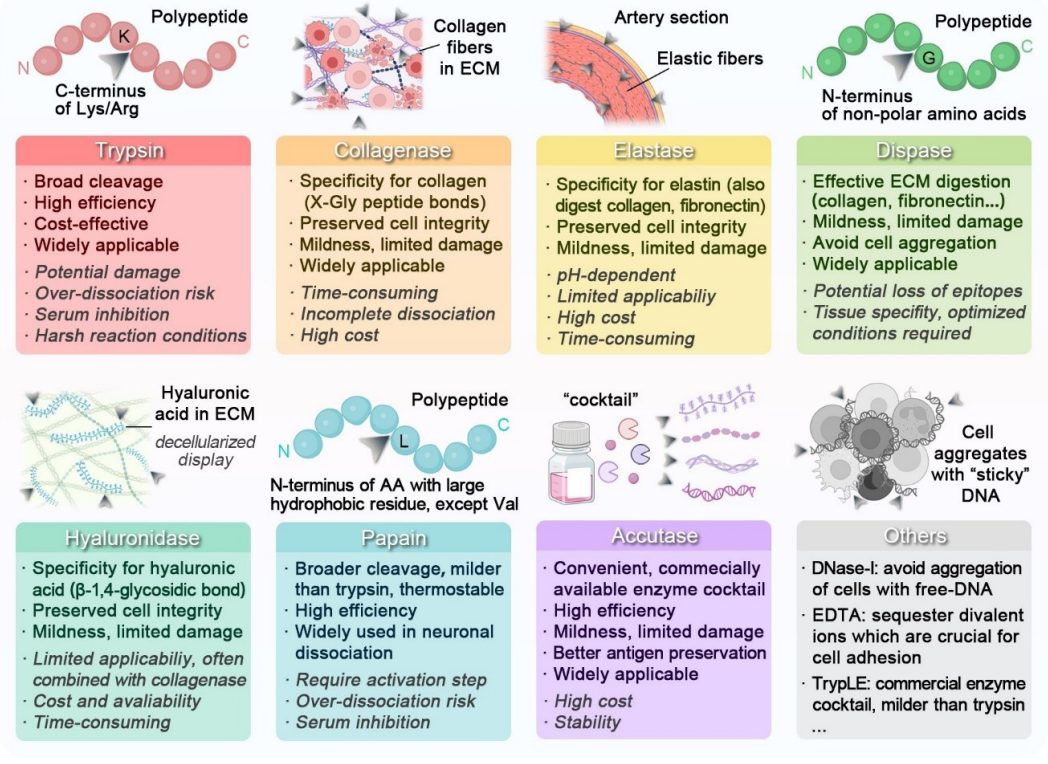

Figure 2. Commonly used enzymes for tissue digestion. A collection of commonly used enzymes for tissue digestion is presented, including trypsin (TrypLE)[24,30–32], collagenase[24,32,33], elastase[34–36], dispase[24,33], hyaluronidase[24,37,38], papain[24,39–41], accutase[24,42], DNase I[24,32], and others. The mechanisms of action for each enzyme or compound are explained, along with a list of some of their respective advantages and disadvantages.

cases, it is indicated that this kind of mechanical force is also able to affect cell stemness, which is essential for organoid culture.

Conversely, enzymatic digestion, by releasing cells from their native environment, can disrupt the niche interactions that regulate stem cell fate. For instance, it is well studied that intestinal organoids depend entirely upon cell niche signals that include Wnt secretion[50,51]. Yet, this method allows for the enrichment of specific cell populations, such as cancer stem cells, through the use of specific surface markers, which can be particularly useful in studies focused on the cancer stem cell hypothesis. Additionally, the single-cell nature of the cell suspension post-digestion may facilitate the *de novo* organization of cells into organoids, potentially leading to a more homogenous population in terms of stemness.

Preservation of tumor heterogeneity

In the context of cancer research, the preservation of tumor heterogeneity is a critical aspect when culturing patient-derived organoids, as it allows for a more accurate representation of the original tumor's complexity and diversity, as well as for the development of personalized medicine approaches[52]. Mechanical dissociation and enzymatic digestion are two techniques that can impact this preservation. Both of them have been employed to generate organoids from patient-derived tumor tissues, with studies demonstrating the successful capture of tumor heterogeneity using both methods[53].

Mechanical dissociation preserves the spatial organization of the tissue to a greater extent than enzymatic digestion, potentially maintaining the heterogeneity of the tumor by reducing the disruption of cell–cell and cell-matrix interactions. However, the physical forces during chopping, grinding, or using a cell strainer, could potentially select for more mechanically resilient cells, possibly introducing a bias towards certain subpopulations within the tumor[21,22,54].

The process of enzymatic digestion is generally more efficient in generating a single-cell suspension. It is beneficial for initiating organoid cultures from a diverse range of cells found in tumors[55], but can disrupt the tumor's natural architecture in the meantime, potentially reducing the heterogeneity of the resulting organoids. Several factors can influence the dissociation process. For example, the use of EDTA to chelate calcium can disrupt cell-to-cell adhesion[56]. Furthermore, enzymatic digestion can introduce selective pressures, favoring the release of more aggressive or rapidly proliferating cells[57]. This selection bias could potentially alter the representation of the original tumor heterogeneity.

Long-term culture implications

The implications of mechanical dissociation and enzymatic digestion on long-term organoid culture are multifaceted. The choice between mechanical dissociation and enzymatic digestion also carries implications for the long-term culture of organoids.

Mechanical dissociation of tissues can potentially preserve the microenvironment, which is crucial for maintaining tissue-specific characteristics. This preservation is advantageous for the stability of long-term organoid cultures. In the protocol described by Dekkers et al., mechanical shearing is utilized to dissociate breast organoids into fragments, which is a preferred method for most organoid cultures due to its higher efficiency in growing into new organoids compared to single-cell passaging[58]. This method is crucial for the propagation of organoids and the establishment of clonal cultures, especially after genetic manipulation, where maintaining genetic integrity is vital. However, there are several challenges associated with this method. First, larger tissue fragments might hinder the uniform distribution of nutrients and oxygen, which is essential for the health of organoid cultures. Over time, this could impact the overall health and viability of the cultures. Second, the mechanical process itself can introduce physical stress, which might affect the longevity of the organoids in culture. Lastly, as noted by Fumagalli et al.[59], the accumulation of cellular debris and dead cells within the organoids can pose a significant challenge for long-term cultures. This may necessitate regular passaging or the development of innovative techniques to ensure the health and genetic stability of the organoids.

Enzymatic digestion, by providing a more homogenous starting population, may facilitate the establishment of organoid cultures with a more uniform growth rate and phenotype. The single-cell suspension also allows for the possibility of clonal culture, which can be advantageous for genetic and phenotypic studies. However, the loss of tissue architecture and microenvironmental cues may require careful consideration of the culture conditions to support long-term self-renewal and differentiation potential. While enzymatic digestion can support the long-term expansion of organoids, as demonstrated by Hu et al.[60], it is also essential to consider the potential for genetic drift and the selection of specific clones over time, which can alter the original characteristics of the organoids.

In cancer research, the long-term culture of organoids is particularly important for studying tumor evolution, drug resistance, and metastasis. Both mechanical and enzymatic methods have been used to generate organoids for long-term studies, with researchers carefully optimizing culture conditions to maintain the fidelity of the organoids to the original tumor over time, as practiced by Song group, who optimized glioblastoma organoid (GBO) culture by avoiding single-cell dissociation, thus preserving the native cytoarchitecture and cell–cell interactions. They propagated GBOs by cutting them into smaller pieces (approximately 0.5 mm in diameter) to prevent necrotic cell death in the inner core. This approach facilitated efficient organoid formation and growth in a defined, serum-free medium, enhancing nutrient and oxygen diffusion without the need for repeated mechanical dissociation. Moreover, it maintains the heterogeneity of the original tumor and is less prone to clonal selection, allowing for the reliable generation of GBOs that closely mimic the characteristics of the parental tumors over extended periods[29].

Application in cancer research

Drug screening and therapy development

PDOs have emerged as a cornerstone in the field of drug screening and personalized medicine[11,61,62], particularly due to their ability to recapitulate the genetic and phenotypic landscape of individual tumors. The employment of mechanical dissociation or enzymatic digestion in the establishment of organoid cultures ensures that the patient's tumor heterogeneity is preserved, which is crucial for accurate drug response profiling. This approach has been successfully utilized in high-throughput drug screening platforms, where organoids serve as a test bed for evaluating the efficacy and toxicity of various therapeutic agents[61,63].

The advantage of mechanical dissociation lies in its ability to

preserve cell viability and clonality, which is crucial for maintaining the genetic stability of cell lines used in drug screening assays. As illustrated by Jocob et al., well-preserved tumor cell viability and largely-maintained molecular signatures of corresponding parental tumors were observed in the GBOs[29]. Likewise, Shi's study in completely mechanics-dissociated skin tumor organoids culture showed around 80% concordance rate of mutation sites between the clinical tissues and organoid samples from the same patient, while the complete overlap of mutation sites among the patients compared to their respective mutation site numbers was not very high, being less than 40%. Additionally, morphological inter-patient heterogeneity was observed[64].

However, the inherent heterogeneity of the source tissue in space during mechanical dissociation may lead to inconsistencies in the cellular composition and niche that are subsequently encapsulated, resulting in changes in cell production within the cultured organoids, different survival rates, and inter-organoid heterogeneity[21,38]. This non-reproducibility constitutes a significant disadvantage compared to organoids produced by enzyme dissociation.

On the other hand, enzymatic digestion is gentler on cells and can yield a higher cell count with minimal damage, which is beneficial for high-throughput screening of drug candidates. Moreover, it is effective in generating a more homogenous population of cell[65], thus guaranteeing the reproducibility and controllability required by large-scale drug screening. In addition, gene editing is easily accessible in organoid derived from enzyme-digested single-cell suspensions, which allow modeling specific cancer in organoid culture. Verissimo et al. employed colorectal cancer (CRC) organoids with a CRISPR-introduced oncogenic *KRAS* mutation to evaluate the drug response of mutant KRAS on EGFR-RAS-ERK pathway inhibitors[66]. Likewise, Peng et al. recently established a genome-scale library of open reading frames in the diffuse gastric cancer (DGC) model of Cdh1−/−RHOAY42C/+ organoids to identify candidate mechanisms of resistance to focal adhesion kinase (FAK) inhibition[67].

Modeling tumor microenvironment

Organoids provide an unparalleled opportunity to model the tumor microenvironment and metastasis, given their capacity to mimic the spatial and cellular organization of tumors[68,69].

The maintenance of the native tissue architecture and cellular interactions in organoids derived from mechanics-dissociated tissue source allows for a more accurate representation of the *in vivo* tumor milieu. This is particularly important for studying the crosstalk between tumor cells and the surrounding stroma, which plays a critical role in tumor progression and metastasis. Shi et al identified immune cells in the culture of skin tumor organoids from patient tumors without enzymatic dissociation and further revealed that metformin can modulate the expression of downstream genes through immune signaling pathways[64]. Likewise, Zhao et al. introduced a novel High-Grade Serous Ovarian Cancer (HGSOC) organoid system that preserves the immune microenvironment and vascular structures, showing a significant response to cisplatin and further emphasizing the role of organoids in advancing our comprehension of metastatic processes and therapeutic potentials[70]. In addition, partial preservation of immune cells was also analyzed in some GBOs. Compared with the parental tumors, the downregulation of blood- and immune-related genes that are main elements constituting the microenvironment was observed over time[29]. These studies provide evidences that in the initial stage of organoid culture, the capacity of mechanical dissociation in partial preservation of microvasculature and immune cells is definitely beneficial for the recapitulation of the native tumor microenvironment, opening the window for further investigations for therapeutic responses and resistance.

Conversely, enzymatic digestion, while offering a controlled approach to study tumor cell behavior *in vitro*, can lead to a loss of stromal and immune cells, which are crucial for maintaining the original tumor context, resulting in organoids that do not accurately represent the complexity of the tumor ecosystem[71]. It is conceptualized that cancer cells grow independently of their niche[72], which means that *in-vitro* culture media may have more impact on non-cancer cells. Given that enzymatic dissociation has more capacity in disrupting cell niche, consequently, especially in long-term culture, the diversity of cells within the organoid initiated from multi-cellular unit or a single-cell suspension generated by enzymatic dissociation relies more on the viability of non-cancer cells. To better reflect the native cellular heterogeneity in tumor, the use of organoid long-term culture systems in which the behavior of cells in relation to niche factors should be optimized and validated *ex vivo*. However, so far, as mostly reported, such conventional cancer organoids generated with enzymatic dissociation contain only malignant cancer cells, failing to retain stromal components, including immune cells, whose interactions with epithelium can dictate tumorigenesis, tumor growth and responses to external influences[26,71,73].

As noted above, organoids initiated by enzyme-digested single-cell suspensions are less able to preserve the tumor microenvironment. However, these organoids can be co-cultured with immune cells or endothelial cells to investigate the complex interactions within the tumor microenvironment, providing insights into angiogenesis, immune evasion, and therapy resistance[74,75]. In most reported studies, immune cells from blood or patient tumors have been reconstituted with heterologous established cancer cell lines in organoid cultures[76]. For instance, Neal et al., employed an air–liquid interface method to propagate patient-derived organoids with native embedded immune cells (T, B, NK, and macrophages)[68]. The development of organ-on-a-chip systems that incorporate organoids has further enhanced the ability to model the microenvironment for metastasis, offering a dynamic platform for studying the metastatic cascade in a controlled and physiologically relevant manner[77].

Cancer organoid model

The use of cancer organoids has significantly advanced our comprehension of tumor biology, especially the molecular underpinnings of cancer initiation, progression, and metastasis, such as the identification of cancer stem cells and the elucidation of signaling pathways that drive tumorigenesis[78]. Especially the advent of PDO cultures is pivotal for dissecting the tumor biology on an individual human level. These cultures, often established through mechanical dissociation or enzymatic digestion, offer a physiologically relevant model that closely mirrors the complexity of human cancers.

The mechanical dissociation method preserves the cellular heterogeneity and allows for the isolation of tumor cells with distinct characteristics, such as the maintenance of intricate intercellular interactions and the preservation of the original tissues' spatial architecture. Mechanical dissociation is lauded for

its ability to preserve the integrity of the tumor microenvironment, enabling the retention of cellular phenotypes that are sensitive to the microenvironment, including cells with high metastatic potential, cells at various stages of the cell cycle, and cells exhibiting drug resistance mechanisms, therefore, offering a nuanced view into the tumor's cellular composition and allowing researchers to explore the tumor's adaptive strategies and its dynamic response to therapeutic interventions. It also allows researchers to study cell–cell interactions and identify rare cell populations within the tumor mass. It is notable, in most cases that cancer patients require rapid treatment, saving time due to the comparatively simple operation and direct process make this method a compelling choice for seeking personalized treat treatment strategies.

On the other hand, the sequential use of different enzymes or the alternative combination of enzymes, such as a combination of hyaluronidase and collagenase[79], can cater to the specific requirements of certain cancer types. Furthermore, gene editing can be easily integrated into the organoid culture derived from enzyme-digested tissue source, introducing targeted mutations to generate specific cancer organoid model for studying the biology and the response to therapeutics of a specific cancer type[80]. This practicability is better suited for building a more comprehensive model for a specific cancer type based on a wide patient population.

Methodological comparisons and optimization

Efficacy in different cancer types

Mechanical dissociation and enzymatic digestion are two primary methods for the preparation of organoid cultures, each with distinct advantages and limitations that can vary significantly across different tissues (Table 1).

In lung cancer research, enzymatic digestion has emerged as a preferred method that can effectively breaks down the extracellular matrix, allowing for the isolating tumor cells, particularly for the derivation of lung cancer organoids[102]. The use of enzymes such as dispase and collagenase allows for the gentle release of cells from the tumor mass, maintaining the stemness of cancer cells, which is crucial for the subsequent self-renewal and differentiation capabilities of organoids. However, in cancers with a dense stroma or high intercellular adhesion, such as pancreatic ductal adenocarcinoma[122,123], mechanical dissociation may be more effective in preserving the cellular integrity and tumor heterogeneity. In the context of colorectal cancer, mechanical dissociation has been traditionally favored due to its simplicity and effectiveness in generating viable organoids from biopsy samples[124,125]. However, recent advancements in enzymatic cocktails have improved the efficiency of organoid generation from colorectal cancer tissues, providing a more physiologically relevant model for studying tumor heterogeneity[85].

Combining mechanical and enzymatic approaches

As for most reported studies, the integration of both methods is more versatile in various tissue-derived organoid culture, where the synergy of mechanical dissociation and enzymatic digestion lies in the complementary nature of these techniques. A preliminary mechanical fragmentation using a scissors, scalpel, or razor blades can reduce tissue integrity, then followed by enzymatic digestion of the tissue fragments to further break down the ECM and generate a single-cell suspension or clumps of cells by using enzymes.

In this integration, based on different cancer types and tissue characters, the mechanical forces can be tailored, while enzymatic cocktails can also be optimized. For instance, the combination of both methods was optimized for long-term culture of organoids derived from either normal human breast tissues or breast cancer tissues[58]. The two-step approach not only accelerates the dissociation process but also ensures a single-cell suspension that is conducive to the formation of organoids with high genetic stability and widely applied in establishment of patient-derived cancer organoids for drug-screening[61]. It can also be particularly useful in cancers with a dense extracellular matrix, such as pancreatic cancer[122,126], where a purely enzymatic approach may be insufficient to access the tumor cells. As described in the procedure for dissociation of the guinea pig pancreas into individual cells, enzymatic digestion with pure collagenase, chymotrypsin, and hyaluronidase were employed, an interposed chelation of divalent cations by EDTA is utilized, and gentle shearing terminated the process[88]. Most studies including these listed above combined both methods before culturing the organoids.

Mechanical dissociation can preserve the tissue's native architecture, but may lead to organoids of varying sizes, which can impact drug penetration and response uniformity. Enzymatic digestion allows for the isolation of pure cell populations, on the other hand, can disrupt tissue architecture, potentially leading to the loss of certain microenvironmental cues. To flip both coins on heads, Han's group strategically combined two methods to sequentially generate organoids. In brief, they applied mechanical fragmentation to grow an initial organoid (IO) derived from the patient's tissue, then treat the IOs with enzymatic digestion or mechanical dissociation respectively, to generate IO-derived organoids[127–129]. The preservation of tissue architecture in IOs derived from mechanical dissociation provides a more accurate representation of the *in vivo* tumor microenvironment, crucial for understanding disease progression and therapeutic responses. Enzymatic digestion allows for the generation of a large number of IO-derived organoids from a limited sample, which is particularly valuable for patient-derived tumor samples that are often scarce. Notably, the heterogeneity and complexity of these IO-derived organoids were well maintained by this strategy. For instance, Glioblastoma (GBM) IO-derived organoids exhibit a heterogeneous cellular composition, comprising a total of 20 cell types[129]. Additionally, the presence of viable and active immune cells was observed in Intrahepatic cholangiocarcinoma (ICC) IO-derived organoids[128]. Last but not least, the uniform-sized ovarian cancer IO-derived organoids from single-cell suspensions allows themselves for high-throughput drug screening and personalized chemotherapy resistance tests[127]. Han's group's attempts above offer a powerful strategy to harness the benefits of both methods in organoid culturing.

In summary, the choice between mechanical and enzymatic methods, or how to combine these two methods, may depend on the specific requirements of the following study, such as the need for single-cell analysis versus the maintenance of tissue architecture, as well as the preservation of the extracellular matrix interactions in source tissue, which are pivotal in cancer progression and metastasis, while it can significantly influence the success of organoid formation and their representativeness of the original tumor. Future research should continue to explore and

Table 1. Dissociation methods for common cancers and the utility of mechanical dissociation

Source of tissue	Dissociation methods	Mechanical dissociation
Intestinal Organoids	• Chopping into ~5 mm pieces[81] • Cutting tissues into 5 mm pieces; mechanically separating crypts in HBSS–EDTA[82] • Mincing tissue to ~ 1 mm pieces with scalpels[83] • Digesting with Liberase™ DH and hyaluronidase[84] • Mincing into small pieces and enzymatically digesting with collagenase IV[85] • Finely mincing of human colorectal tissues and digesting with collagenase Type 1[86] • Chopping rectal cancer samples to 1 mm pieces in PBS-DTT buffer; resuspending vigorously in ADF medium; settling for crypt inspection by microscopy, repeating until no crypts detected[87]	+++
Liver Organoids	• Mincing the tissue into pieces of roughly 0.5 mm^3 using fine scissors, pipetting the sample up and down to remove red blood cells and fat, then digesting with collagenase and dispase II for mouse and collagenase D for human[88] • Mincing into pieces (~ 0.25–1 cm^3) and incubating at 37 °C with the digestion solution[89] • Collagenase-accutase digestion after mechanical dissociation[60, 89–91] • Isolating hepatocytes from mice or human adult liver via two-step collagenase digestion; mechanically fragmenting and re-seeding organoids 14 days post-seeding[91]	+
Pancreatic organoids	• Mincing into small pieces, then digesting with collagenase II and further digested with TrypLE[92] • Mincing into pieces, washing with 10 mL AdvDF+++, and digesting with collagenase II[93, 94] • Mincing into small portions using a scalpel, then digesting with digest medium including collagenase XI, DNase, and Y27632[95] • Mincing the tissue into pieces of roughly 0.5 mm^3 using fine scissors; Pipetting the sample up and down to remove red blood cells and fat. Digesting with medium containing collagenase and dispase II for mouse and collagenase D for human[88]	+
Kidney organoids	• Chopping into small pieces with surgical blade, shaking for 25 min, pipetting ~ 25 times to dissociate the tissues into single cells[96] • Mincing into ~ 1-mm^3 pieces, following digestion with AdDF+++ containing collagenase and Y-27632[97] • Isolating tubular fragments from human cortical kidney or mouse kidney tissue through collagenase digestion[98] • Mincing into small pieces and digesting with collagenase II with ROCK inhibitor Y-27632 dihydrochloride, following enzymatic digestion in TrypLE Express. Subsequently, centrifuged pellets were pipetted up and down to further dissociate tissue fragments[99]	++
Lung organoids	• Pipetting up and down to passage lung organoids. If single cells are not required, or enzymatic digestion is not suitable for downstream applications, mechanical dissociation is recommended[100] • Dissecting pleura and large airways; processing into single-cell suspension with dispase, collagenase I, and DNase I[101] • Digesting human tumor specimen with DNase I and collagenase/dispase in DMEM/F12 medium[102] • Mincing tumor samples with scissors; digesting with collagenase II and Y-27632, preceded by TrypLE Express and Y-27632[103] • Mincing non-small-cell lung cancer biopsies with scissors; digesting in collagenase type II in Advanced DMEM/F12 with gentle shaking[104]	+
Brain organoids	• Either mildly disaggregating into small cell clusters by incubation in StemPro™ Accutase™ or cutting into 0.5–1 mm pieces using tweezers and scalpels[105]. • Dissociating patient tissue samples by either finely mincing or enzymatic digesting into single-cell suspensions[106, 107] • Cutting into 1 mm in diameter pieces using fine dissection scissors, and later cutting into 0.5 mm in diameter pieces for propagation[29] • Cutting glioma specimens into 1–2 mm^3 pieces with dissection scissors and suspending in Short-Term Glioma Organoid Medium[108]	+++
Gastric organoids	• Mechanically dissociating mouse stomach with micro-dissecting scissors and fine forceps; cutting into pieces < 5 mm^2; shaking in DPBS (without Ca^{2+} and Mg^{2+}, with EDTA); followed by pipetting up and down[109] • Cutting human samples into ~ 5 mm pieces; incubating with chelating solution; pipetting up and down to extract glands[16, 110] • Mincing epithelial tissue with surgical razors; digesting fragments with collagenase type I[111] • Mincing gastric cancer specimens following enzymatic digestion with dispase II and collagenase XI[112, 113], or with EDTA and TrypLE[13], or Liberase TH and TrypLE Express[114], or collagenase and hyaluronidase[115]	++
Breast/Female genital organoids	• Mechanically shearing Stage IV high-grade serous cancer (HGSC) specimens[28] • Mincing mouse mammary glands into 1 mm pieces; digesting with collagenase and hyaluronidase[116] • Cutting human ovary cancer tissues into 3–5 mm^3 pieces; digesting remaining large pieces in Advanced DMEM/F12 with RHO/ROCK pathway inhibitor and collagenase[117] • Mechanically dissociating human breast cancer tissue samples with scalpels or razor blades; obtaining single-cell suspensions or cell clumps through enzyme digestion using collagenase, DNase, dispase, hyaluronidase, trypsin (TrypLE), or enzyme mixes like liberase[61]	++
Male genital organoids	• Finely cutting prostate cancer samples with scissors to produce small tissue fragments, termed "aggregates"[118] • Mincing mouse or human prostate lobes into small pieces (~ 1–5 mm^3) with a scalpel, followed by digestion in collagenase II with Y-27632[8] • Mechanically dissociating prostate cancer samples with scissors or generating a cell suspension through further digestion with Collagenase II[118–120] • Cutting human testis cancer specimens into approximately 1 mm^3 pieces and culturing in hanging drops[121]	++

optimize these combined approaches to further advance our understanding of complex diseases and develop more effective therapies.

Future directions in method optimization

As organoid technology advances, there is a growing interest in refining the dissociation methods to better recapitulate the in vivo tumor microenvironment.

The integration of enzymatic digestion with advanced technologies, such as fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS), has enabled the isolation of specific cell populations from the dissociated tissue[130, 131]. This has been particularly valuable in studies aimed at understanding the heterogeneity within tumors and the identification of cancer stem cells[132]. Bioprinting technology has advanced the precision of cell placement in three-dimensional spaces, which is critical for the formation of patient-derived organoids. This method can potentially improve mechanical dissociation by pre-patterning tissues in a way that allows for more uniform and predictable dissociation outcomes[133, 134]

Additionally, the integration of microfluidic systems for controlled mechanical dissociation presents an exciting prospect for the precise manipulation of tumor tissues at the microscale. This approach could potentially reduce the physical stress on cells, preserving their viability and functional status for organoid formation[135]. Dynamic culture systems based on microfluidics can even apply precisely controlled mechanical forces to organoids, aiding in their dissociation[136]. Microfluidic technology provides a tool for the precise control of the cellular microenvironment, which is essential for the optimization of enzymatic digestion processes. Moreover, these systems allow for the precise delivery of enzymes and the regulation of reaction conditions, such as temperature and pH, which can significantly enhance the efficiency and specificity of enzymatic digestion[137].

The advent of organ-on-a-chip technology also heralds a new era in organoid culture, where the co-culture of multiple organoids within a microfluidic device can mimic the complex interplay between different organs and the tumor[138, 139]. This system could provide a more holistic understanding of cancer progression and therapeutic responses. Organ-on-a-chip systems further enhance this by providing a dynamic environment that simulates the physiological conditions of the human body, which can be particularly beneficial for optimizing enzymatic digestion by controlling the microenvironmental factors such as nutrient flow and waste removal[140].

The CRISPR-Cas9 system offers a means to genetically modify cells within organoids[141], which can impact their adhesion properties and response to mechanical forces. By targeting genes that regulate cell–cell or cell-matrix interactions, researchers can modulate the ease of mechanical dissociation. Additionally, gene editing may be used to introduce mutations that affect specific cellular responses to enzymatic digestion, thus enabling the development of cancer organoids with step-wise enzymatic digestion.

Challenges and considerations

Standardization and reproducibility

The mechanical dissociation and enzymatic digestion techniques used in PDO cultures are crucial for the standardization and reproducibility of cancer research.

Mechanical dissociation, although rapid and cost-effective, can lead to inconsistent cell yields and viability due to its reliance on physical forces that may vary between procedures[142] and spatiotemporal specificity by tumor tissue source[143]. On the other hand, enzymatic digestion offers a more controlled approach but requires optimization of the enzymatic cocktail composition and incubation conditions for different tissue types, as these factors can significantly affect the efficiency of cell dissociation[144]. Standardizing these methods is essential to ensure that organoid cultures accurately represent the patient's tumor, enabling reliable comparisons across studies.

Reproducibility in organoid culture is further complicated by the inherent heterogeneity of patient tumors and the potential for genetic drift during *in vitro* expansion[119]. To address these challenges, it is necessary to establish rigorous protocols for dissociation methods, including the use of standardized equipment, controlled environmental conditions, and consistent cell passage practices. Additionally, thorough validation of organoid cultures through genetic and phenotypic characterization is required to confirm their fidelity to the original tumor samples[145, 146].

Scaling up for high-throughput applications

Scaling up organoid cultures for high-throughput applications presents significant challenges, particularly in the context of mechanical dissociation and enzymatic digestion. High-throughput techniques require the processing of large numbers of samples in a manner that is both efficient and maintains the integrity and viability of the resulting organoids. Automation of dissociation methods can help to address this issue, but the development of such systems must balance the need for consistent cell disruption with the preservation of cellular phenotypes[117]. Wang et al. attempted to scale up intrahepatic cholangiocarcinoma (ICC) by culturing tissue-fragment derived organoids followed by enzymatic digestion to generate single-cell derived organoids ultimately, whilst the microenvironment preserved in mechanics-dissociation initiated tissue-fragment derived organoids could be mostly inherited to single-cell derived organoids[128]. Of note, the retaining of immune cell in their process indicates that the tissue dissociation methods applied in the initiation stage from tissue to organoid may play a decisive role in the preservation of tumor microenvironment.

On the other hand, the scalability of enzymatic digestion is limited by the need for precise control of enzymatic activity and the potential for batch-to-batch variability in enzyme preparations. To overcome these challenges, researchers are exploring the use of defined and synthetic extracellular matrix components that can provide a more consistent and reproducible environment for organoid dissociation and culture[147].

The development of microfluidic devices and bioprinting technologies offers promising avenues for the miniaturization and parallelization of organoid culture processes, potentially enabling the high-throughput screening of drug responses and genetic perturbations in a more controlled and physiologically relevant manner[90].

Strategic organoid dissection for cancer modeling

Mechanical dissociation and enzymatic digestion both have been instrumental in advancing our understanding of solid tumor

organoid biology, with each offering unique advantages depending on the context of use (Fig. 1b). Ideally, the dissociation protocol is individualized for the tissue-derived organoid of interest and evaluated relative to both optimal and representative cell yield, as well as based on a balance between cost-effectiveness and experimental fidelity.

In the realm of organoid-based cancer modeling, the devil is in the details, whilst in the term of its ultimate objective-curing cancer, the death is at the speed. Strategic use of these methods can significantly influence the quality and outcomes of cancer research and so require considering multiple factors.

Firstly, the selection of the appropriate dissociation method should be predicated on the specific requirements of the experiment and the characteristics of the tissue. For instance, when the preservation of tumor heterogeneity and fast drug test for patients are required, mechanical dissociation with a better performance to maintain the *in vivo* tumor milieu and a speedy process can be chosen. When the preservation of cellular viability is crucial or single-cell suspension is required, enzymatic digestion with a carefully optimized cocktail of enzymes can be employed.

Secondly, the efficiency and reproducibility of enzymatic dissociation often surpass those of mechanical methods, enabling uniform processing of tissue samples. In addition, enzymatic dissociation also facilitates the use of DNase to remove DNA from necrotic cells, reducing the viscosity of the dissociation medium and improving cell suspension quality. When enzymatic digestion is chosen, optimizing its process is essential. By fine-tuning the concentration of enzymes, the duration of exposure, and the temperature, researchers can minimize enzyme usage, thereby reducing costs.

Thirdly, the integration of mechanical dissociation, while faster and less expensive, should be approached with an understanding of its limitations in terms of cell yield and viability. Combining mechanical and enzymatic methods may strike a balance, improving cell yield while maintaining cell integrity.

Furthermore, if possible, bulk processing can reduce the cost per sample. Scaled operations can reduce the preparation and processing time for individual samples.

Lastly, the development of organ-on-a-chip systems and the incorporation of physiological flow rates can make up the shortfalls of these tissue dissociation methods to some extent and so improve the characteristics of organoids, making them a better representative of *in vivo* conditions. This advanced approach, while complex, can lead to more accurate modeling of cancer progression and response to treatment.

In summary, the strategic application of mechanical dissociation and enzymatic digestion in organoid-based cancer modeling involves a multifaceted approach. It requires careful consideration of the experimental goals, optimization of dissociation conditions, combination of methods for enhanced cell yield, and the use of advanced culture systems to create more physiologically relevant models. By doing so, researchers can achieve a balance between cost-effectiveness and the generation of robust, representative cancer models that can advance our understanding of cancer biology and facilitate the discovery of new therapeutics.

Conclusion

The dissection of PDOs through mechanical dissociation and enzymatic digestion stands as a cornerstone in the advancement of cancer research. Mechanical dissociation, with its capacity to maintain the tissue's native architecture and cellular heterogeneity, offers a methodological advantage in preserving the tumor microenvironment's fidelity. This physical approach minimizes the disruption of cell–cell interactions and ECM, thereby enhancing the organoids' ability to accurately model the complexity of human cancers. Conversely, enzymatic digestion, through the selective degradation of the ECM, provides a chemical means of cell isolation. This method, while potentially leading to a more thorough dispersion of cells, must be meticulously optimized to avoid the loss of critical microenvironmental cues. The choice between these two dissociation techniques is not merely academic but has profound implications for the integrity and representativeness of PDOs in cancer modeling.

The optimization of these methods is essential for the standardization of PDO cultures, ensuring the reliability and reproducibility of research outcomes. The integration of cutting-edge technologies, such as bioprinting and organ-on-a-chip systems, alongside CRISPR-Cas9 system, is set to enhance the efficiency and throughput of organoid production. These innovations are poised to deepen our molecular understanding of tumors and facilitate the development of targeted therapies.

The future of PDOs in precision oncology hinges on the refinement of these dissociation techniques, which will enable more accurate predictions of treatment responses and the personalization of therapeutic strategies. As the field progresses, the standard and the scalability of organoid culture for high-throughput applications must be addressed to fully realize the potential of PDOs in transforming cancer therapy. The ongoing evolution of organoid technology, driven by advancements in biomaterials and genetic engineering, promises to further refine these models, offering a more physiologically relevant representation of the tumor microenvironment.

Research ethics and patient consent

Not applicable

Availability of data and material

Not applicable

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Shichao Duan, Haijun Li: conceptualization, supervision, writing–review. Jing Ren, Mengli Liu: writing original draft, writing–review, editing. Mingjie Rong: visualization. Xuan Zhang, Gang Wang, Yihan Liu: writing original draft.

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