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Culture of vibrating microtome tissue slices as a 3D model in biomedical research

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Abstract

The basic idea behind the use of 3-dimensional (3D) tools in biomedical research is the assumption that the structures under study will perform at the best in vitro if cultivated in an environment that is as similar as possible to their natural in vivo embedding. Tissue slicing fulfills this premise optimally: it is an accessible, unexpensive, imaging-friendly, and technically rather simple procedure which largely preserves the extracellular matrix and includes all or at least most supportive cell types in the correct tissue architecture with little cellular damage. Vibrating microtomes (vibratomes) can further improve the quality of the generated slices because of the lateral, saw-like movement of the blade, which significantly reduces tissue pulling or tearing compared to a straight cut. In spite of its obvious advantages, vibrating microtome slices are rather underrepresented in the current discussion on 3D tools, which is dominated by methods as organoids, organ-on-chip and bioprinting. Here, we review the development of vibrating microtome tissue slices, the major technical features underlying its application, as well as its current use and potential advances, such as a combination with novel microfluidic culture chambers. Once fully integrated into the 3D toolbox, tissue slices may significantly contribute to decrease the use of laboratory animals and is likely to have a strong impact on basic and translational research as well as drug screening.

Keywords 3D models, Organotypic culture, Tissue slices, Vibrating microtomes, Microfluidics

The dimensions of biomedical research tools

Researchers address their experimental questions by employing a variety of models, which lie on a wide spectrum in terms of tractability and physiological relevance. Animal studies, at one pole, provide valuable insights into the pathophysiology of most biological process in a (manipulable) systemic environment. On the downside, animal experiments can be expensive, time-consuming, technically challenging, and their translational value may be severely compromised by intrinsic species-specific differences, not to mention the substantial ethical dilemma of causing animal pain or suffering. At the other end of the scale, simple two-dimensional cell culture systems are unbeatable in terms of convenience, accessibility, and readout throughput. Regrettably, they fail short in terms of physiological significance due to the lack of essential features typical for the in vivo situation, such as the presence of extracellular matrix (ECM) and proper spatial and signal-based interactions with other cells or tissues. It is therefore not surprising that massive effort has been put in developing experimental tools combining the advantages (and avoiding as far as possible the drawbacks) of both approaches. Such tools, collectively designated as three-dimensional (3D) models, indeed provide a more natural culture environment, besides facilitating studies with the biological material of the relevant species, thus increasing the translational relevance of the study [1–3].

Current 3D models include a wide variety of approaches. In a simple form, primary cells or cell lines are guided towards 3D aggregates by providing a natural or synthetic scaffold. This basic principle, however,

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can be expanded to create advanced and biologically relevant multicellular spheroids [4]. Organoids, in contrast, are complex structures based on the long-term culture of stem cells or primary cells and ordered differentiation of their progeny under the influence of a cocktail of growth factors and/or chemicals in order to recapitulate the structural and functional properties of multiple adult organs [5, 6]. Organ-on-chip models integrate mechanical (such as shear or strain stress) and chemical (growth factors, cytokines) cues and include tailored sensing of the culture environment regarding aspects as medium flow rate, temperature, pH, partial pressure of gases, and mechanical forces, among many others [7–9]. Organ-on-chip are a particularly promising strategy for assessing the safety and efficacy of chemicals and pharmaceuticals [10]; the devices can harbor simple (cell lines) or complex (organoids) biological structures [11], and can be designed to allow communication between cell types of different organs, in what has been called multiorgan chips. Another recent advance is bioprinting, in which 3D printing-like techniques are used to combine cells, growth factors, and/or biomaterials to create 3D cell aggregates resembling tissues or organs [12, 13]. Due to ever improving cell culture conditions and chip manufacturing methods as well as a growing variety of printing techniques and an apparently unlimited availability of suitable biological and scaffold materials, the combination of 3D-based methods as organoids, organ-on-chip, tissue slices, and bioprinting (see Table 1 for a comparison of their main features), is likely to lastly bridge the gap between cell culture and a living organism [1, 14], significantly improving biomedical research and reducing the use of laboratory animals.

From Warburg's shaving razor to precision-cut tissue slices

Tissue slices are a 3D model par excellence [14] and have been extensively used to address numerous research questions, including the study of intermediate metabolism in the liver or transport processes in the kidney. More recently, however, this method has been somewhat overshadowed by the general excitement around the organoid/organ-on-chip/bioprint trio [1–3]. Originally developed as an improvement of whole organ cultures, tissue slices have been in continuous use at least since the early 1900s, and thus decades before the establishment of the first cell line. Otto Warburg, for instance, generated tissue slices with a hand-guided razor blade for his Nobel prize-winning research on tumor metabolism [15]. Initially simple hand-held equipment, the devices improved over the decades to meet the need for uniform slice thickness, important for experiment reproducibility, and low sample waste when using small organs. A simple microtome for slice preparation from fresh tissue, described in 1944 [16], was followed by motor-driven [17] or hand-operated choppers [18], which reduced the time necessary for section preparation and did not require intensive training. A further improvement was the development of precision-cut tissue slices, namely the Krumdieck/Alabama [19] and later the Brendel/Vitron [20] slicers. With these apparatuses, which have been continuously improved regarding precision and ease of operation, slices are produced by mechanically moving an immobilized tissue cylinder across a microtome blade; the slice thickness can be adjusted within a rather large range of ~ 100 to 1000 µm, and tissue slicing is very rapid, with one slice produced every 3 to 4 s [21–23]. Both apparatuses seem to produce slices of comparable quality, as demonstrated by direct comparison of rat liver slices [24].

Slices should be cut at a thickness permitting efficient gas and metabolite exchanges; in most studies this ranges between 100 and 400 µm (see also Tables 2 and 3). Slices made too thick may show ischemic injury in the slice core, while slices made too thin may have a large proportion of damaged cells at their surface as compared to the total amount of healthy cells. Importantly, the thickness of precision-cut slices is much more constant, the number of damaged cells is greatly reduced, and the induction of immune responses is reduced compared to previous methods [25, 26]. Also, cultivation of such slices over prolonged periods (up to several weeks) has included different techniques, such as roller-tube cultures, culture on semipermeable membranes at the air–liquid interface, or embedding in 3D gels on culture dishes [27]. More recently, culture under continuous flow [28] or in microfluidic chambers [29] was reported.

Several features contributed to a high popularity of "precision-cut slices", including: a) the ECM and all or at least most supportive cell types are already present in the correct tissue architecture; b) all cell types are isogenic; c) there is no enzymatic dissociation, thus preserving cell surface proteins (however, tissue damage during slicing can induce immune responses); d) it is amenable for imaging; e) a large number of slices can usually be obtained from a single organ; f) it is an accessible, unexpensive and technically rather simple procedure. Therefore, slice production and culture methods were next extensively improved and adapted to different requirements and became an essential part of the toolbox in most fields of biomedical research, including neuroscience [96], lung [97] and liver [98] diseases, and host–pathogen interactions [99].

Table 1 Comparison of the key features of 3D systems

	Organoids	Organ-on-chip	Bioprinting	Tissue slices
Attainable complexity	Scalable, highly complex cellular composition by the combination of primary cells, stem cells and their progeny possible	Scalable, highly complex cellular composition and culture environments are possible	Highly defined and controlled assembling of cell types and matrices possible	Retain the original tissue architecture and complexity
Cell damage	Low or absent, but necrotic cores possible	Low or absent	Potentially high (temperature, shear stress)	Damage of adjacent cells unavoidable
Long-term culture	Virtually unlimited due to passaging (subcultivation)	Weeks to months, depending on the specific cell turnover and matrix properties	Depends on the specific cell turnover and matrix properties	Usually days to weeks
Non-preparative sampling	Supernatant, 3D imaging. Access to the apical surface may be difficult	Supernatants (compartment-wise, but limited volumes), sensor readouts; 3D imaging challenging	Supernatant, 3D imaging	Supernatant, 3D imaging
User-friendliness	Requires rather complex cell culture medium and additives	Sophisticated culture devices can be quite costly, time-consuming, and challenging to operate	Requires complex technologies, may be challenging in terms of operation and costs	Easy operation but requires recurrent tissue supply

Table 2 Selection of studies culturing vibrating microtome-generated slices from different organs or tissues

Organ/ tissue	Species	Slice thickness	Culture time (max)	Culture system features	Purpose	Ref
Brain	rt	300 µm	8 weeks	On insert, ALI, lentiviral infection	Protocol for creating hippocampal slices	[30]
	ms	110 µm	4 weeks	On insert, different Co-cultures	Protocol for co-cultures	[31]
	hu	250-350 µm	6 weeks	On insert, submerged,	Protocol for creating cortical slices	[32]
Spinal cord	rt	350 µm	14 days	On insert, ALI	Model development	[33]
Oculomotor nerve	ms	400-450 µm	72 h	On insert, submerged,	Study of oculomotor nerve outgrowth	[34]
Retina	ms, rt	40 – 170 µm	4 weeks	Submerged, in a LumiCycle	Study of circadian oscillations	[35]
	pg	250-300 µm	48 h	Within a gelatine sandwich, submerged	Morphometry and viability of photorecetors	[36]
	fs	150 µm	5 days	On coverslips, agarose coated	Method validation, interaction among retinal cells	[37]
	rt	125 µm	3 weeks	Plasma clot technique for cultivation	Electrophysiological recordings (patch clamp)	[38]
Olfactory epithelium	rt	400 µm	5 days	On coated inserts, submerged	Method for assessing olfactory development and function	[39]
Heart	rt, ms, hu, pg, dg	100-400 µm	7 days	ALI	Model development	[40]
	hu, ms	380 µm	4 days	In chip, dynamic, with sensors	Shippable model for pre-clinical drug testing and basic research	[41]
	hu, pg	300 µm	6 days	Submerged, electrical stimulation, media oxygenation	Model for drug testing and gene therapy	[42]
	ms	300 µm	6 days	On insert, ALI	Model for gene therapy, gene transfer efficiency, cell tropism, and toxicity	[43]
	hu	400 µm	48 h	On insert, ALI	SARS-CoV-2 infection model	[44]
	hu	300 µm	14 days	Submerged, application of pre- and afterload	Analysis of contraction force and kinetics	[45]
	pg	400-500 µm	48 h	On PDMS pillars, ALI, with/without insert, static and dynamic	Study epicardial cell physiology and activation	[46]
Lung	ms	275 µm	5 days	On insert, ALI	Model for circadian timing in lung, role of Clara cells and glucocorticoids	[47]
	hu, ms	500 µm, 300 µm	5 days	Submerged, rolling, co-culture with transfected fibroblasts	Multidimensional immunolabeling and 4D time-lapse imaging of vital slices	[48]
	hu, ms	500 µm, 300 µm	14 days	Submerged	Wnt-induced repair, 4D confocal live tissue imaging	[49]
	ms	150 µm	15 days	Submerged	Study of small airway smooth muscle contraction	[50]
	rt	500 µm	12 h	Stretcher to mimic breathing mechanic	Analysis of response to cigarette smoke	[51]
	pg, hm, ct	350 µm	4 days	Submerged	Check for susceptibility for SARS-CoV-2	[52]
	ms	300 µm	4 days	Submerged	Study of pulmonary fibrosis disease mechanisms	[53]
Salivary gland	hu	35 µm and 50 µm	14 days	On insert, ALI	Slice culture model development	[54]
	ms	50 µm	2 days	Submerged	Slice culture model development with emphasis on imaging	[55]

Table 2 (continued)

Organ/ tissue	Species	Slice thickness	Culture time (max)	Culture system features	Purpose	Ref
Small Intestine	ms	250 µm	6 days	Slices covered with collagen and medium on top	Model for interactions of different cell types in the intestine	[56]
	ms	250 µm	48 h	Slices covered with collagen and medium on top	Neuronal regulation of goblet cell production by	[57]
Colon	hu	250 µm	3 days	On collagen-covered slices, submerged, co-culture with *S. typhimurium*	Host microbial interactions, influence of oxygen availability	[58]
Prostate	hu	200-300 µm	10 days	Submerged, hypoxia	Prostate tissue model	[59]
Pancreas	ms	100 µm	12 weeks	On insert, ALI; electrophysiology	β-cell physiology modell establishment	[60]
Liver	hu, rt	100-400 µm	28 days	On insert, ALI	Fibrosis model	[61]
	ms	100-250 µm	5 days	Submerged	Model for chronic liver diseases	[62]
	ms, hu	250 µm	5 days	Submerged	Improve slice culture, fibrosis drug therapy testing	[63]
Spleen	ms	230 µm	4 days	Submerged	Protocol, method validation	[64]
Thymus	ms	400–500 µm	Several days	On insert, submerged; overlayed with thymocyte cell suspension	Model for studying T cell development	[65]
Femur	rt	300-400 µm	3 weeks	On insert	Study enchondral osteogenesis	[66]
Meibomian gland	ms	150 µm	21 days	Submerged	Model development, effect of melanocortins on secretion	[67]
Endometrium	hu	200 µm	48 h	Submerged	Modulation of endometrial PGE2 synthesis	[68]

ALI Air–liquid interface, *hu* human, *rt* rat, *ms* mouse, *pg* pig, *dg* dog, *hm* hamster, *ct* cat

Vibrating microtomes enter the stage

In a recent survey, Dewyse and colleagues [98] report that while the majority of precision-cut liver slices is still generated with the Krumdieck or Brendel slicers, vibrating blade microtomes are gaining popularity. These devices were originally developed at Oxford Laboratories in California [100] and later marketed by different companies including Leica, currently the owner of the brand name "vibratome". The hallmark of the apparatus is the lateral, saw-like movement of the blade as it progresses, which significantly reduces tissue pulling or tearing compared to a straight cut (Table 4). In a typical setup, fresh tissue samples (either tissue pieces or punch-generated tissue cores) are embedded in low gelling temperature agarose blocks (Fig. 1A) and attached with contact glue to a holder within the cutting chamber of the vibratome (Fig. 1B). The holder is raised or lowered to adjust the thickness of the section as a sharp blade moves and cuts in a plane parallel to the sample's surface. During cutting, both the sample and the blade edge are immersed in an aqueous buffer, resulting in the formation of free-floating sections, which can be immediately imaged, fixed and histologically processed, enzymatically dissociated for obtaining individual cell populations, or employed for cultivation and manipulation in vitro.

Finding the suitable vibratome settings for the tissue of interest and establishing a protocol for an optimal slicing procedure can be time-consuming. Factors to be considered are the rigidity or elasticity of the tissue itself, the type of supportive embedding material, and the settings of the instrument, including the desired slice thickness, the angle and amplitude of the blade, and its propulsion speed. The downstream processing of the slices also needs to be considered, as the dehydration associated to sample fixation can lead to shrinkage in slice thickness of over 50% [103]. While in principle any tissue can be sliced with this technique, samples that are very soft, contain hard components, or are rich in elastic elements are less suitable than homogeneous samples and may require extensive protocol improvement. Although some studies focused on the impact of specific device settings and the type of sample (fresh vs. fixed) on slice properties [104], or on the impact of the viscoelastic properties of the embedding structure on slice viability and quality [105], the diversity of sample types makes it difficult to provide exact recommendations regarding the device settings. As a general rule, soft and elastic tissues should be

Table 3 Non-exhaustive selection of studies culturing vibrating microtome-generated slices from different tumor types

Tumor type	Species	Slice thickness	Culture time (max)	Culture system features	Purpose	Ref
Lung	hu	300 µm	6 months	Implanted into mice (xenograft)	Model for primary tumor expansion and xenograft production	[69]
	sh, ms	300 µm	1 month	Submerged	Standardized slice model for viral infection gene therapy	[70]
	ms	160-250 µm	3 days	ALI, titanium grid, rotation	Tumor drug testing model	[71]
Oral squamous cell carcinoma	hu	350–450 µm	8 days	Chorioallantoic membrane (CAM)	Establishing slices on CAM as a tumor model	[72]
Gastrointestinal (various)	hu	250 µm	7 days	On insert, submerged	Protocol, method evaluation	[73]
Prostate	hu	200-300 µm	10 days	Submerged, hypoxia	Prostate tumor model	[59]
	ms	300 µm	6 days	With/without insert and strainer	Establishing a chemotherapy model	[74]
	hu	250 µm	9 days	On insert, ALI	Model for immune microenvironment studies	[75]
	hu	350 µm	96 h	On insert, submerged	Model development	[76]
	hu	350 µm	96 h	On insert, submerged	Assessing culture effects by transcriptome profiling	[77]
	hu	250 µm	9 days	On insert, ALI	Model development	[78]
	hu	250 µm	4 days	On insert, ALI	Tumor immunology studies	[79]
	hu	300 µm	15 days	On insert, ALI	Method development	[80]
	hu	250 µm	6 days	On insert, submerged	Interaction of tumor cells with immune microenvironment	[81]
Liver	hu	200-300 µm	3 days	Submerged	Comparison of slicing devices	[82]
	hu, ms	200-300 µm	7 days	On insert, submerged	Drug discovery, immuno-oncology	[83]
	hu	250 µm	4 days	On insert, submerged	Immune checkpoint ligands and chemotherapy response	[84]
	hu	250 µm	6 days	On insert, submerged	Establish CarT-cell treatment model	[85]
	hu	250 µm	3 days	On insert, submerged	Neutralizing antibodies and CAR-T cells in cancer therapy	[86]
Bladder	hu	300 µm	2 days	On insert, submerged, on a rotating plate	Method for studying oncolytic viruses	[87]
Kidney	hu	300 µm	1 week	Submerged	Characterization of the tumor immune environment	[88]
Uterine leiomyoma	hu	500 µm	3 weeks	On alginate scaffold discs	Model development	[89]
Breast	hu	300 µm	7 days	Submerged with/without rotating platform	Model development, comparison with manual slicing	[90]
	hu	250 µm	7 days	Submerged	Model evaluation	[91]
	hu	250 µm	3 days	Submerged	Establishing a chemotherapy model	[92]
Breast (xenograft)	hu	200 µm	4 days	Submerged	Drug testing	[93]
Breast and prostate PDX models	hu	300 µm	2 weeks (breast), 1 week (prostate)	In chip, submerged, shear stress, perfusion	Cancer on chip platform for predicting drug response	[94]
Head and Neck Squamous Cell Carcinoma	hu	300 µm	5 days	Rotating platform	Model for evaluation of treatment response to radiation and chemotherapy	[95]

ALI Air–liquid interface, *PDX* Patient-derived xenograft, *hu* Human, *ms* mouse, *sh* sheep

cut at higher amplitude and lower mechanic deflection and propulsion than more rigid tissue types. Researchers may initially apply the settings used in previous publications employing the same tissue (see Tables 2 and 3 for numerous examples), but optimal slicing conditions frequently need to be established empirically.

By directly comparing a vibratome with a Krumdieck tissue slicer, Zimmermann and colleagues [82] showed

Table 4 Cutting by pressing and sliding: lessons from cheese and salami slicing

Cutting of soft materials, be it human flesh by the surgeon, meat or vegetables by the chef, or tissue samples by the histologists, is made considerably easier by sliding the blade rather than just pressing it against the surface of the object to be cut. This principle also holds for the common "paper cut", the painful rendezvous between the skin and a thin paper sheet. The phenomenon has been modelled with a wide variety of materials, and different explanations have been provided. Atkins and colleagues [101] lively review the mechanics of cutting and define our study object as a material in which sectioning creates a floppy offcut that is not permanently deformed and has negligible bending resistance. These authors demonstrate (by cutting cheddar cheese and salami) that the greater the "slice/push ration", the lower the necessary cutting forces. Reyssat and colleagues [102] focus on the role played by shear forces on gelatin blocks and reveal that the sliding action creates a critical local tension at the contact site, in contrast to a strong global tension caused by pressing only. Thus, under slicing (= blade vibration) conditions, there is less global deformation and material damage, resulting in better preserved slices – in our context the key motivation for employing vibrational cutting

that the vibratome, while requiring longer operation time, is superior in terms of accuracy and reproducibility. However, the technique can also be applied in fixed or cleared tissue samples, and has been integrated into numerous imaging platforms [106, 107]. Importantly, the technical features of the apparatuses are being constantly improved. For example, Wang and colleagues determined that the sectioning quality of soft materials can be enhanced by using higher sectioning frequency, blade oscillation amplitude, and lower sample feed rate [108]. As a result, the same group developed a novel high-frequency vibrating microtome allowing high-speed cutting without compromising slice quality, and successfully applied it to organ-wide imaging [109].

As a gentle cutting technique, it is particularly suitable for creating fresh tissue slices to be further cultured. Upon identification of appropriate sample thickness and other cutting parameters, optimal culture conditions can be established. Tissue slices generated with a vibrating microtome have been cultured under a large variety of conditions: simply submerged in culture medium, on membrane-coated inserts, or at the air–liquid interface,

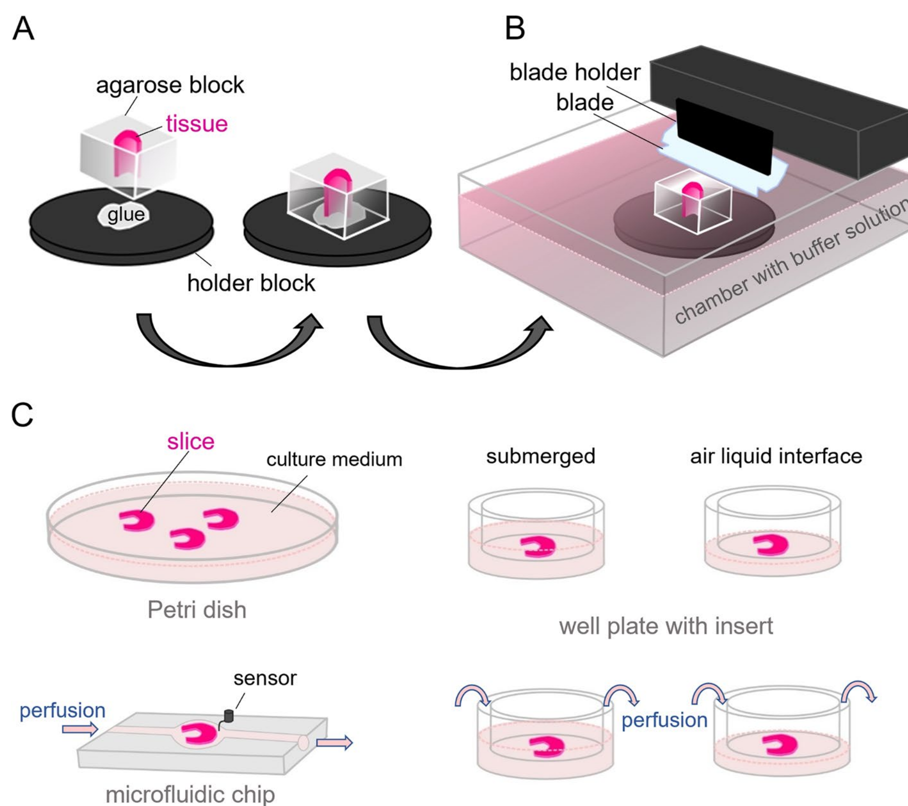

Fig. 1 Working principle of tissue slice culture. **A** The sample is embedded in agarose and sticked to the block holder (left). **B** Side-view of a typical vibratome cutting chamber (right). **C** Typical culture methods employed downstream of slice generation: submerged in culture medium, on insert submerged or at the air–liquid interface, in each case with or without perfusion, and in a microfluidic culture chamber. Figures were created using Krita (https://krita.org)

in each case with or without perfusion, or in microfluidic devices, among others (Fig. 1C). Such systems have been widely used for housing and manipulating tissue slices for a variety of purposes and experimental questions. A non-exhaustive overview of investigations involving the culture of vibrating microtome-derived normal and tumor tissues is given in Tables 2 and 3, respectively.

Perspectives and conclusions

Vibrating microtome sectioning is an outstanding tool for creating tissue slices suitable for a wide variety of studies and amenable for numerous cutting-edge imaging technologies [14, 110]. Latest downstream applications include tissue regeneration studies [53, 63] or the evaluation of cancer treatment strategies like gene therapy, where the transfection efficacy in a complex environment can be assessed [43]. Furthermore, immunotherapy precision and the invasion of Car T-cells can be tracked [86]. As immune cells are present in a natural architecture within the slices, they are a suitable model for the investigation of host–pathogen interactions [52, 99, 111], host-microbiome interactions [58] and drug safety assessment [42, 71]. Nevertheless, it should not be omitted that this method also comes with several potential drawbacks, including the increased appearance of apoptotic or necrotic areas directly at or close to areas damaged by the blade (including anoikis induced by ECM removal), disruption or clogging of vessels, depletion of specific cell types (for instance by migration into the medium), and considerably reduced supply and removal of substrates and metabolites compared to the uncut tissue. These problems may represent a significant challenge and require considerable improvement of tissue slice culture techniques.

Overall, this strategy will benefit from diverse technical improvements and subsequent developments.

Vibrating microtome technical improvements

While vibrating microtomes are easy to use and, compared to standard tissue choppers, more sample-gently, slice preparation is more time-consuming. The time needed for sample processing may indeed be especially critical for enzyme-rich (pancreas) or highly metabolic (liver) organs. To overcome this issue, e.g. for liver samples, sophisticated media can be combined with low temperatures during slicing, thus improving slice quality and viability [63]. Therefore, an automated temperature control of sample holder and media would be favorable, as for the most systems manual addition of crushed ice remains necessary. During sample preparation, the rigidity of relatively soft and flexible tissue types like skin, intestine, or lung needs to be increased. While appropriate stabilizing agents like low melting point agaroses are available, getting the slices completely rid of their remnants remains an issue. During sequential sample processing, usually slices end up floating around in the sample chamber filled with media, while the blade is already beginning to move to generate the next slice. Here, the inclusion of a medium stream gadget in the instruments to transport the floating slices away from the blade, thereby preventing sample damage and facilitating slice transfer to culture systems, would be desirable. Although most operators work with antibiotics in the media, it may be favorable to work without these additives for some applications. As the footprint of some devices is rather small, sterile working conditions can be readily achieved by placing the vibrating microtome under a sterile hood. In contrast, there is currently no vibrating microtome available containing a self-sterilization function or including a sterile working chamber. Finally, as for the regular microtomes, working safety is a critical issue and accidents may occur. Therefore, a corresponding cap to cover the razor blade when not in use, as well as an easily accessible emergency stop button would be useful additions.

Combination with other 3D-models and downstream applications

3D models shouldn't be seen as stand-alone techniques, as only the combination of different approaches may result in a physiologically relevant model. Tissue slices combined with organ-on-chip technology enable sensor implementation into the culture device and a tight control of parameters [41]. This enables sophisticated manipulation of culture conditions and thereby mimic homeostatic or dysbiotic conditions. Organ-on-chip/microfluidics systems may also allow reproducing one of the key properties of tissues in vivo, the continuous nutritional supply, gas exchange, and removal or transport of metabolites and growth factors via capillarization. These processes maintain important biophysiochemical gradients alongside the endothelial-epithelial axis, and its implementation in in vitro models is essential for improving the translational value of the studies. Furthermore, certain cell types may require perfusion as they respond towards the corresponding shear stress with an increased barrier function and morphological adaptions [112]. Especially in case of linear perfusion, microfluidic devices can help to decrease the amount of media consumption. With regard to specific applications, dynamic cultivation used in infection experiments might contribute to prevent microbial overgrowth [113] or provide more in vivo-like infection conditions, for instance when studying invasion mechanisms [114].

From a practical point of view, as most tissue slices are cultured on inserts, perfusable plates are one option for

dynamic cultivation conditions, and the same applies to chip-systems containing a porous membrane as separator for perfusable compartments. In such systems, one compartment contains the tissue slice, and the other one can be perfused. Depending on the tissue type, site-specific, ubiquitous, unidirectional, or bidirectional perfusion can be applied. However, perfusion similar to that in vivo is difficult to achieve in microfluidic chambers, as vessel anastomosis in bioreactors is usually missing. Also, the multicellular tissue slices are exposed to a single media type, and a site-specific application of shear stress can hardly be realized. This could negatively affect cell viability and function of tissues not exposed to shear stress under physiological conditions (for instance, interstitial tissue).

Co-culture of slices with cell lines opens up a broad spectrum of investigations. For instance, fibroblasts added to preliminary injured tissue slices can be used for the investigation of fibrosis mechanisms [48]. In another study, Car T-cell and genetically engineered macrophage invasion in tumor tissue slices were examined [85]). Not only mammalian cells, but also pathogens as SARS-CoV-2 have been co-cultured with tissue slices in order to determine cell type susceptibility for the virus and thereby identify potential treatment targets [44, 52].

Tissue slices can be combined with animal models as well. Frequently, the initial manipulation takes place in vivo, and the subsequent generation of tissue slices greatly amplifies the number of samples available for further in vitro treatments. In this way, the number of animals used in experiments can be reduced. However, the experimental setup can also be designed inversely, as tissue slices of one species can be implanted into another one in the form of xenografts [69].

Sample analysis and logistics

Co-evolving imaging technologies of live tissue imaging (4D) enable whole sample analysis, time-lapse recording of viable tissue slices enabling thereby e.g., in-tissue observation of cell migration and tissue regeneration [49]. The analysis of living and fixed tissue slices via cLSM and light-sheet microscopy permits a 3D-reconstruction of native and manipulated tissue in all its complexity [30, 55]. Of course, preparative downstream analysis of vital tissue, including single cell analysis and studies on ECM function, production and regeneration, so far mainly performed with tissue chopper-generated slices, can be carried out on vibrating microtome-generated slices as well [115–117]. Similarly to slides generated with standard microtomes, vibrating microtome slices would significantly benefit from cryopreservation methods, as hundreds or thousands of slices may be created from a single organ, cryopreserved, and used on demand [118]. Notably, test platforms combining precision-cut slices with cryopreservation for assessing drug response of hepatic tumors [119] or assessing the immune response of the lung [120] have been recently reported.

To conclude, there is great potential for the combination of vibrating microtome tissue slices with microfluidic culture devices, which have been greatly improved in the context of organ-on-chip methods regarding the modulation of specific culture conditions and the use of miniaturized sensors. In particular, the correct tissue-like spatial organization, multicellularity and the presence of native ECM of such slices in combination with tightly controlled culture conditions will provide a unique model for assessing organ physiology and testing the effects of substances in vivo.

Abbreviations

3D Three-dimensional
ECM Extracellular matrix

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Authors' contributions

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