ESSAY

The third dimension bridges the gap between cell culture and live tissue

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Abstract | Moving from cell monolayers to three-dimensional (3D) cultures is motivated by the need to work with cellular models that mimic the functions of living tissues. Essential cellular functions that are present in tissues are missed by 'petri dish'-based cell cultures. This limits their potential to predict the cellular responses of real organisms. However, establishing 3D cultures as a mainstream approach requires the development of standard protocols, new cell lines and quantitative analysis methods, which include well-suited three-dimensional imaging techniques. We believe that 3D cultures will have a strong impact on drug screening and will also decrease the use of laboratory animals, for example, in the context of toxicity assays.

Most of the cell-based data-harvesting efforts that drive the integration of cell biology, mathematics, physics and engineering are based on the assumption that cell monolayers reflect the essential physiology of real tissues. However, it is generally recognized that the flat and hard plastic or glass substrates that are commonly used for cell culture are not representative of the cellular environment found in organisms. In fact, tissue-specific architecture, mechanical and biochemical cues and cell–cell communication are lost under such simplified and highly biased conditions.

Although a wealth of information on cellular behaviour in a physiologically relevant context is provided by three-dimensional (3D) data analysis in living animals (such as developing embryos), an alternative *ex vivo* approach is offered by 3D cell cultures. Studies spanning over two decades of research have shown that growing cells within 3D scaffolds reduces the gap between cell cultures and physiological tissues. A permissive context allows the preservation of a differentiated tissue-specific phenotype in cultured cells[1]. Following the lessons of the pioneers of this field, we argue that a 3D approach to cell biology has the potential to improve the physiological relevance of cell-based assays and advance the quantitative modelling of biological systems from cells to organisms. Equally important, the increased predictive power of cell-based drug and toxicity screenings could contribute to reduce the number of animals that are used for testing purposes by the pharmaceutical industry and governmental institutions[2].

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Innovations both in sample handling and imaging techniques are fundamental in order to fully exploit the benefits of the third dimension in the life sciences. In fact, 3D samples are typically highly scattering and several hundreds of micrometres thick, and challenging for conventional microscopy. Moreover, minimizing photobleaching and light-induced damage is essential for large living samples. On the basis of our own experience in modern imaging techniques, we contribute to the discussion on 3D cell biology by reviewing recent developments in this field.

Lessons from cell biology

Cells within a tissue interact with neighbouring cells and with the extracellular matrix (ECM) through biochemical and mechanical cues. Cell–cell and cell–ECM interactions establish a 3D communication network that maintains the specificity and homeostasis of the tissue[3]. Key events in the life cycle of a cell, such as proliferation, migration and apoptosis, are regulated by organizing principles (as defined by M. Bissell and collaborators) that are determined by the cellular context[4]. 3D cell cultures that re-establish such physiological cell–cell and cell–ECM interactions can mimic the specificity of real tissues better than conventional two-dimensional (2D) cultures. 3D cultures are currently used in a broad range of cell biology studies, including tumour biology, cell adhesion, cell migration and epithelial morphogenesis.

Tumour biology. A perturbation of the normal healthy microenvironment of a cell triggers epigenetic processes in the cell that contribute to neoplastic transformation. Bissell and collaborators used 3D cultures within reconstituted basement membrane (rBM, also known as Matrigel) to show the substantial role of the extracellular context in tumorigenesis. 3D rBM cultures allow distinction between malignant and normal mammary epithelial cells (MECs). Whereas tumorigenic MECs maintain their invasive phenotype and form disorganized colonies, normal MECs differentiate into polarized hollow spherical monolayers (acini) that resemble the original breast tissue[5]. Further rBM-based assays have shown that the malignant phenotype of human breast cancer cells can be reverted to a normal morphology by inhibiting β1 integrin receptors[6] or downregulating epidermal growth-factor receptors[7]. Strikingly, there is a bidirectional correlation between these two receptor classes that is not revealed by 2D culture[7]. Recent studies provide evidence that changes in the mechanical properties of the ECM (for example, an increase in stiffness) can promote neoplastic transformation by perturbing the extracellular signal-regulated kinase (ERK)/Rho mechanoregulatory circuit of breast epithelial cells[8].

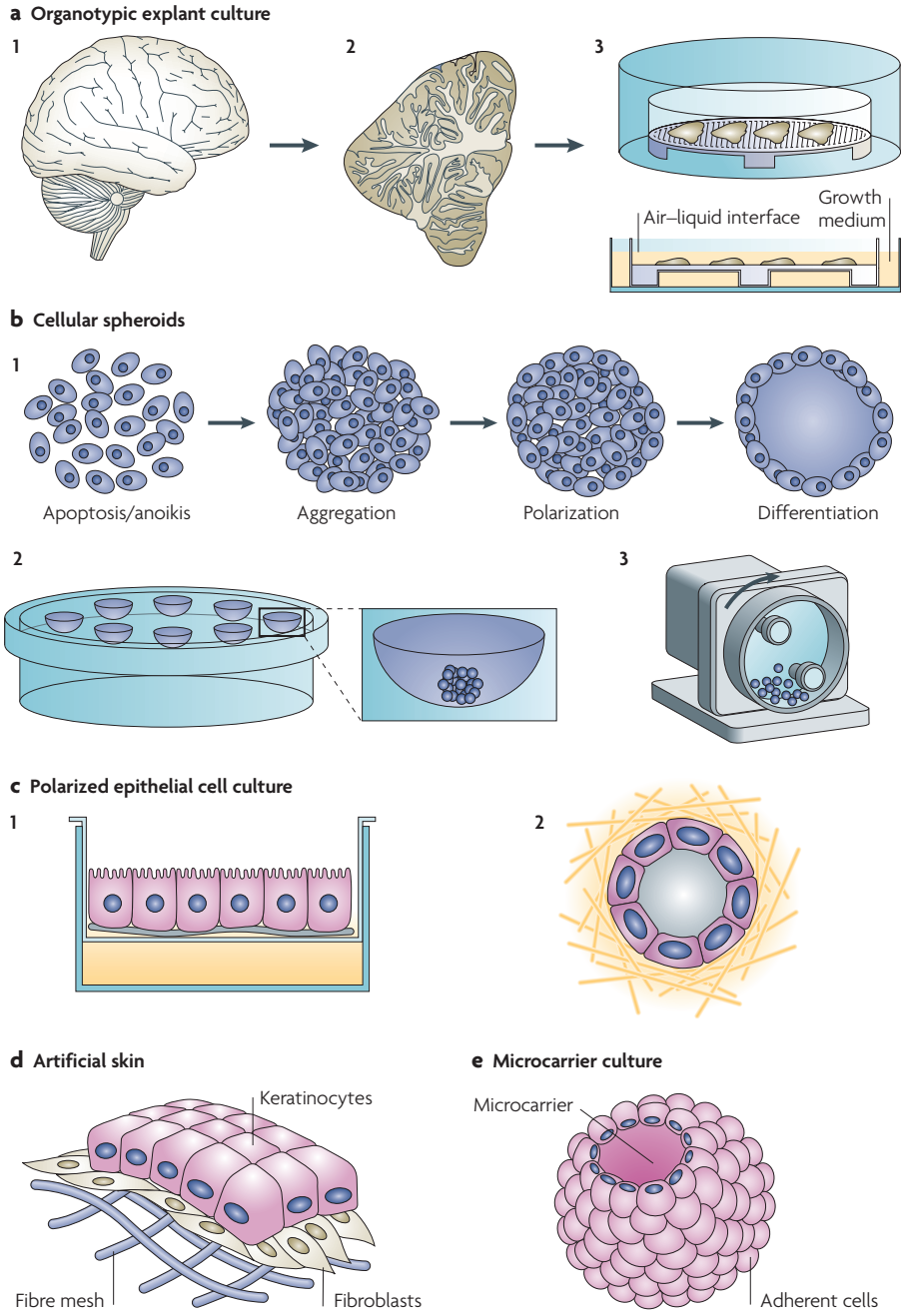

Figure 1 | **Three-dimensional culture models. a** | Organotypic explant culture. Dissected organ slices (1, 2) are placed on porous substrates, supported by a metal grid and cultured at the air–liquid growth medium interface (3). **b** | Cellular spheroids. By aggregating into large (several hundreds of micrometres) spheroids, cells can re-establish mutual contacts and specific microenvironments that allow them to express a tissue-like phenotype. Endothelial cells cluster and form a differentiated hollow monolayer spheroid (1) . Spheroids can be obtained by culturing cells at specific concentrations within a 'hanging drop' (2) or in rotating-wall vessels (3). With both methods, the cells cluster by gravity and aggregate. **c** | Polarized epithelial cell cultures. Cells grown at the air–medium interface on porous membranes form polarized monolayers (1). Three-dimensional (3D) clonal growth of Madin–Darby canine kidney (MDCK) cells in hydrated collagen gel yields spherical monolayered cysts (2), which are fully polarized (see also FIG. 3d). Their baso-lateral surface is in contact with the gel, and the apical side faces the fluid-filled internal cavity. **d** | Artificial skin. Primary fibroblasts are cultured in petri dishes and are subsequently seeded onto a biodegradable fibre mesh. After several weeks in culture, keratinocytes (for example, extracted from the foreskin) are placed onto the new dermal tissue and form an epidermal layer. **e** | Microscaled materials (microcarriers). Beads derived from dextran, gelatine, glycosaminoglycans and other porous polymers can be used as a 3D support for the culture of anchorage-dependent animal cell lines. Part **b** modified with permission from REF. 45 © (2003) Wiley.

The high plasticity of tumour cells was demonstrated by time-lapse imaging of cultures within collagen I scaffolds. Invasive cancer cells can switch from a mesenchymal (spindle-shaped cells) to an amoeboid (ellipsoid-shaped cells) motility pattern[9]. Such a process is triggered by the inhibition of integrins or of the proteolytic activity of matrix-metalloproteinases[10,11]. Both integrins and matrix-metalloproteinases are potential drug targets. These studies, therefore, highlight the ability of cancer cells to elude pharmacological treatment by changing their migratory behaviour from mesenchymal to amoeboid and suggest the development of improved therapeutic strategies.

Cell adhesion and migration. 3D cell cultures can provide deeper insights into cell adhesion and migration mechanisms. Fibroblasts that migrate on a 2D substrate have a different shape and a different distribution of transmembrane adhesion proteins compared with fibroblasts within 3D collagen[12–14]. Assays in 3D ECM cultures assess the diversity of cell migration strategies. Depending on the specific biological situation, cell migration can be of mesenchymal or amoeboid type, individual or collective (that is, clusters and multicellular sheets)[9]. Biophysical experiments have highlighted differences between 2D and 3D cell migration. On flat surfaces, the speed with which cells migrate is related to the strength of the cell-surface adhesion, as determined by integrin-dependent anchorage.

Whereas the maximum migration speed on 2D substrates is reached in regimes of intermediate adhesiveness[15], a more complex behaviour is observed inside 3D matrices. In fact, within ECM gels, the migration speed of cells in addition depends on the sterical and mechanical properties of the matrix[16]. New theoretical models include the matrix compliancy as an additional parameter and improve our understanding of the migration of tumour and healthy cells in tissues[17].

Epithelial morphogenesis. The dichotomy between 2D and 3D cell culture is highlighted by the investigation of epithelial morphogenesis[18]. Epithelial Madin–Darby canine kidney (MDCK) cells cultured in collagen I or rBM start an intrinsic differentiation programme and form spherical luminal monolayers (cysts), which resemble rudimentary kidney suborgans[18,19]. Following chemical stimulation (for example, with hepatocyte growth factor (HGF)), MDCK cysts undergo partial de-differentiation

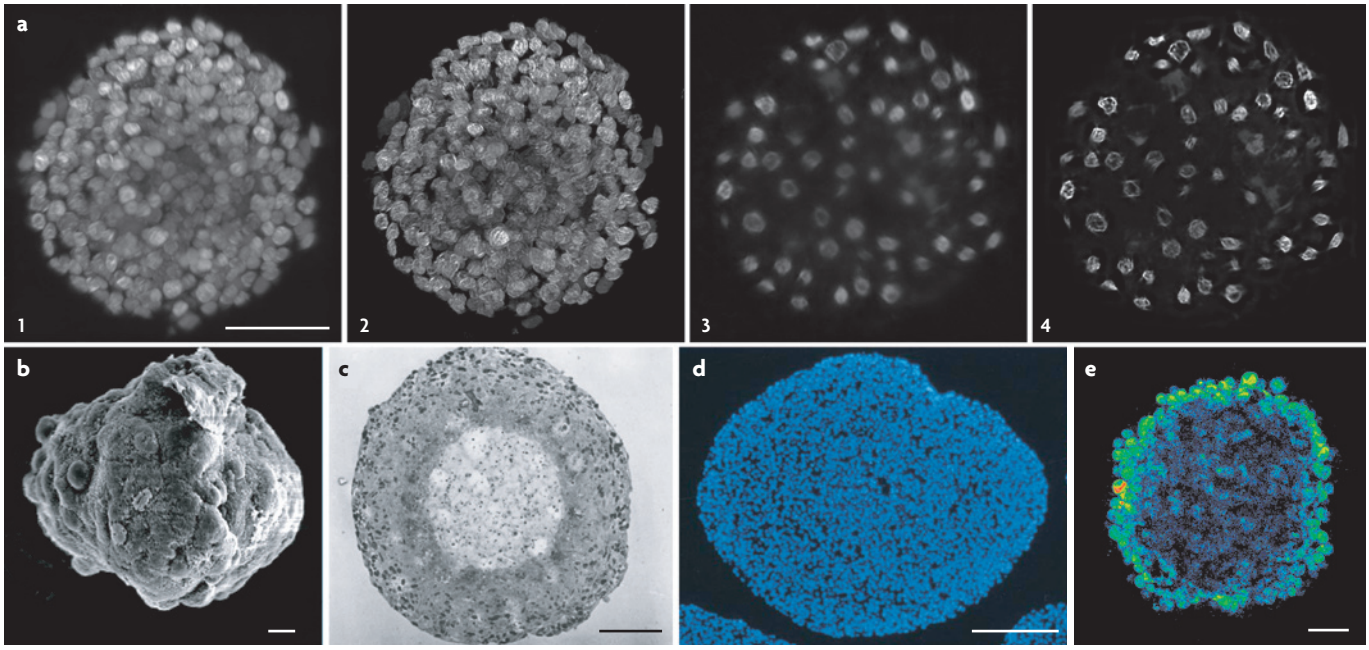

Figure 2 | **Imaging of cellular spheroids.** Examples of cellular spheroids imaged with different microscopy techniques. **a** | Light-sheet-based fluorescence microscopy. Part (1) shows the maximum projection of a stack obtained from a multiview reconstruction of twelve stacks recorded along different angles (0° to 330°) with an incremental step of 30°. Objective lens Carl Zeiss Apochromat 40×, 0.8 numerical aperture. The stack comprises 400 slices at an axial spacing of 0.5 micrometres. The nuclei were stained with Draq5 (excitation 647 nm, emission 670 nm). Part (2) shows the maximum projection of the same dataset shown in (1) after deconvolution. The subnuclear organization of chromatin is clearly visible. Part (3) shows a cross section of the spheroid. The cells in the spheroid's internal core are clearly visible. Part (4) shows the same image as in (3) after deconvolution. Data derived from REF. 65. This spheroid was formed by ~800 BXPC3 human pancreatic cancer cells cultured using the hanging-drop method. Scale bar, 50 µm. **b** | Scanning electron micrograph of human hepatoma cell line (HepG2). Scale bar, 10 µm. **c** | Electron microscopy cross-section of a V-79 chinese hamster lung cell spheroid. Scale bar, 250 µm. **d** | Immunohistochemical staining of the nuclei of a Rat1-T1 spheroid (diameter ~900 µm). Scale bar, 250 µm. **e** | Autofluorescence images of a multicellular tumour spheroid obtained with multiphoton microscopy. Scale bar, 100 µm. Part **a** courtesy of M. Marcello, A. Schrödel and M. Löhr, German Cancer Research Center, Heidelberg, Germany. Part **b** modified with permission from REF. 45 © (2003) Wiley. Part **c** modified with permission from REF. 83 © (1973) Rockefeller University Press. Part **d** modified with permission from REF. 84 © (2000) Histochemical Society. Part **e** courtesy of W. W. Webb, Cornell University, USA.

(a partial epithelial–mesenchymal transition) and develop branching tubules[20,21]. Cysts and tubules are fully polarized structures: cells have an apical surface facing the cavity lumen and a basolateral surface that contacts neighbouring cells and the ECM. Interestingly, MDCK cells that grow within the gel create their polarization cue autonomously, starting from a nearly isotropic situation. By contrast, in 2D cultures, it is the underlying substrate that provides an inherently anisotropic cue for the establishment of the polarization axis[22]. Moreover, MDCK monolayers grown on a flat substrate are only partially polarized because the nutrients have limited access to the basal side[23].

The experimental conceptual framework for the morphogenesis of MDCK cells was recently translated into a computational model. In the model, independent software representations ('software agents') of individual cells interact through axioms with their local environment, which can be an empty space, matrix or other cells. The simulation successfully reproduced the phenotypes arising from four different *in vitro* culture conditions (ECM-embedded culture, 2D monolayer, inverted cyst and collagen overlay)[24]. 3D culture of mammary epithelial cell lines (for example, the MCF-10A line) has been extensively used to investigate how the biochemical and physical properties of the ECM drive morphogenesis and homeostasis of mammary epithelial acini[4]. Studies show that apoptosis has a primary (but not an exclusive) role in acinar lumen formation by selectively clearing the cells that are located internally. Interestingly, lumen formation is not hindered by inhibiting cell death through overexpression of the anti-apoptotic protein B-cell lymphoma-2 (BCL2). Only the contemporary activation of proliferative oncogenes triggers filling of the luminal space[25]. Thus, the well-ordered architecture of epithelial organoids in 3D matrices is remarkably robust against switching off isolated regulatory mechanisms.

Gene expression. Pioneering experiments performed by Bissell and collaborators showed that the ECM affects chromatin structure, and thereby gene expression, in mammary epithelial cell lines[26]. It is now acknowledged that cells cultured in 3D have different gene expression levels compared with their 2D counterparts. Melanoma cells cultured on flat substrates upregulate and downregulate other genes compared with melanoma cells cultured in 3D as spheroids. Interestingly, the genes that are upregulated in the spheroids are also found to be upregulated in tumours[27]. Other studies show that the properties of the substrate influence the expression of integrins both at the level of mRNA and protein biosynthesis. Mammary epithelial cells that are cultured on flat plastic surfaces dramatically upregulate the expression of mRNA that codes for β1 integrins. By contrast, culturing on rBM induces expression levels of mRNA that are comparable with those in the breast tissue[28].

Box 1 | **Light-sheet-based fluorescence microscopy**

Conventional and confocal epi-fluorescence microscopy uses the same lens for both fluorescence excitation and detection. Confocal theta fluorescence microscopy[75] introduced the use of separate lenses for illumination and detection. In theta microscopes, the optical axes are arranged at an azimuthal angle of 90°, which improves the axial resolution instrumentally by a factor between five to ten and results in an almost spherical point spread function.

The wide-field implementation of the theta principle illuminates the specimen along an entire plane with a light sheet. The use of light sheets for imaging purposes has been known for more than 100 years. However, its applicability for high-resolution fluorescence light microscopy has not been realized until recently. One implementation of a light-sheet-based fluorescence microscope (LSFM) is the single plane illumination microscope (SPIM) at the European Molecular Biology Laboratory[65,80,81]. This system relies on two independent processes: excitation of a fluoropohore and observation of a fluorescence signal. Optical sectioning arises from the overlap between the focal plane of the fluorescence detection system and the central plane of the excitation light sheet. Moreover, as only the fluorophores within the light sheet are excited, the illumination causes no damage either above or below the plane of focus. Therefore, light-sheet-based fluorescence microscopes are well suited for the imaging of sensitive biological objects over long periods of time. They also perform particularly well with long-working-distance lenses. Because the numerical aperture (NA) of the illumination system is much smaller than that of the detection system, light-sheet-based microscopes have a good penetration depth. Millimetre-sized specimens can be observed in their totality. Importantly, light-sheet-based fluorescence microscopy takes advantage of state-of-the-art charge-coupled device (CCD) cameras and easily records up to 10 megapixel frames per second with a dynamic range of 10–12 bits.

A further increase of resolution and information content can be obtained by observing the same specimen multiple times but along different directions. Parts of the sample that would otherwise be hidden or obscured along one direction now become visible. In a further step, the image stacks that are independently recorded along different angles are combined to yield a single fused image stack. Interestingly, its resolution relies mainly on the lateral resolution in the original single-sided data stacks[83]. The three-dimensional resolution is therefore dominated by the much better lateral resolution and becomes isotropic along all directions.

When observing green fluorescent protein, a lens with an NA of 0.16 will provide a lateral resolution of 2 μm and an axial resolution of 7 μm (approximately six and four times better than conventional and confocal microscope, respectively), whereas an NA of 1.0 will provide a resolution of 0.3 μm and 0.9 μm, respectively. In a multiple-view recording, the axial resolution can drop to 2 μm and 0.3 μm. Because of the high signal-to-noise ratio, image processing procedures such as deconvolution work extremely well[65]. This can result in isotropic resolutions of 1.4 μm and 0.2 μm for the two objective lenses mentioned above.

Lessons from developmental biology

Cell–cell and cell–environment interactions are essential for tissue morphogenesis in developing embryos. Time-lapse analysis of 3D cell trajectories *in vivo* with confocal microscopy has provided new insights into such interactions. Large shear forces induced by blood flow are necessary for correct heart development in zebrafish[29]. *In vivo* studies on chick embryos revealed that the fate and the position of individual cells during somite boundary formation do not exclusively rely on gene expression patterns[30]. Instead, multiple guidance cues are involved, among them mechanical forces within the developing tissue. Thus, previous models that assumed strict molecular 'pre-programming' have to be revised by taking into account bidirectional communication between cells and the surrounding microenvironment.

The application of developmental biology concepts to cancer biology is providing new insights into the role of the microenvironment in cancer progression. Green fluorescent protein (GFP)-labelled invasive human melanoma cells were transplanted into the neural crest of an embryonic chick model and analysed after several days of incubation. Instead of forming a tumour, melanoma cells responded to the embryonic microenvironment and reversed to a normal phenotype, as confirmed by the detection of melanocyte and neuronal markers that were not expressed at the time of transplantation[31].

Lessons from drug screening

The need for quantitative and physiologically relevant cellular systems is particularly perceived in the drug-screening process. Currently, a drug candidate that enters Phase I trials will reach the bedside with a probability of just 8%[32]. A high failure rate and rising costs are serious challenges for pharmaceutical companies.

Cell-based assays are expected to improve the success rate at the early stages of the drug-discovery process by providing a cell-specific response, which is missing in the target-orientated approach[33]. Reproducing the phenotype of the target tissue in cultured cells is essential for obtaining reliable biomedical data[34,35]. However, monolayer cell cultures lose their tissue-related functions rapidly, seriously impairing the predictive power of such assays. For example, primary hepatocytes cultured as a monolayer become undifferentiated and die within 4 days[36]. Strikingly, the biosynthesis of drug-metabolizing enzymes, which is essential for toxicity assays in pharmaceutical research (toxic side effects are one of the most common reasons for the failure of a drug candidate), is among the first functions to be lost[36]. Studies have shown that re-establishing (at least partially) the original mechanical and chemical milieu preserves liver-specific functions for longer periods of time. This is achieved by embedding primary hepatic cells within 3D matrices such as collagen I (REF. 37), rBM[37], synthetic peptide scaffolds[38], or keeping them under a constant perfusion flow[39].

Methods for 3D cell biology

Several experimental and technical challenges have to be solved before researchers can take full advantage of 3D cell biology. The currently available 3D biological models include intact animals (both embryos and adults), organ slices and cell cultures in ECM gels. They provide tissue-specific information at different and complementary levels of complexity. Reliable methods for culturing such demanding specimens for longer periods of time are needed, as well as improved imaging techniques that can penetrate the depth of thick and highly scattering samples with minimal photodamage.

Living whole animals and organs. Embryos provide data on the behaviour of cells in their original physiological environment. Culture of fruitfly, medaka (or Japanese killifish) and zebrafish embryos *in vitro* allows some flexibility in the setting of environmental parameters. By contrast, mammalian embryos (for example, mouse embryos) require tightly controlled temperature and oxygenation to maintain their viability during an experiment. Static culture systems that are suitable for time-lapse imaging of pre- and post-implantation embryos have been developed[40].

Organ explant slices can be cultured on a semiporous membrane (FIG. 1a) or embedded in 3D collagen gel[41]. Organotypic slice culture preserves the cytoarchitecture and the cellular differentiation of the original tissue. Applications include the study of brain physiology[42] and ureteric bud branching morphogenesis[43].

3D cell cultures. The heterogeneity and low transparency of whole animals and organs is challenging for imaging and data collection. 3D cell cultures represent a simplified reductionist model.

Cellular spheroids are simple 3D systems, which take advantage of the natural tendency of many cell types to aggregate (FIG. 1b). Spheroids from a broad range of cell types are produced by the hanging-drop technique[44,45] or by using rotating-wall vessel cultures[46] (FIG. 1b). Spheroids can be obtained from single cultures or co-cultures (mono- or multicellular spheroids, respectively). The most obvious advantage of spheroids is that they do not require external scaffolds to aggregate. Cellular spheroids are the system of choice for therapeutically orientated biomedical studies[47–49]. They have applications in biotechnology[50] and are straightforward to apply in high-throughput screens[51,52]. The simple spherical geometry allows for relatively easy modelling of dynamic processes, such as growth and invasiveness of solid tumours[53,54].

Models of polarized epithelial tissues are obtained by culturing non-transformed immortalized epithelial cell lines under 3D conditions. Examples are the aforementioned MDCK cells or the mammary epithelial MCF-10A cells. These cells grow as polarized monolayers on microporous membranes[55] (FIG. 1c), or as hollow spherical monolayers within ECM gels[23] (FIG. 1c). The tissue counterparts of these suborgan structures are common to most of the epithelial organs and are known as 'acini' in mammary tissue as well as lungs and 'tubules' in the kidney. More complex epithelial structures, such as the human skin, have been developed from 3D cultures on membrane inserts or by using microscale materials such as supporting fibre meshs[56] (FIG. 1d).

A systematic collection of methods that helps scientists in the transition from monolayer cultures to 3D systems (that is, a 3D cell biology blueprint[57]) is still lacking, despite the recent publication of detailed protocols[58,59]. A closer collaboration between tissue engineers and cell biologists is required to fill this gap[57].

Large cellular aggregates require a careful control of the gas exchange as well as the diffusion of soluble nutrients and chemical agents[57]. Biochemical analysis is complicated by the lower cell density and the additional steps required to separate the cells from the matrix[60]. Because most of the currently available ECM gels are extracted from animals or cultured cells, quality control is difficult. For example, the amount of undesired soluble components varies between batches, which reduces the reliability and reproducibility of the assay. Progress is achieved with fully synthetic fibrous biopolymer scaffolds, and gels of self-assembling synthetic oligopeptides are now available for 3D cell cultures (for example, the commercially available Puramatrix). At pH and temperature conditions that are compatible with that of tissue culture, the oligopeptide building blocks form a well-defined scaffold made of nanometre-sized fibres. Nanometre-sized fibres and pores are essential to ensure a true 3D environment for the cell[61,62]. A further advantage is that such gels can be custom-tailored with specific amino-acid sequences that are recognized by the cell's adhesion receptors[63].

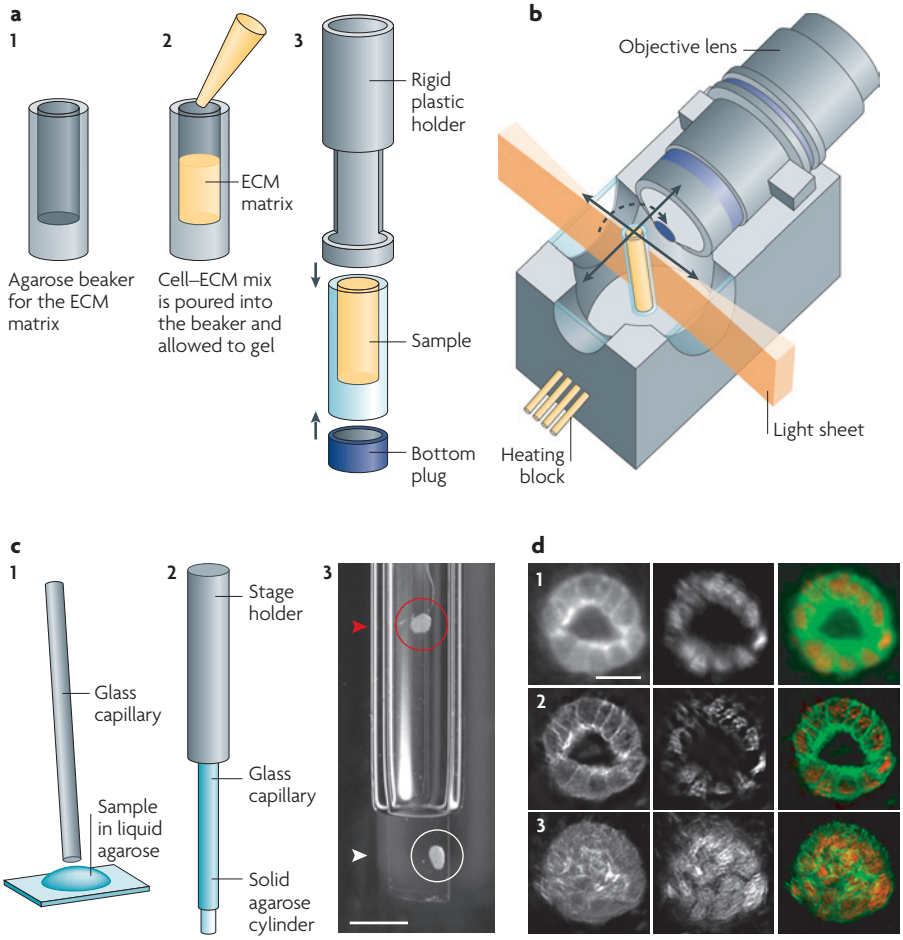

Figure 3 | **Three-dimensional cell biology with light-sheet-based fluorescence microscopy.** **a** | Preparation of a stable, optically clear agarose beaker for three-dimensional (3D) cell cultures. Liquid agarose is poured into a template and forms a small beaker (1). A cell–ECM-gel mix is deposited into the beaker (2). A plastic holder ensures stability for time-lapse studies (3). **b** | Autoclavable cell culture chamber for SPIM. An integrated heater maintains the temperature at 37 ± 0.1°C. The objective lens is immersed into the medium. **c** | Living specimens are embedded in agarose for multiview imaging. The specimen is placed into a droplet of liquid agarose and sucked into a glass capillary (1). As soon as the agarose has hardened, the sample is pushed out of the capillary and imaged (2). Image of a real specimen (cellular spheroid; white circle) (3). A second sample within the capillary is visible (red circle). The white arrow points to the boundary of the agarose. The red arrow indicates the boundary of the capillary. Scale bar, 1 mm. **d** | MDCK cells that express green fluorescent protein-tagged actin were cultured in a 3D collagen I gel for ~3 days and formed small cysts. An isolated cyst embedded in agarose according to (**c**) was imaged with a Carl Zeiss Apochromat 40×, 0.8 NA water immersion lens. Six views of the cyst were recorded and subsequently fused into a higher-resolution image. (1) Left to right: cross section of the cyst, nuclei stained with Draq5 and the two channels merged. (2) Deconvolution of (1). (3) Maximum projections of the deconvolved stacks. Left to right: actin, nuclei and the two channels merged. Scale bar, 20 µm. ECM, extracellular matrix; MDCK, Madin–Darby canine kidney; NA, numerical aperture; SPIM, single plane illumination microscopy.

Imaging methods for 3D cell biology. A dynamic quantitative analysis of 3D biological structures requires an excellent signal-to-noise ratio, optical sectioning capability (that is, a real, as opposed to an estimated, resolution along the optical axis), large field of view, good spatial resolution, a fast image stack recording rate and a low sample fluorophore excitation level[64]. Currently, confocal fluorescence microscopy represents the commercially available state-of-the-art advanced imaging of moderately thick specimens. However, as in conventional wide-field epifluorescence, in confocal imaging the excitation light illuminates the entire object and extends photobleaching and phototoxic effects to all planes. A further shortcoming of confocal fluorescence microscopy is the limited penetration depth, when using high numerical aperture lenses. These two limitations prevent a systematic application to *in toto* studies of large 3D samples[65].

Two-photon and multiphoton microscopy seem to be attractive when observing large specimens, mainly because of their twofold penetration depth compared with confocal microscopy[66,67]. Multiphoton microscopy is often regarded as the technique of choice for imaging 3D samples[40,68,69]. However, it allows only a low resolution[70] and requires relatively large light intensities that cause bleaching and phototoxic effects in the focal plane.

Tomographic techniques, such as optical coherence tomography (OCT)[71] and optical projection tomography (OPT)[72], were specifically developed to image large 3D samples such as organs[73], tumour spheroids[74] and developing embryos[64]. In the tomographic approach, an object is imaged along multiple angles and the different views are merged into one final dataset. Cross-correlation of the single views allows the reconstruction of an isotropic image at an improved resolution. Even though OPT and OCT work well with large samples, they have limitations in terms of spatial resolution and recording rate.

In recent years, significant efforts have been devoted to enhancing the spatial resolution of optical microscopy. Confocal theta fluorescence microscopy[75], 4Pi-confocal[76], I^5M (REF. 77) and stimulated emission depletion (STED)[78,79] fluorescence microscopy are impressive when resolving subcellular molecular distributions in fixed cells. However, apart from theta microscopy, these techniques are less suitable for imaging highly scattering, living samples that are several hundreds of micrometres thick, such as spheroids (FIG. 2) or cells cultured in ECM matrices, as they rely on the preservation of the phase relationship that is required for the generation of well-defined interference patterns.

Progress in optical microscopy has to overcome the challenges that prevent high-resolution imaging of thick and highly scattering 3D samples. In this respect, a new implementation of light sheet-based fluorescence microscopy — single plane illumination microscopy (SPIM; BOX 1, FIG. 3)[65,80,81], which combines fluorescence optical sectioning with some aspects of tomography — is a promising approach.

> “Progress in optical microscopy has to overcome the challenges that prevent high-resolution imaging of thick and highly scattering 3D samples.”

Concluding remarks

Owing to their physiological relevance, 3D cellular models have the potential to become a fundamental research tool in cell biology. They will certainly foster the integration process of data from genomics, proteomics and molecular cell biology. This will result in models of tissues and organisms with enhanced predictive power. Most importantly, drug discovery and clinical research should be able to take advantage of the third dimension by generating physiologically relevant screening assays. Large-scale toxicity screenings like the recently started ‘registration, evaluation and authorization of chemicals’ programme of the European Union (REACH) will require thousands of laboratory animals. Organotypic 3D cell cultures could provide an alternative to animal models. This is attractive for both ethical and economical reasons.

Recent advances in imaging technology and establishment of standard protocols address some of the obstacles that have prevented the observation of live cells that grow in a more natural physiological 3D environment. Certainly, a big challenge for the next decade in life sciences will be the further exploitation of the third dimension.

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Competing interests statement

The authors declare competing financial interests.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BCL2

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