



Label-free technology and patient cells: from early drug development to precision medicine

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Drug development requires physiologically more appropriate model systems and assays to increase understanding of drug action and pathological processes in individual humans. Specifically, patient-derived cells offer great opportunities as representative cellular model systems. Moreover, with novel label-free cellular assays, it is often possible to investigate complex biological processes in their native environment. Combining these two offers distinct opportunities for increasing physiological relevance. Here, we review impedance-based label-free technologies in the context of patient samples, focusing on commonly used cell types, including fibroblasts, blood components, and stem cells. Applications extend as far as tissue-on-a-chip models. Thus, applying label-free technologies to patient samples can produce highly biorelevant data and, with them, unique opportunities for drug development and precision medicine.

Introduction

Two significant challenges to current drug development are the interindividual variability in drug effectiveness, and lack of translatability of preclinical results. Simultaneously, modern medicine is shifting towards personalized or precision medicine, which proposes to use individual characteristics of a specific patient or subpopulation to tailor drug prescriptions, thereby decreasing risks of ineffective dosing or adverse effects [1]. Challenges to achieve this are associated with a generally perceived lack of understanding of the molecular details of drug action and of pathological processes in the human individual. This is brought about to a large degree by insufficient physiological representability of the model systems and assays used in drug research. Traditional drug research has relied on a target-focused approach by screening compounds in *in vitro* assays. Such assays traditionally use reporter systems, for instance radiolabeled or fluorescent probes, dyes, and reporter gene constructs, all of which are modifications that can influence target pharmacology (Box 1). In addition, cellular models and cell systems are often selected based on habit and technical feasibility rather than on disease relevance,

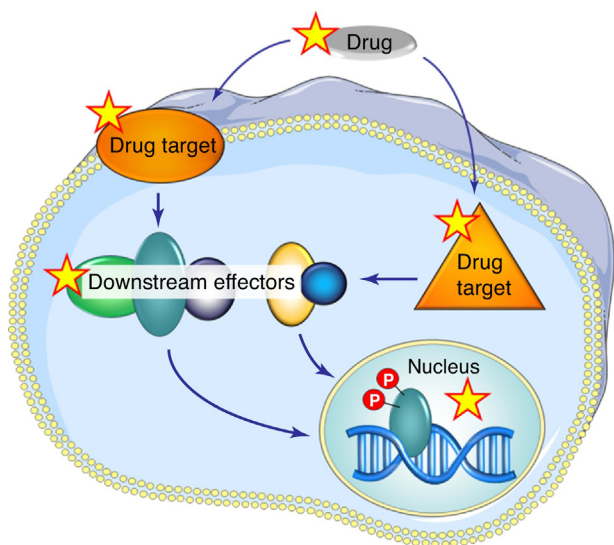
resulting in physiologically less representative heterologous or recombinant cell lines. Such renewable *in vitro* cell sources have been essential in facilitating drug discovery and have merits for studying target or drug action in more detail. However, both assay and model systems are factors that can contribute to an eventual lack of clinical effectiveness and, thus, to issues experienced in drug development to date, such as high attrition rates [2]. To fully comprehend the mechanisms underlying pathologies, drug response, and its variation in individuals, functional characterization on a physiologically relevant molecular and cellular level is essential. Hence, the focus is shifting to more physiologically appropriate cellular models and readout systems. Specifically, patient-derived cells offer great opportunities when used directly as a model system. Novel label-free cellular assays are a new type of phenotypic assay that can result in molecular-level understanding of complex biological processes in their native environment [1,2]. Applying such assays to human primary cells can increase the physiological relevance of the results [3–5]. In this review, we highlight the reach of these possibilities by focusing on the application of one such label-free cellular assay, based on impedance, to some of the most common types of human primary cells derived from patient samples.

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BOX 1

Traditional label-based versus label-free assays

Traditional label-based assays follow drug effects and cellular functions by the chemical attachment of a 'label' to the drug molecule, drug target, or downstream effectors. These can comprise, for instance, radiolabeled or fluorescent probes or dyes. Reporter-based assays introduce specifically regulated gene promoters as biomarkers for specific events. Commonly used reporter genes involve visually identifiable characteristics, such as fluorescent and luminescent proteins (Figure 1). Label-free assays do not require any such modifications because they measure cellular changes by alternative detection means, without the need to introduce chemical or bioengineered modifications.



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FIGURE 1

Traditional label-based assays. Stars highlight where effects are often measured by introducing labels or reporters. Image constructed using components from Servier Medical Art by Servier (www.servier.com/Powerpoint-image-bank).

Advantages of label-free cellular assay technologies

The two currently most-used forms of label-free cellular biosensors are impedance- or optics-based biosensors. Extensive reviews on the detection principles are provided elsewhere [6–8]. In short, the ECIS, xCELLigence, and CellKey systems use an electrode array biosensor to measure impedance changes in a cell monolayer (Fig. 1). Optical systems, such as the EPIC and BIND, use resonant waveguide grating to detect dynamic mass redistribution in cells. Both optical and impedance methods detect a spectrum of cellular changes, from cell adhesion to life cycle processes, such as proliferation, growth, and death; as well as pathogen infections and response; cell migration; and signaling, such as receptor signaling or cell–cell communication [6]. Hence, these label-free assays are also known as phenotypic assays.

In this review, we focus on impedance-based assays, which are applicable to a range of samples, are highly versatile and can integrate many assays into one (Fig. 2). For instance, such assays

record a variety of cellular parameters, including proliferation, adhesion, and cellular morphology, in one combined read-out in real-time (Fig. 1 and Fig. 2a). This is a particular advantage over many traditional assays, which often interrogate one aspect only of a given pathway or a cellular response (e.g., second messenger accumulation). Impedance-based assays offer the distinct advantage of a direct read-out of drug action in real-time. Although there are also traditional assays that record specific functions in real-time (e.g., Ca^{2+} -mobilization assays), impedance measurements offer the benefits of real-time measurements in both acute (e.g., direct receptor signaling) and chronic settings (e.g., cellular proliferation). Besides recording the abovementioned cellular functions, impedance-based label-free assays also provide some specialist applications, such as electrical stimulation for pore formation (Fig. 2d) and co-culture without contact (Fig. 2h), although these can require specialized recording or plate equipment (Fig. 2b,e,h). Overall, impedance-based assays have already been successfully applied to an extensive list of targets, including important drug target classes, such as G-protein-coupled receptors (GPCRs) [6,9], nuclear receptors [10], and receptor tyrosine kinases [11]. Applications extend as far as toxicity screens on cardiac function [12] and migration of cancer cells in 3D cultures [13] (Fig. 2b,e). Furthermore, almost any cell type can be studied. Examples include standard recombinant cell lines, primary and stem cells, both adherent as well as suspension cell types [6,9,14] (Table 1). This is because, in comparison to many traditional assays, label-free technologies offer a sensitive, less-invasive detection methodology that monitors drug effects on a whole cell. Furthermore, without the need for tagging, labeling, or recombinant expression, cellular functions can be studied in a more physiological context, including a vast amount of endogenously expressed targets and pathways. Simultaneously, sensitivity is often high enough to distinguish subtle changes in mechanisms of action in, for example, GPCR signaling bias [6,14]. Receptors are linked to various downstream signaling pathways, a feature termed 'signaling pluridimensionality'. Ligands can be biased towards one or some particular downstream pathways, potentially resulting in different pharmacological effects. For instance, closely related agonists of the β_2 -adrenergic receptor induce subtly yet distinctly different response signatures as a consequence of such bias [15,16].

Hence, as several reviews have already summarized, label-free technologies can offer distinct advantages for drug development. They capture compound action in a dynamic time-resolved manner, allow for the identification of leads independent of prior assumptions of signaling pathways, and enable the use of more-native systems at higher throughput. As a cell phenotypic screen, they can be used for target identification, compound screening, lead selection, investigating the mechanism of action, and testing drug safety and toxicity [14,17]. In this review, we particularly focus on applications involving patient cells. This offers opportunities for both drug development and precision medicine research by sensitively detecting an extensive variety of pharmacological effects under minimally invasive conditions in a clinically relevant endogenous context of primary cells, and even patient samples. Nowadays, such samples are increasingly available to support research, for instance by their systematic collection in biobanks.

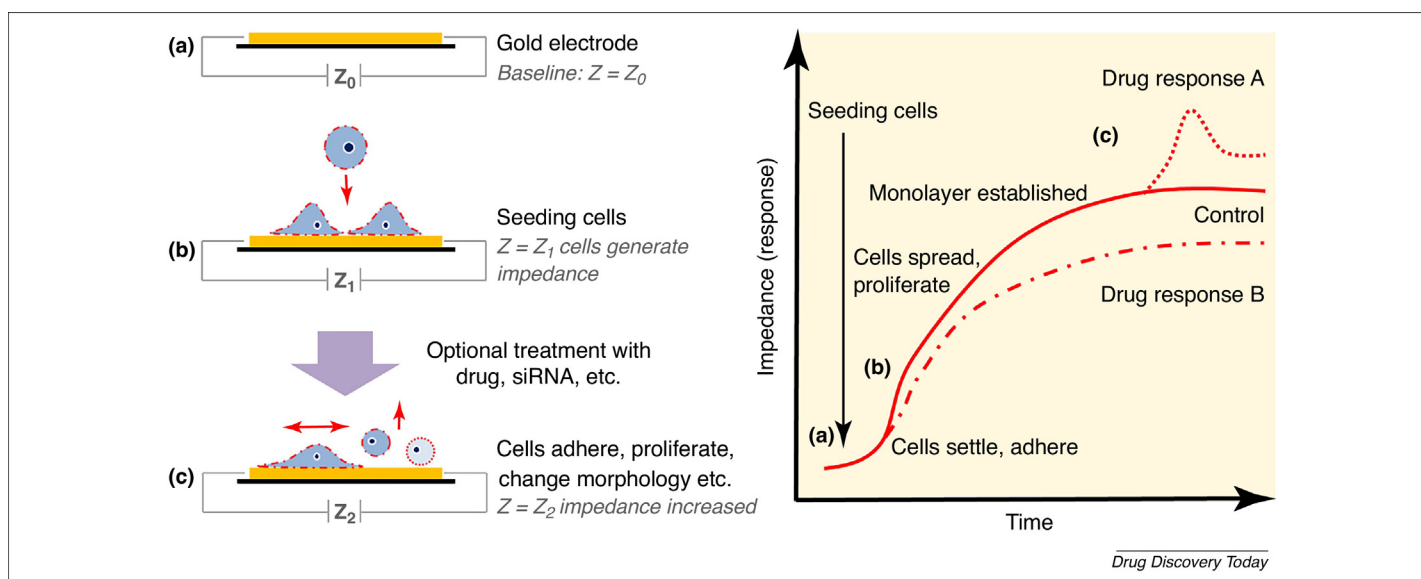


FIGURE 1

Principle of impedance-based label-free cellular assays. Cell attachment to gold electrodes generates impedance by changing the local ionic environment at the electrode–solution interface. Relative changes in impedance (Z) are recorded in real-time. (a) Before the seeding of cells, baseline impedance is Z_0 . (b) As cells adhere to the electrodes, impedance increases proportionally. (c) Changes in cell number, adhesion, viability, and morphology are directly reflected in the impedance profile. Impedance-based label-free cellular assays can detect a range of cellular events, including cell proliferation, division, growth, death, migration, and signaling. All these parameters can, in turn, be affected by drugs. For instance, depending on the moment of drug treatment, drugs can result in response A by initiating receptor signaling or drug response B by decreasing overall proliferation.

Advantages of primary human cells

Over the past decades, numerous biobanks have emerged to support medical research by the programmed storage of biological material and corresponding data. These biomaterials include tissues, (stem) cells, blood, and serum, all of which have had a critical role in medical research. These materials are actively used from translational and personalized medicine research to target and drug discovery [18,19]. For human physiology, primary

human cells are considered a better model system than the more traditional cellular models, such as rodent, recombinant, or immortalized nontissue specific human cell lines, and even better than *in vivo* rodent models [20–22]. Although the abovementioned cellular models have merits, for instance ease of use or to attain initial understanding of pathways, their physiological relevance is questioned increasingly. In recombinant cell lines, target overexpression, differences in intracellular metabolic

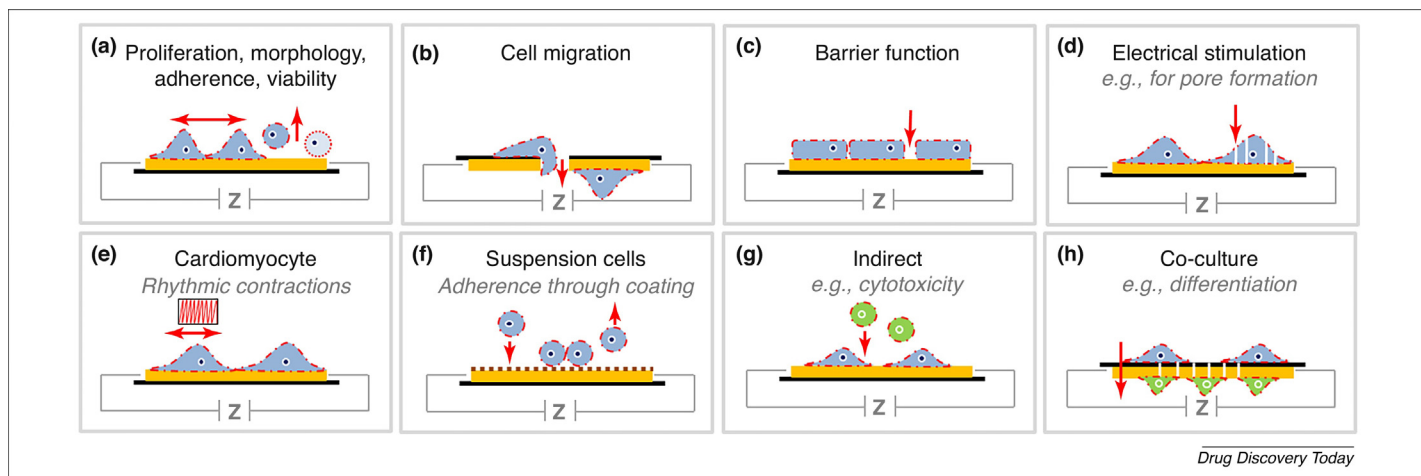


FIGURE 2

Typical applications of impedance-based label-free cellular assays. (a) General label-free cellular assay formats are capable of monitoring many cellular functions, such as adherence, proliferation, viability, and morphology. Additional specialized assay applications exist, for instance, to (b) monitor cell migration (e.g., through a porous membrane, xCELLigence); (c) measure barrier functionality, for instance, in a wound scratch assay; (d) apply electrical impulses, for example, to increase cellular permeability (ECIS); and (e) measure (cardio-)myocyte contractility (xCELLigence CARDIO system). (f) Besides adherent cells, label-free cellular assays are also applicable to suspension cells and capable of monitoring interactions between two cell types, for instance by (g) cytotoxicity of effector cells on another type of target cell or (h) cell–cell communication without any cellular contact (xCELLigence co-culture set-up).

TABLE 1

Application examples of impedance-based label-free cellular technology to patient samples and stem cell related types

Type	Subtype	Technology	Material source	Refs	
Blood components	Antibodies	xCELLigence	Patients with type I diabetes or type II diabetes and healthy controls	[37]	
	PBMCs	xCELLigence	From healthy volunteers but tested on patient material	[32,33]	
	Plasma and cells therein	ECIS	Healthy volunteers versus trauma patients	[35]	
		ECIS	Patients with Hantavirus cardiopulmonary syndrome	[36]	
	Monocytes	ECIS	Patients with peripheral vascular disease and abdominal aortic aneurysm	[31]	
		Neutrophils	ECIS	Critically ill patients with sepsis	[34]
	Cancer cells and related cells	Serum	ECIS	Patients with scleroderma	[27]
		$\gamma\delta$ T cells	xCELLigence	Healthy volunteers and patients with B-cell ALL	[60]
		Glioblastoma cells	xCELLigence	Paired tumoral and peritumoral tissue samples from patients with glioblastoma	[54]
			xCELLigence	Malignant melanoma of the ciliary body from a female patient	[55]
Malignant pleural effusions		xCELLigence	Patients with solid tumors	[59]	
Mesenchymal chondrosarcoma cells		xCELLigence	Newly established cell line from patient	[56]	
Mononuclear cells		xCELLigence	Normal controls and patients with breast cancer	[61]	
Myxofibrosarcoma cells		xCELLigence	Patient with myxofibrosarcoma	[58]	
Non-small cell lung carcinoma cells		xCELLigence	Patient with non-small cell lung carcinoma	[57]	
Normal and neoplastic mammary cells		xCELLigence	Patient-derived primary human breast cancer epithelial cells	[8]	
Ovarian cancer cells	xCELLigence	Patient with serous ovarian cancer and patient with endometrioid peritoneal cancer	[53]		
Chondrocytes	Chondrocytes and cartilage tissue	xCELLigence	Patients with OA	[32]	
	Fibroblasts	Benign prostatic hyperplasia	xCELLigence	Patients with benign prostatic hyperplasia	[26]
		Dermal	ECIS	Patients with scleroderma and normal controls	[27]
		Orbital	ECIS	Patients with or without Graves' disease	[24]
		Synovial	xCELLigence	Patients with RA or OA	[28–30]
iPSCs and similar stem cell types	Adipose stromal/stem cells	ECIS	Healthy human donors of varying age groups	[51]	
		xCELLigence	Female patients undergoing liposuction, model for obesity	[52]	
	iPSC cardiomyocytes	xCELLigence	Healthy human donors or commercial from Cellular Dynamics ^a	[12,43–45]	
	iPSC retinal pigment epithelium	ECIS	Patient with AMD and unaffected sibling	[47]	
	Mesenchymal stromal/stem cells	ECIS and xCELLigence	From bone marrow (three donors) and adipose tissue (two donors)	[48]	
		xCELLigence	From endometrial lining of the uterus of premenopausal women	[49]	
		xCELLigence	Healthy human donors	[50]	
Myoblasts	Skeletal muscle myoblasts and myotubes	xCELLigence	Patients with OA	[33]	
		xCELLigence	Patients with chronic heart failure and age- and gender-matched healthy donors	[62,63]	
		xCELLigence			

^a www.cellulardynamics.com/products/cardiomyocytes.html.

conditions, and products from other genes can modify cellular responses [5]. Well-established cell lines derived from a patient with a particular disease can be more representative of that specific pathological condition. However, these are generally immortalized cell lines derived from one particular patient sample a long time ago. Prolonged cell culture frequently leads to problems, such as contamination or genotypic and phenotypic instability. These issues unfortunately contribute to irreproducibility in preclinical research, which is an increasingly well-recognized problem [23].

In general, primary cells express signaling pathways and retain many cellular functions that are seen *in vivo*, thus providing a more relevant context. Tissue or cell samples from healthy or patient volunteers are even more representative of (patho)physiology and closer to the situation in the clinic.

Application to patient samples and primary human cells

Many patient-related biomaterials can and have already been studied using impedance-based label-free technologies, of which some prominent examples are discussed here. The sample types most commonly studied include fibroblasts and blood

components, but applications also extend to endothelial, epithelial, and stem cells (Table 1). In these examples, label-free impedance-based assays are used to monitor a range of cellular effects, including specific functions, such as migration, epithelial barrier function, or cardiomyocyte beating (Fig. 2). Overall, the highlighted examples show that impedance-based label-free technology is highly versatile with an extensive range of applications.

Fibroblasts

The earliest applications of label-free assays to fibroblasts date back more than two decades. In one early example, by comparing morphological changes of orbital fibroblasts from patients with and without Graves' disease versus dermal fibroblasts, prostaglandin E2 was shown to have a significant role in Graves' disease pathology (Fig. 2a). The authors chose ECIS over traditional light microscopy after testing both methodologies head to head, because ECIS offered insight into the subtle, rapid cellular changes, especially into the underlying kinetics, of this disease [24].

Since then, label-free cellular assays have been applied to other types of fibroblast. Fibroblasts are in fact the most common cell type in human connective tissue and can often retain memory of

their previous tissue context, thus giving rise to numerous fibroblast types (Table 1). They are also among the most commonly used clinical and biobanked samples [25].

For instance, Nolte *et al.* demonstrated a potential strategy against hyperproliferating fibroblasts by treating fibroblasts from patients with benign prostatic hyperplasia with small interfering (si)RNA against the transcription factor serum response factor. Effects on cell proliferation and growth inhibition were detected with the xCELLigence (Fig. 2a) [26]. Another notable study involved dermal fibroblasts and sera from patients with scleroderma, which is discussed below [27].

Finally, in a clinically relevant setting, synovial fibroblasts from patients with rheumatoid arthritis (RA) or osteoarthritis (OA) obtained during knee surgery were investigated. In the most recent studies, Lowin *et al.* used xCELLigence to show that the endocannabinoid system is involved in regulating inflammatory effects in RA [28]. This suggested a potential treatment for RA with synthetic cannabinoids, demonstrated in a later study [29]. Similar studies showed further contributors to the pathogenesis of RA that modify the cellular functions and adhesion of synovial fibroblasts, the most recent of which are included in Table 1 [30]. The relevance and implications of these findings for potential treatment options are of translational value because the cells were obtained from patients with the disease.

Blood cells

Blood is an easily obtained patient material and, thus, is often biobanked [25]. Hence, various types of blood components or cells are used in medical research and have been investigated using impedance-based label-free cellular assays.

Several studies involving monocytes have been published. Interestingly, monocytes are often measured indirectly by quantifying their effect on another cell type. A layer of adherent target cells is grown on the electrodes, after which they are exposed to the effector cells, here monocytes, which induce cytotoxicity in the target cells, for instance (Fig. 2g). Lee *et al.* used ECIS to reveal differences between patients with peripheral vascular disease and with abdominal aortic aneurysm to find better methods for targeted therapy. Monocytes of patients with peripheral vascular disease induced higher endothelial barrier dysfunction [31] compared with those from patients with abdominal aortic aneurysms.

Another particularly useful type of blood cell are peripheral blood-derived mononuclear cells (PBMCs). Hopper *et al.* showed that PBMCs enhanced osteoarthritic human chondrocyte migration, which could be the basis for a treatment strategy for OA. PBMCs were derived from healthy volunteers, whereas chondrocytes and cartilage tissue explants were from patients undergoing total knee replacement. Here, the migration and chemokinetic potential of the cells were measured using a specialized migration assay format of the xCELLigence (Fig. 2b) [32]. Later, it was shown that PBMCs also enhanced the migration and chondrogenic differentiation of multipotent mesenchymal stromal cells (MSCs) from knees of patients with OA [33].

Other types of blood component have also been assayed using label-free technology, although most studies again relied on an indirect measurement through effects on another cell type. For instance, neutrophils from critically ill patients with sepsis were found to reduce endothelial barrier integrity to a greater extent

than untreated normal neutrophils in an ECIS assay [34]. Human serum was also used in some studies. In an early example by Huang *et al.*, ECIS was used to demonstrate differences in micromotions of dermal fibroblasts from patients with scleroderma and from normal controls, as well as the effect of sera from patients on fibroblast behavior [27]. Rahbar *et al.* measured the effects of plasma samples from healthy volunteers and severely injured trauma patients on human endothelial cells using ECIS. Material of patients with low plasma colloid osmotic pressure caused an increase in cell permeability [35]. In a similar manner, plasma samples of patients with Hantavirus cardiopulmonary syndrome were shown to induce the loss of cell–cell adhesion in epithelial and endothelial cells in ECIS [36]. Finally, Jackson *et al.* used xCELLigence to demonstrate that anticalcium channel autoantibodies from patients with type 1 diabetes mellitus inhibited the adherence of Rat insulinoma cells, while antibodies from patients with type 2 diabetes mellitus and from healthy controls did not [37].

The reason why all these blood components are measured indirectly is twofold. On the one hand, studying their effect on the function of other cell types provides more physiological context. On the other hand, many of the cell types involved are suspension cells. Label-free technology was long deemed incompatible with suspension cells, because the detection mechanism positioned at the bottom of the well requires cells to adhere [7]. However, several studies demonstrated that suspension cells are also amenable to label-free technologies using either optical or impedance-based biosensors. Interestingly, impedance-based assays appear less susceptible to decreased cellular adherence than do optical biosensors [7] and, hence, are potentially applicable to a broader range of cell types. Examples include various types of blood cell, one notably involving personal cell lines. For instance, CellKey was used to directly measure GPCR signaling in monocytes, neutrophils, and PBMCs, although these were not patient material [38,39]. xCELLigence was applied to lymphoblastoid cell lines (LCLs) from participants of The Netherlands Twin Register to show effects of single nucleotide polymorphisms on GPCR signaling [9,40]. On these occasions, increased cell densities and usage of adherence-mediating agents were sufficient to allow measurements (Fig. 2f). LCLs are a preferred choice for storing genetic material, including in biobanks of renowned consortia, such as the International HapMap project [25,41].

iPSC and common stem cell types

Stem cells carry great promise for rendering physiologically more relevant cell models, in particular induced pluripotent stem cells (iPSCs). By reprogramming of fibroblasts into a pluripotent state, for example, iPSCs can be derived that maintain the disease genotype and phenotype indefinitely. These iPSCs then provide a source of models for an expansive range of adult differentiated cells, possibly even for each individual patient, which has the potential to personalize drug discovery [42]. Many of the cell types derived from such iPSCs can be investigated using label-free technology. A specific type of application has been developed for xCELLigence, namely a cardiomyocyte-based biosensor. Safety pharmacology studies that evaluate potential cardiac (adverse) effects of drug candidates are an essential part of drug development. The xCELLigence RTCA Cardio System detects the beating rhythm of cardiomyocytes (Fig. 2e) and has been applied to

human iPSC-derived cardiomyocytes (hiPS-CMs) on several occasions to investigate risks of drug-induced arrhythmia and general cardiotoxicity, of which the most recent publications are listed in Table 1 [12,43–45]. Rhythmic beating is essential for cardiomyocyte function, but has traditionally been hard to investigate in simple *in vitro* assays. Phenotypic measurements of native cellular systems are more suited for this [46]. The xCELLigence Cardio System capturing cardiac beating was the most sensitive of various tests for detecting compounds with known clinical cardiac risk [43], and can be used to evaluate potential clinical drug candidates [12].

Another stem cell-based study involved iPSC-derived retinal pigment epithelium (RPE) as a disease model-on-a-chip of age-related macular degeneration (AMD). In general, epithelial and endothelial cells are often studied using label-free technology, and some specific assay formats related to formation and disruption of monolayers have been developed for these (e.g., barrier function, Fig. 2c). Here, RPE cells from a patient with inherited AMD and an unaffected sibling were examined using an ECIS electrical wound-healing assay. Real-time monitoring over a 25-day period demonstrated the establishment and maturation of RPE layers on the microelectrode arrays, in which spatially controlled damage to the cell layer was introduced to mimic AMD. Thus, label-free technology can also be used to measure long-term effects and is suited for tissue-on-a-chip technology. This offers translational value by enabling real-time, quantitative, and reproducible patient-specific studies [47].

Another stem cell type of interest are MSCs, which are attractive candidates for tissue engineering because of their wide mesodermal differentiation potential. Angstmann *et al.* compared ECIS and xCELLigence in a search for standardized quality control assays to monitor differentiation and high-throughput screening that is both non-invasive and time-resolved. The authors studied MSCs isolated from two different tissues of various donors, namely bone marrow and adipose tissue. Impedance measurements were used to discriminate osteogenic from adipogenic differentiation, which showed modulating effects of extracellular matrix components [48]. Label-free assays were also used to establish culture conditions for expansion of endometrial MSC (eMSC) isolated from endometrial lining of the uterus of premenopausal women [49] or to test MSC labeling by a new type of nanoparticle [50].

In another instance, ECIS was used to monitor proliferation and osteogenic differentiation of human adipose stem cells (hASC) from donor populations of different ages. This assay could be used to predict the osteogenic potential for patient-specific bone tissue engineering [51]. Finally, Berger *et al.* studied molecular mechanisms in human obesity in hASCs from liposuctions of female patients. By studying lipid uptake and adipocyte differentiation with xCELLigence, the authors identified several dysregulated adipocyte-specific genes involved in fatty acid storage or cell adhesion [52].

Other cell types

Label-free assays are suited for almost any cell type and have been applied to numerous others besides the most commonly bio-banked samples highlighted above.

A further category of particular interest are cancer and related cell types. Here, impedance-based cellular assays are often used to

measure migratory and invasive properties (e.g., Fig. 2b), which are key characteristics of any (metastatic) cancer type. For instance, xCELLigence was used to monitor the motility of primary human normal mammary cells versus patient-derived breast cancer epithelial cells [8], migration in various ovarian cancer patient samples [53] and proliferation and response to kinase inhibitors in glioblastoma samples from patients [54]. Others have evaluated (potential) treatment options on a patient's malignant melanoma cells [55] and on a newly established mesenchymal chondrosarcoma cell line from a patient [56]. Two other publications used xCELLigence for characterization of newly established cell lines from patient samples, offsetting them against parental tumor tissue or traditionally used carcinoma cell lines [57,58]. Finally, Ruiz *et al.* applied xCELLigence to patients' own cancer cells for the *in vitro* selection of the most promising treatment, in this case for human carcinoma cells from malignant pleural effusions [59]. This is an illustrative example of possible applications in precision medicine.

Impedance-based technologies are also suited to test potential cell-based therapies (Fig. 2g). Seidel *et al.* demonstrated the therapeutic potential of $\gamma\delta$ T cells for antibody-based immunotherapy in pediatric patients with B-lineage acute lymphoblastic leukemia (ALL). $\gamma\delta$ T cells were derived from healthy blood donors as well as from a patient with common ALL. xCELLigence was used to measure $\gamma\delta$ T cell lysis in a breast adenocarcinoma cell line in real-time, and outperformed the traditional endpoint assay [60]. In a similar manner, others have studied the ability of mononuclear cells from normal patients and those with breast cancer to kill different breast cancer cell lines in the presence or absence of trastuzumab [61].

Myoblasts from muscle biopsy samples are another cell type of interest. In a recent example, Sente *et al.* studied pathological mechanisms of heart failure. Using xCELLigence, they observed myoblast adiponectin signaling, differentiation, proliferation, and viability in primary myoblasts and myotubes from patients with chronic heart failure and age- and gender-matched healthy donors [62,63].

From drug discovery to precision medicine

As a result of their versatility, label-free assays and patient cells, when combined, can be utilized at various stages of medicines research. As a cell-phenotypic screen, label-free assays are well suited for target identification, compound screening, and lead selection. Likewise, they allow the investigation of mechanisms of action and the testing of drug efficacy and safety [14,17]. In this review, we have provided typical examples involving patient cells, which offer increased physiological context. Given that such patient samples are often in limited supply, this set-up is not regularly used for screening drug candidates, for example, but rather for understanding disease mechanisms and testing potential treatments. This was done by Lowin *et al.* in the context of RA to identify drug targets, subsequently test compounds, and define possible treatments [28,41]. In a more integrated approach, the combination of patient cells and label-free assays resulted in tissue-on-a-chip technology, as demonstrated by Gamal *et al.* [47]. It is to be expected that the advent of stem cell technology will radically change the availability of patient-derived materials [42,64], which would allow further integration of label-free assays. This would be

an ideal starting point for the advancement of precision medicine, if patient-derived material can be made available readily, on demand, and in larger quantities. However, the question arises whether label-free technologies can be developed that take the three-dimensionality of advanced cellular models and organoids into account [65–67]. In drug safety and toxicity research, iPSC-derived cardiomyocytes can be used in a label-free setting to evaluate potential cardiac (adverse) effects of drug candidates [12,43]. Finally, the combination of patient cells and label-free technology can be used for clinical compound selection, for instance by measuring patient cell responses *in vitro* as means of selecting the most promising treatment. This has been demonstrated by profiling drug treatment responses of patient-derived malignant pleural effusions in a study by Ruiz *et al.* [59], with the aim to provide drug treatment of cancer in a personalized manner.

Concluding remarks

Physiologically more-appropriate cellular models and readout systems are needed to increase representability and translational value. Patient-derived cells can provide pathological and physiological context, and biobanking has increased the availability of human primary samples for research. Label-free impedance-based assays can and have been applied to a range of such samples. These assays increase the physiological representability by omitting reporter-based modifications and measuring physiological cell function in real-time. Thus, combining label-free assays with human primary samples offers a uniquely biorelevant set-up for the purposes of drug development and precision medicine.

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