Take it personal! Genetic differences in G protein-coupled receptors as studied with label-free technology

Hillger, J.M.

CHAPTER 2

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

Julia M. Hillger

Wai-Ling Lieuw

Laura H. Heitman

Adriaan P. IJzerman

Drug discovery today, 2017. pii: S1359-6446(17)30153-8.

DOI: http://dx.doi.org/10.1016/j.drudis.2017.07.015

Abstract

Drug development requires physiologically more appropriate model systems and assays to increase understanding of drug action and pathological processes in the human individual. 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 it unique opportunities for drug development and precision medicine.

Introduction

Two significant challenges in today's drug development are the inter-individual 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 sub-population to tailor drug prescriptions, hereby decreasing risks of ineffective dosing or side-effects [1]. Challenges to achieve this are in a generally perceived lack of understanding of the molecular details of drug action and of pathological processes in the human individual. This, in turn, is to a large degree brought about by insufficient physiological representability of 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 may influence target pharmacology (**Box 1, Fig. 1**). In addition, cellular

BOX 1: Traditional label-based versus label-free assays.

Traditional label-based assays follow drug effects and cellular functions by chemical attachment of a "label" to the drug molecule, drug target or downstream effectors. These can consist of 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.

Label-free assays do not require any such modifications as they measure cellular changes by alternative detection means, without the need for introducing chemical or bioengineered modifications.

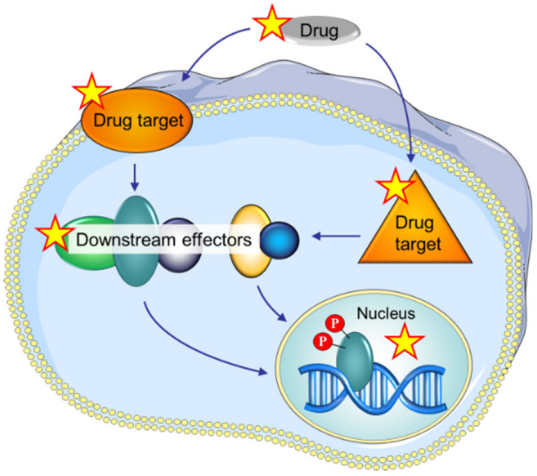

Figure 1. Traditional label-based assays. Stars highlight where effects are often measured by introducing labels or reporters. Image constructed using components from Servier Medical Art by Servier (*http://www.servier.com/Powerpoint-image-bank*).

models and cell systems are often selected based on habit and technical feasibility rather than disease relevance, resulting in physiologically less representative heterologous or recombinant cells lines. Such renewable in vitro cell sources have been essential in facilitating drug discovery and certainly 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 the issues experienced in the 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 onto 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 able to acquire the molecular-level understanding from complex biological processes in their native environment [1, 2]. Applying them to human primary cells can increase physiological relevance [3-5]. In this review, we highlight the realm of these possibilities, by focusing on the application of one type of such label-free cellular assays, based on impedance, on some of the most common types of human primary cells derived from patient samples.

Advantages of Label-free cellular assay technologies

The two currently most used forms of label-free cellular biosensors are impedance- or optics-based. 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. 2**). 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 wide 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 broad range of samples, are highly versatile and can integrate many assays into one (see also **Fig. 3**). 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. 3A**). 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).

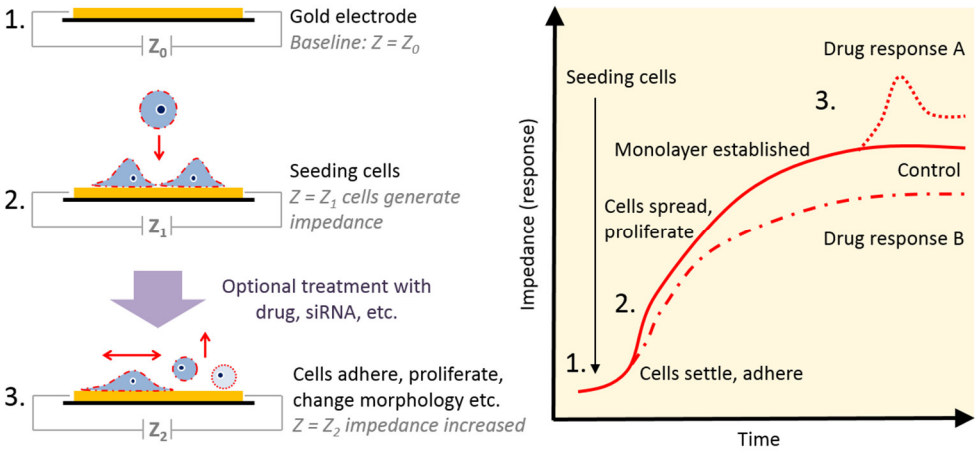

Figure 2. Principle of impedance-based label-free cellular assays. Cell attachment to gold electrodes generates impedance by changing the local ionic environment at the electrode-solution interface. Relative changes in impedance (Z) are recorded in real-time. **1.** Prior to the seeding of cells, baseline impedance is Z0. **2.** As cells adhere to the electrodes, impedance increases proportionally. **3.** Changes in cell number, adhesion, viability and morphology are directly reflected in the impedance profile. Impedance-based label-free cellular assays can detect a wide 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.

Impedance-based assays offer the distinct advantage of a direct read-out of drug action in real-time. While there are also traditional assays which record specific functions in real-time (e.g., Ca^{2+}-mobilization assays) impedance measurements offer the benefits of real-time measurements in both acute (eg. direct receptor signalling) 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. 3D**) and co-culture without contact (**Fig. 3H**), though these may require specialized recording or plate equipment (**Fig. 3B, 3E, 3H**). Overall, impedance-based assays have already successfully been applied to an extensive list of targets, including highly 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. 3B** and **3E**). 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] (see also **Table 1**). This is because in comparison to many traditional assays, label-free technologies offer a sensitive, less invasive detection methodology that monitors drug

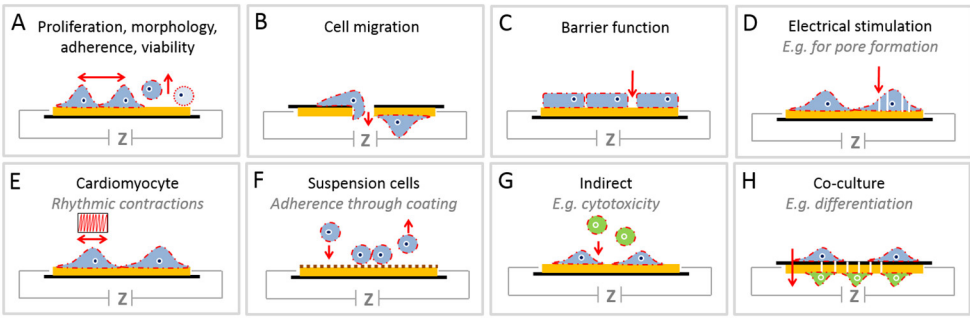

Figure 3: Typical applications of impedance-based label-free cellular assays. (**A**) General label-free cellular assay formats are capable of monitoring many cellular functions such as adherence, proliferation, viability and morphology. Additional specialized assay applications exist for instance to (**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 e.g. 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 actual cellular contact (xCELLigence co-culture set-up).

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 e.g., GPCR signaling bias [6, 14]. Receptors are linked to various downstream signaling pathways, 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 for the β2- adrenergic receptor induced subtly yet distinctly different response signatures as a consequence of such bias [15, 16].

Hence, as a number of 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 identification of leads independent of prior assumptions of signaling pathways and enable the use of more native systems at higher through-put. As a cell-phenotypic screen, they can be used for target identification, compound screening, lead selection, investigating 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 both for 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.

Advantages of primary human cells

Over the past decades, numerous biobanks have emerged to support medical research by programmed storage of biological material and corresponding data. These biomaterials include tissues, (stem) cells, blood, and serum, all of which have played 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 much better model system than the more traditional cellular models such as rodent, recombinant, or immortalized non-tissue specific human cell lines, and even better than *in-vivo* rodent models [20-22]. While the mentioned cellular models certainly 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 conditions and products from other genes could 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 for (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 employed to monitor a wide range of cellular effects, including specific functions such as migration, epithelial barrier function or

Table 1. Application examples of impedance-based label-free cellular technology to patient samples and stem-cell related types.

Type	Subtype	Technology	Material source	Reference
Blood components	Antibodies	xCELLigence	Type I diabetes patients, type II diabetes patients and healthy controls	[37]
	PBMCs	xCELLigence	From healthy volunteers but tested on patient material	[32, 33]
	Plasma and cells therein	ECIS	Healthy volunteers vs. trauma patients	[35]
		ECIS	Hantavirus Cardiopulmonary Syndrome patients	[36]
	Monocytes	ECIS	Patients with peripheral vascular disease and abdominal aortic aneurysm	[31]
	Neutrophils	ECIS	Critically ill septic patients	[34]
	Serum	ECIS	Scleroderma patients	[27]
Cancer cells and related cells	γδ T cells	xCELLigence	Healthy volunteers and B-lineage acute lymphoblastic leukemia patients	[60]
	Glioblastoma cells	xCELLigence	Paired tumoral and peritumoral tissue samples from glioblastoma patients	[54]
	Malignant melanoma cells	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 breast cancer patients	[61]
	Myxofibrosarcoma cells	xCELLigence	Myxofibrosarcoma patient	[58]
	Non-small-cell lung carcinoma cells	xCELLigence	Non-small-cell lung carcinoma patient	[57]
	Normal and neoplastic mammary cells	xCELLigence	Patient-derived primary human breast cancer epithelial cells	[8]
	Ovarian cancer cells	xCELLigence	Serous ovarian cancer patient and endometrioid peritoneal cancer patient	[53]
Chondrocytes	Chondrocytes and cartilage tissue	xCELLigence	Osteoarthritic patients	[32]

Fibro-blasts	Benign prostatic hyperplasia	xCELLigence	Benign prostatic hyperplasia patients	[26]
	Dermal	ECIS	Scleroderma patients and normal controls	[27]
	Orbital	ECIS	Patients with or without Graves' disease	[24]
	Synovial	xCELLigence	Patients with rheumatoid arthritis or osteoarthritis	[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 (CDI; *http://www.cellulardynamics.com/products/cardiomyocytes.html*)	[12, 43-45]
	iPSC Retinal pigment epithelium	ECIS	Age-related macular degeneration patient 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 human uterus of premenopausal women	[49]
		xCELLigence	Healthy human donors	[50]
		xCELLigence	Osteoarthritic patients	[33]
Myo-blasts	Skeletal muscle myoblasts and myotubes	xCELLigence	Chronic heart failure patients and age and gender-matched healthy donors	[62, 63]

cardiomyocyte beating (Fig. 3). 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 to over two decades. In one early example, prostaglandin E2 was shown to play a significant role in Graves' disease pathology by comparing morphological changes of orbital fibroblasts from patients with and without Graves' disease versus dermal fibroblasts (**Fig. 3A**). The authors chose ECIS over traditional light microscopy after testing both methodologies head to head, as it offered insight into the subtle, rapid cellular changes, especially into the underlying kinetics [24].

Since then, label-free cellular assays have been applied to other types of fibroblasts. 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 employed clinical and biobanked samples in general [25]. For instance, Nolte *et al.* demonstrated a potential strategy against hyper-proliferating fibroblasts by treating fibroblasts from benign prostatic hyperplasia patients with small interfering RNA against the transcription factor serum response factor. Effects on cell proliferation and growth inhibition were detected with the xCELLigence (Fig. 3A) [26]. Another notable study involved dermal fibroblasts and sera from scleroderma patients, which is discussed later [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 ones, Lowin *et al.* used the 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 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 as the cells were obtained from patients with the disease.

Blood cells

Blood is an easily obtained patient material and is thus 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 for instance cytotoxicity in the target cells (Fig. 3G). Lee *et al.* used ECIS to reveal differences between patients of peripheral vascular disease and abdominal aortic aneurysm to find better methods for targeted therapy. Monocytes of peripheral vascular disease patients induced higher endothelial barrier dysfunction [31].

Another particularly useful type of blood cells are peripheral blood-derived mononuclear cells (PBMCs). Hopper *et al.* showed PBMCs enhanced osteoarthritic human chondrocyte migration, which could be the basis for a treatment strategy for OA. The PBMCs were derived from healthy volunteers, while chondrocytes and cartilage tissue explants were from patients undergoing total knee replacement. Here, migration and chemokinetic potential of the cells

were measured using a specialized migration assay format of the xCELLigence (**Fig. 3B**) [32]. Later it was shown that PBMCs also enhanced migration and chondrogenic differentiation of multipotent mesenchymal stromal cells (MSCs) from knees of OA patients [33].

Other types of blood components have been assayed using label-free technology as well, although most of them again rely on an indirect measurement through effects on another cell type. For instance, neutrophils from critically ill septic patients were found to reduce endothelial barrier integrity to a greater extent than untreated normal neutrophils in an ECIS assay [34]. Human serum was also employed 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 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 loss of cell-cell adhesion in epithelial and endothelial cells in ECIS [36]. Finally, Jackson *et al*. employed xCELLigence to demonstrate that anti-calcium channel autoantibodies from patients with type I diabetes inhibit the adherence of Rat insulinoma cells, while antibodies from type II diabetes patients and healthy controls did not [37].

The reason that all these blood components are measured indirectly is twofold. On 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, as the detection mechanism positioned at the bottom of the well requires cells to adhere [7]. However, a number of studies demonstrated that suspension cells are amenable to label-free technologies as well, with both optical and impedance-based biosensors. Interestingly, impedance-based assays appear less susceptible to decreased cellular adherence than optical biosensors [7], and hence potentially applicable to an even broader range of cell types. Examples include various types of blood cells, one notably involving personal cell lines. For instance, CellKey was used to directly measure GPCR signaling in monocytes, neutrophils and PBMCs, though these were not in fact patient material [38, 39]. The 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. 3F). LCLs are in fact used as 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 e.g. fibroblasts into a pluripotent state, 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. For one of these, a specific type of application has been developed for the xCELLigence, namely a cardiomyocyte-based biosensor. Safety pharmacology studies that evaluate potential cardiac (side) effects of drug candidates are an essential part of drug development. The xCELLigence RTCA Cardio System detects the beating rhythm of cardiomyocytes (Fig. 3E). It 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 in fact 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. 3C). 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 a spatially controlled damage to the cell layer was introduced to mimic AMD. Apparently, label-free technology can be used to measure long-term effects, and is apparently 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 due to their wide mesodermal differentiation potential. Angstmann *et al.* compared ECIS and xCELLigence in search for standardized quality control assays to monitor differentiation and high-throughput screening that is both non-invasive and time-resolved. They 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 human uterus of premenopausal women [49] or to test MSC labelling 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. Studying lipid uptake and adipocyte differentiation with the 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 virtually any cell type and have in fact been applied to numerous others besides the most commonly biobanked samples highlighted above.

A further category of particular interest are cancer and related cell types. Here, impedance-based cellular assays are most often used to measure migratory and invasive properties (e.g. **Fig. 3B**), which are key characteristics for any (metastatic) cancer type. For instance, the 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 patients' glioblastoma samples [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 the xCELLigence for characterization of newly established cell lines from patient samples, offsetting them versus parental tumor tissue or traditionally used carcinoma cell lines [57, 58]. Finally, Ruiz *et al.* applied the xCELLigence to patients' own cancer cells for *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. 3G). Seidel *et al.* demonstrated the therapeutic potential of γδ T cells for antibody-based immunotherapy in pediatric patients with B-lineage acute lymphoblastic leukemia (ALL). γδ T cells were derived from healthy blood donors as well as from a patient with common-ALL. The xCELLigence was used to measure γδ T cell lysis in a breast adenocarcinoma cell line in real-time, and outperformed the traditional endpoint assay [60]. In a similar manner, others studied the ability of mononuclear cells from normal and breast cancer patients 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 the xCELLigence, they observed myoblast adiponectin signaling, differentiation, proliferation and viability in primary myoblasts and myotubes from chronic heart failure patients and age- and gender-matched healthy donors [62, 63].

From drug discovery to precision medicine

Due to 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 andlead selection. Likewise they allow the investigation of mechanisms of action and the testing of drug efficacy and safety [14, 17]. In this review we provided typical examples involving patient cells, which offer increased physiological context. As such patient samples are often in limited supply, this set-up is not so much used for e.g., screening drug candidates but rather for understanding disease mechanisms and testing potential treatments. This was done by Lowin *et al*. in the context of rheumatoid arthritis to identify drug targets, to subsequently test compounds and to 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 a 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. In this light 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 (side) 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.

Conclusion

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 wide range of such samples. These assays indeed increase 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.

References

1. Kojima, R., D. Aubel, and M. Fussenegger, *Novel theranostic agents for next-generation personalized medicine: small molecules, nanoparticles, and engineered mammalian cells.* Curr Opin Chem Biol, 2015. **28**: p. 29-38.
2. Moller, C. and M. Slack, *Impact of new technologies for cellular screening along the drug value chain.* Drug Discov Today, 2010. **15**(9-10): p. 384-90.
3. Verdonk, E., et al., *Cellular dielectric spectroscopy: a label-free comprehensive platform for functional evaluation of endogenous receptors.* Assay Drug Dev Technol, 2006. **4**(5): p. 609-19.
4. McGuinness, R., *Impedance-based cellular assay technologies: recent advances, future promise.* Curr Opin Pharmacol, 2007. **7**(5): p. 535-40.
5. Eglen, R. and T. Reisine, *Primary cells and stem cells in drug discovery: emerging tools for high-throughput screening.* Assay Drug Dev Technol, 2011. **9**(2): p. 108-24.
6. Fang, Y., *Combining label-free cell phenotypic profiling with computational approaches for novel drug discovery.* Expert opinion on drug discovery, 2015. **10**(4): p. 331-43.
7. Lieb, S., et al., *Label-free analysis of GPCR-stimulation: The critical impact of cell adhesion.* Pharmacological research : the official journal of the Italian Pharmacological Society, 2016. **108**: p. 65-74.
8. Mandel, K., et al., *Characterization of spontaneous and TGF-beta-induced cell motility of primary human normal and neoplastic mammary cells in vitro using novel real-time technology.* PLoS One, 2013. **8**(2): p. e56591.
9. Hillger, J.M., et al., *Getting personal: Endogenous adenosine receptor signaling in Lymphoblastoid Cell Lines.* Biochem Pharmacol, 2016.
10. Czajka, A.A., et al., *Family of microRNA-146 Regulates RARbeta in Papillary Thyroid Carcinoma.* PLoS One, 2016. **11**(3).
11. Chanakira, A., et al., *Hypoxia Differentially Regulates Arterial and Venous Smooth Muscle Cell Migration.* PLoS One, 2015. **10**(9): p. e0138587.
12. Zhang, X., et al., *Multi-parametric assessment of cardiomyocyte excitation-contraction coupling using impedance and field potential recording: A tool for cardiac safety assessment.* J Pharmacol Toxicol Methods, 2016. **81**: p. 201-16.
13. Febles, N.K., A.M. Ferrie, and Y. Fang, *Label-free single cell kinetics of the invasion of spheroidal colon cancer cells through 3D Matrigel.* Analytical chemistry, 2014. **86**(17): p. 8842-9.
14. Rocheville, M., et al., *Mining the potential of label-free biosensors for seven-transmembrane receptor drug discovery.* Progress in molecular biology and translational science, 2013. **115**: p. 123-42.
15. Fang, Y. and A.M. Ferrie, *Label-free optical biosensor for ligand-directed functional selectivity acting on beta(2) adrenoceptor in living cells.* FEBS Lett, 2008. **582**(5): p. 558-64.
16. Stallaert, W., et al., *Impedance responses reveal beta(2)-adrenergic receptor signaling pluridimensionality and allow classification of ligands with distinct signaling profiles.* PLoS One, 2012. **7**(1): p. e29420.
17. Fang, Y., *Label-free drug discovery.* Frontiers in pharmacology, 2014. **5**(52).

18. Artene, S.A., et al., *Biobanking in a constantly developing medical world.* ScientificWorldJournal, 2013. **2013**: p. 343275.
19. Astrin, J.J. and F. Betsou, *Trends in Biobanking: A Bibliometric Overview.* Biopreserv Biobank, 2016. **14**(1): p. 65-74.
20. Al-Ahmad, A., et al., *Nature-inspired antimicrobial polymers--assessment of their potential for biomedical applications.* PLoS One, 2013. **8**(9): p. e73812.
21. Seok, J., et al., *Genomic responses in mouse models poorly mimic human inflammatory diseases.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(9): p. 3507-12.
22. Schulz, S., et al., *Interactive fibroblast-keratinocyte co-cultures: an in vivo-like test platform for dental implant-based soft tissue integration.* Tissue engineering. Part C, Methods, 2012. **18**(10): p. 785-96.
23. Freedman, L.P., et al., *Reproducibility: changing the policies and culture of cell line authentication.* Nature methods, 2015. **12**(6): p. 493-7.
24. Reddy, L., et al., *Assessment of rapid morphological changes associated with elevated cAMP levels in human orbital fibroblasts.* Exp Cell Res, 1998. **245**(2): p. 360-7.
25. Nicola Daniele, M.C., Claudio Pellegrini, Entela Shkëmbi and F. Zinno, *Biobanks and Clinical Research: An "Interesting" Connection.* Peertechz Journal of Cytology and Pathology, 2016. **1**(1): p. 034-043.
26. Nolte, A., et al., *Small interfering RNA transfection against serum response factor mediates growth inhibition of benign prostatic hyperplasia fibroblasts.* Nucleic Acid Ther, 2013. **23**(1): p. 62-70.
27. Huang, C.N., et al., *Sera from patients with scleroderma inhibit fibroblast micromotions monitored electrically.* J Rheumatol, 1999. **26**(6): p. 1312-7.
28. Lowin, T., et al., *Anti-inflammatory effects of N-acylethanolamines in rheumatoid arthritis synovial cells are mediated by TRPV1 and TRPA1 in a COX-2 dependent manner.* Arthritis Res Ther, 2015. **17**(321): p. 015-0845.
29. Lowin, T., G. Pongratz, and R.H. Straub, *The synthetic cannabinoid WIN55,212-2 mesylate decreases the production of inflammatory mediators in rheumatoid arthritis synovial fibroblasts by activating CB2, TRPV1, TRPA1 and yet unidentified receptor targets.* J Inflamm, 2016. **13**(15): p. 016-0114.
30. Bohm, M., et al., *alpha-MSH modulates cell adhesion and inflammatory responses of synovial fibroblasts from osteoarthritis patients.* Biochem Pharmacol, 2016. **116**: p. 89-99.
31. Lee, E.S., et al., *Monocytic adhesion molecule expression and monocyte-endothelial cell dysfunction are increased in patients with peripheral vascular disease versus patients with abdominal aortic aneurysms.* J Surg Res, 2012. **177**(2): p. 373-81.
32. Hopper, N., et al., *Peripheral blood derived mononuclear cells enhance osteoarthritic human chondrocyte migration.* Arthritis Res Ther, 2015. **17**: p. 199.
33. Hopper, N., et al., *Peripheral blood derived mononuclear cells enhance the migration and chondrogenic differentiation of multipotent mesenchymal stromal cells.* Stem Cells Int, 2015. **2015**: p. 323454.
34. Fox, E.D., et al., *Neutrophils from critically ill septic patients mediate profound loss of endothelial barrier integrity.* Crit Care, 2013. **17**(5): p. R226.

35. Rahbar, E., et al., *Endothelial glycocalyx shedding and vascular permeability in severely injured trauma patients.* J Transl Med, 2015. **13**: p. 117.
36. Bondu, V., et al., *Elevated cytokines, thrombin and PAI-1 in severe HCPS patients due to Sin Nombre virus.* Viruses, 2015. **7**(2): p. 559-89.
37. Jackson, M.W. and T.P. Gordon, *A novel impedance-based cellular assay for the detection of anti-calcium channel autoantibodies in type 1 diabetes.* J Immunol Methods, 2010. **361**(1-2): p. 31-6.
38. Leung, G., et al., *Cellular Dielectric Spectroscopy: A Label-Free Technology for Drug Discovery.* Journal of the Association for Laboratory Automation, 2005. **10**(4): p. 258-269.
39. Molecular Devices Inc., *Analyzing Endogenous Receptors in Non-Adherent Cell Lines and Primary Cells with the CellKey Small Sample 96W Microplate.* CellKey System Application Highlight 5, 2008.
40. Hillger, J.M., et al., *Phenotypic screening of cannabinoid receptor 2 ligands shows different sensitivity to genotype.* Biochemical pharmacology, 2017.
41. Welsh, M., et al., *Pharmacogenomic discovery using cell-based models.* Pharmacological reviews, 2009. **61**(4): p. 413-29.
42. Hosoya, M. and K. Czysz, *Translational Prospects and Challenges in Human Induced Pluripotent Stem Cell Research in Drug Discovery.* Cells, 2016. **5**(4).
43. Doherty, K.R., et al., *Structural and functional screening in human induced-pluripotent stem cell-derived cardiomyocytes accurately identifies cardiotoxicity of multiple drug types.* Toxicol Appl Pharmacol, 2015. **285**(1): p. 51-60.
44. Chaudhari, U., et al., *Identification of genomic biomarkers for anthracycline-induced cardiotoxicity in human iPSC-derived cardiomyocytes: an in vitro repeated exposure toxicity approach for safety assessment.* Arch Toxicol, 2016. **90**(11): p. 2763-2777.
45. Hu, N., et al., *High-performance beating pattern function of human induced pluripotent stem cell-derived cardiomyocyte-based biosensors for hERG inhibition recognition.* Biosens Bioelectron, 2015. **67**: p. 146-53.
46. Li, X., et al., *Cardiotoxicity screening: a review of rapid-throughput in vitro approaches.* Arch Toxicol, 2016. **90**(8): p. 1803-16.
47. Gamal, W., et al., *Real-time quantitative monitoring of hiPSC-based model of macular degeneration on Electric Cell-substrate Impedance Sensing microelectrodes.* Biosensors and Bioelectronics, 2015. **71**: p. 445-455.
48. Angstmann, M., et al., *Monitoring human mesenchymal stromal cell differentiation by electrochemical impedance sensing.* Cytotherapy, 2011. **13**(9): p. 1074-89.
49. Rajaraman, G., et al., *Optimization and scale-up culture of human endometrial multipotent mesenchymal stromal cells: potential for clinical application.* Tissue engineering. Part C, Methods, 2013. **19**(1): p. 80-92.
50. Skopalik, J., et al., *Mesenchymal stromal cell labeling by new uncoated superparamagnetic maghemite nanoparticles in comparison with commercial Resovist--an initial in vitro study.* Int J Nanomedicine, 2014. **9**: p. 5355-72.
51. Nordberg, R.C., et al., *Electrical Cell-Substrate Impedance Spectroscopy Can Monitor Age-Grouped Human Adipose Stem Cell Variability During Osteogenic Differentiation.* Stem Cells Transl Med, 2016. **7**: p. 2015-0404.

52. Berger, E., et al., *Pathways commonly dysregulated in mouse and human obese adipose tissue: FAT/CD36 modulates differentiation and lipogenesis.* Adipocyte, 2015. **4**(3): p. 161-80.
53. Jacob, F., et al., *The glycosphingolipid P(1) is an ovarian cancer-associated carbohydrate antigen involved in migration.* British journal of cancer, 2014. **111**(8): p. 1634-45.
54. Cruceru, M.L., et al., *Signal transduction molecule patterns indicating potential glioblastoma therapy approaches.* OncoTargets and therapy, 2013. **6**: p. 1737-49.
55. Li, J., et al., *The proliferation of malignant melanoma cells could be inhibited by ranibizumab via antagonizing VEGF through VEGFR1.* Mol Vis, 2014. **20**: p. 649-60.
56. de Jong, Y., et al., *Inhibition of Bcl-2 family members sensitizes mesenchymal chondrosarcoma to conventional chemotherapy: report on a novel mesenchymal chondrosarcoma cell line.* Lab Invest, 2016. **96**(10): p. 1128-37.
57. Bartscht, T., et al., *The Src family kinase inhibitors PP2 and PP1 effectively block TGF-beta1-induced cell migration and invasion in both established and primary carcinoma cells.* Cancer Chemother Pharmacol, 2012. **70**(2): p. 221-30.
58. Lohberger, B., et al., *The novel myxofibrosarcoma cell line MUG-Myx1 expresses a tumourigenic stem-like cell population with high aldehyde dehydrogenase 1 activity.* BMC Cancer, 2013. **13**: p. 563.
59. Ruiz, C., et al., *Culture and Drug Profiling of Patient Derived Malignant Pleural Effusions for Personalized Cancer Medicine.* PLoS One, 2016. **11**(8).
60. Seidel, U.J., et al., *γδ T Cell-Mediated Antibody-Dependent Cellular Cytotoxicity with CD19 Antibodies Assessed by an Impedance-Based Label-Free Real-Time Cytotoxicity Assay.* Frontiers in immunology, 2014. **5**: p. 618.
61. Kute, T., et al., *Understanding key assay parameters that affect measurements of trastuzumab-mediated ADCC against Her2 positive breast cancer cells.* Oncoimmunology, 2012. **1**(6): p. 810-821.
62. Sente, T., et al., *Primary skeletal muscle myoblasts from chronic heart failure patients exhibit loss of anti-inflammatory and proliferative activity.* BMC cardiovascular disorders, 2016. **16**: p. 107.
63. Sente, T., et al., *Tumor necrosis factor-alpha impairs adiponectin signalling, mitochondrial biogenesis, and myogenesis in primary human myotubes cultures.* Am J Physiol Heart Circ Physiol, 2016. **310**(9): p. 26.
64. Rony, I.K., et al., *Inducing pluripotency in vitro: recent advances and highlights in induced pluripotent stem cells generation and pluripotency reprogramming.* Cell proliferation, 2015. **48**(2): p. 140-56.
65. Lee, J., et al., *Nonmediated, Label-Free Based Detection of Cardiovascular Biomarker in a Biological Sample.* Adv Healthc Mater, 2017. **21**(10): p. 201700231.
66. Smout, M.J., et al., *A novel high throughput assay for anthelmintic drug screening and resistance diagnosis by real-time monitoring of parasite motility.* PLoS neglected tropical diseases, 2010. **4**(11): p. e885.
67. Shin, S.R., et al., *Label-Free and Regenerative Electrochemical Microfluidic Biosensors for Continual Monitoring of Cell Secretomes.* Adv Sci, 2017. **4**(5).