ELSEVIER

Impact of new technologies for cellular screening along the drug value chain

Clemens Möller and Mark Slack

Evotec AG, Schnackenburgallee 114, 22525 Hamburg, Germany

High-information screening formats, using more physiologically relevant cellular models and readout approaches, are slowly replacing traditional, target-orientated approaches in drug discovery programs. With improved access to primary cells, as well as label-free, non-intrusive methods of compound interrogation (such as automated electrophysiology), high-thoughput screening facilities have to adapt to more complex assay scenarios. The implementation of novel cellular systems, readout technologies and data management in a drug discovery company are essential to improve the current falling productivity evident in recent years throughout the pharmaceutical industry.

Introduction

Under intense pressure to improve on drug pipelines and reduce the attrition associated with screening 'the high hanging fruits' and new post-genomic targets, a general overhaul of the established drug discovery philosophy is now evident. Indeed, the disappointing productivity [1–3] witnessed over the past decade in terms of new chemical entities approved and new drug targets has lagged far behind the development of new technologies and progress in understanding in disease biology. In this context, a whole variety of buzzwords are evident, such as 'systems biology' and 'translational medicine'. Each of these terms attempts to define the search for novel chemical matter, not in a 'one gene – one drug' manifestation but in terms of the whole organism. In a wider context, this drug developmental strategy is slowly being mirrored with a general change in thinking in terms of assay development.

The original 'one target – one drug – one effect' pharmacological profile is now generally accepted as an oversimplistic model. This is giving way to a growing appreciation for the need to study the interaction of small compounds with their respective targets in the more complex native environment.

Analysis of a modern day drug discovery and development company predominantly shows a typical mix of biochemical and cell-based assays used in the hit finding stage. Such systems are generally artificial in their composition, in terms of either the buffer composition and protein source or the nature and origin of the cellular expression system. Retrospective analysis of high-throughput screening (HTS) campaigns will show that the majority of these were based on the use of fluorescent probes or reporters as indirect readout technologies. Although these methods are universal and straightforward to apply to a diverse target base, such systems are open to their fair share of artifacts (or irrelevance) and have to be queried in terms of the ability to report all hit populations. Indeed, over the past several years, there has been a slow acceptance that such systems can be biased in the hit populations that they ultimately identify; thus, screening teams are now looking at new methods and technologies that can remove this bias and present the target in more physiological systems. To that end, we see two major areas of innovation: the use of primary and human stem cells as research tools for compound screening and the implementation of direct biophysical techniques for the study of membrane receptors and ion channels in particular.

Stem cells

Probably no other area is currently experiencing so much interest and expansion as that of stem cell technologies. Over the past few years, an explosion in application and basic understanding of these rudimentary cell populations has been evident. Indeed, at the time of writing, a large number of stem cell programs are close to entering into clinical trials (www.clinicaltrials.gov). From a

Corresponding author:. Möller, C. (clemens.moeller@evotec.com), Slack, M. (mark.slack@evotec.com)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.02.010

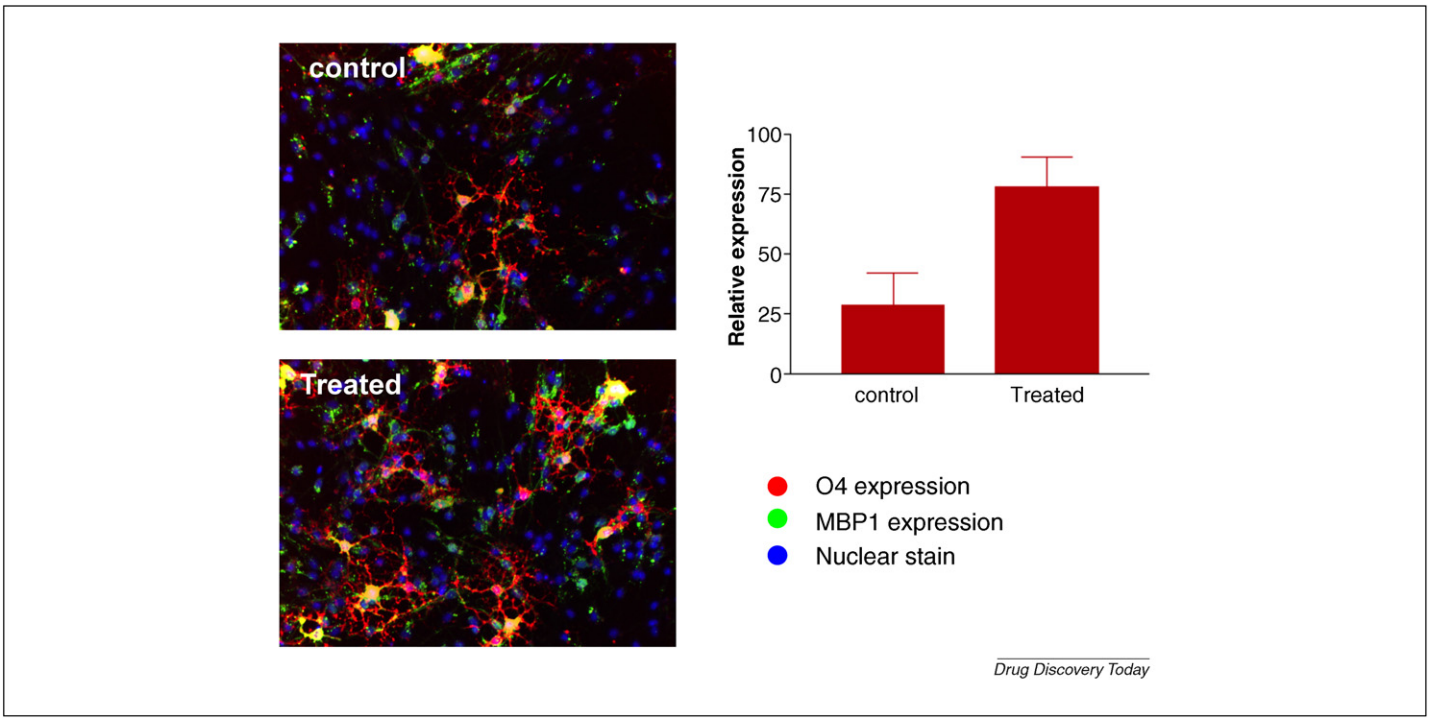

FIGURE 1

Differentiation of neuronal stem cells toward oligodendrocytes. Neural stem cells are differentiated, by the addition of platelet-derived growth factor, toward oligodendrocytes. Using an automated confocal imaging reader, the detection of oligodendrocyte markers (O4, red) and myelin-basic protein (MBP, green) can be quantified. Cell nuclei are stained with Draq5 (blue).

clinical standpoint, the emphasis for the majority of these programs has been on allogenic applications in a disease background, administering modified stem cell populations into patients to alleviate symptoms. It is currently too early to say how successful these approaches will be, but for some of the approaches, early indications look promising [4,5]. In the context of modern drug discovery, stem cell technologies have the potential to offer unlimited amounts of unique cell populations, expressing disease-relevant targets in disease-relevant tissue types. Stem cell applications can thus be divided into two main branches: first, direct clinical application in symptomatic patients, and second, novel tissue types for drug development in HTS paradigms. Irrespective of either approach, the supply of fully differentiated and functional cell populations is often limiting and incomplete, and lack of detailed and efficient protocols has inclined researchers to address this bottleneck via HTS approaches [6–8]. Using neurospheres as a model, Liu and colleagues were able to screen more than one million compounds. The identified molecules were subsequently shown to differentiate neural stem cells into distinct neuronal lineages or maintain them in a proliferative state. The implementation of high-content screening (HCS) technologies in this context has been essential, and at Evotec, we are utilizing the Opera HCS platform with a selection of stem cell assays for HTS purposes (Fig. 1). Using a similar approach, a small compound was identified that can direct the *in vitro* development of stem cells toward insulin-expressing beta cells [9], which opens the door for future drug development programs in the field of diabetes.

Difficult ethical issues are associated with the generation of and access to suitable human embryonic stem (hES) cell lines. Landmark investigations have shown that it is possible to induce the reversal of somatic-tissue-derived cell lines into stem cell populations, termed 'induced pluripotent stem cells' (iPS) [10]. These have been shown to be similar in their phenotype and epigenetic status to ES cell lines but, importantly, show subtle differences in their gene expression profiles [11]. The sudden availability of stem cell populations from affected patients via iPS technologies now enables the directed differentiation and production of cell types normally affected in the same patient population [12,13], such as dopaminergic neurons in Parkinson's disease or striatal neurons in Huntington's patients. Indeed, Ebert *et al.* [14] were able to successfully develop iPS cells from skin fibroblasts isolated from a child suffering with spinal muscular atrophy and subsequently develop a subpopulation of these into motor neurons that retained the disease phenotype. Such cells are a valuable resource for novel approaches in the treatment of these diseases and can be applied to drug discovery cascades. Although a variety of iPS cell lines are now available, these are often produced from different cell lineages via the application of different protocols. Perhaps unsurprisingly, these cell lines – although similar in their pluripotency potential – show significant differences in their ability to generate teratomas *in vivo* [15]. In addition, in an effort to increase the normal low efficiency of the iPS process, many groups are using dysregulation of the gatekeeper function of p53. The apparent increase in iPS efficiency comes at a price, however; namely, the increased likelihood of taking potentially mutagenic mutations through to the final iPS cell line [16,17].

HCS

The term 'high-content screening' has developed from a combination of advancements in automated microscopy and software

applications aimed at detailed image analysis. Unlike standard HTS approaches, in which one readout (e.g. luminescence, fluorescence or absorbance) is used to address one target or effect, high-content approaches, by definition, can address multiple readouts simultaneously [18]. The high information content delivered offers the investigator the opportunity to obtain a variety of parametric data sets on an individual compound basis, which, in turn, enables more informed decisions to be made. The enormous benefits that such an approach can offer do not come without a price, of course. To achieve maximum leverage from the immense data set, a comprehensive understanding of the underlying biology and concepts on a whole-organism level are paramount [19,20]. Indeed, in applications of HCS to human stem cell populations and systems biology, efforts are being made to establish the entire complex of signaling pathways and how they interact to govern stem cell development [21]. Although this information is extremely important, the complexity that this reveals cannot be translated to modern day drug discovery in simple terms.

Although HCS is, at present, mainly reserved for an HTS supporting role, it is widely used in the setting of target validation and hit qualification [22,23]. In a hit finding scenario, the potential of this technology to identify novel chemical matter in complex signaling pathways has been demonstrated, and HCS is starting to enjoy growing precedence as a forefront HTS technology [24–26]. Not unexpectedly, an HCS application can alleviate bottlenecks in the drug discovery cascade for protein targets and complexes that achieve their mechanism of action via cellular redistribution (either within the cytoplasm or from one organelle to another, such as nuclear import and export), which cannot be accessed via other technology platforms [27]. G-protein-coupled receptor (GPCR) drug development, a mainstay of pharmaceutical productivity, has also benefited via the addition of HCS to an already comprehensive assay suite. In recognition of native GPCR signaling and cell-dependent signal trafficking, HCS enables the assessment of ligand activation to various key pathways in parallel [28,29], aiding in the selection of compounds for further medicinal chemistry optimization.

In a pharmaceutical company, despite the examples above, most data sets acquired by HCS groups are used to address off-target mechanisms of compounds with emphasis on preliminary toxicity or to understand mechanism of action [30]. Using eight different parameters covering various aspects of nuclear morphology through to membrane integrity and mitochondria membrane potential, Abraham *et al.* [22] were able to develop an HCS assay format in a HepG2 cell line that could effectively support lead optimization through predictive toxicology. Further developments concerning toxicity profiling have seen HCS taking advantage of primary cell cultures including human hepatocytes [31] and neurons with the development of neurite outgrowth assays an indicator of potential neurotoxicity [32]. There is currently a general movement toward the use of primary cell systems for drug development and discovery, and these are being adapted to the HCS field to deliver more physiological data sets and provide access to new disease models [25,33,34].

Label-free drug screening employing cellular assays

A historical analysis of drug development programs over the past decades, where compound screening using an *in vitro* assay system was influential in the original hit identification process, will show that the described assay (whether biochemical or cellular in nature) encompassed the use of reporter systems for the target in question. Such approaches mostly require the use of fluorescent dyes or reporter gene constructs and the manipulation of cells, which can often influence or bias the subsequent target pharmacology. In addition, for the same cell-based assays, the selection of the cell line for expression of the target has been based more on habit and technical feasibility (e.g. HEK293 and CHO) than disease relevance. This, no doubt, will have contributed to the subsequent lack of efficacy for some of these compounds in later *in vivo* testing.

Recognizing the influence that such artificial systems can have on signaling cascades and correct target expression and physiology, the use of so-called 'label-free methodologies' is currently gaining increased importance. In a cellular context, two principle technologies are evident, based first on changes in electrical impedance across a cell monolayer after functional interaction with a ligand, and second on the refractive index changes or resonance waveguide grating. The second approach detects small changes in refracted light upon conformational changes at the solution substrate interface. These principle technologies and others are reviewed elsewhere [35,36]. Although these technologies offer the ability to investigate receptor signaling in more physiologically relevant cell backgrounds, with endogenous expression, the nature of the signal is firstly unspecific. Any compound or ligand that produces a mass redistribution within the cell coupled to a morphological change will be identified as a hit irrespective of the target. To realize the full potential of these technologies, an in-depth pharmacological understanding of the target and a comprehensive toolbox of validation compounds are necessary to specifically assign the observed signal to the target of choice.

Label-free approaches have yet to make it as a mainstay HTS platform. There are various factors contributing to this, including the instrument and reagent cost and, in particular, the difficulty in procuring a sufficient supply of primary cells to make the label-free approach more meaningful than the use of recombinant engineered cells. Nevertheless, they are widely employed in the areas of hit expansion and understanding the mechanism of action, as well as in target validation. Akin to HCS approaches, label-free can deliver high-content data for a variety of cellular events. Using an impedance-based platform, Jahnke and colleagues have recently been able to specifically follow the intercellular hyperphosphorylation of Tau protein implicated in the development of Alzheimer's disease. The positive effects of small compounds on reducing this effect could also be efficiently monitored and quantified [37] and could be verified via other approaches. Probably the area of greatest exposure for label-free technologies has centered on the characterization of GPCR signaling cascades and compound testing [38,39]. Additional examples demonstrate the possibility of simultaneously tracking the effects of various compounds on the multitude of pathways activated by receptor tyrosine kinase (such as EGF) [40].

Ion channel screening

Patch-clamp electrophysiology provides a direct readout of ion channel function

Ion channels are generally considered an underexploited target class (e.g. Ref. [41]). With manual patch-clamp electrophysiology

[42,43], which is generally referred to as the 'gold standard' method of investigating ion channel function, a functional read-out of ion currents that provides a direct insight into the function of ion channels is possible. Such a direct biophysical measurement of protein function is not available for other target types, although the major disadvantage of this method of observation has traditionally been the extremely low throughput of manual patch clamping. A direct functional screening of a larger number of compounds has, therefore, until recently not been possible, and electrophysiology had been limited to being a follow-up technique to indirect (e.g. fluorescence- or ligand-based) high-throughput screening techniques.

Automated patch-clamp electrophysiology – current and future

With the advent of automated patch-clamp electrophysiology approximately five years ago, this major bottleneck in ion channel research has been alleviated. However, the high cost per data point screened and the still relatively low throughput and efficiency of these devices still represent hurdles for primary screening by automated patch clamping.

The first generation of automated patch-clamp instruments merely focus on the parallel formation of the high-resistance seals between a substrate and the cell and are able to automatically establish the recording conditions, apply required voltage stimulation protocols to the cells being studied and deliver test solutions to the cells. Instruments of the first generation are summarized in Table 1. Currently, second-generation automated platforms are available to the ion channel researcher. In addition to the features of the first-generation formats, further developments have improved not only the throughput but also data consistency and quality. Some instruments have achieved the increase in capacity via parallelization (e.g. Sophion QPatch HT, 48 channels). A development that has additionally increased the success rate of patch robots is the measurement from an ensemble (a population) of cells simultaneously at one recording site [44]. In these second-generation-type instruments, each individual recording well has numerous holes that can potentially generate a seal with a cell and, thus, each amplifier records from a number (a population) of cells, automatically averaging their currents. Linear leak currents from non-occupied holes are subsequently corrected. In this way, the overall success rate for the patch-clamp planar technology is greatly improved and biological variability between individual cells (e.g. poorly expressing cells) is averaged out. It has also been shown that this technique enables multiplexing using different cell types (e.g. two different targets being expressed and patch clamped simultaneously) [45]. Molecular Devices (now MDS) were the first to introduce this technique under the name Population Patch Clamp™ on the second-generation IonWorks, termed IonWorks Quattro. Each well has 64 holes, thus achieving an overall patch success rate of nearly 100%. On the success of this, other companies introduced similar techniques on their machines or are close to launching such instruments, including Sophion (QPatch HTX as a further development of the QPatch HT, 48 channels), Cellectricon (Dynaflow HT) and Fluxion Biosciences (IonFlux, 64 recordings in parallel). Interestingly, data from the QPatch HTX with a fluid exchange time below 10 ms have been shown, which makes this instrument suitable to record from and screen ligand-gated channels including $P2X_3$ and the fast-desensitizing nAChR α7-channels in high throughput [46].

Additional modifications have addressed the accelerated delivery of test solutions to the recording site, enabling the investigation of fast-desensitizing ligand-gated ion channels. Cellectricon are offering a chip-based laminar flow system called Dynaflow Pro as an add-on to manual patch-clamp rigs. This simplifies the solution exchange in manual patch clamping and is considered of particular advantage for automated recording of concentration-response curves and for recording from fast-desensitizing ligand-gated ion channels. Cellectricon are currently engaged in collaboration with AstraZeneca, employing their know-how with laminar-flow solution control, to develop an automated planar-chip-based patch-clamp system [47]. The current design is based on a chip with simultaneous measurement of six cells per fluid delivery position, enabling rapid solution exchange, suitable to automatically record from fast-desensitizing ligand-gated ion channels.

Other advances in increasing the throughput in ion channel drug discovery include 'lab-on-a-chip' approaches, which introduce a fluid delivery and recording system on a chip, enabling electrophysiological readouts from cells [48]. A different approach

TABLE 1

Automated patch-clamp platforms

Instrument	Number of channels: total/parallel read	Company	Remark
First-generation instruments			
PatchXpress®	16/16	Axon, now MDS	
IonWorks HT	384/48	Essen instruments, now marketed by MDS	Only low-resistance seals
QPatch 16	16/16	Sophion	
Port-a-Patch	1/1	Nanion	
PatchLiner	16/2, 4 or 8	Nanion	
PatchBox	1/1	Flyion	
FlyScreen 8500	3–6/3–6	Flyion	
Second-generation instruments			
QPatch HT	48/48	Sophion	
IonWorks Quattro	384/48	MDS	Population Patch™
QPatch HTX	48/48	Sophion	
SynchroPatch 98	98/16	Nanion	
IonFlux-16	96/16	Fluxion Biosciences	
IonFlux HT	384/64		

to increase the information content in electrophysiological recordings is being followed by microelectrode array (MEA) systems, which enable the recording of electrical activity of, for example, neuronal and cardiac tissue [49]. The continuous improvement of the technology and the cell culture protocols associated with MEAs has made this a versatile technique being potentially closer to *in vivo* conditions than plain single-cell preparations.

Target-based screening by automated patch-clamp electrophysiology

The improved success rates of existing automated patch-clamp electrophysiology robots, together with ever-increasing parallelization on patch-clamp chips and improved parallelization of individual experiments, has made the screening of large compound collections more feasible. Focused libraries have been screened in the size of tens of thousands of compounds on the IonWorks [50], and we ourselves routinely perform ion channel profiling of up to 20 000 cpds using the IonWorks Quattro, in a time frame of a few weeks.

The direct functional screening of libraries on voltage-gated ion channels can be expected to provide a major breakthrough for pharmacological research on several ion channel targets in the near future. Voltage-gated sodium channels, for example, have recently gained particular interest as targets for the treatment of pain [51,52]. The local anesthetic binding site of this channel type is sensitive to several small molecules, which have traditionally yielded high hit rates in other (e.g. fluorescence-based) high-throughput screens. Because of the high conservation of the local anesthetic binding site between several sodium channel subtypes, however, these hits have so far suffered from low subtype selectivity. Consequently, marketed sodium channel drugs are rarely subtype selective and suffer from potential side-effects stemming from their activity on the whole complement of sodium channels. Some functional selectivity governed by a strong state- or use-dependence has so far been achieved, as opposed to pure pharmacological selectivity. We (and others) suspect that the high hit rate from traditional non-functional screens has masked compounds acting on less conserved binding positions. Binding sites located at the voltage paddles, which have recently been shown to provide subtype-selective binding positions for toxins [53], could thus become attractive, less conserved binding positions for selective sodium channel blockers. It can be speculated that a direct functional electrophysiological screen of diverse libraries on a sodium channel subtype could provide novel starting points for interesting chemistry, potentially yielding pharmacologically subtype-selective sodium channel blockers.

Selectivity and safety profiling by automated patch-clamp electrophysiology

One of the major applications of patch-clamp electrophysiology in the pharmaceutical industry in past years has been selectivity testing against the cardiac human ether-a-gogo-related (hERG) ion channel. This ion channel has a major role during the repolarization of the cardiac action potential, and it is well established that dysfunction or blockade of this ion channel can lead to potentially lethal arrhythmias of the torsades de pointe type (for a recent review, see e.g. Ref. [54]). Several drugs had to be withdrawn from the market because of their potential to induce this type of arrhythmia by a blockade of the hERG ion channel, which has, therefore, become a major concern in drug discovery and development. Consequently, regulatory guidelines now require compounds to be tested on hERG interactions during preclinical testing. Traditional patch clamping has not been able to deliver the throughput of compound testing on hERG required during preclinical development cycles and, thus, hERG tests have been one of the first major beneficiators of automated patch clamping in the pharmaceutical industry [55–59]. Although medicinal chemistry iteration cycles involving automated patch-clamp hERG tests have become a standard in many drug discovery programs, other mechanisms for cardiac side-effects are being increasingly understood, and assay systems that deliver more relevant high-content safety information, in addition to pure hERG effects, are being sought after. Relatively straightforward is the set-up of assays for off-target testing against the major cardiac ion channels (particularly NaV1.5, CaV1.2, Kv4.3/KChIP2, KCNQ1/KCNE1, Kv1.5 and Kir2, besides hERG) on automated patch-clamp devices. A more novel approach that is still evolving is the use of stem-cell-derived cardiac myocytes in safety pharmacology and patch-clamp electrophysiology [60,61]. Protocols to differentiate stem cells into cardiac myocytes have been developed (e.g. Refs [62–64]) but still seem technically challenging, particularly on a large scale. Such protocols will, we hope, in the near future, provide a nearly unlimited source of homogenous cardiac myocytes, which can be used for electrophysiology [63,65,66]. Recently, the first stem-cell-derived cardiac myocytes (albeit from mouse) have been applied to automated patch clamping, and action potential recordings from these cells have been shown (http://www.nanion.de/pdf/Port-a-Patch_Cardiomyocytes.pdf). The suitability of these for safety pharmacology investigations needs to be addressed further, however.

Atomic force microscopy in drug discovery and development

Atomic force microscopy (AFM) is arguably one of the most exciting recent developments for life science research. Invented in 1986 as a further development of the scanning tunneling microscope, the heart of the instrument is an atomically sharp tip attached to a sensitive cantilever [67]. By scanning a surface with the tip and monitoring the force between the surface and the tip, the instrument enables one to visualize surfaces of biological specimens (e.g. whole cells as well as their components) [68], in buffer conditions at high resolution. Even more attractive is the possibility of resolving substructures of individual membrane proteins [69], their interactions with the environment [70] or the effects of agents on their intermolecular interactions [71]. Besides imaging, AFM offers the possibility of measuring inter- and intramolecular forces at the nanometer scale [72,73]. Employing functionalized AFM tips, specific interactions of single molecules with the surface of living cells can be probed. In addition, cells have been used to functionalize AFM cantilevers, allowing cell adhesion to surfaces to be probed [74] (specifically, the adhesion between two cells [75]).

In another application, by using the AFM probe as an indenter at the micro- and nanometer scale, Stolz and coworkers have probed the elasticity of healthy and diseased cartilage on a functional level

[76,77]. Excitingly, early changes of osteoarthritis disease onset were only detectable at the nanometer scale. Compared with standard methods, such as histology staining or micron-sized indentation testing, this tool might speed up the testing of compound effects against osteoarthritis in model systems.

HTS has been a major driving force in past years in drug discovery at most pharmaceutical and many biotechnology companies worldwide. Most often, as discussed above, the required increased throughput is achieved by parallelization of readout technologies. Currently available commercial cantilever-based atomic force instrumentation, such as the JPK/nAmbition's ForceRobot® 200/500, promise to deliver greater than 80 000 force curves of individual molecules per 24 hours in unattended operation, with automatic variation of recording conditions such as temperature or loading rate, from a single cantilever. This instrument offers the particular advantage of simplifying the setup and calibration of an experiment with an individual cantilever, without the need for an experienced AFM operator. However, an instrument capable of high-throughput compound screening by a parallelized AFM technique is apparently not yet commercially available, although companies have successfully introduced AFM-related nanotechnology systems positioned to address the requests of drug discovery research. This includes the Nano eNabler™ technology by BioForce Nanosciences, a system that enables the microfluidic surface patterning for, for example, cell cultures. Nanotechnology methods related to AFM are, thus, becoming valuable additions to the drug discovery and development toolbox.

Concluding remarks

In the face of declining productivity, pharmaceutical companies are assessing and integrating new technologies into their drug discovery and development platforms to reduce final drug attrition rates. A major approach is to assess target function in a cellular background that more closely reflects the target tissue with processes that more closely mimic the (patho)physiological state of the target. Modern screening platforms now encompass a variety of different readout technologies filed to the target in hand and represent a distancing from earlier, more indirect readout approaches.

At present, the impact of such strategic changes on productivity remains difficult to assess. It will become more evident as preclinical programs advance into clinical trials. Over the coming years, it will be interesting to monitor the effect of the introduction of these new and exciting approaches within the pharmaceutical industry and justify the increased R&D spent [1,78,79]. In a continuation of the governing philosophy, we are certainly set to see the routine implementation of these and other new and emerging technologies.

References

1 David, E. *et al.* (2009) Pharmaceutical R&D: the road to positive returns. *Nat. Rev. Drug Discov.* 8, 609–610
2 Prous, J.R. and Khurdayan, V.K. (2007) The story so far in R&D. *Drug News Perspect.* 20, 7–15
3 Zambrowicz, B.P. and Sands, A.T. (2003) Knockouts model the 100 best-selling drugs – will they model the next 100? *Nat. Rev. Drug Discov.* 2, 38–51
4 Geffner, L.F. *et al.* (2008) Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. *Cell Transplant.* 17, 1277–1293
5 Krause, K. *et al.* (2009) Percutaneous intramyocardial stem cell injection in patients with acute myocardial infarction: first-in-man study. *Heart* 95, 1145–1152
6 Borowiak, M. *et al.* (2009) Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell* 4, 348–358
7 Desbordes, S.C. *et al.* (2008) High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. *Cell Stem Cell* 2, 602–612
8 Liu, Y. *et al.* (2009) Identification of small-molecule modulators of mouse SVZ progenitor cell proliferation and differentiation through high-throughput screening. *J. Biomol. Screen.* 14, 319–329
9 Chen, S. *et al.* (2009) A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat. Chem. Biol.* 5, 258–265
10 Takahashi, K. *et al.* (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872
11 Chin, M.H. *et al.* (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 5, 111–123
12 Dimos, J.T. *et al.* (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321, 1218–1221
13 Park, I.H. *et al.* (2008) Disease-specific induced pluripotent stem cells. *Cell* 134, 877–886
14 Ebert, A.D. *et al.* (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 457, 277–280
15 Miura, K. *et al.* (2009) Variation in the safety of induced pluripotent stem cell lines. *Nat. Biotechnol.* 27, 743–745
16 Kawamura, T. *et al.* (2009) Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* 460, 1140–1144
17 Marion, R.M. *et al.* (2009) A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* 460, 1149–1153
18 Bertelsen, M. (2006) Multiplex analysis of inflammatory signaling pathways using a high-content imaging system. *Methods Enzymol.* 414, 348–363
19 Durr, O. *et al.* (2007) Robust hit identification by quality assurance and multivariate data analysis of a high-content, cell-based assay. *J. Biomol. Screen.* 12, 1042–1049
20 Young, D.W. *et al.* (2008) Integrating high-content screening and ligand-target prediction to identify mechanism of action. *Nat. Chem. Biol.* 4, 59–68
21 Muller, F.J. *et al.* (2008) Regulatory networks define phenotypic classes of human stem cell lines. *Nature* 455, 401–405
22 Abraham, V.C. *et al.* (2008) Application of a high-content multiparameter cytotoxicity assay to prioritize compounds based on toxicity potential in humans. *J. Biomol. Screen.* 13, 527–537
23 Haasen, D. *et al.* (2008) Pharmacological profiling of chemokine receptor-directed compounds using high-content screening. *J. Biomol. Screen.* 13, 40–53
24 Antczak, C. *et al.* (2009) Live-cell imaging of caspase activation for high-content screening. *J. Biomol. Screen.* 14, 956–969
25 Wolff, M. *et al.* (2008) Activation and translocation of glucokinase in rat primary hepatocytes monitored by high content image analysis. *J. Biomol. Screen.* 13, 837–846
26 Vogt, A. *et al.* (2005) The benzo[c]phenanthridine alkaloid, sanguinarine, is a selective, cell-active inhibitor of mitogen-activated protein kinase phosphatase-1. *J. Biol. Chem.* 280, 19078–19086
27 Kwon, Y.J. *et al.* (2007) High-content classification of nucleocytoplasmic import or export inhibitors. *J. Biomol. Screen.* 12, 621–627
28 Haasen, D. *et al.* (2006) G protein-coupled receptor internalization assays in the high-content screening format. *Methods Enzymol.* 414, 121–139
29 Heilker, R. *et al.* (2009) G-protein-coupled receptor-focused drug discovery using a target class platform approach. *Drug Discov. Today* 14, 231–240
30 O'Brien, P.J. *et al.* (2006) High concordance of drug-induced human hepatotoxicity with *in vitro* cytotoxicity measured in a novel cell-based model using high content screening. *Arch. Toxicol.* 80, 580–604
31 Ainscow, E.K. *et al.* (2008) Investigations into the liver effects of ximelagatran using high content screening of primary human hepatocyte cultures. *Expert Opin. Drug Saf.* 7, 351–365
32 Radio, N.M. *et al.* (2008) Assessment of chemical effects on neurite outgrowth in PC12 cells using high content screening. *Toxicol. Sci.* 105, 106–118
33 Evensen, L. *et al.* (2009) Mural cell associated VEGF is required for organotypic vessel formation. *PLoS One* 4, e5798

34 Hu, M. *et al.* (2007) High content screen microscopy analysis of A beta 1-42-induced neurite outgrowth reduction in rat primary cortical neurons: neuroprotective effects of alpha 7 neuronal nicotinic acetylcholine receptor ligands. *Brain Res.* 1151, 227–235

35 Shiau, A.K. *et al.* (2008) Back to basics: label-free technologies for small molecule screening. *Comb. Chem. High Throughput Screen.* 11, 231–237

36 Xi, B. *et al.* (2008) The application of cell-based label-free technology in drug discovery. *Biotechnol. J.* 3, 484–495

37 Jahnke, H.G. *et al.* (2009) An impedimetric microelectrode-based array sensor for label-free detection of tau hyperphosphorylation in human cells. *Lab Chip* 9, 1422–1428

38 Lee, P.H. *et al.* (2008) Evaluation of dynamic mass redistribution technology for pharmacological studies of recombinant and endogenously expressed g protein-coupled receptors. *Assay Drug Dev. Technol.* 6, 83–94

39 Peters, M.F. *et al.* (2007) Evaluation of cellular dielectric spectroscopy, a whole-cell, label-free technology for drug discovery on Gi-coupled GPCRs. *J. Biomol. Screen.* 12, 312–319

40 Fang, Y. *et al.* (2005) Characteristics of dynamic mass redistribution of epidermal growth factor receptor signaling in living cells measured with label-free optical biosensors. *Anal. Chem.* 77, 5720–5725

41 Dunlop, J. *et al.* (2008) High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. *Nat. Rev. Drug Discov.* 7, 358–368

42 Hamill, O.P. *et al.* (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* 391, 85–100

43 Neher, E. and Sakmann, B. (1976) Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature* 260, 799–802

44 Finkel, A. *et al.* (2006) Population patch clamp improves data consistency and success rates in the measurement of ionic currents. *J. Biomol. Screen.* 11, 488–496

45 Dale, T.J. *et al.* (2007) Population patch clamp electrophysiology: a breakthrough technology for ion channel screening. *Mol. Biosyst.* 3, 714–722

46 Friis, S. *et al.* (2009) Characterization of compounds on nicotinic acetylcholine receptor alpha7 channels using higher throughput electrophysiology. *J. Neurosci. Methods* 177, 142–148

47 Dabrowski, M. (2009) Global ion channel initiative. *Ion Channel Retreat June 29–July 1, 2009* See also the Cellectricon web site

48 Dahan, E. *et al.* (2008) Rapid fluidic exchange microsystem for recording of fast ion channel kinetics in *Xenopus* oocytes. *Lab Chip* 8, 1809–1818

49 Stett, A. *et al.* (2003) Biological application of microelectrode arrays in drug discovery and basic research. *Anal. Bioanal. Chem.* 377, 486–495

50 Castle, N. *et al.* (2009) Sodium channel inhibitor drug discovery using automated high throughput electrophysiology platforms. *Comb. Chem. High Throughput Screen.* 12, 107–122

51 Amir, R. *et al.* (2006) The role of sodium channels in chronic inflammatory and neuropathic pain. *J. Pain* 7, S1–S29

52 Dib-Hajj, S.D. *et al.* (2009) Voltage-gated sodium channels in pain states: role in pathophysiology and targets for treatment. *Brain Res. Rev.* 60, 65–83

53 Schmalhofer, W.A. *et al.* (2008) ProTx-II, a selective inhibitor of NaV1.7 sodium channels, blocks action potential propagation in nociceptors. *Mol. Pharmacol.* 74, 1476–1484

54 Sanguinetti, M.C. and Tristani-Firouzi, M. (2006) hERG potassium channels and cardiac arrhythmia. *Nature* 440, 463–469

55 Guo, L. and Guthrie, H. (2005) Automated electrophysiology in the preclinical evaluation of drugs for potential QT prolongation. *J. Pharmacol. Toxicol. Methods* 52, 123–135

56 Kutchinsky, J. *et al.* (2003) Characterization of potassium channel modulators with QPatch automated patch-clamp technology: system characteristics and performance. *Assay Drug Dev. Technol.* 1, 685–693

57 Pugsley, M.K. (2005) Methodology used in safety pharmacology: appraisal of the state-of-the-art, the regulatory issues and new directions. *J. Pharmacol. Toxicol. Methods* 52, 1–5

58 Sorota, S. *et al.* (2005) Characterization of a hERG screen using the IonWorks HT: comparison to a hERG rubidium efflux screen. *Assay Drug Dev. Technol.* 3, 47–57

59 Tao, H. *et al.* (2004) Automated tight seal electrophysiology for assessing the potential hERG liability of pharmaceutical compounds. *Assay Drug Dev. Technol.* 2, 497–506

60 Kettenhofen, R. and Bohlen, H. (2008) Preclinical assessment of cardiac toxicity. *Drug Discov. Today* 13, 702–707

61 Steel, D. *et al.* (2009) Cardiomyocytes derived from human embryonic stem cells – characteristics and utility for drug discovery. *Curr. Opin. Drug Discov. Devel.* 12, 133–140

62 Bin, Z. *et al.* (2006) Efficient cardiomyocyte differentiation of embryonic stem cells by bone morphogenetic protein-2 combined with visceral endoderm-like cells. *Cell Biol. Int.* 30, 769–776

63 Igelmund, P. *et al.* (1999) Action potential propagation failures in long-term recordings from embryonic stem cell-derived cardiomyocytes in tissue culture. *Pflugers Arch.* 437, 669–679

64 Passier, R. and Mummery, C. (2005) Cardiomyocyte differentiation from embryonic and adult stem cells. *Curr. Opin. Biotechnol.* 16, 498–502

65 Shang, L.L. *et al.* (2006) Analysis of arrhythmic potential of embryonic stem cell-derived cardiomyocytes. *Methods Mol. Biol.* 330, 221–231

66 van Ginneken, A.C. and Fijnvandraat, A.C. (2007) Electrophysiological properties of embryonic stem cells during differentiation into cardiomyocyte-like cell types. *Methods Mol. Biol.* 403, 211–217

67 Binnig, G. *et al.* (1986) Atomic force microscope. *Phys. Rev. Lett.* 56, 930–933

68 Drake, B. *et al.* (1989) Imaging crystals, polymers, and processes in water with the atomic force microscope. *Science* 243, 1586–1589

69 Muller, D.J. *et al.* (1995) Imaging purple membranes in aqueous solutions at sub-nanometer resolution by atomic force microscopy. *Biophys. J.* 68, 1681–1686

70 Butt, H.J. (1992) Measuring local surface charge densities in electrolyte solutions with a scanning force microscope. *Biophys. J.* 63, 578–582

71 Moller, C. *et al.* (2000) Reversible loss of crystallinity on photobleaching purple membrane in the presence of hydroxylamine. *J. Mol. Biol.* 301, 869–879

72 Muller, D.J. *et al.* (2002) Stability of bacteriorhodopsin alpha-helices and loops analyzed by single-molecule force spectroscopy. *Biophys. J.* 83, 3578–3588

73 Oesterhelt, F. *et al.* (2000) Unfolding pathways of individual bacteriorhodopsins. *Science* 288, 143–146

74 Helenius, J. *et al.* (2008) Single-cell force spectroscopy. *J. Cell Sci.* 121, 1785–1791

75 Benoit, M. *et al.* (2000) Discrete interactions in cell adhesion measured by single-molecule force spectroscopy. *Nat. Cell Biol.* 2, 313–317

76 Aigner, T. *et al.* (2009) Nanomedicine: AFM tackles osteoarthritis. *Nat. Nanotechnol.* 4, 144–145

77 Stolz, M. *et al.* (2009) Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy. *Nat. Nanotechnol.* 4, 186–192

78 Collier, R. (2009) Drug development cost estimates hard to swallow. *CMAJ* 180, 279–280

79 Weiss, D. *et al.* (2009) The 'big pharma' dilemma: develop new drugs or promote existing ones? *Nat. Rev. Drug Discov.* 8, 533–534