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Streamlining the Drug Discovery Process by Integrating Miniaturization, High Throughput Screening, High Content Screening, and Automation on the CellChip™ System

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Abstract. **A major bottleneck to the early stages of drug discovery is the absence of integration of high throughput screening (HTS) with smarter assays that screen ''hits'' from HTS to identify leads (High content screening, HCS). We propose a solution using novel fluorescent engineered protein biosensors integrated into a miniaturized live-cell-based screening platform (CellChip™ System) that markedly shortens the early drug discovery process. Microarrays of selectively localized living cells, containing engineered fluorescent biosensors, serve to integrate HTS and HCS onto a single platform. HTS ''hits'' are identified using one biosensor while reading the whole chip array of cells. The high-biological content information is then obtained from probing target activity at inter-cellular, sub-cellular and molecular levels in the ''hit'' wells. HCS assays yield temporal-spatial dynamic maps of the drug-target interaction within each living cell. We predict that a new platform incorporating HTS and HCS assays that are automated, miniaturized, and information-rich will dramatically improve the decision making process in the pharmaceutical industry and optimize lead compounds during the early part of the drug discovery process. There is an opportunity to establish a new paradigm for drug discovery based on integration of fluorescence technology, micropatterning of living cells, automated optical detection and data analysis, and a new generation of knowledge building bioinformatics approaches. The technology will have an expansive impact spanning the fields of drug discovery, biomedical research, environmental monitoring, life sciences, and clinical diagnostics. The integrated CellChip™ Platform with miniaturized tissue-specific microarrayed cells capable of providing inter-cellular and sub-cellular spatio-temporal information in response to drug-cell, toxin-cell, or pathogen-cell interactions will serve to enhance the decision making process in drug discovery, toxicology, and clinical diagnostics.**

Key Words. **drug discovery, CellChip, high content screening, fluorescence, patterning, sensors, microarrays, bioinformatics, tissue engineering**

Introduction

Bottlenecks in early drug-discovery

A major obstacle to the early stages of drug discovery is smarter lead compound optimization. Drug discovery can now be categorized as a series of processes that can be measured by the number of candidates identified in a given period with a defined level of resources. Productivity and speed become critical discovery performance metrics. The discovery process is typically defined as being composed of four distinct, yet related, processes: (1) target identification/validation, (2) lead identification, (3) lead optimization, and (4) discovery/development interface, with early drug discovery encompassing the first three of these processes. The successes in Target Identification due to the application of DNA sequencing and genomics databases have created serious bottlenecks downstream in the drug discovery pipeline (Figure 1) (Giuliano, 1997). These constrictions exist at target validation, lead identification (which includes high throughput screening (HTS)), and lead optimization. The pharmaceutical industry has eased the bottleneck at the Lead Identification step by employing HTS to test large libraries against a growing list of targets. The critical constriction at Lead Optimization is getting worse as the speed of HTS increases. Speed alone is insufficient to identify optimal lead compounds emerging at the end of the early discovery pipeline prior to evaluation in expensive animal models. Higher biological content information on the effect of the compounds on cellular targets and cellular processes is required.

One of the critical bottlenecks, lead optimization begins with the identified ''hits'' from HTS and focuses on the construction of a limited number of structural variations of the ''hit'' molecule coupled to biological evaluation for improvements in specificity, activity,

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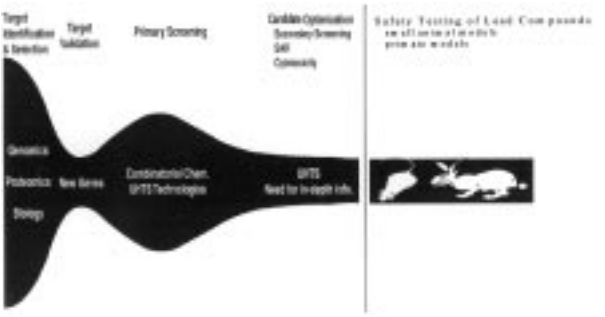

Fig. 1. Critical bottlenecks in the 'drug development pipeline' can potentially lead to too few well-qualified and too many poorly qualified lead compounds being tested in animal models.

selectivity and potency criteria. The rate at which ''qualified leads'' can be successfully generated from this process is dependent on the integration of chemical and biological data on each compound. A reduction in the time devoted to lead optimization can best be achieved by decreasing the number and length of feedback loops between chemists and biologists and increasing the depth of the biological information.

Industry analysts have predicted that the pharmaceutical industry will need to increase the number of products launched by 4-fold, to at least 24 to 36 drugs per annum, each earning more than 1 billion, to achieve the forecasts for growth in global sales of 7% annually. The industry has reduced the average drug development project from 12 years to 8 years and is currently assessing and incorporating technologies that, in the future, will further reduce this to a period of 4 years. With these reductions in development project duration, the drug discovery process becomes the major time consuming component of the entire drug discovery and development pipeline. This requires an increase in efficiency and effectiveness to realize the promise of an increase in the number and quality of targets. Easing the bottleneck at lead optimization with technology capable of providing high content biological data for chemical compound evaluation can help achieve the target of reducing Lead Optimization from a process taking 24–36 months to a period of 12–18 months.

State of the art in early drug discovery

Radioactive assays and solution-based fluorescence assays have dominated the lead identification and lead optimization phases of compound development in industry standard microplates (96, 384, 1536 wells). These assays provide only limited information on the potency, and specificity of the compounds because drug target activity is often assessed using isolated targets and not whole, living cells. Recently primary and secondary screening assays have employed living cells that provide responses that are increasingly based on fluorescence read-outs. These live cell assays provide an averaged population response from cells cultured in industry standard microplates to screen for target activity and cellular toxicity of the lead compounds. The ''hits'' from these assays serve as binary indicators of the potency, target activity, toxicity, and bioavailability of test compounds resulting in their classification as ''lead compounds'' for safety, toxicity, and efficacy testing in animal models.

However, a new approach providing deep, functional knowledge generation at the lead optimization phase is required to move from binary to multiple bit biological readout data. Sophisticated, multi-color fluorescence, cell based assays can provide critical cellular and molecular spatio-temporal target activity information that will result in ''highly qualified hits'' being moved downstream into the animal testing phase. The implementation of ultra high throughput screening strategies is leading to a higher number of ''poorly qualified hits'' being passed down the pipeline towards expensive *in vivo* animal studies. Therefore, in addition to HTS, it is vital to provide a rich array of assays in living cells that provide ''deep biology'' information pertaining to the specificity, toxicity, bioavailability, and potency of candidate compounds. This, in turn, leads to better decision making in selecting the optimum compounds as leads for further safety and efficacy testing in animal models.

A new paradigm in drug-discovery

The one property common to all drug candidate compounds is their target, the living cell. Therefore, we are using live cells and novel fluorescence-based biosensors to serve as real time fluorescent reporters for analysis of pharmaceutical drug candidate compounds and as sentries of toxic agents of threat (chemical and biological warfare agents). We use an integrated systems approach to obtain high biological content data from an automated miniaturized HTS/HCS platform (CellChip^{TM} System) using our engineered biosensor cells as reporters of drug and toxin activities (Figure 2):

High content screening. Normal cell function involves the dynamic spatio-temporal distribution and activity changes of biomolecules, therefore developing an understanding of how lead compounds affect cellular function requires tools that provide temporal and spatial information of target activity within, on, and between cells. High content screens designed to extract deep biological information using advanced fluorescent protein biosensors will provide the desired ''high-content information'' by directly measuring the effects of drugs/toxins on complex molecular processes such as signal transduction pathways, and cellular functions like mitosis, phagocy-

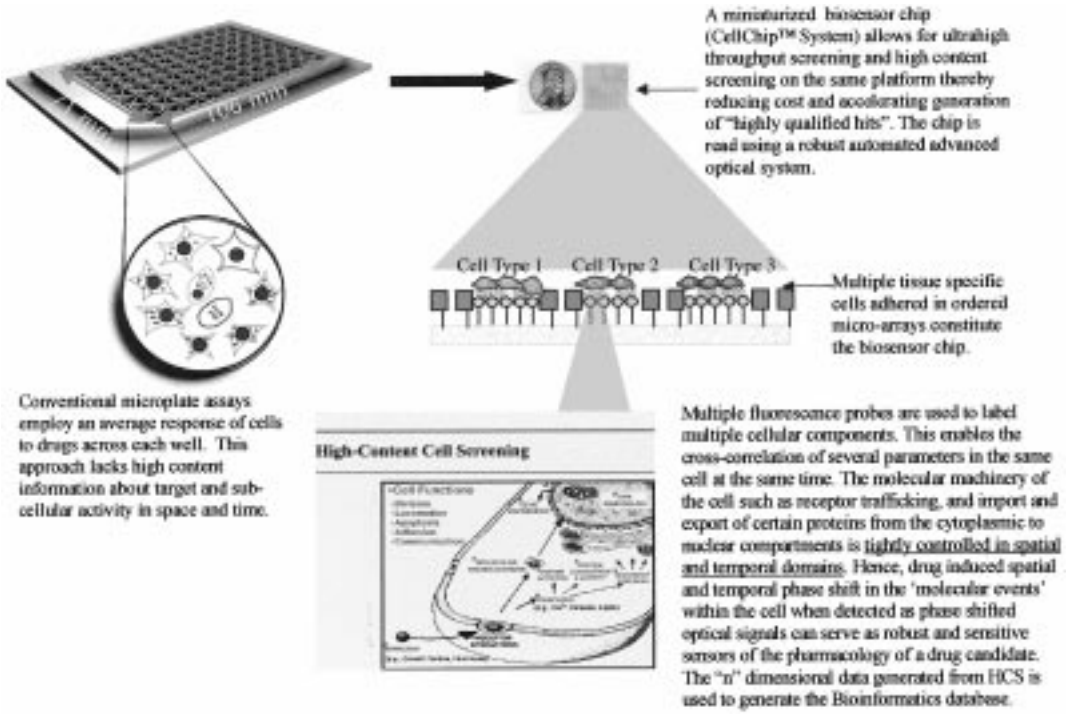

Fig. 2. *Coupled HTS and HCS on an automated and miniaturized CellChip™ system yields deep biological knowledge of the spatio-temporal target activity in response to test compounds or toxins. The HCS information is used to populate and develop a Bioinformatics database to manage and analyze the enormous amount of multidimensional information that will be used to build the cellular knowledge database critical to the new paradigm in drug discovery.*

tosis, endocytosis, exocytosis, locomotion, apoptosis, and cell-cell communication. These novel assays provide spatial and temporal information of target activity in, on and between cells. Used in conjunction with the state-of-the-art HTS assays, our HCS assays will enable classification of a subset of ''hits'' into ''leads.'' Therefore, HCS used in conjunction with novel fluorescent reagents, many of which are engineered to be expressed within living cells, enables the automated extraction of multiparameter information from single cells and cell populations (Giuliano et al., 1997).

A significant component of HCS is the development of fluorescent protein biosensors designed to sense and report the intracellular spatio-temporal changes in target activity in response to lead compounds. Proteins mediate the majority of intracellular chemical reactions. As such, tagging them with fluorescent reagents enables their use as optical reporters of the dynamic distribution of specific reactions, kinetics of reactions, and post-translational modifications. The protein component of the biosensor serves as a highly selective molecular-recognition moiety containing a fluorescent molecule attached, in many cases, proximal to the active site of the protein, thus serving to transduce the environmental changes into fluorescence signals for optical readout of target localization and activity (Giuliano et al., 1995; Giuliano and Taylor, 1998). New fluorescent protein biosensors along with other fluorescent reagents utilized for HCS are depicted in Figure 3. Our multiparameter approach combines several key reagents in single cells to yield multidimensional information on the modulation of cell function by drug candidates or toxins. Our efforts to engineer sensor cells to express fluorescent protein biosensors where the fluorescent component is an autofluorescent derivative of the green fluorescent protein (GFP) and its spectral mutants, further strengthens HCS as the cells can then produce the biosensor(s) and become reagents in themselves.

An example class of HCS involves the quantitation of translocation of cytoplasmic ligand-receptor complexes following receptor activation (Figure 4). This figure depicts a single parameter high-content assay being performed on a population of engineered sensor cells.

Automation. The ArrayScan™ System (Ding et al., 1998; Conway et al., 1999) is a fully automated platform for high-content screens performed in several formats including microplates and chips. The instrument scans microplates and CellChips™ and acquires multicolor fluorescence images of cells at sub-cellular resolution.

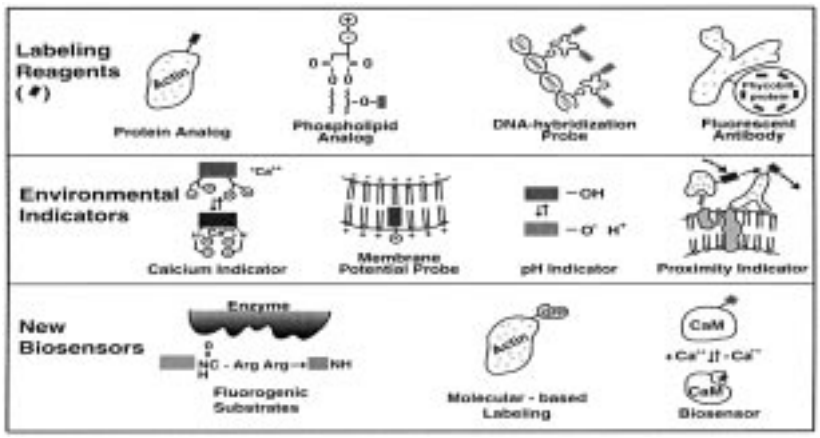

Fig. 3. *Fluorescent reagents for smart drug discovery. Classes of these fluorescent reagents include labeling reagents that measure the distribution and amount of molecules in living cells, as well as environmental indicators to report signal transduction events in space and time.*

The system comprises robotic hardware for multiple microplate handling, automated fluorescence excitation and emission optics, solid-state camera, a dedicated processor, on-board algorithms for fluorescence image feature extraction, and database management capabilities (Figure 5). Additionally, an environmental-chamber with controlled temperature, humidity, and gas supports the use of live cells in the ArrayScan^{TM} System.

Miniaturization. Miniaturization is one of the major forces driving improved productivity in early drug discovery. (HTBD, 1998; Kapur et al., 1999; Service, 1998). Market research indicates that miniaturized cellular test beds with footprints the size of postage stamps will capture 50% of the microplate market within 4 years. The advantages offered by miniaturization include: (1) a combined HTS and HCS platform with a single pass read of the HTS data from all ''wells'', (2) higher throughput, (3) reduced processing time, (4) increased number of tests run in a massive parallel format on one substrate, (5) smaller reagent volumes, (6) conservation of new chemical entity volumes (NCE) and, (7) reduced waste. The integration of such advantages translates into a dramatic reduction of cost and acceleration of productivity in candidate compound testing. For example, migrating assays from 96-well microplate to 1536-well microplate versions reduces the reagent cost by 100 fold. Migration from the microplate format to microarrayed cells on chips will dramatically drop the volume of reagents and the cost of plate

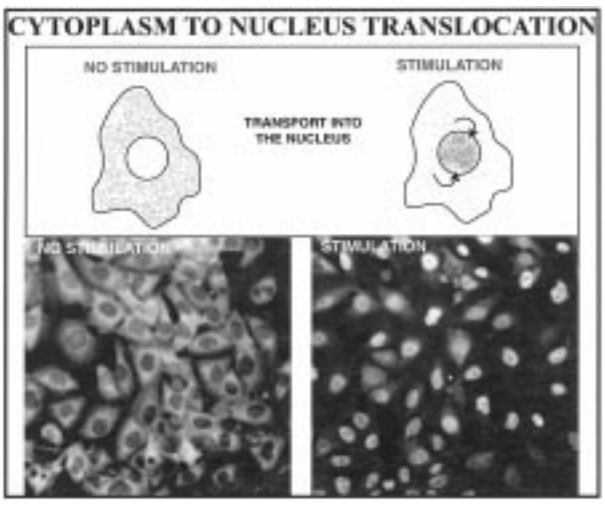

Fig. 4. *Measurement of drug induced redistribution of intracellular molecules. The effect of drug treatment is indicated by the translocation of a steroid receptor-GFP chimera that is synthesized within the living cells. The ArrayScan^{TM} system's automated image analysis software quantitates these responses for both steady-state and for time-dependent, live cell studies.*

Fig. 5. *The ArrayScan^{TM} system. An automated platform produced by Cellomics^{TM}, Inc. for HCS. A plate stacking robot is shown here delivering a microplate to the sample compartment for HCS. The ArrayScan^{TM} system allows evaluation of high content screens on microplate and prototypic CellChip^{TM} platforms.*

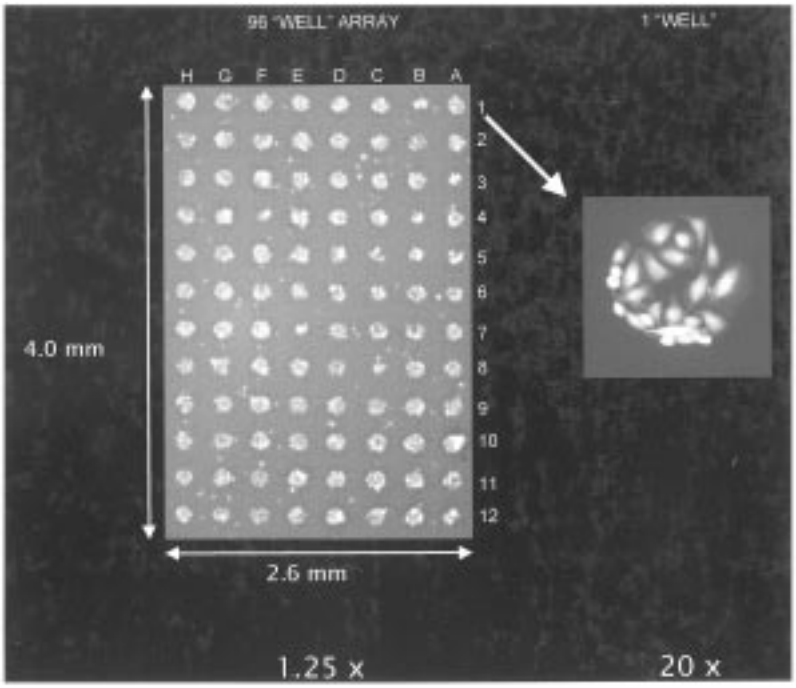

Fig. 6. *Mammalian cells arrayed on the CellChip™ Platform in a* 12×8 *array format covering a macroscopic footprint orders of magnitude smaller than a 96 well microplate. At low magnification the whole chip can be analyzed for HTS (changes in activity over time), while at higher magnifications individual wells and cells can be analyzed in a HCS mode (changes in activity over space and time).*

handling (Figure 6). Furthermore, developing chips microarrayed with tissue specific cells will have a tremendous impact in screening the potency, specificity, toxicity, and efficacy of test compounds against a ''tissue-like'' ensemble leading to higher predictive relevance of the live cell data.

Developing multiple cell based test-beds microarrayed in preferred and known addresses on chips will enable the use of these 'microscale tissues' as powerful indicators and predictors of the *in vivo* performance of the lead compound or toxin using HCS. The evolution of a miniaturized cell-based drug/toxin discovery and identification platform, the CellChip™ System, will march in parallel with other miniaturization technologies aimed at shrinking bench-top instruments into their hand-held miniaturized versions (Marshall, 1999; Ding et al., 1998). Furthermore, the combination of HTS and HCS onto a single platform (Figure 7) will dramatically shorten the pipeline. This combined platform reduces the data capture, processing, and analysis times and provides a complete cell-based screening platform. Developing technologies to enable arrays of multiple cells on glass or plastic chips, with each cell carrying its own reagents in the form of our novel fluorescent protein biosensors, adds an *n*th dimensional power to a complete drug screening platform. Furthermore, reagent and assay technology developments made on today's platform will migrate directly to the next generation miniaturized platform.

CellChip™ System as an Automated HTS/ HCS Integrated Platform

Platform

The miniaturized platform consists of single or multiple engineered cells types microarrayed in predetermined spatial addresses on an optically clear substrate of choice (glass and polymers). Self-assembled hetero-monolayers coupled with arrayed cell-specific ligands are used to array single or multiple cell types on the miniaturized platfrom (Kapur, 1999). Such arrays of live single and multiple cells function as interrogators and reporters of drugs, organics, organisms, and pathogens. Each cellular domain can be accurately defined from one cell diameter to several hundred cell diameters. In one prototype, a 12×8 array of domains cover a 10×10 mm area on either glass or optically clear flexible plastic (Figure 8). These domains can be populated either with a single cell type, or with multiple cell types by adhesive self-sorting from a mixed cell suspension, according to selective adhesive interactions with particular cell-specific ligands coupled to the individual domains (Figure 9).

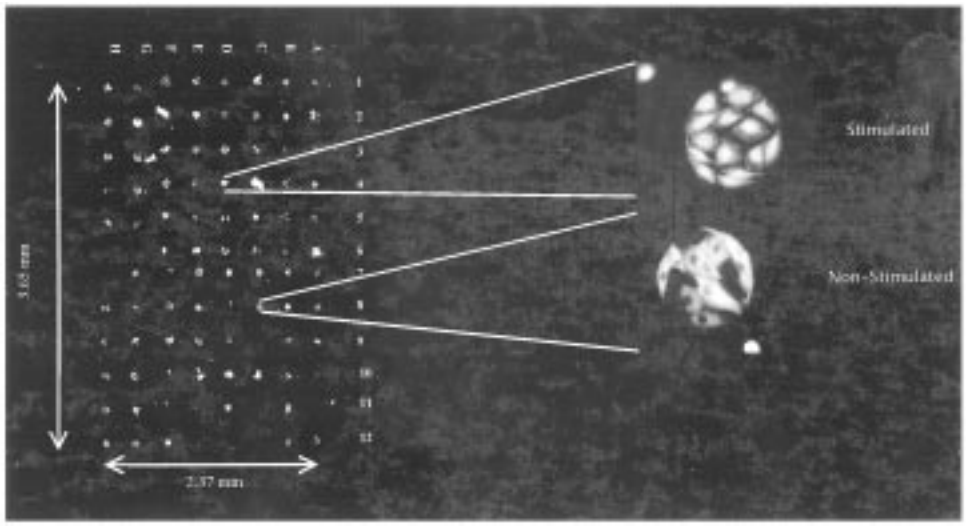

Fig. 7. *Combining HTS and HCS on the same platform. The massive parallelization achievable with miniaturization is shown in a simple simulation on this surface modified glass platform. The HTS is simulated here to detect ''hits'' on the miniaturized chip platform. Lack of fluorescence signals in wells H6, C9, C12, and D12, for example, indicate ''non-hits''. HCS measurements are then made only on the ''hit'' wells to gain more in-depth information to produce more ''highly qualified hits''. Further depth and breadth of information can be obtained by arraying multiple organ specific cells on a single chip and fluidically addressing each domain with a reagent of choice.*

The ability to engineer the CellChip™ Substrate with two or more cell types adds a very powerful dimension to the use of these chips for profiling a drug candidate across several tissue and organotypic cells. In one model of a 12×8 array of cytophilic islands, fluidic addressing of each cellular domain, where each domain represents one cell type, enables addressing 96 different cell types with one drug candidate compound on one chip. This massive parallelization of assays on the CellChip™ System will increase the throughput in both HTS and HCS modes.

High content screens on the CellChip™ platform

Cytoplasm to nuclear translocation of intracellular molecules

This class of cell-based screens tests the ability of chemical entities (candidate drug compounds, toxins, pathogens, and other organisms) to induce or inhibit transport of intracellular molecules such as transcription factors and stress associated proteins from the cytoplasm

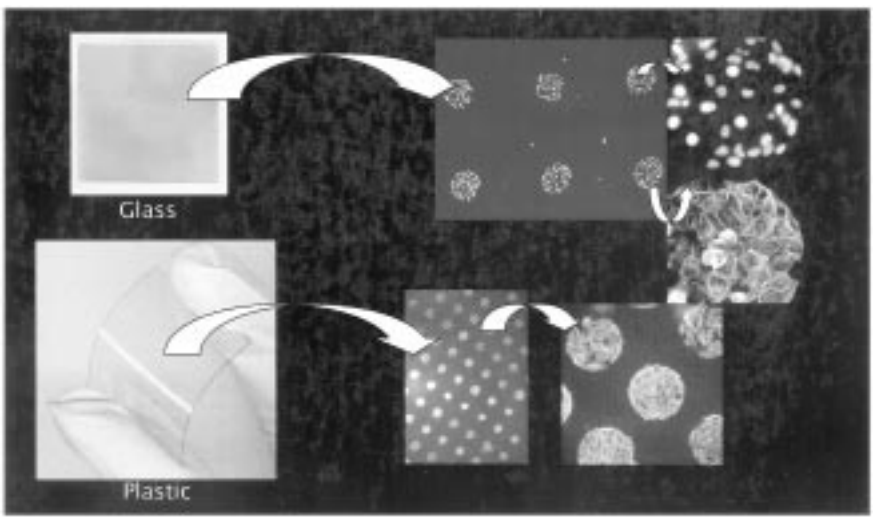

Fig. 8. *The CellChip™ platform on glass and thermoplastic substrates.*

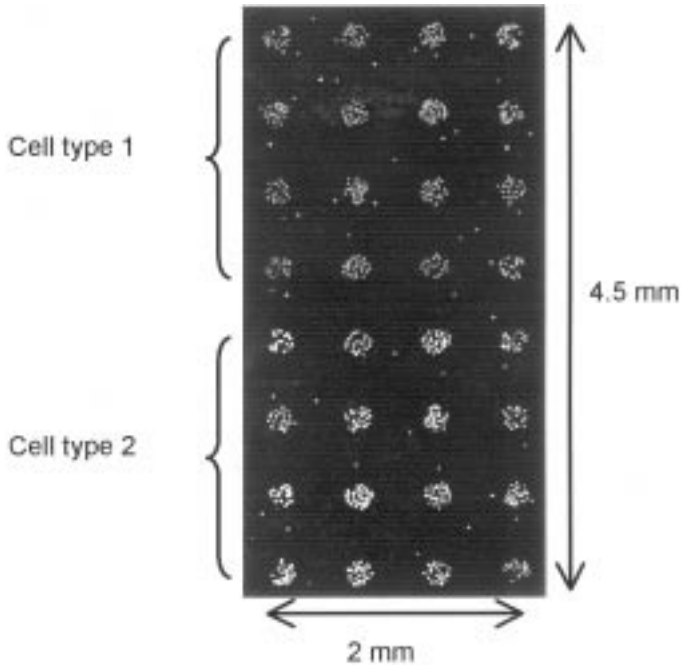

Fig. 9. *Cell specific macromolecule mediated generation of multicellular CellChip™ Plates. A-20 (red stained cells), and X-16 (green stained cells) lymphocytic cell lines were mixed together in medium and plated on the engineered CellChip™ system resulting in self-sorting and adhesion of the two cell types onto cytophilic islands bearing the cell-specific ligand.*

into the nucleus. Sensor cells arrayed on the CellChip™ platform are treated with a combination of chemical entities. The assays can be run as ''fixed endpoint'' or dynamic live cell assays. In a fixed endpoint assay the array of sensor cells is treated with a chemical fixative and labeled with a nucleic acid probe at one color of fluorescence and an antibody against the transcription factor or stress associated protein labeled with another color. The test consists of measuring the fluorescence from the antibody in the nucleus (the nucleus being defined by the nucleic acid probe), versus the cytoplasm defined by the cell domain outside of the nucleus (Ding et al., 1998). Proprietary algorithms enable quantitation of the kinetics and amount of transcription factor or stress protein translocation into the nucleus over time. Quantitation of changes in the spatial and temporal distribution of key molecular events by chemical entities as afforded by the combination of the ArrayScan™ system and high content screens is unparalleled. The CellChip™ platform adds the power of combining HCS and increased throughput.

Cytoplasm to nuclear translocation of transcription factor NF-κB

The NF-κB transcription factor pathway is associated with the activation of many cellular defense genes (Siebenlist, 1995). This pathway can be activated by tumor necrosis factor-alpha (TNFα), a pro-inflammatory cytokine. NF-κB is normally found in an inactive state in the cytoplasm coupled with IκBα, a member of the IκB inhibitor protein family. In this complexed form, a nuclear localization sequence found on NF-κB is masked by the IκBα, preventing nuclear translocation of NF-κB, DNA binding, and transcriptional activation (Baeuerle, 1988). The activation of the TNFα receptor initiates a cascade of events which includes the specific phophorylation of Ser32 and Ser36 residues in IκBα, the specific destruction of the phosphorylated IκBα protein by proteasomes, resulting in the release of NF-κB, and translocation of intact NF-κB to the nucleus (Beg, 1993).

Using the CellChip™ System, we have measured the effects of TNFα on the NFκB nuclear translocation response. Mammalian cells were patterned on the CellChip™ Platform in complete serum supplemented medium and maintained in a controlled 5% CO_2 and 37 °C environment. The cells were rinsed after a short incubation, followed by overnight incubation in fresh medium. The following day, appropriate cellular domains on the CellChip™ Platform were incubated with a pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α). Post exposure to TNF-α the cells were fixed, permeabilized and labeled for the NF-κB p65 domain and the nucleus (Cellomics ''HitKit™'' Catalog #K01-0001-1). As seen in Figure 10, there is a

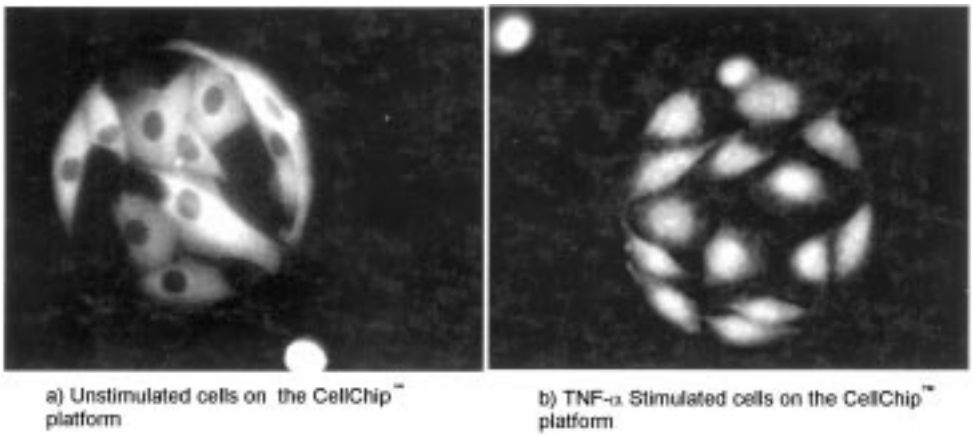

Fig. 10. *Mammalian cells on the CellChip™ Platform employed in a high content Screen for transcription factor activation. As a result of stimulation with a pro-inflammatory factor such as tumor necrosis factor-alpha, there is a spatial redistribution of nuclear factor kappa B. There is a 4-fold increase in nuclear intensity post stimulation, as quantified with ArrayScan™ system.*

redistribution of the transcription factor to the nucleus as a result of stimulation. There is up to a 4-fold increase in normalized nuclear intensity and the normalized ratio between nuclear and cytoplasmic intensities post-translocation. This 4-fold increase is equivalent to the results obatined in the microplate platform (Ding et al., 1999).

Cytoplasm to nuclear translocation of stress proteins

Most mammalian cells will respond to a variety of environmental stimuli with the induction of a family of proteins called stress proteins. Anoxia, amino acid analogues, sulfhydryl-reacting reagents, transition metal ions, decouplers of oxidative phosphorylation, viral infections, ethanol, antibiotics, ionophores, non-steroidal antiinflammatory drugs, and metal chelators are all inducers or translocators of cell stress proteins (Bachelet et al., 1998). Upon induction these proteins play a role in folding and unfolding proteins, stabilizing proteins in abnormal configurations, and repairing DNA damage (Ketis and Karnovsky, 1988).

Using the CellChip™ System, we have also measured the effects of drugs on the translocation response of stress proteins. Mammalian cells were patterned on the CellChip™ platform in complete serum supplemented medium and maintained in a controlled 5% CO_2 and 37 °C environment. The cells were rinsed after a short incubation, followed by overnight incubation in fresh medium. The following day, the appropriate cellular domains on the CellChip™ Platform were incubated with a non-steroidal anti-inflammatory drug. Following exposure to the drug, the cells were fixed, permeabilized and labelled for the stress protein and the nucleus (Cellomics ''HitKit™'' under development). As seen in Figure 11, there is a substantial redistribution of the stress protein after stimulation. In the control cells, the protein is localized in the cytoplasm with basal levels in the nucleus. After stimulation there is up to a 3-fold increase in normalized nuclear intensity corresponding to translocation of the cytoplasmic protein into the nucleus.

Drug-induced Temporal and Spatial Dynamics of the Microtubule Cytoskeleton within Single Living Tumor Cells

Microtubules are cytoskeletal components that play pivotal roles in a variety of cellular processes ranging from mitosis and cell motility to intracellular transport and establishment of cell shape. Within the cell, microtubules assume a characteristic radial array emanating from a centrally located microtubule organizing center, or centrosome. Immediately preceding mitosis, the interphase microtubule array disassembles and remodels to form the mitotic spindle. Microtubules are inherently dynamic structures that undergo rapid and frequent changes in length, particularly at the plus end, in a process termed dynamic instability. The dynamic nature of microtubules is essential for many of the processes they mediate, including mitosis, and many anti-mitotic compounds specifically alter microtubule dynamics. Such changes in dynamics are manifested as either an overall loss of microtubule polymer as a result of depolymerization, or an increase in polymer due to enhanced microtubule stabilization through an inhibition of dynamic instability.

To measure the effects of anti-mitotic drugs on the microtubule cytoskeleton in living cells a high-content

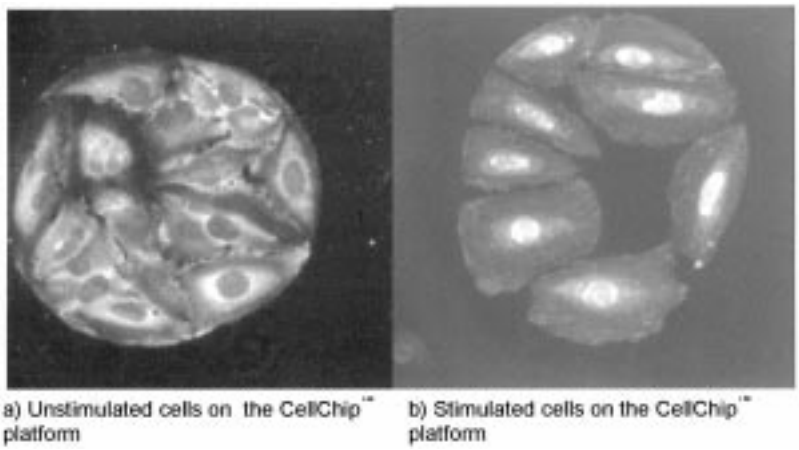

Fig. 11. *Mammalian cells on the CellChip™ platform employed in a high content screen for activation of stress-associated protein. Post stimulation with non-steroidal anti-inflammatory drug, there is a spatial redistribution of stress associated protein. There is a 2–3 fold increase in normalized nuclear intensity post stimulation.*

screen of microtubule assembly has been developed for the ArrayScan™ system. The reporter of intracellular microtubule assembly is a fluorescent probe whose temporal and spatial distribution in living cells is measured both in the absence and presence of potential lead compounds. The ArrayScan™ system produces a time series containing images of the microtubule cytoskeleton. Images are collected prior to drug treatment, and then continuously from minutes to hours after the addition of drug. The ArrayScan™ system analyzes the cell images concurrent with their acquisition. Automated feature extraction quantifies the dynamics of the microtubule cytoskeleton during the experiment and a time series of values representing the dynamic assembly-state of microtubules is returned to the user. Temporal and spatial variations in these values provide the user with important data concerning the efficacy and bioavailability of the drug. Hence, the assay is designed to take a validated anticancer drug target, the microtubule cytoskeleton, from the test tube into the living cell where the true activity of potential lead compounds can be approached and quantified. In parallel, a proprietary algorithm extracts features quantifying the shape and size of the nucleus. This results in a measured effect of potential lead compounds on both cytoskeletal dynamics and nuclear morphology.

We have used the CellChip™ system to analyze the effects of anti-microtubule drugs on both microtubule organization and nuclear morphology. Mammalian cells were patterned on the CellChip™ Platform in complete serum supplemented medium and maintained in a 5% CO_2 and 37 °C environment. The cells were rinsed after a short incubation, followed by overnight incubation in fresh medium. The following day, the cells were treated with anti-mitotic drugs, paclitaxel, or curacin-A to induce microtubule disruption. The cells were incubated with the drugs in a controlled 5% CO_2 and 37 °C environment. Post-treatment, the cells were fixed, permeabilized and labeled for the microtubule cytoskeleton and the nucleus (Cellomics ''HitKit™'' under development). As seen in Figure 12, both paclitaxel and curacin-A have dramatic effects on microtubule organization compared to untreated cells. Paclitaxel inhibits microtubule dynamics, resulting in the formation of thick microtubule bundles with increased labeling intensity. In addition, paclitaxel interferes with cell cycle progression, and after prolonged incubation induces apoptosis. Apoptotic cells are identified based upon the nuclear fragmentation that is observed in paclitaxel treated cells. In contrast to the effects seen with paclitaxel, curacin A increases microtubule dynamics and results in depolymerization of most microtubules within the cell. As a result, a diffuse microtubule-labeling pattern is observed, rather than the typical interphase microtubule array. In some cases, small amounts of microtubule polymer remain that most likely represent populations of stabilized microtubules normally present in cells. Unlike paclitaxel, curacin-A does not have a significant effect on nuclear morphology, suggesting that it does not induce this stage of apoptosis. The differential effect of the two drugs on nuclear morphology is simultaneously detected and quantitated. This enables a 2 parameter (microtubule organization and nuclear morphology) high content screen to be conducted on any given set of cells.

Summary

The cell is the next frontier in early drug discovery. The n-bit language of cellular physiology is a combinatorial

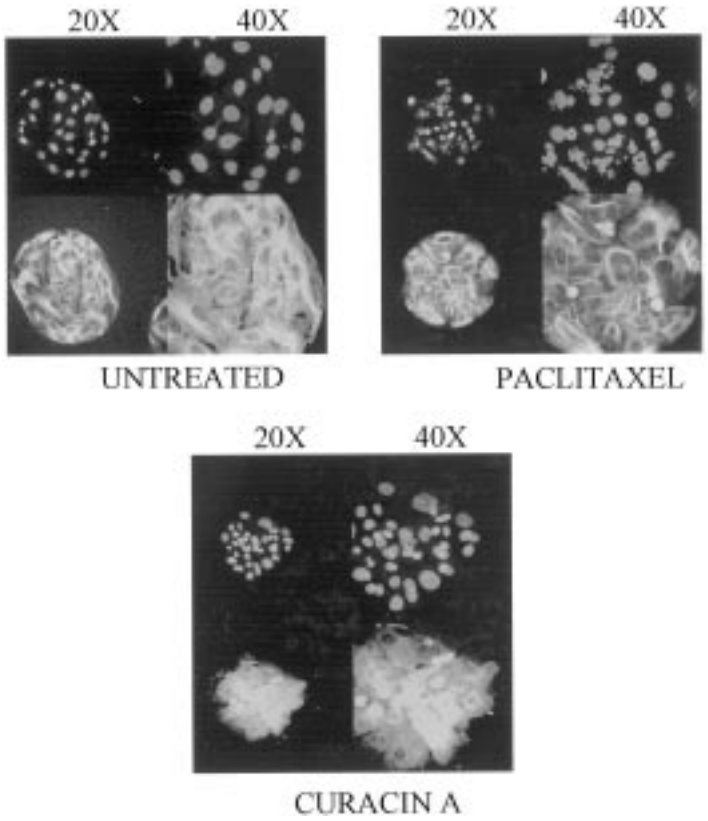

Fig. 12. *Mammalian cells on the CellChip™ platform employed towards a high content screen for microtubule stability. Post-treatment with anti-mitotic drugs, paclitaxel and curacin, there is a spatial redistribution of microtubules. The microtubule bundling induced by paclitaxel, and microtubule de-polymerization induced by curacin are detected and quantitated by a proprietary algorithm.*

integration of the 4-bit nucleotide language of the DNA, and the 20-bit language of amino acids. High content screens on living cells are used to dissect this complex language and provide deep biological information on target distribution and activity in space and time to provide better decision making systems in profiling candidate compounds. Multiparameter, multicellular information from an automated miniaturized system such as the CellChip™ system enables the coupling of HCS and HTS on a single robust platform. This yields faster and more efficient winnowing of ''leads'' from ''hits'' thereby optimizing the selection of the compounds at the end of the early discovery pipeline prior to evaluation in animal models. Early investment in high content information from cell based systems can thereby reduce the cost and time associated with animal and clinical trials.

The massive parallelization of assays foreseeable with the microarrayed multicellular CellChip™ platform, and the conservation of new chemical entities and other reagents due to the small volume of each reaction ''well'' will together serve to simultaneously increase throughput and reduce costs. With the pharmaceutical industry marching steadily to the drumbeat of ''smaller, cheaper, and faster,'' the CellChip™ System is leading the way with ''smaller, cheaper, faster, and better.''

Based on the high content information obtained from high content screens conducted on live cells on the CellChip™ platform, we propose Cellome's Law (analogous to Ohm's Law)* *Cellome's Law* states that resistance to low cost-high speed drug discovery, R, is equal to the volume of the reaction wells, V, divided by the information content, I:

$$R = V/I.$$

Reduction in volume of reaction wells (through miniaturization) and increase in information content from reaction wells (through coupled HTS/HCS) will

*Ohm's Law states that the electrical resistance [R] equals the voltage [V] divided by the current [I]:$R = V/I$.

serve to reduce the resistance to achieving low cost-high speed drug discovery.

Thus Cellome's Law can be restated as:

low cost-high speed drug discovery =
miniaturization coupled with high content screening

Future Developments

The Cellomics^{TM} Knowledge Base

The Cellomics Knowledge Base is a bioinformatics platform that applies techniques for managing, analyzing, and making predictive decisions on the vast amount of multiparametered cellular information generated through high content screens. The database is designed to capture and analyze the complex spatio-temporal patterns and interrelationships between key biomolecules within cells. The database organizes multiparameter information connecting multiple intracellular molecular processes and hence enables the eventual construction of a ''wiring diagram'' of intracellular pathways of pharmacological and diagnostic relevance.

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