Bioengineering in Cell and Tissue Research

Gerhard M. Artmann · Shu Chien (Eds.)

# Bioengineering in Cell and Tissue Research

With 342 Figures

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The cover figure represents a shape transformed red blood cell (echinocyte) as calculated by Prof. Reinhard Grebe, University of Technology, Compiegne, France.

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# Introduction

The idea of publishing this book on "*Bioengineering in Cell and Tissue Research*" was originated by Gerhard M. Artmann, with the goal of writing about our dreams and making the reader dream with the authors and be fascinated. The book is meant to have life and spirit, and to become a pioneer in technology and sciences, especially the life science. The chapters in this book are written by excellent scientists on advanced, frontier technology and address scientific questions that need considerable thinking in terms of engineering. The aims are to provide the readers, including students, faculty, and all scientists working in academia and industry, new information on bioengineering in cell and tissue research to enhance their understanding and innovation.

This book is composed of six sections that cover a broad hierarchy from genes to the universe. These sections are Genes, Genome and Information Network; Cell and Tissue Imaging; Regenerative Medicine and Nanoengineering; Mechanics of Soft Tissues, Fluids and Molecules; Bioengineering in Clinical Applications; and Plant and Microbial Bioengineering.

*Section I* on "*Genes, Genome and Information Network*" contains three chapters:

*Chapter 1* on "*Reporter Genes in Cell-based Ultra-High Throughput Screening*" by Stefan Golz presents the processes used to identify target-specific, disease-relevant genes or gene products devoid of side effects with the aid of ultra-high throughput screening (uHTS). It discusses how to set up a drug discovery pipeline starting from target identification to finally delivering molecules for clinical development. This chapter presents many of the large arsenal of technologies available for researchers in industry and academia to generate data in support of a functional link between given genes and a disease state. The author concludes that experimental testing of candidate drug compounds remains the major route for the lead drug discovery process, which has been aided by uHTS using targeted assays together with the design of combinatorial chemistry libraries. Converting the knowledge of the target mechanism and underlying molecular recognition principles into robust and sensitive assays is a prerequisite of successful uHTS screening.

*Chapter 2* on "*Gene Arrays for Gene Discovery*" by David Ruau and Martin Zenke provides an overview of the strategies for gene discovery using gene arrays.

A wealth of genetic information has become available due to the determination of the DNA sequences of the entire genomes of human and other biological organisms and the advancement of microarray technology that measures simultaneously the expression of thousands of genes. As a result, bioinformatic tools have been developed to determine transcriptional "signatures" of various cell types, tissues and entire organisms in normal and disease states, to apply data mining strategies for gene discovery, and to synthesize and understand complex gene networks. This chapter reviews the data mining strategies for microarray gene expression data, including data pre-processing, cluster analysis, information retrieval from knowledge-based databases, and their integration into microarray data analysis workflows, as exemplified by studies on antigen-presenting dendritic cells that have been treated with transforming growth factor β1.

*Chapter 3* on "*Physical Modulation of Cellular Information Networks*" by Sumihiro Koyama and Masuo Aizawa reviews the effects of physical stimulation on the information networks in cells. The modulating factors applied include electrical potential, hydrostatic pressure, electromagnetic field, shear stress, and heat shock application, and the cellular functions investigated include viability, proliferation, differentiation, gene expression, and protein production, as well as morphological changes. The authors conclude that future work in cellular engineering for physical stimuli-induced gene expression will involve the exploration of specific physical stimulus-responsive promoters and investigations on stress-induced expression mechanisms. They also conclude that the effects of physical stimulation on mammalian cells will have a wide range of applications, particularly in cellular engineering, tissue engineering, and medical engineering.

*Section II* on "*Cell and Tissue Imaging*" contains three chapters:

*Chapter 4* on "*Fluorescence Live-Cell Imaging*" by Yingxiao Wang, John Y-J. Shyy, and Shu Chien surveys the principles and technologies used in performing fluorescence live-cell imaging and their applications in mechanobiology. The coverage includes the uses of fluorescent proteins and their derivatives and fluorescent microscopy and its integration with atomic force microscopy, with the purposes of visualizing intraellular localization of organelles, signaling/structural molecules, gene expression, and post-translational modifications. Techniques are presented for the spatiotemporal quantification of these subcellular and molecular events, e. g., by using fluorescence resonance energy transfer (FRET), fluorescence recovery after photobleach (FRAP), and fluorescence lifetime imaging microscopy (FLIM). The impact of these fluorescence technologies on cardiovascular research in relation to mechanobiology is discussed. It is pointed out that studies of cardiovascular specimens from transgenic animals would reveal new information on the target molecules.

*Chapter 5* on "*Optical Coherence Tomography (OCT)*" by Gereon Hüttmann and Eva Lankenau addresses this emerging technology for three-dimensional imaging of biological tissues. OCT is a new imaging technology that provides higher imaging depth with a high resolution that goes below 10 micrometers and does not rely on the depth of focus of the imaging lens. The basic theory of OCT and its emerging applications in tissue engineering are presented. Compared to technolo-

gies such as ultrasound, X-ray, MRT and electron microscopy, OCT offers a non-contact, non-invasive in-vivo applicability with compact and affordable devices. Coupling with spectroscopy, stains and specially designed probes provides additional means to generate site- and function-specific contrast. Hence, optical imaging and microscopy are well developed technologies now widely used in medicine and biotechnology.

*Chapter 6* on "*Ultrasonic Strain Imaging and Reconstructive Elastography for Biological Tissue*" by Walaa Khaled and Helmut Ermert reviews the technology of imaging relevant to biomechanics and summarizes their work in the field of elastography. The authors discuss some basic principles and limitations in the calculation of displacement estimators that are needed to evaluate strain images and the relative elastic moduli of biological tissues, which are illustrated with results from tissues *in-vitro* and *in-vivo*. The quantitative information obtained on the relative shear modulus seems promising for differential diagnosis of lesions in biological tissues. This chapter closes with a discussion on the prospects and applications of ultrasound elastography. This real-time ultrasound strain imaging system has been used in a clinical study for the early detection of prostate cancer during conventional transrectal ultrasound examinations. The authors feel that elastography holds promise in the in vivo diagnosis of prostate cancer and intravascular diseases.

*Section III* on "*Regenerative Medicine and Nanoengineering*" contains five chapters:

*Chapter 7* on "*Embryonic Stem Cell Derived Somatic Cell Therapy*" by Kurt Pfannkuche, Agapios Sachinidis and Jürgen Hescheler discuss the results from transplantation studies of embryonic stem cell derived somatic cells in animal models. The results are promising for ES cell-based cell therapy of degenerative diseases such as cardiac and neurological diseases. However, clinical application of ES cell-based therapies requires resolving the current barriers regarding safety aspects, purity and quantity of the cells, immunological rejection and ethical issues. Tissue engineering in combination with the ES cells might contribute to the development of new therapeutical concepts for treatment of severe degenerative diseases. Indeed, many important studies and concepts exist to develop strategies for generation of tissue engineered heart valves. The authors discuss cardiac tissue engineering with a special focus on embryonic stem cell derived cardiomyocytes.

*Chapter 8* on "*Collagen Fabrication for Cell-based Implants in Regenerative Medicine*" by Hwal (Matthew) Suh discusses the use of fabricated collagen for cell-based regenerative medicine as applied to skin regeneration, bone reconstruction, esophagus replacement, and liver regeneration, as well as anti-adhesive matrix that promotes wound healing. This chapter provides an overview for the characterization and fabrication of collagen, and the requirements of materials for cell-based implants and biomaterials in cell hybridization. The author points out that a systemized approach with single cell encapsulation by collagen containing signal transduction agents and ligands to attract specific cell adhesive receptors and with stem cell differentiation and proliferation toward the designated target tissue may contribute to the future progress of stem cell-based implant.

*Chapter 9* on "*Tissue Engineering*" by Bernd Denecke, Michael Wöltje, Sabine Neuss and Willi Jahnen-Dechent discusses the approach to tissue engineering by combining cells and biomaterials into functional tissues. The authors recapitulate the basic considerations of cell-material interactions in the development of biological substitutes. The tissue-engineered substitutes are comprised of cells cultured in a natural or nature-like environment that sustain growth and differentiation. The biomaterial scaffolds in the substitute need to provide cell attachment sites and a basic three-dimensional organization resembling the extracellular matrix. This chapter covers the recent advances in material scaffolds and cell-material interactions, and the use of stem cells for tissue engineering. The authors point out that the combination of cells and material scaffolds into tissue-engineered replacements of tissues and organs poses a formidable task because of the hurdles of regulatory approval and commercial viability.

*Chapter 10* on "*Micro and Nano Patterning for Cell and Tissue Engineering*" by Shyam Patel, Hayley Lam, and Song Li focuses on micro and nano technologies that have been used to investigate the regulation of cell functions by micro and nano features in matrix distribution, surface topography and three-dimensional microenvironment. The authors discuss the technologies that can be applied to the fabrication of functional tissue constructs and the recent advances in microfabrication and nanotechnology industry that have enabled many new ways of examining, manipulating and engineering biological processes. This chapter provides information on the uses of this emergent micro and nano patterning technology for the study of cell behavior and the creation of new therapies and engineering of tissues that are functionally similar to native tissues.

*Chapter 11* on "*Integrative Nanobioengineering*" by Andrea A. Robitzki and Andrée Rothermel presents novel bioelectronic tools for real-time pharmaceutical high-content screening in living cells and tissues. The authors discuss new online and real-time diagnostic systems. Miniaturized biomonitoring systems that utilize nano- and micro systems technology, automatic methods and software programs will allow remote measurements of disease-related biomarkers, thus making it possible to perform risk assessments on the patient before actual symptoms occur. This will allow the implementation of personalized prevention program and early individual therapies for people with an increased risk for a certain disease. The authors propose that future screening platforms should provide several technologies, including predictive tests of incompatibility, proteomics and post-genomics, cell and tissue based microsystems, combination with *in silico* models, and bioelectronical cell-based immunological measurements.

*Section IV* on "*Mechanics of Soft Tissues, Fluids and Molecules*" contains six chapters:

*Chapter 12* on "*Soft Materials in Technology and Biology*" by Manfred Staat, Gamal Baroud, Murat Topcu, Stephan Sponagel gives an introduction to the mechanics of soft materials. The authors pointed out that growing interest in flexible structures has brought biomechanics into the focus of engineering. Elastomers and soft tissues consist of similar networks of macromolecules. The chapter provides a brief introduction to the concepts of continuum mechanics, and presented typical

isotropic models of soft materials in technology and biology. The authors discussed the similarities and differences of the thermo-mechanical behavior. For rubber-like materials, a modification of the Kilian network is suggested which greatly simplifies the identification of material parameters. Finally, the dynamical loading of biopolymers and volume changes with phase transitions are considered.

*Chapter 13* on "*Modeling Cellular Adaptation to Mechanical Stress*" by Roland Kaunas aims at elucidating the mechanism underlying the adaptive changes of cells in response to mechanical loading. In arteries, atherosclerotic plaques are preferentially located at sites of low and oscillating wall shear stress; bone and skeletal muscle adapt to compressive and tensile forces, respectively. In all these cases, mechanical loading modulates the form and function of tissues. This chapter describes a new approach for developing an adaptive constitutive model of adherent cells subjected to mechanical stretch. Such a model would provide a framework by which the accelerating accumulation of mechanotransduction data can be interpreted, thus providing a clearer understanding of how cells respond to mechanical loading. To illustrate the concepts, the model is applied toward understanding how endothelial cells adapt to cyclic stretch.

*Chapter 14* on "*How Strong is the Beating of Cardiac Myocytes?*" by Jürgen Trzewik, Peter Linder and Kay F. Zerlin describes the CellDrum technique, which involves the culture of cell monolayers or thin tissue composites under biaxial load conditions for mechanical evaluation. A CellDrum consists of a plastic cylinder sealed on one end with a thin and biocompatible silicon membrane. The membrane allows cell attachment and proliferation under in vitro cell culture conditions. This device can be used to monitor the stress-strain relationship of the cell-membrane composites and has been applied to studying biomechanical properties of cells cultured on CellDrum membranes, including endothelial cells, fibroblasts and cardiomyocytes (self-contracting in monolayer cultures or embedded in collagen I matrices). The relative displacement of silicon membranes attached to cylindrical wells (diameter 16 mm) is measured with non-contact displacement sensors at a resolution in the $\mu$m-range. This system can be adapted for medical applications to determine the mechanical properties of cells/tissues in disease states.

*Chapter 15* on "*Mechanical Homeostasis of Cardiovascular Tissue*" by Ghassan S. Kassab addresses the state of mechanical homeostasis in the cardiovascular system, with special attention to the variations of stresses and strains. This chapter critically evaluates the various hypotheses on cardiovascular homeostasis of stresses and strains, viz., uniform wall shear stress, uniform mean circumferential stress and strain, uniform transmural stress and strain, and biaxial stress and strain. The evidence for these hypotheses is considered in the normal, flow-overload, and pressure-overload cardiovascular system, as well as during its development and in atherogenesis. This chapter considers the implications of these hypotheses on mechanotransduction and on vascular growth and remodeling. The author concludes that there is significant evidence that the internal mechanical factors are narrowly bounded and carefully regulated such that a perturbation of mechanical loading causes adaptive responses to restore mechanical homeostasis.

*Chapter 16* on "*The Role of Macromolecules in Stabilization and Destabilization of Biofluids*" by Björn Neu and Herbert J. Meiselman focuses on the interactions between RBCs as influenced by the presence of macromolecules in the suspending phase. RBCs are chosen to study cell-cell interaction because they are the most numerous cells in blood, have an important function of oxygen transport, and can raise low-shear blood viscosity and adversely affect microvascular blood flow when undergoing excessive aggregation. The authors deal in detail the depletion of macromolecules near the RBC surface because they believe this phenomenon is the most likely mechanism for RBC aggregation. Stabilization of RBC systems via small polymers or covalent attachment of polymers to the membrane surface is also considered. The authors point out that increased attention to the biophysical aspects of cell-cell interactions will yield important new information that may lead to improved disease diagnosis, patient care and advances in cellular- and bio-engineering.

*Chapter 17* on "*Hemoglobin Senses Body Temperature*" by Gerhard M. Artmann, Kay F. Zerlin and Ilya Digel report on a glass-transition like temperature transition occurring under certain conditions in RBCs around body temperature. The chapter was based on an accidental discovery of a temperature transition of RBC passage through small micropipettes many years ago at the University of California, San Diego. Artmann's group followed the biophysical mechanism of this effect since then. The authors' claim that the body temperature of a species was imprinted into the structure of hemoglobin and other proteins has been supported by several recent publications. The writing reflects Dr. Artmann's philosophy and view of life.

*Section V* on "*Bioengineering in Clinical Applications*" contains five chapters:

*Chapter 18* on "*Nitric Oxide in the Vascular System: Meet a Challenge*" by Stefanie Keymel, Malte Kelm, and Petra Kleinbongard reviews several aspects of research on nitric oxide (NO), especially its role in vascular biology and other fields in biology and medicine. Theoretical and experimental studies of NO metabolism and the *in vivo* and *ex vivo* detections of NO by bioassay and biochemical methods are outlined. The authors provide evidence that plasma nitrite is a sensitive marker for eNOS activity and propose that it may be used to monitor the efficacy of therapeutic interventions influencing endothelial function and NO metabolism in future clinical trials. The role of the NOS activity of RBCs as an intravascular source of NO is discussed. The authors point out the importance of using high-resolution intravital microscopy and real-time image acquisition and analysis to visualize the microcirculation, in order to study NO dynamics in relation to RBC rheology in vivo.

*Chapter 19* on "*Vascular Endothelial Responses to Disturbed Flow*" by Jeng-Jiann Chiu, Shunichi Usami, and Shu Chien focuses on the role of non-uniform and irregular distribution of wall shear stress at branches and bends of the arterial tree in the preferential distribution of atherosclerosis. While laminar blood flow and sustained high shear stress in the straight part of the arterial tree up-regulates the expression of genes and proteins in endothelial cells (ECs) to protect them against atherosclerosis, disturbed flow and the associated oscillatory and low shear stress up-regulate pro-atherosclerotic genes and proteins. This chapter summarizes the know-ledge on the effects of disturbed flow on ECs in terms of signal transduction, gene expression, cell structure and function, as well as pathologic implications.

Such investigations serve to elucidate the mechanisms underlying the effects of disturbed flow on ECs, thus facilitating the understanding of the etiology of lesion development in the disturbed flow regions.

*Chapter 20* on "*Why is Sepsis an Ongoing Clinical Challenge?*" by Aysegül Temiz Artmann and Peter Kayser reports on RBC responses during sepsis. Most *in-vivo* studies conducted by introducing microorganisms such as *Escherichia Coli* into experimental animals showed deleterious effects of the infection on RBCs. The authors found in their own *in vitro* studies with whole blood, partly in contrary to earlier studies, that after adding *E. coli*-derived lipopolysaccharides, RBCs lost their capability of aggregation, and this was accompanied by RBC swelling. The authors formulated a new biophysical hypothesis based on colloid osmotic pressure considerations to explain the phenomenon.

*Chapter 21* on "*Bioengineering of Inflammation and Cell Activation*" by Alexander H. Penn, Erik B. Kistler, and Geert W. Schmid-Schönbein discusses the role of inflammation and cell activation in circulatory shock and multi-organ failure. The authors have traced the inflammatory mediators in several forms of shock to the action of digestive enzymes, which are synthesized in the pancreas and act on the intestine. During intestinal ischemia, the mucosal barrier becomes permeable to pancreatic enzymes, allowing their entry into the intestine wall to cause auto-digestion of matrix proteins and tissue cells and production of inflammatory mediators, which are released into the central circulation, lymphatics and the peritoneal cavity to cause multi-organ failure. Inhibition of pancreatic enzymes in the lumen of the intestine serves to attenuate the inflammation in several forms of shock, and this may have major significance in the treatment of a variety of clinical conditions.

*Chapter 22* on "*Percutaneous Vertebroplasty*" by Christianne Vant, Manfred Staat, and Gamal Baroud reviews the two intraoperative complications of this interventional radiology procedure used to treat vertebral compression fractures. While this procedure shows promising results, it can cause complications due to excessive injection pressure, which is an extravertebral problem, or extraosseus cement leakage, which is an intravertebral problem. Current solutions for these complications involve the modification of cement delivery devices and procedure and the modulation of the rheological properties of the cement. Testing in a synthetic model demonstrates the existence of conflicting demands on the cement viscosity, i. e., low-viscosity cement is needed to solve the extravertebral problem, but high-viscosity cement is needed to solve the intravertebral problem. The challenge is to develop biomaterials, techniques and/or devices that can optimize the conflicting demands on cement viscosity.

*Section VI* on "*Plant and Microbial Bioengineering*" contains five chapters:

*Chapter 23* on "*Molecular Crowding*" by Ira. G. Tremmel addresses the effects of a crowded physico-chemical environment on the structure, function and evolution of cellular systems in photosynthetic membranes. The impact of crowding on photosynthetic electron transport has been simulated and analysed, taking into account realistic concentrations and shapes of photosynthetic proteins. The effects of macromolecular crowding may depend on many factors (e. g., background molecules) and there may be opposing effects (e. g., decrease of diffusion vs. increase of thermo-

dynamic activities). Diffusion coefficients for both large and small molecules are reduced 3- to10-fold from those in water, but the mobility of some enzymes is large in mitochondria, suggesting the presence of channels to facilitate protein movement. The findings on movement of molecules through crowded environment raises interesting questions in molecular evolution as to how biological macromolecules have evolved to optimize their function in environments that have evolved to become crowded.

*Chapter 24* on "*Higher Plants as Bioreactors*" by Fritz Kreuzaler, Christoph Peterhänsel, and Heinz-Josef Hirsch discusses the use of gene technology with C3-type plants to optimize $CO_2$ fixation for the production of biomass and bio-energy. As a result of photosynthesis utilizing $CO_2$, ATP is generated to provide the energy to do work in biological organisms. In higher plants, the important biochemical pathways involved in $CO_2$ reduction and assimilation are C3 and C4. Because the loss of fixed $CO_2$ by photorespiration is large in C3 plants, but virtually none in C4 plants, it is desirable to modify the plants from C3- to C4-type. The authors discuss the strategies of integrating new biosynthetic pathways into C3 plants by modifying the photorespiratory pathway or the $CO_2$-fixating enzyme Rubisco in plant chloroplasts. Among the various possibilities to produce more biomass to deal with the progressive increase in demand, the best way is to use gene technology to improve the efficiency of photosynthesis and $CO_2$-fixation, thus producing more biomass with better quality.

*Chapter 25* on "*Controlling Microbial Adhesion*" by Ilya Digel discusses the use of a surface engineering approach to manipulate microbial adhesion to surfaces. Microbes have a tendency to colonize surfaces, and this complex adhesion process is affected by many factors, including the characteristics of the bacteria, the target material surface, and environmental factors such as the presence of macromolecules, ions or bactericidal substances. A better understanding of these factors would enable the control of the adhesion process. The controllable stimulation or inhibition of microbial adhesion can be achieved either by physical adsorption or by chemical grafting of functional groups onto a suitable matrix. The feasibility and applicability of these methods have been proved in ethanol production using laboratory-scale bioreactors. Such surface engineering approach exploiting the propensity of microbes to adhere to surfaces can serve many biotechnological purposes.

*Chapter 26* on "*Air Purification by Means of Cluster Ions Generated by Plasma Discharge*" by Kazuo Nishikawa and Matthew Cook discusses ways to purify air pollution by using cluster ions. The authors review the growing need for the removal of harmful molecules in the air due to increasing pollution. The removal of airborne particles allow for an improvement in indoor air quality and a reduction in illnesses caused by airborne viruses, bacteria, and fungi and due to allergic bronchial asthma. The authors discuss the research on air purification through applying a plasma discharge into the atmosphere and creating ozone and radicals of strong chemical reactivity, and they present a novel plasma discharge technology, which can produce positive and negative "cluster" ions at a normal atmospheric pressure and characterize the resultant cluster ions. A series of experiments have been performed to prove

the air purification effects of such cluster ions, with close attention paid to airborne harmful microbes and cedar pollen allergens.

*Chapter 27* on "*Astrobiology*" by Gerda Horneck discusses this relatively new area that attempts to reveal the origin, evolution and distribution of life on Earth and throughout the Universe in the context of cosmic evolution. A multidisciplinary approach is required involving astronomy, planetary research, geology, paleontology, chemistry, biology and others. Astrobiology extends the boundaries of biological investigations beyond the Earth, to other planets, comets, meteorites, and space at large. Focal points are the different steps of the evolutionary pathways through cosmic history that may be related to the origin, evolution and distribution of life. Increasing data on the existence of planetary systems in our Galaxy support the assumption that habitable zones are not restricted to our own Solar System. From the extraordinary capabilities of life to adapt to environmental extremes, the boundary conditions for the habitability of other bodies within our Solar System and beyond can be assessed. Astrobiology has the potential to give new impulses to biology.

In summary, the twenty-seven chapters in this book on "*Bioengineering in Cell and Tissue Research*" provide state-of-the-art knowledge on Genes, Genome and Information Network; Biological Imaging; Regenerative Medicine and Nanoengineering; Mechanics of Soft Tissues, Fluids and Molecules; Clinical Applications; and Plant and Microbial Bioengineering. The book is intended to stimulate the reader to think about these problems and create innovative solutions. The authors and the editors would be most gratified if these aims are achieved.

Chu Chien

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**Part III Regenerative Medicine and Nanoengineering**

**Part VI Plant and Microbial Bioengineering**

# Part I
# Genes, Genome and Information Network

# Chapter 1
# Reporter Genes in Cell Based ultra High Throughput Screening

**Stefan Golz**

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**Abstract** Pharma research in most organizations is organized in discrete phases together building a "value chain" along which discovery programs process to finally drug candidates for clinical testing. The process envisioned to identify target-specific modulators lacking several side effects. Following a technical assessment of the targets "drugability", the probability to identify small molecule modulators, and technical feasibility target-specific assays are developed to probe the corporate compound collection for meanful leads. "High-Throughput-Screening" (HTS) started roughly one decade ago with the introduction of laboratory automation to handle the different assay steps typically performed in microtiter plates. Today a large arsenal of screening technologies is available for researchers in industry and academia to set up uHTS or HTS assays. Here the use of reporter genes offer an alternative for following signal transduction pathways from receptors at the cell surface to nuclear gene transcription in living cells.

## 1.1 Introduction

The modern drug research process has reversed the classical pharmacological strategy. Today, research programs are initiated based on biological evidence suggesting a particular gene or gene product to be a meaningful target for small molecule drugs useful for therapies. The process envisioned to identify target-specific modulators lacking several side effects. Also, it allows setting up a linear drug discovery process starting from target identification to finally delivering molecules for clinical development. One central element is lead discovery through high-throughput screening of comprehensive corporate compound collections. Pharma research in most organizations is organized in discrete phases together building a "value chain" along which discovery programs process to finally drug candidated for clinical testing (Hüser et al. 2006).

This pipeline is fueled by targets suggested from external or in-house generated data suggesting a gene or gene product to be disease relevant. Today a large arsenal

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of technologies is available for researchers in industry and academia to generate data in support of a functional link between a given gene and a disease state.

## 1.2 From Gene to Target

Following a technical assessment of the targets "drugability" (Hopkins and Groom, 2002), the probability to identify small molecule modulators, and technical feasibility target-specific assays are developed to probe the corporate compound collection for meanful leads. Lead discovery in the pharmaceutical industry today still depends largely on experimental screening of compound collections. To this end, the industry has invested heavily in expanding their compound files and established appropiate screening capabilities to handle large numbers of compounds within a reasonable period of time. "High-Troughput-Screening" (HTS) started roughly one decade ago with the introduction of laboratory automation to handle the different assay steps typically performed in microtiter plates. HTS technologies during the last decade have witnessed remarkable developments. Assay technologies have advanced to provide a large variety of various cell-based and biochemical test formats for a large spectrum of disease relevant target classes (Walters and Namchuk, 2003). In parallel, further miniaturization of assays volumes and parallelization of processing have further increased the test throughput. The ultra-high-throughput is required to fully exploit big compound files of $>1$ million compounds and is performed entirely in 1536-well plates with assay volumes between $5-10\,\mu$l. This assay carrier together with fully-automated robotic systems allow for testing in excess of 200,000 compounds per day. The comprehensive substance collection, together with sophisticated screening technologies, have resulted in a clear advantages in lead discovery especially for poorly druggable targets with a poor track record in the past.

## 1.3 Screening Assay Classes

Today a large arsenal of screening technologies is available for researchers in industry and academia to set up uHTS or HTS assays with high reproducibilty and accuracy. The assay technologies can be divided into three differrent classes:

1. Cellular growth and proliferation assays have been employed to search for therapeutics in anti-infectives and cancer. The ease use, the possibility for adaptation to high-throughput formats and the direct relevance to disease pathology of these growth assays were the reasons for the wide use in drug discovery processes. The difficulty to discriminate pharmacologically from cytotoxic effects is the main problem for an assay principle with unclearly defined targets. As a consequence cellular growth and proliferation assays have a higher risk to fail even in toxicological testing in late stages.

2. Biochemical bioassays are an experimental approach for the testing of isolated enzymes or receptors in which the activty of a purified protein is monitored directly.
3. Cell based bioassays are a different experimental approach for pharmacological assays and are widely used in drug discovery processes. Recently, molecular biology has revolutionized cell based bioassays by providing recombinant cell lines containing readout technologies amenable to ultra high-throughput formats. The functional readout of this assay format allows, to monitor all possible drug-receptor interactions, including allosteric modulation and allow the screening of different pharmacological target classes as G-protein coupled receptors, Ion-channel or transporter. The timescale of the assay type ranges from seconds, e. g. hormone-stimulated $Ca^{2+}$ signals, to few hours for reporter gene readouts. This assay type is target biased and allows the discrimination and differentiation of target-specific signals from general phenotypic effects. Optical assays rely on absorbance, fluorescence or luminescence as readouts. The used readout technologies are comprising fluorescent $Ca^{2+}$ indicators and different reporter genes. The reporter genes used in ultra high-throughput screening can be divided into two different classes: photoproteins & luciferases and fluorescent proteins.

The choice of cellular screening approach has an enormous impact both on the development and implementation of the HTS or uHTS for a target. The availability and behavior of the cells, together with the amplitude and reproducibility of the signal attainable against that cellular background, can all determine whether primary cells or cell lines, both native and engineered, are selected. Primary cells of human origin are arguably the most physiologically relevant model system and several selected primary cell types, human and other species, are commercially available and amenable to HTS or uHTS respectively. In general, primary cells cannot be obtained at the scale necessary for HTS or uHTS, and thus primary cell screens are positioned in the screening paradigm as low-throughput secondary assays. Transformed cell lines of mammalian origin (e. g. CHO, HEK 293) are the most commonly used cell-based uHTS assay formats. The advent of molecular and cell biology techniques to clone and express human proteins has provided access to cell lines with high expression levels of the target of interest. Cell lines can be engineered to express or over-express a target of interest. Expression can be transient or stable and several expression systems can be employed depending on the nature of the cell line and the target. Stable cell lines are most commonly generated by plasmid transfection infection. Stable expression of the target is the approach of choice for HTS and uHTS in the most drug discovery processes.

## 1.4 Reporter Gene Classes

Bioluminescence is the light produced in certain organism as a result of luciferase- or photoprotein mediated reactions. Numerous marine and terrestrial organisms are bioluminescent, while the biochemistry and molecular biology of the underlying