



**20th Annual Conference
of the
German Society
for Cytometry**



Date:

October 13 - 15, 2010

Location:

Leipzig Kubus
Helmholtz-Centre for
Environmental Research Leipzig

Scientific Committee

PD Dr. Susann Müller
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Prof. Dr. Annette Beck-Sickinger
Dr. Wolfgang Beisker
PD Dr. Lars Blank
Prof. Dr. Dirk Bumann
Prof. Dr. Thomas Egli
Dr. Elmar Endl
Dr. Thomas Kroneis
Prof. Dr. Leoni Kunz-Schughart
Prof. Dr. James Leary
Prof. Dr. Andreas Radbruch
Prof. Dr. Andrea Robitzki
Prof. Dr. Ulrich Sack
Dr. Alexander Scheffold
Frank Alexander Schildberg
Dr. Stephan Schmid
Prof. Dr. Günter K. Valet
Dr. Torsten Viergutz
Prof. Dr. Christian Wilhelm
Dr. Nicole I. zur Nieden

Organising Committee

Dr. Elmar Endl
PD Dr. Susann Müller
Christine Süring

General Information

www.dgfz.org

Dear Colleagues,

The program committee is cordially inviting you to participate in the

20th Annual Conference of the DGfZ

hosted at the Helmholtz – Centre for Environmental Research Leipzig-Halle GmbH, Germany.

This years conference is based on many topics and two of them have to be highlighted. First we want to celebrate the 20th anniversary of the Annual Conference which is a good reason to look back for a moment. We can be proud of the major achievements attained by the founders and members of the DGfZ over these last twenty years. The ideas and developments are now part of the daily routine in clinics and experimental design in basic research in countless laboratories world wide, names of German cytometrists are closely related with many topics in single-cell analytics.

The second remarkable new aspect of the conference is the attempt to start a discussion on how developments in nanotechnology might provide new advantages for single-cell analytics. 'Cytometry goes Nano' for example means that scientists are challenged by getting resolution below the μm -Scale within the cell to better understand cellular functions on a well resolved molecular level. Going nano not only means the dimensions of the object under investigation, but also the dimensions of the materials and tools used to perform a more accurate analysis of the cells. New nano-technologies, and the corresponding changes in physical, molecular and biological characterisation of cells, might therefore be a reasonable part of upcoming instrumentation technologies. Setting up a dialogue between Nanoscience and Cytometry is an experiment itself and we invite you to participate in that experiment.

I would especially like to remind young scientist of the fact that the DGfZ conferences are well known for the relaxed and creative atmosphere and famous for discussion and the close contact to experienced scientists in many fields. So it is the opportunity for first time visitors to make new friends and for the regular participants to network with old friends.

We hope we set up an interesting and stimulating program. Please, enjoy the conference.

Yours Sincerely,

Susann Müller
President

20th Annual Conference of the German Society for Cytometry

October 13-15, 2010
Helmholtz-Centre for Environmental Research, Leipzig



Wednesday, 13/Oct/2010

12:30PM - 1:30PM

OPENER: CYTOMETRIC RESEARCH IN GERMANY – TWENTY YEARS OF EXPERIENCE

Chair: Susann Müller

Chair: Laura Teodori

Speaker: Günther Valet

Speaker: Andreas Radbruch

1:30PM - 3:00PM

AGING IN HEALTH AND DISEASE

Chair: Leoni Kunz-Schughart

3:30PM - 5:00PM

REGENERATION

Chair: Ulrich Sack

Chair: Nicole I. zur Nieden

5:25PM - 6:55PM

NANOMEDICINE

Chair: Annette G. Beck-Sickinger

Chair: Andrea A. Robitzki

6:55PM - 9:00PM

CORE FACILITY MANAGER WORKSHOP

Chair: Elmar Endl

Chair: Torsten Viergutz

7:05PM - 9:30PM

Get Together and Poster Setup/Session

Thursday, 14/Oct/2010

9:00AM - 9:30AM

CYTOMETRY GOES NANO

Wolfgang Fritzsche

9:30AM - 11:00AM

NANOTOOLS

Chair: James Leary

Chair: Ulrich Sack

11:30AM - 1:00PM

NEW DEVELOPMENTS IN NANOINSTRUMENTATION

Chair: Dieter Weiss

Chair: Wolfgang Beisker

2:00PM - 3:30PM

IMMUNOLOGY: AUTOIMMUNE DISEASES

Chair: Alexander Scheffold

3:00PM - 5:30PM

POSTER SESSION

Chair: Wolfgang Beisker

Chair: Torsten Viergutz

5:30PM - 7:00PM

Mitgliederversammlung

7:00PM - 8:00PM **OPEN SPECIAL LECTURE**

Chair: Susann Müller

8:00PM - 11:59PM **DINNER**

Friday, 15/Oct/2010

9:00AM - 10:30AM **OMICS ON SORTED CELLS AND ORGANELLES**

Chair: Dirk Bumann

Chair: Susann Müller

11:00AM - 12:30PM **NANOBIOTECHNOLOGY**

Chair: Lars M. Blank

1:30PM - 3:00PM **WATER**

Chair: Thomas Egli

Chair: Christian Wilhelm

3:30PM - 5:00PM **THE KLAUS GOERTTLER SESSION**

Chair: Thomas Kroneis

Chair: Stephan Schmid

Chair: Frank Alexander Schildberg

Wednesday, 13/10/2010:

Tutorials: 8:30 - 11:15

Tutorial 1: Messung des Immunstatus - Saal2

Tutorial 2: Basiswissen Durchflusszytometrie - Saal1/CD

Tutorial 3: Investigation of flow cytometric data: gating and clustering - Saal1/AB

Tutorial 4: Publication in Cytometry Part A - Guidelines, quality requirements and new formats - Saal1/AB

Thursday, 14/10/2010: 1:00pm - 2:00pm

Lunch-Session

Partec GmbH

Life Technologies

Beckman Coulter GmbH

Friday, 15/10/2010: 12:30pm - 1:30pm

Lunch-Session

Millipore GmbH

BD Biosciences

20th Annual Conference of the German Society for Cytometry

October 13-15, 2010
Helmholtz-Centre for Environmental Research, Leipzig



Wednesday, 13/Oct/2010

8:00am – 12:30pm Registration and poster setup

12:30PM - 1:30PM KUBUS, SAAL 1

Opener: Cytometric Research in Germany – twenty years of experience

Chair: Susann Müller

Chair: Laura Teodori

Speaker: Günther Valet "20 years DGfZ: Past and future concepts"

Speaker: Andreas Radbruch "Immunologie und Zytometrie: 35 Jahre Ko-Evolution"

1:30PM - 3:00PM KUBUS, SAAL 1

Aging in Health and Disease

Chair: Leoni Kunz-Schughart

1:30 – 2:00 Thomas von Zglinick, Institute for Ageing and Health,
Newcastle University, United Kingdom
"Is cell senescence a cause of mammalian ageing?"

2:00 – 2:30 Alexander Navarrete Santos, Halle
"A flow cytometry approach for the detection of amyloid β -
oligomers in cerebrospinal fluid"

2:30 – 2:45 Sabrina Gundermann, Deutsches
Krebsforschungszentrum (DKFZ), Germany
"Fibroblasts: the driving force for skin aging?"

2:45 – 3:00 Sandra Heller, Universität Leipzig, Germany
"Assay of cell lines devoid of mtDNA compared to their
parental wild type"

3:00pm - 3:30pm *Coffee break*

3:30PM - 5:00PM

KUBUS, SAAL 1

Regeneration

Chair: Ulrich Sack

Chair: Nicole I. zur Nieden

- 3:30 - 4:00 Nicole I. zur Nieden, Fraunhofer Institute for Cell Therapy and Immunology, Germany
"Cell surface marker expression in embryonic stem cells overexpressing of a stable form of beta-catenin"
- 4:00 – 4:20 Laura Teodori, BIORAD-TOSS, Agenzia Nazionale Energia, Ambiente e Sviluppo Economicamente Sostenibile, Italy
"Production of skeletal muscle constructs for in vivo transplantation: preliminary results"
- 4:20 – 4:40 Marcin Frankowski, Physikalisch-Technische Bundesanstalt Berlin, Germany
"Statistical methods for quantitative assessment of cells viability based on flow cytometry and microscopy"
- 4:40 – 5:00 Maja-Theresa Dieterlen, Heart Center Leipzig, Germany
"Phosphoflow cytometry-based therapeutic drug monitoring after heart transplantation"

5:00pm - 5:25pm Coffee break

5:25PM - 6:55PM

KUBUS, SAAL 1

Nanomedicine

Chair: Annette G. Beck-Sickinger

Chair: Andrea A. Robitzki

- 5:25 – 5:55 Christoph Alexiou, Friedrich-Alexander-Universität, Erlangen-Nürnberg, Germany
"Cancer Therapy with Magnetic Nanoparticles"
- 5:55 – 6:15 Elmar Endl, Universitätsklinik Bonn, Institut für Molekulare Medizin, Germany
"Laser-activated gold-nanoparticles as a potential new treatment modality for age-related macular-degeneration"
- 6:15 – 6:35 Matthias Bartneck, RWTH-University Hospital Aachen, Germany
"Nanoparticle surface chemistry determines the endocytic uptake and the activation stage of human macrophages"

6:35 – 6:55 Ulrike Taylor, Institute of Farm Animal Genetics (FLI),
Germany
"Effect of gold nanoparticles on reproductive cells of model
animals"

6:00pm – 9:00pm *Get together*

6:55PM - 9:00PM KUBUS, SAAL 1

Core Facility Manager Workshop

Chair: Elmar Endl

Date: Thursday, 14/Oct/2010

9:00AM - 9:30AM KUBUS, SAAL 1

Cytometry goes Nano Wolfgang Fritzsche

9:30AM - 11:00AM KUBUS, SAAL 1

Nanotools

Chair: James Leary

Chair: Ulrich Sack

9:30 - 10:15 James Leary, Purdue University, United States of
America
"Nanotools for Bionanotechnology and Nanomedicine"

10:15 – 10:30 Sabine Klein, Institut für Nutztiergenetik Mariensee (FLI),
Germany
"Visualization and quantification of colloidal and intracellular
gold nanoparticles by confocal microscopy"

10:30 – 10:45 Elmar Endl, Universitätsklinik Bonn, Institute for Molecular
Medicine, Germany
"Purification of Human Embryonic Stem Cell Cultures by Laser
activated Gold Nanoparticles"

10:45 – 11:00 Michael Angstmann, University of Applied Science
Mannheim, Germany
"Monitoring human mesenchymal stem cell differentiation in
live cell chips"

11:00am - 11:30am *Coffee break*

11:30AM - 1:00PM KUBUS, SAAL 1

New Developments in Nanoinstrumentation

Chair: Dieter Weiss

Chair: Wolfgang Beisker

- 11:30 – 12:00 Maxime Dahan, Laboratoire Kastler Brossel, France
"Exploring the dynamic properties of supramolecular assemblies in live cells with single molecule imaging"
- 12:00 – 12:30 Petra Schuille, Biotechnologisches Zentrum der TU Dresden, Germany
"Fluorescence Correlation Spectroscopy: An analytical tool for systems biology"
- 12:30 – 13:00 James Leary, Purdue University, United States of America
"Dual modality MRI/NIRF in-vivo imaging of multifunctional nanomedical systems"
- 1:00pm - 2:00pm *Lunch*

LUNCH-SESSION: KUBUS, SAAL 1

- 1:05 – 1:25 Partec GmbH: Ines Nasdala
"A New Level of High-Performance Flow Cytometry"
- 1:25 – 1:45 Life Technologies: Paola Paglia
"Breakthrough Technology for Cellular Analysis: The Attune™ Acoustic Focusing Cytometer from Applied Biosystems®"
- 1:45 – 1:55 Beckman Coulter GmbH: Andreas Wicovsky
"Solid-Phase Cytometry: Introducing the Compucyte Laser Scanning Technology"

2:00PM - 3:30PM KUBUS, SAAL 1

Immunology: Autoimmune Diseases

Chair: Alexander Scheffold

- 2:00 – 2:30 Karsten Kretschmer, Center for Regenerative Therapies Dresden, Germany
"Molecular and Cellular Pathways of Foxp3+ Regulatory T Cell Generation"
- 2:30 – 2:50 Hyun-Dong Chang, Deutsches Rheuma-Forschungszentrum Berlin (DRFZ),
"Pathogenic and Protective Immunological Memory"
- 2:50 – 3:10 Andreas Grützkau, DRFZ Berlin, Ein Leibniz Institut, Germany
"Global cytometric profiling as a new tool for disease and therapy monitoring and clinical biomarker discovery"
- 3:10 – 3:30 Alexander Scheffold, Miltenyi Biotec GmbH, Germany

"High-sensitivity detection and analysis of rare cells by integrated magnetic enrichment and flow-cytometric analysis using the MACSQuant® Analyzer"

3:30PM - 5:30PM

Poster Session

Chair: Torsten Viergutz
Chair: Wolfgang Beisker

5.30PM - 7.00PM

KUBUS, SAAL 1 CD

Mitgliederversammlung

7:00PM - 8:00PM

KUBUS, SAAL 1 CD

Open Special Lecture

Chair: Susann Müller

7:00 – 8:00 Michael Nüsse, Johann-Wolfgang-Goethe-Universität
Frankfurt am Main, Germany
"From Cyto- to Archaeometry: Studies on Roman Coins in their
Archaeological Context"

8:00PM-11:59PM

CONFERENCE DINNER KUBUS, SAAL 1

Date: Friday, 15/Oct/2010

9:00AM - 10:30AM

KUBUS, SAAL 1

Omics on sorted Cells and Organelles

Chair: Dirk Bumann
Chair: Susann Müller

9:00 - 9:30 Dirk Bumann, Biozentrum, University Basel, Switzerland
"Studying pathogen properties in infected tissues using flow
cytometry"

9:30 – 9:50 Frank Schmidt, University of Greifswald, Germany
"Profiling of the adaptation of Staphylococcus aureus to
internalization by human epithelial cells: A functional genomics
approach"

9:50 – 10:10 Christin Koch, Helmholtz Centre for Environmental
Research - UFZ, Germany
"Describing microbial population dynamics in biogas reactors
using flow cytometry"

10:10 – 10:30 Thomas Ernst Schmid, Klinikum rechts der Isar, Germany
“Changes in cell cycle and Y-H2AX phosphorylation kinetics
after irradiation with 20 MEV protons”

10:30am – 11:00am *Coffee break*

11:00AM -12:30PM KUBUS, SAAL 1

Nanobiotechnology

Chair: Lars M. Blank

11:00 – 11:30 Jan Regtmeier, Universität Bielefeld, Germany
“Single cell analysis on a chip: from protein expression to cell
heterogeneity”

11:30 – 11:50 Lars M. Blank, Laboratory of Chemical Biotechnology, TU
Dortmund, Germany
“A new reactor concept - the Envirostat”

11:50 – 12:10 Ali Kinkhabwala, Max Planck Institute of Molecular
Physiology, Germany
“Precision Biology with Yeast”

12:10 – 12:30 Petra S. Dittrich, ETH Zurich, Switzerland
“Single cell analysis on microfluidic platforms ”

12:30pm - 1:30pm *Lunch*

LUNCH-SESSION: KUBUS, SAAL 1

12:40 - 1:00 Millipore GmbH: Wolfgang Vukovich
“Microcapillary Flowcytometry: The Power of Guava”

1:00 - 1:20 BD Biosciences: Jens Fleischer
“FACSAria III – In a Perpetual State of the Art”

1:30PM - 3:00PM KUBUS, SAAL 1

Water

Chair: Thomas Egli

Chair: Christian Wilhelm

1:30 - 2:00 Thomas Egli, Swiss Federal Institute of Aquatic Science
and Technology (eawag), Switzerland
“SODIS: sunlight for drinking water disinfection. Flow cytometry
and other methods reveal the respiratory chain as the main
target in enteric bacteria”

- 2:00 – 2:20 Gerrit Jan van den Engh, BD Bioscience/Cytopeia, United States of America
“Phytoplankton analysis by measuring fluorescence action spectra in flow cytometry”
- 2:20 – 2:40 Sabine Kleinsteuber, Helmholtz Centre for Environmental Research - UFZ, Germany
“Resolution of natural microbial community dynamics by community fingerprinting, flow cytometry and trend interpretation analysis”
- 2:40 – 3:00 Susanne Dunker, University of Leipzig, Germany
“Flow cytometry as a tool to measure phytoplankton diversity and physiological activity with taxonomic resolution”
- 3:00pm – 3:30pm *Coffee break*

3:30PM - 5:05PM KUBUS, SAAL 1

The Klaus Goerttler Session

Chair: Thomas Kroneis
Chair: Stephan Schmid
Chair: Frank Alexander Schildberg

- 3:30 – 3:55 Kathleen M. Gillespie, University of Bristol, Clinical Science, United Kingdom
“Maternal microchimerism: the importance of single cells in health and disease”
- 3:55 – 4:20 Ingo Müller, Universitätsklinikum Hamburg-Eppendorf, Germany
“Clinical potential of mesenchymal stem cells in immune modulation and regenerative medicine”
- 4:20 – 4:45 Marc Beyer, Universitäten Bonn, LIMES Institute, Germany
“Regulatory T cells: major players in the tumormicroenvironment”
- 4:45 – 4:55 Antje Dietrich, TU Dresden, Germany
“Fiber-optic confocal live-imaging to monitor vascular hyperpermeability in tumor and normal tissue”
- 4:55 – 5:05 Mirjam Ingargiola, TU Dresden, Germany
“Target-specific therapy testing in spheroids to examine new Cetuximab-based radiotherapeutic approaches”

Sessions

Aging in Health and Disease

Knowledge about the basic biology of cell and tissue aging is of utmost importance because age is the single biggest risk factor for many severe diseases such as cancer, cardiovascular disease, type II diabetes, or dementia. There is considerable overlap in the underlying pathways that contribute to both ageing and age-related diseases. Consequently, therapies targeting the mechanisms of aging are also considered to counteract these pathologies. According to apoptosis, aging of cells has long been assumed a programmed process driven by biological self-destruct mechanisms. Today, however, aging is understood as an inherently stochastic yet progressive impairment of functions in molecules, cells and organs due to the limited capacity to remove gene effects by natural selection which results in a loss of adaptive response of cells to stress. One major model of cellular aging is replicative senescence, where somatic cells irreversibly lose their proliferative capacity after a more or less constant number of cell divisions. Cell senescence is heterogeneous, with large differences in lifespan between organisms, cell types, and individual cell lineages. It is related to the loss of DNA sequences at the ends of chromosomes (telomeres) and seems to be triggered by random mitochondrial dysfunction. Major determinants of cell aging at the single-cell level that are responsible for the cell-to-cell variation in replicative lifespan will be discussed in this session. The mitochondrial theory as well as other mechanistic aspects of cellular aging ranging from the role of telomeres, telomerases and cell senescence in health and disease to mitochondrial biogenesis and the mitochondrion nucleus cross-talk during senescence and immortalization will be of interest.

Regeneration

Analysis of cellular function and phenotypes is a profound requirement for research in regeneration. Besides analysis, for application of cells and cellular products in regenerative medicine, methods for cell-specific separation and preparation are required. In this session, application of cytometric methods to research in and medical application of cellular therapies are covered.

Nanomedicine

Nanomaterials find a wide range of applications not only in science and medicine, but also in modern society. Beside their use as diagnostics and drug delivery system, they appear in surface paintings, food additives and cosmetics. However, at present there is growing concern over the safety of nanomaterials with respect to occupational, consumer and environmental exposures. The assessment of nanoparticle cellular interactions is fundamental for adequate risk assessment of engineered nanoparticles. Cytometric techniques are definitely one approach to address this question on a cellular level.

The session is aimed at bringing together scientists from different disciplines of the nanotoxicology field to present their current research findings and discuss the potential impact of nanomaterials on human health and the environment.

Cytometry goes Nano

Nanotechnology describes a trend of using units and/or tools of nanoscale dimensions for various applications in technology. It enables novel approaches not possible with larger structures. For life sciences, these novel methods allow the control down to dimensions of single biomolecules. Directed transport of effectors, intracellular sensing, or cell manipulation are examples where nanotechnology assists in biological and biomedical research or even already in diagnostics and therapy. Because these techniques usually aim at the individual cell level it holds a special potential for cytomics. The presentation will give an overview over applied nano-approaches in biomedical and cell biology applications. It will then focus on nanoparticles (especially metal ones) and their applications for various kinds of cell and biomolecular studies as well as manipulations.

Nanotools

Reliable miniaturized test methods and tools for manipulation and analysis of cells intend to resolve and understand cellular and subcellular processes in health and disease, in research and applied sciences. Here, we focus on the application of nanotools which aim to break usual boundaries in resolution and information usually predetermined by traditional fluorescent dye assays.

New Developments in Nanoinstrumentation

New technologies and instrumentation in microscopy and cytometry have always driven scientific progress and open new horizons. With special respect to Nanotechnologies, sensitivity and resolution has to be driven to its limits. High detection sensitivity up to single photons has to be combined with high spatial resolution in order to achieve new insights. Special attention has to be given to the surface structure of these particles.

Immunology: Autoimmune Diseases

Breakdown of immune tolerance mechanisms lead to pathologic immune responses, such as autoimmunity, allergy or chronic inflammation. This session will focus on cytometric analyses helping to identify good and bad cellular players in this process, to define protective and pathologic mechanisms and to develop strategies for therapeutic intervention, e.g. transfer of protective cell populations.

Omics on sorted Cells and Organelles

The acquisition of information at the gene, transcription, and protein levels are important additions to the concept of cytomics. Together, cytomics and functional -omics promise to generate new breakthroughs in understanding the regulation of pathways and determination of specific functions on the molecular level. The session will invite ideas to resolve functions of single cells, sub-populations and populations to reveal interactions between individual cells even beyond the organismic level.

Nanobiotechnology

The production of valuable products (from pharmaceuticals to bulk chemicals) is compromised by cell population heterogeneities. To minimize rationally cell-to-cell variations and thereby improving rate and yield of the desired product,

one needs a detailed understanding of single cell responses. Techniques used to quantify these responses on nano scale and their contribution to biotechnology is the theme of this session.

Water

Water is a most precious resource of mankind. It is used as drinking water, in agriculture, for industry and recreation. The phytoplankton, growing in open water resources, is a sensitive marker for climate change and water pollution. This session focus on how cytometry can be used to ensure water quality and to determine ecological disturbances.

The Klaus Goerttler Session

A newly designed session at the Annual Conference of the DGfZ, the Goerttler session will be chaired by three notable young researchers elected by a board of the DGfZ. The Goerttler session represents a novel concept of approaching young talented scientists and will be held at every following DGfZ conference, introducing young academics to the scientific cytometry community. This first Goerttler Session chairs are the nominees of last year's Goerttler Prize: Thomas Kroneis, Frank Schildberg and Stephan Schmid. Although approaching the field of cytometry through the eyes of a chemist, a biologist and a physician, the overall idea of this session was chosen to be the "bench to bedside" principle. As part of the concept all three chairs of the Goerttler session were asked to invite speakers from their field of research, therefore enabling them to intensify their own topics, to meet outstanding researchers, and to set up an exciting session. As this conference deals with special aspects of single cell analysis, the talks in the Goerttler session will relate single-cell issues to different aspects of medicine.

Poster Session

During the poster session you have the chance to describe your work in a short presentation and to explain the significance of your results during a discussion with the chairs. The time limit is 5 min. The best poster presenter will get a poster price (certificate and 200 Euro).

Sessions for Communication

Workshop: Core Facility management

The aim of the workshop is to share knowledge and experience among people that run or work within a flow or image core facility. Talks and discussion should be of interest for anyone managing daily life in a core facility or especially for people thinking about setting up a core facility. Refreshments will be there during the workshop.

Thursday, 14/10/2010: 1:00pm - 2:00pm

LUNCH-SESSION

A NEW LEVEL OF HIGH-PERFORMANCE FLOW CYTOMETRY

Ines Nasdala
Partec GmbH, Germany

BREAKTHROUGH TECHNOLOGY FOR CELLULAR ANALYSIS: THE ATTUNE™ ACOUSTIC FOCUSING CYTOMETER FROM APPLIED BIOSYSTEMS®

Paola Paglia, PhD
Cytometry Systems, Life Technologies

The Attune™ Acoustic Focusing Cytometer is the first cytometer to use sound waves to precisely align cells prior to sampling, and brings significant performance advantages compared to traditional flow cytometry. Incorporating breakthrough acoustic focusing technology allows users the ability to quickly process dilute samples and to exquisitely control transit times in the laser beam, yielding increased sensitivity without sacrificing throughput. As a result, the Attune™ Cytometer excels at rare event detection and provides lower signal variation in populations. Data is provided to compare results with conventional cytometers using hydrodynamic focusing. This presentation will describe the design and applications of the Attune™ Cytometer. Technologies, Germany

SOLID-PHASE CYTOMETRY: INTRODUCING THE COMPUCYTE LASER SCANNING TECHNOLOGY

Andreas Wicovsky
Beckman Coulter GmbH, Germany

Laser Scanning Cytometry (LSC) uses laser-based opto-electronics and automated analysis capabilities to simultaneously and rapidly measure biochemical constituents and evaluate cell morphologies. LSC technology combines quantitative analysis with flow cytometry and therefore provides a measure of precision and visualization that is available in no other cellular analysis system. Beckman Coulter, Inc. and CompuCyte Corporation have entered into a global distribution agreement, under the terms of which Beckman Coulter will have the right to distribute CompuCyte laser scanning cytometers in cellular analysis markets worldwide.

Time: Friday, 15/10/2010: 12:30pm - 1:30pm

LUNCH-SESSION

MICROCAPILLARY FLOWCYTOMETRY: THE POWER OF GUAVA

Wolfgang Vukovich
Millipore GmbH, Germany

Guava using its patented Microcapillary Flowtechnology enables direct volumetric measurements and absolute cell counting. Guava has been on the market since 10 years building small benchtop analysers running without sheat fluid and no need for laser alignment.

Instruments range from 1 Laser instruments and 3 colours to machines with 2 Lasers and 6 colours as well as FSC and SSC.

Hand in Hand with a dedicated easy to use software and 96 well automation Guava provides a powerful tool for researchers both in the Academic and in the Biotech field.

FACSARIA III – IN A PERPETUAL STATE OF THE ART

Jens Fleischer
BD Biosciences, Germany

BD introduces the BD FACSAria™ III cellsorter, offering enhanced multicolor capabilities with up to 6 lasers in a benchtop instrument. The new “-Xmount” optical table enables up to 5 fibre-launched lasers in addition to the near-UV diode laser. With the addition of an octagon the BD FACSAria III can now measure up to 18 colors and 2 scatter parameters. The “next generation flowcell” supports more laser pinholes and offers enhanced DNA resolution. New lasers are the 561 nm YG laser for excitation of mCherry and better PE resolution, and the 445 nm blueviolet laser for an optimal CFP excitation. All existing FACSAria platforms can be upgraded with the new features to FACSAria II and FACSAria III level, demonstrating ongoing support for installed instruments since 2003 and allowing researchers to adapt their existing instruments to an expanding range of applications.

13/10/2010: Tutorials: 8:30 - 11:15

Tutorial 1: 8:30 – 11:15 Saal2 Messung des Immunstatus

Dagmar Riemann, Martin Luther University, Germany

Tutorial 2: 8:30 - 11:15 Saal1CD Basiswissen Durchflusszytometrie

Andreas Lösche, University Leipzig, Germany

Tutorial 3: 9:00 – 10:30 Saal1 AB

Investigation of flow cytometric data: Gating and Clustering

Claudio Vallan¹, Ingo Fetzer²,¹Celeza GmbH, Switzerland; ²Helmholtz Centre for Environmental Research - UFZ

Tutorial 4: 10:30 – 11:15 Saal1 AB

Publication in Cytometry Part A – Guidelines, quality requirements and new formats.

Attila Tárnok, Cardiac Center GmbH; University of Leipzig, Germany

Tutorial 1: 8:30 - 11:15 Messung des Immunstatus

Lymphozyten sind ein wichtiger Bestandteil der weißen Blutkörperchen und wesentlich an der Immunabwehr beteiligt. Die Lymphozytendifferenzierung im Blut erlaubt die Bestimmung der Anzahl von T-Lymphozyten (Helferzellen und zytotoxische Zellen), B-Lymphozyten (verantwortlich für die Antikörperbildung) und natürlichen Killerzellen (Virus- und Tumorabwehr). Auch wenn im Blut nur ein geringer Anteil der im Körper vorhandenen Lymphozyten zirkulieren, erlaubt die Messung der Blutlymphozytenzahlen doch Rückschlüsse auf normale, verminderte oder erhöhte Lymphozytenwerte. Angewendet wird die Untersuchung u.a. beim Verdacht auf einen Immundefekt, bei der Überwachung von immunsuppressiven Therapien (z.B. bei Transplantatempfängern) oder zur Abklärung einer Lymphozytose. Im Workshop wird die Durchführung und Auswertung einer Lymphozytentypisierung mittels monoklonaler Antikörper und Mehrfarben-Immunfluoreszenz besprochen. Eingegangen wird besonders auf Fehlermöglichkeiten beim Ansetzen der Probe bzw. bei der Messung am Durchflusszytometer. Beispielfhaft erfolgt die Interpretation der Absolutzahlen (Zellen/Mikroliter).

Tutorial 2: 8:30 - 11:15 Basiswissen Durchflusszytometrie

Die Durchflusszytometrie hat in den letzten Jahren durch die Etablierung neuer Methoden und die Entwicklung neuer Fluoreszenzfarbstoffe sowohl im klinischen Bereich als auch bei der Optimierung biotechnologischer und verfahrenstechnischer Prozesse eine immer breitere Anwendung gefunden. Das Spektrum reicht von einfachen standardisierten Applikationen bis zu komplexen Multiparameter und -color Analysen und Sortierungen. In dem Tutorial werden die Grundlagen der Durchflusszytometrie und der Umgang damit dargestellt. Das Prinzip der Durchflusszytometrie (Flüssigkeitssystem, Optik, Signalverarbeitung) wird erläutert und Anwendungsbeispiele werden gezeigt. Schwerpunkt des Tutorials ist die Darstellung der Planung und Durchführung durchflusszytometrischer Experimente:

- wie gehe ich an eine Analyse/Sortierung heran
- Anforderungen an die Zellen

- Probenvorbereitung
- Auswahl der Fluoreszenzfarbstoffe
- Durchführung der Analyse/Sortierung.

Besonders behandelt wird auch die Auswertung und Beurteilung der gemessenen Daten.

Tutorial 3: 9:00 - 10:30 Investigation of flow cytometric data: Gating and Clustering

The increase in number of parameters that can be measured by modern flow cytometers drastically increases the complexity of the analysis of acquired data and raises need for new approaches. Correct compensation, adequate transformation and algorithms helping identifying discrete cell populations are a prerequisite for an accurate evaluation of flow cytometric data.

The workshop will present the possibilities given by three software packages:

- FlowJo has outstanding capabilities to be employed for “standard” analyses and for fast and automated pre-processing of the data. Appropriate techniques for gating and transformation will be elucidated as well as FlowJo’s capabilities of visualization of the data.

- More complex and customized analyses can be performed with packages available from Bioconductor, an open source project based on the R statistical programming language. We will give a short introduction to the R language and a brief overview of the available flow cytometry analysis packages with particular emphasis on the clustering software.

- The ‘Dalmatian-plot-analysis’ software is currently being developed in house at the Helmholtz Centre for Environmental Research. This is an easy to apply method to identify gradual temporal or treatment dependent changes of cell communities. The method is based on the combination of image analysis with a multivariate approach. The general idea is to calculate similarities of scatter plots derived from cytometric measurements and visualize their changes. Similarities between two scatter plots are calculated as the rate of overlap between occurring cell clusters indicated by sort gates. The gates are extracted from the scatter plots and transformed into bitmap pictures. By following simple image calculation, overlays of all picture combinations, from the resulting 'Dalmatian plots', are produced and similarities from the overlap rates are then estimated using a modified Jaccard-Index S. For the final visualization of similarity changes ordination methods as non-metric multidimensional scaling (n-MDS) can be used. Two different approaches can be applied. The first possibility is to only include gate position and disregard cells abundances covered by the gates. Looking only at presence-absence of clusters enables to give equal priority to emerging clusters and allows all gates to be judged independently of the abundance of the cells therein. This might be of advantage given that often highly abundant cells in a distinct cluster are not necessarily those most relevant for a studied process, but represent functional, non-important generalists. In a second possibility cell abundances can be included for calculating similarities. Here relative cell numbers within gates are translated into gray values. This approach is suitable when cell numbers may correlate with occurring temporal changes or an applied treatment.

Tutorial 4: 10:30 - 11:15 Publication in Cytometry Part A - Guidelines, quality requirements and new formats.

Topics covered (among others):

- Basic requirements for manuscripts
- Data display
- Statistics
- Supplementary material
- MIFlowCyt
- Criteria for manuscripts on new technology evaluation
- Publication of software developments
- New innovative manuscript formats: OMIPs, communications, commentary
- Guidelines for reviewers
- Guidelines for guest editors of regular and virtual issues

**ABSTRACTS
(ORAL PRESENTATIONS)**

Wednesday, 13/Oct/2010

12:30pm - 1:30pm

OPENER: CYTOMETRIC RESEARCH IN GERMANY – TWENTY YEARS OF EXPERIENCE

Chair: Susann Müller

Chair: Laura Teodori

Speaker: Günther Valet

20 years DGfZ: Past and future concepts

Speaker: Andreas Radbruch

Immunologie und Zytometrie: 35 Jahre Ko-Evolution

Chair: Leoni Kunz-Schughart

IS CELL SENESENCE A CAUSE OF MAMMALIAN AGEING ?

Thomas von Zglinicki

Institute for Ageing and Health, Newcastle University, United Kingdom

Cell senescence is characterized by the loss of proliferative capacity after a (generally well reproducible) number of cell divisions. However, recently it has become clear that senescence is much more than just a permanent growth arrest observed in cells cultured in vitro.

Senescent cells develop a completely different phenotype, including mitochondrial dysfunction, production of reactive oxygen species and secretion of pro-inflammatory cytokines and other bioactive substances.

The signalling pathways governing this phenotypic change are triggered as late responses to lasting DNA damage. Moreover, senescent cells are found in tissues of ageing animals and humans at significant frequencies. In many tissues, these frequencies increase with ageing and decrease under caloric restriction, which postpones ageing.

This suggests that cell senescence might be among the causes of organismic ageing. We are beginning to unravel the complex signalling pathway networks that govern the changes to the senescent phenotype in the hope to find novel targets for preventive intervention.

A FLOW CYTOMETRY APPROACH FOR THE DETECTION OF AMYLOID β-OLIGOMERS IN CEREBROSPINAL FLUID

Alexander Navarrete Santos

Department of Cardiothoracic Surgery, Martin Luther University Halle-Wittenberg,
Halle, Germany

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia with an average survival of 7 years after prognosis. Ongoing clinical studies point out promising possibilities for the treatment of this disease. For ideal therapy and timely conservation of essential cognitive functions, however, a diagnostic tool for the early detection of AD is a pre-requisite. In this regard, there are evidences pointing to Aβ oligomers as the neurotoxic species in AD. For example the concentration of these structures in human brain was found to be up to 70-fold higher in AD patients than in non-demented controls. Furthermore, it was shown that the severity of the disease correlates with the oligomer concentration rather than with the number of plaques (a hallmark of AD in the brain). Actually, a reliable method for the measurement of oligomers in cerebrospinal fluid (CSF) does not exist. In our work we developed a reliable method for the detection of Aβ oligomers in CSF. The method is based on the measurement of fluorescence energy transfer signals (FRET) from two different Aβ binding antibodies labelled with Alexa fluor 488 and Alexa fluor 594 (donor/acceptor pair). The detection of the oligomers is achieved by flow cytometry. The accuracy of the method for the discrimination between AD and healthy controls is ongoing.

FIBROBLASTS: THE DRIVING FORCE FOR SKIN AGING?

Sabrina Gundermann, Hans-Jürgen-Stark, and Petra Boukamp
Deutsches Krebsforschungszentrum (DKFZ), Germany

One presently favoured “aging hypothesis” is the telomere hypothesis of cellular aging. For human skin, telomere loss was postulated as a major driving force for the aging process. However, we recently demonstrated that neither the continuously proliferating epidermis nor the largely resting dermal fibroblasts show significant age-dependent decline (Krunic et al., 2009). Instead, we now show that UV-dependent telomere loss is more crucial and more likely contributes to skin cancer development (Krunic et al., in prep.).

It is further proposed that the epidermal stem cells decline in number or lose functional competence with age thereby cause the aging phenotype. We however, propose that not the epidermis but the dermis with its matrix and cellular components, the dermal fibroblasts are responsible for skin aging in humans. Their age-dependent modulation has in turn consequences on epidermal function and leads to the known phenotype of the aging skin, i.e. loss of rete ridges, thinning of the epidermis, reduced barrier function, and impaired wound healing.

To test our hypothesis, we studied fibroblasts from skin of different aged donors (in vivo aging) and in the first step compared the in vivo aged fibroblasts with fibroblasts aged by continuous culturing close to cellular senescence (in vitro aged fibroblasts). These studies demonstrated that the in vivo aged fibroblasts and in vitro aged fibroblasts differ in their telomere length, in their gene expression profile, and also in their ability to support epidermal regeneration in organotypic cocultures (OTCs). From these findings we postulate that in vivo aging and in vitro aging are two independent processes.

Most importantly, when studying fibroblasts from different aged donors, we did not yet identify senescent cells. Instead, we found a massive increase in α -smooth muscle actin in in vivo aged fibroblasts correlating with an increased number of differentiated fibroblasts, so called myofibroblasts. Additionally, gene expression array analyses showed that fibroblasts from aged donors overexpressed a number of common extracellular matrix proteins and also expressed matrix proteins not found in fibroblasts from a young donor. Verification of the expression of these proteins in the OTCs as well as in skin sections from different aged donors confirmed our expression analysis, thus strongly arguing for the functional relevance of these proteins for the skin aging process.

Altogether, we conclude that it may not be the accumulation of senescent fibroblasts that may be causal for skin aging but rather that the fibroblasts differentiate into myofibroblasts. Thereby they express a different set of proteins, which modifies the extracellular matrix and in turn modifies the paracrine interaction with the keratinocytes that cause the phenotypic characteristics associated with skin aging.

ASSAY OF CELL LINES DEVOID OF MTDNA COMPARED TO THEIR PARENTAL WILD TYPE

Sandra Heller, Susanna Schubert, Peter Seibel
Universität Leipzig, Germany

The mitochondrion, one important organelle of eukaryotic cells harbours important biochemical processes and acts as key-player in the ageing process and the programmed cell death.

The human mitochondrial genome displays a size of 16569bp and contains 37 genes that are important for normal mitochondrial function. Apart from genes for rRNA and tRNA the mitochondrial DNA (mtDNA) encodes 13 polypeptides that are essential enzymatic subunits of the respiratory chain. Genes of other mitochondrial peptides are located in the nuclear genome so that these peptides have to be transported into mitochondria.

The respiratory chain consists of the four enzyme complexes NADH:ubiquinone oxidoreductase (complex I), succinate:ubiquinone oxidoreductase (complex II), ubiquinol:cytochrome c oxidoreductase (complex III) and cytochrome c oxidase (complex IV) that function as electron transport complexes and the ATP synthesising complex ATP synthase respectively. Only complex II is completely encoded by the nuclear genome whereas genes of the other respiratory chain complexes are located on the nuclear and mitochondrial genome.

Cells without mitochondrial DNA are termed ρ 0-cells according to the genetics of yeast. Therefore, cells with ρ 0-genotype lack a functional respiratory chain and require metabolic supplementation for cell viability. Cells devoid of mitochondrial DNA are generated by cultivating them on growth medium that includes chemicals like ethidiumbromide or ditercalinium that interfere with DNA replication. A new method takes advantage of a restrictionendonuclease directed to the mitochondrial matrix. This allowed us to generate ρ 0-cells without the toxicological side effects caused by the chemical method.

Chair: Ulrich Sack

Chair: Nicole I. zur Nieden

CELL SURFACE MARKER EXPRESSION IN EMBRYONIC STEM CELLS OVEREXPRESSING A STABLE FORM OF BETA-CATENIN

Nicole I. zur Nieden^{1,2}

¹Department of Cell Biology & Neuroscience and Stem Cell Center, University of California Riverside, Riverside, CA 92521, USA; ²Fraunhofer Institute for Cell Therapy and Immunology, Department of Cell Therapy, Applied Stem Cell Technologies Unit, Perlickstrasse 1, 04103 Leipzig, Germany

Although immune-compatible autologous adult stem cells have been proven to repair bone defects, the practical use of these cells is limited by the scarcity of available progenitors or the quality of their genetic composition. New approaches to skeletal repair involving pluripotent stem cells have thus emerged. Our lab has previously explored the in vitro osteogenic differentiation of murine, non-human primate and human embryonic stem cells (ESCs) as a model of skeletal development from pluripotent stem cells. One of the major bottlenecks on the path to successful clinical implementation of ESC technology in the area of degenerative bone diseases, however, is the poorly defined strategies that are currently available to induce directed differentiation of ESCs into osteoblasts.

In the past, we have identified the Wnt/beta-catenin (CtnB) signaling pathway as a major regulator of osteogenesis, which may be manipulated to enhance mineralization and maturation as well as early mesendodermal specification. We have found that Wnt signaling cross-talks to a variety of other signaling pathways, such as the ones activated by nitric oxide, bone morphogenetic protein-2 and retinoic acid, in the regulation of these processes.

PRODUCTION OF SKELETAL MUSCLE CONSTRUCTS FOR IN VIVO TRANSPLANTATION: PRELIMINARY RESULTS

Laura Teodori

BIORAD-TOSS, Agenzia Nazionale Energia, Ambiente e Sviluppo Economicamente Sostenibile, CR-Casaccia. Rome, Italy

Dario Coletti[°], Barbara Perniconi^{°°}, Alessandra Costa* and Laura Teodori*

[°]Unité de Vieillessement, Stress, Inflammation, Université Pierre et Marie Curie.

Paris, France. ^{°°}Dipartimento di Istologia, Sapienza Università di Roma. Rome, Italy

*BIORAD-TOSS, Agenzia Nazionale Energia, Ambiente e Sviluppo Economicamente Sostenibile, CR-Casaccia. Rome, Italy

For reconstructive interventions in patients suffering from muscle deficits due, for example, to congenital abnormalities, trauma or surgery, tissue engineered skeletal muscle may offer a significant benefit along with reduced donor-site morbidity and a virtually unlimited supply of tissue. The goal of the present work was to generate functional, implantable skeletal muscles containing different tissues pre-assembled in vitro on a scaffold derived from cadaveric mice muscle. Cadaveric murine hind limbs muscles, Tibialis anterior and Extensor Digitorum Longus, were decellularized by incubation in detergent solution for different times. Myoblasts and/or fibroblast cell lines were injected into the decellularized muscle matrix and the constructs cultured for two weeks in order to induce myogenesis in vitro. Cell viability was assessed by vital dyes. Homolog muscles were removed by microsurgery in wild type mice and the constructs were transplanted to replace them. We then tested the fate of the constructs in vivo in regards to histocompatibility, bioactivity, degradability, toxicity. The results demonstrated that the decellularization procedure produced acellular scaffolds from cadaveric skeletal muscle with preserved extracellular matrix and anatomical patterning including vascular architecture. The scaffolds were suitable for cell culture of various types of cells in vitro and the cells were viable for several weeks, demonstrating the feasibility of such an approach for transplant intervention. The extent on the correct anatomical cell-type specific localization and the degree of muscle fiber formation are presently under investigation together with the preservation and immunoresponse induced by the construct transplant. This study represents the first molecular characterization of the bioactivity of scaffolds derived from decellularized muscle and of the feasibility of such approach for transplantation intervention.

STATISTICAL METHODS FOR QUANTITATIVE ASSESSMENT OF CELLS VIABILITY BASED ON FLOW CYTOMETRY AND MICROSCOPY

Marcin Frankowski, Andreas Kummrow, Nicole Bock, Christine Werner,
Rainer Macdonald, Jörg Neukammer

Physikalisch-Technische Bundesanstalt Berlin, Germany

Metrology for health underpins the more reliable and efficient exploitation of diagnostic and therapeutic techniques. Quantitative instead of only qualitative diagnostics is required in order to validate e.g. viability of cells. The examined methods included in this paper are optical microscopy and flow cytometric analysis.

Assessment of suitable methods to validate viability of selected cells is based on a key parameter which is the fraction of cells surviving the sorting/enrichment procedure (like FACS or MACS). An approach used here employs testing the metabolic activity with molecules that become fluorescent in living cells (calcein-AM) complemented by dead cells staining (ethidium). However, the usually sparse populations of target cells relevant for regeneration could essentially limit an application of high throughput flow cytometric cell counting. Thus, the quantitative wide-field microscope imaging is assumed as an efficient compromise solution by providing statistically significant information on an ensemble of rare target cells produced by a certain enrichment technique. Sorted target cells were characterized by morphological parameters in correlation with fluorescent labels. In parallel, a high throughput flow cytometric analysis was performed to evaluate the algorithms applied for image analysis.

PHOSPHOFLOW CYTOMETRY-BASED THERAPEUTIC DRUG MONITORING AFTER HEART TRANSPLANTATION

Maja-Theresa Dieterlen, Sara Klein, Katja Eberhardt,
Hartmuth B. Bittner, Stefan Dhein, Friedrich W. Mohr, Markus J. Barten

Heart Center Leipzig, Germany

Backgrounds: Preclinical and clinical development as well as routine clinical use of immunosuppressants rely on measurements of blood concentrations of these drugs, rather than on the biologically more relevant quantitation of the effects of these drugs on immune cell function. Pharmacodynamic monitoring as an approach of therapeutic drug monitoring (TDM) directly reflects the biologic effects of drugs. We used phosphoflow cytometry to detect phosphorylated ribosomal protein S6 (p-RPS6), a downstream product of the mTOR-pathway, to assess the specific effects of drug-induced target inhibition. Thus, we validated a novel pharmacodynamic assay to measure the individual drug effects of mTOR-inhibitors after heart transplantation.

Methods: Blood from healthy volunteers was incubated with different clinical relevant concentrations of the immunosuppressive drugs Sirolimus (SRL), Ciclosporin A (CsA), mycophenolic acid (MPA) and Dexamethasone (DEX). Whole blood was then stimulated, and p-RPS6 was measured by phosphoflow cytometry. Assay-specific parameters like inter-individual, intra-assay and inter-assay variabilities for p-RPS6 were determined. Additionally, we investigated if storage period and storage temperature of whole blood samples influences phosphorylation status of RPS6.

Results: Phosphoflow analysis revealed that addition of mTOR-inhibitors like SRL suppressed phosphorylation of RPS6 in human T cells, whereas CsA, MPA and DEX as known from their mechanism of action did not inhibit mTOR-related RPS6-phosphorylation. We determined the assay-specific IC₅₀ for sirolimus at 19.8nM. The maximum inhibitory effect (I_{max}%) of SRL on S6 phosphorylation in T-cells was obtained at 91.9%. Reproducibility of this assay was verified with slide-based cytometry. Inter-assay and intra-assay coefficients of variation ranged from 0.12 to 0.25 and 0.03 to 0.05 respectively. Intra-individual variability ranged from 14.0 to 38.0. Human heparinised whole blood samples can be stored at 4°C up to 2h and at room temperature up to 24h after withdrawal without any significant changes in phosphorylation of RPS6. **Conclusions:** In this study, we established a specific phosphoflow-based whole-blood assay to assess drug effects on the mTOR-pathway through measuring p-RPS6. We validated and characterized assay parameters, and demonstrated that p-RPS6 could serve as a biomarker for monitoring effects of mTOR-inhibitors in T cells. Future studies in HTx recipients will show if this assay has the potential to dose mTOR-inhibitors in combination therapies more safely without losing the efficacy.

Chair: Annette G. Beck-Sickinger

Chair: Andrea A. Robitzki

CANCER THERAPY WITH MAGNETIC NANOPARTICLES

Christoph Alexiou

Friedrich-Alexander-Universität, Erlangen-Nürnberg, Germany
Department of-Rhino-Laryngology, Head and Neck Surgery, Section for
Experimental Oncology and Nanomedicine (Else Kröner-Fresenius-
Professorship), University Hospital Erlangen, Germany

Cancer is a leading cause of death worldwide and 7.9 million deaths (around 13% of all deaths) occurred in 2007. Deaths from cancer worldwide are projected to continue rising, with an estimated number of 12 million in 2030 [1]. Chemotherapy is an important therapeutic column in treatment of cancer, but the systemic administration can lead in patients often to severe negative side effects. This disadvantage is caused by the application-to-tumor-dose-ratio due to the insufficient drug dose in the respective tumor region. Therefore new therapeutic methods have to be developed to improve the effectiveness of this therapy and to reduce adverse effects. With Magnetic Drug Targeting (MDT) chemotherapeutic agents bound to magnetic nanoparticles can be directed to desired body compartments using an external magnetic field. Superparamagnetic iron oxide nanoparticles are frequently used in medical research for new drug carriers (Fe_3O_4). These particles are biodegradable and they are still in use as contrast agent in magnetic resonance imaging (MRI) [2].

LASER-ACTIVED GOLD-NANOPARTICLES AS A POTENTIAL NEW TREATMENT MODALITY FOR AGE-RELATED MACULAR-DEGENERATION

Ina Hahn², Florian Levold¹, Nicole Eter²,
Elmar Endl¹

¹Universitätsklinik Bonn, Institut für Molekulare Medizin, Germany;

²Universitäts-Augenklinik Bonn, Germany

Background and purpose: In the last decade nanomaterials have increasingly become subject to investigation for use in anti-tumor and anti-angiogenic therapy. In our study we use laser-activated gold nanorods which are known to be nontoxic and to have excellent absorption properties in the near infrared (NIR) as a potential new treatment modality for choroidal neovascularization in age-related macular-degeneration.

Methods: Gold nanorods were coated with both, polyethylenglycol (PEG) to increase biocompatibility and peptides to bind specifically on new growing endothelial cells. Primary retinal endothelial cells and retinal pigment epithelial cells (ARPE-19) were incubated in vitro with gold nanorods. Uptake of nanoparticles was studied by light- and electron microscopy. Cells incubated with and without nanorods were irradiated with a NIR-laser. Afterwards cell death and apoptosis and the influence on proliferation were investigated by various cytometric methods. The impact of intracellular or membrane-bound localization of nanorods on irradiation efficiency was analysed by the use of light- and fluorescence microscopy and flow cytometry. Nanorod accumulation in the eye and general biodistribution in vivo was studied histologically in a laser-induced CNV mouse model.

Results: Light microscopy and electron microscopy demonstrated binding and intracellular uptake of coated nanoparticles. Immediately after laser irradiation no effect on cells in terms of cell death could be observed. However, 24h after irradiation a high amount of apoptotic cells could be found and proliferation was reduced. Laser treatment alone without nanoparticles showed no effect on viability and proliferation of neither pigment epithelial cells nor endothelial cells. PEG-coating was observed to be crucial to bypass filter organs and increase circulation time in blood and thereby height accumulation in target tissue. Both, PEG-coated nanoparticles and peptide and PEG-coated plus peptide-coated nanoparticles accumulated exclusively within the areas of choroidal neovascularization and not in healthy tissue.

Conclusion: Specific hyperthermic cell elimination via apoptosis and reduction of cell proliferation can be achieved using biofunctionalized laser-activated gold nanoparticles. Further studies in animals are under way.

NANOPARTICLE SURFACE CHEMISTRY DETERMINES THE ENDOCYTIC UPTAKE AND THE ACTIVATION STAGE OF HUMAN MACROPHAGES

Matthias Bartneck¹, Heidrun Keul²,
Gabriele Zwadlo-Klarwasser¹, Jürgen Groll²

¹RWTH-University Hospital Aachen, Germany; ²DWI e.V. and Institute of Technical and Macromolecular Chemistry, RWTH Aachen University

I*Introduction*

Gold nanoparticles (AuNP) are one of the most promising candidates for the application of nanotechnology in biology and medicine due to their simple manufacture and the possibility to control size, shape and surface chemistry precisely. The efficacy of these particles may however be limited due to the high phagocytic activity of macrophages resulting in phagocytic uptake or inflammation. We investigated the effects of AuNP on MΦ activation by studying phenotypic changes and cytokine expression.

Materials and methods

Gold nanorods (AuNR) were synthesized by the seeded growth procedure, followed by exchange of the stabilizer cetyl-trimethylammoniumbromide (CTAB) with poly(ethylene oxide) (PEO) bearing OH, COOH and -NH₂ as endgroups. Cells and different concentrations of nanoparticles were incubated with 5% human serum. The uptake of the different AuNR was visualized using seedless deposition and cytospin preparations and validated by Transmission Electron Microscopy (TEM). To investigate long-time effects of internalized AuNP on MΦ differentiation and activation, monocytes were cultured with gold nanoparticles for seven days. Gene expression and phenotype were determined using TaqMan-based Real-Time PCR and flow cytometry.

Results and discussion

CTAB-coated AuNP and commercial AuNS were phagocytized after 15 minutes. PEO-stabilization resulted in an inhibition of particle phagocytosis. After longer times of incubation (48 h), however, also PEO OH-stabilized AuNP were internalized by monocytes and MΦ. In mature macrophages, AuNP were still located inside the cells. Nanoparticles altered the expression of genes, cytokines and of the number of cells expressing the surface markers CD163 and 27E10. The intracellular presence of AuNS led to a classically activated (M1) proinflammatory MΦ phenotype indicated by an increased amount of 27E10⁺ cells and enhanced expression of IL1β. The presence of PEO-NH₂-stabilized AuNR in cell culture medium increased the number of CD163⁺ cells up to 40% M2 macrophages whereas PEO-COOH rods induced the M1 phenotype (up to 30 % increase in the number of 27E10⁺ cells). PEO-stabilized nanorods led to a bipolarization of macrophages with an increased number of CD163⁺ cells and enhanced IL1β expression.

Conclusion

Our findings show that phagocytosis of AuNP can be inhibited by modification of surface chemistry, however, only for a limited period of time. Furthermore, the presence of the particles has strong effects on the activation state of human MΦ in vitro. Thus, in vivo AuNP may lead to chronic inflammation in particular in tissues where these particles accumulate. Nevertheless, our data suggest that chemical modifications of gold nanoparticles might have immunomodulatory potency.

EFFECT OF GOLD NANOPARTICLES ON REPRODUCTIVE CELLS OF MODEL ANIMALS

Ulrike Taylor¹, Svea Petersen², Annette Barchanski², Wiebke Garrels¹,
Wilfried Kues¹, Stephan Barcikowski², Sabine Klein¹, Detlef Rath¹

¹Institute of Farm Animal Genetics (FLI), Germany;

²Laser Zentrum Hannover e.V.

Gold nanoparticles (AuNP) possess excellent optical properties, recommending them as biomarkers for optical imaging and intervention in in vivo applications. If produced by laser ablation in liquids, the particles are of high stability and homogeneity, and can readily be functionalised with biomolecules. However, a solid understanding of their toxicity is still wanting. Therefore, these studies aimed to explore the potential reprotoxicity of laser generated AuNP. Bovine spermatozoa (7 bulls, 3 replicates/bull) were diluted in medium containing AuNP (0.5, 5, or 50 μM Au) and incubated for 2h (Fig. 1C). Electron microscopical studies confirmed close association of AuNP with the sperm membrane. Evaluation of membrane integrity and morphology revealed no difference between treated and untreated spermatozoa. However, 50 μM Au led to a drop in motility by 22% ($p < 0.05$) compared to control sperm. In a further study approximately 10 pL of a nanoparticle dispersion containing 250 μM Au ($n=93$) were injected into one blastomere of two-cell-stage murine embryos (Fig. 1B). Embryos injected with water alone ($n=79$) served as controls. Successful AuNP-injection was confirmed using confocal microscopy (CM). No abnormal development related to morphology and timing of cleavage was observed. A total of 66.2% of the water-injected embryos developed to blastocysts, compared to 62.4% after injection of AuNP giving no indication of significant differences between groups. In conclusion, these data indicate that AuNP can have a detrimental effect on germline cells if used in concentration above 50 μM . Current investigations, including IVF with AuNP-exposed spermatozoa, qRT-PCR of development-relevant genes in blastocysts and transfer of injected embryos into recipient animals to study functionality and potential long-term effects, may gain a better understanding of AuNP toxicology.

Thursday, 14/Oct/2010

9:00pm - 9:30pm

CYTOMETRY GOES NANO

Wolfgang Fritzsche

Nano Biophotonics Department, Institute of Photonic Technology,
Jena ,Germany

Nanotechnology describes a trend of using units and/or tools of nanoscale dimensions for various applications in technology. It enables novel approaches not possible with larger structures. For life sciences, these novel methods allow the control down to dimensions of single biomolecules. Directed transport of effectors, intracellular sensing, or cell manipulation are examples where nanotechnology assists in biological and biomedical research or even already in diagnostics and therapy. Because these techniques usually aim at the individual cell level it holds a special potential for cytomics.

The presentation will give an overview over applied nano-approaches in biomedical and cell biology applications. It will then focus on nanoparticles (especially metal ones) and their applications for various kinds of cell and biomolecular studies as well as manipulations.

Thursday, 14/Oct/2010

9:30pm - 11:00pm

NANOTOOLS

Chair: James Leary

Chair: Ulrich Sack

NANOTOOLS FOR BIONANOTECHNOLOGY AND NANOMEDICINE

James Leary

Purdue University, United States of America

In order to perform tasks at the cellular and subcellular level (e.g. “nanosurgery”) we need to develop “nanotools” that are sophisticated, multifunctional, multicomponent nanomachines. These nanomachines are characterized by the careful ordering of atoms and molecules, often thermodynamically self-assembling nanostructures, to perform highly specialized and “programmable” (in the sense of being able to control a sequence of events in a specific order) functions. Unlike conventional tools which are used to make smaller objects from bigger one in a “top-down” approach, nanotechnology uses nanotools to build larger objects from smaller ones in a “bottoms up” approach”.

To characterize these suboptically sized nanomachines, we need to use a variety of nano-metrology technologies (“nanotools”). To study the interaction of these nanomachines with cells we use many of the normal tools of cytometry (e.g. flow and image cytometry, confocal microscopy, fluorescence microscopy, transmission electron microscopy, cell tracking velocimetry, Raman spectroscopy, and surface plasmon resonance spectroscopy) but sometimes in different ways.

VISUALIZATION AND QUANTIFICATION OF COLLOIDAL AND INTRACELLULAR GOLD NANOPARTICLES BY CONFOCAL MICROSCOPY

Sabine Klein¹, Svea Petersen², Ulrike Taylor¹, Csaba Laszlo Sajti²,
Detlef Rath¹, Stephan Barcikowski²

¹Institut für Nutztiergenetik Mariensee (FLI), Höltystr. 10, 31535 Neustadt, Germany; ²Laserzentrum Hannover e.V., Hollerithallee 8, 30419 Hannover

Their superior optical properties and the high biocompatibility favor gold nanoparticles (AuNP) as biomarkers for the visualization of various ligands in cellular systems. The extraordinary high quantum yield of the surface plasmon resonance scattering and the stable scattering cross section over prolonged time periods are not granted by any other marker system. In the current study we demonstrate the size dependent quantification of laser generated AuNP in dispersion and in a bovine endothelial cells by laser scanning confocal microscopy down to the single particle level. Shifted scattering and luminescence properties of the AuNPs inside the cells are described. For AuNP larger than 60 nm a reliable quantification at the single particle level was confirmed by mass spectrometry. AuNP conjugated to a Cy5 tagged TAT sequence, had been visualized by overlapping intracellular AuNP scattering and Cy5 fluorescence and thus verified the cellular uptake of bio-functionalized laser generated AuNP in fibroblasts.

PURIFICATION OF HUMAN EMBRYONIC STEM CELL CULTURES BY LASER ACTIVATED GOLD NANOPARTICLES

Elmar Endl¹, Franziska Winter², Stefanie Terstegge²,
Andreas Dolf¹, Barbara Rath², Philip Koch², Oliver Brüstle²

¹Universitätsklinik Bonn, Institute for Molecular Medicine; ²Institute of Reconstructive Neurobiology, Life & Brain Center, University of Bonn

In human embryonic stem cell (hESC) research the long term goal is to eventually transplant cells such as neuronal precursors that are derived by differentiation of hESCs into a patient.

Of major concern thereby are remaining undifferentiated, pluripotent hESCs as these cells can induce tumour formation after they have been transplanted. hESCs express the tumour related antigens (Tra)-1-60 and Tra-1-81, which are strongly associated with a pluripotent state of the cells. Hence these surface markers were the targets for nanogold-coupled monoclonal antibodies and subsequent laser exposure in order to selectively eliminate pluripotent hPSCs. Success of the ablation was investigated by flow cytometrical means and quantification of gold particle binding was controlled by cytospin preparation.

The method of selected cell targeting and elimination itself was initially tested on animal cell lines from different origins achieving depletion rates of more than 95%.

Applying the method to hESCs proofed that populations of solely undifferentiated, pluripotent hESCs could be eliminated using biotinylated antibodies in combination with streptavidin-coated 25 nm nanogold particles. The damage mechanism is based on physical parameters and can therefore be varied more precisely than chemical methods by choosing appropriate particle size, pulse width and pulse energy and easily be controlled in time and space.

Additional studies on potential side effects showed that efficient elimination of hPSC can be achieved, while co-treated hPSC-derived neural precursors maintain their normal proliferation and differentiation potential. In vivo transplantation of treated and untreated cultures revealed that laser ablation can dramatically reduce the risk of teratoma formation. Laser-assisted photothermolysis thus represents a novel contact-free method for the efficient elimination of hPSC from in vitro differentiated hPSC-derived somatic cell populations.

MONITORING HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION IN LIVE CELL CHIPS

Michael Angstmann^{1,2}, Irena Brinkmann³, Karen Bieback³,
Dirk Breitzkreutz¹, Christian Maercker^{1,2}

¹University of Applied Science Mannheim, Germany; ²German German Cancer Research Center (DKFZ), Heidelberg; ³Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University

For their wide mesodermal differentiation potential mesenchymal stromal/stem cells (MSCs) are attractive candidates for tissue engineering. However, standardized quality control assays are not firmly established which are non-invasive and continuous over time. We have developed a non-invasive cell-based assay in live cell chips to discriminate osteogenic and adipogenic differentiation lineages of MSCs by monitoring electrical impedance during cell adhesion and growth, employing two different systems and using fibroblasts and keratinocytes as controls. Adhesion and growth related impedance profiles of non-induced MSCs roughly resembled those of fibroblasts, whereas keratinocytes differed significantly. For differentiation studies, impedance profiles were recorded comparing MSCs from bone marrow and adipose tissue, either non-induced or induced for osteogenesis or adipogenesis, for 5 to 14 days. Additionally, differentiation modulating effects of extracellular matrix components were analyzed. Compared to MSCs in undifferentiated state, osteogenic induction of MSCs revealed initially rapid and continuously rising impedance, in part corresponding to mineralized calcium matrix formation. Conversely, adipogenic induction caused shallower initial slopes and eventually declining profiles, corresponding to more compact, adipocyte-like cells with numerous lipid vacuoles. Precoating with either collagen type I or IV apparently favored osteogenesis and laminin or fibronectin adipogenesis. Impedance recordings correlated well to the extent of differentiation evaluated by histochemical staining. Overall, our data demonstrate that impedance profiling offers a suitable basis for standardized real time noninvasive high-throughput screening of MSC properties. It further enables testing of the influence of diffusible factors or ECM composites on MSC differentiation or maintenance of stemness, thus substantiating therapeutic application.

Thursday, 14/10/2010:

11:30am - 1:00pm

NEW DEVELOPMENTS IN NANOSTRUMENTATION

Chair: Dieter Weiss

Chair: Wolfgang Beisker

**EXPLORING THE DYNAMIC PROPERTIES OF SUPRAMOLECULAR
ASSEMBLIES IN LIVE CELLS WITH SINGLE NANOPARTICLE IMAGING
AND MANIPULATION**

Maxime Dahan

Laboratoire Kastler Brossel, France

In recent years, several experiments have demonstrated the potential of nanoparticles to study biochemical reactions at the single molecule level and to probe the formation and maintenance of supramolecular assemblies in live cells. In a first part, I will discuss the principles and methods of single quantum dot (QD) tracking, a labeling and imaging tool originally introduced to investigate the diffusion properties of membrane proteins. I will present our current effort to go beyond membrane dynamics and make QD imaging a standard technique in cell biology. First, I will discuss how QDs can be internalized into live cells, how their colloidal properties affect their intracellular behavior and how QDs can be targeted to specific biomolecules or organelles. Next, I will show the results of recent experiments on the motion of individual molecular motors kinesin and myosin V in the cytoplasm of live cells. These experiments give access to important parameters such as the velocity, the processivity or stepping characteristics of the motor, directly in its cellular environment. I will also describe our effort to combine QD tracking with emerging high-resolution imaging techniques and show how we could access detailed morphological information on subcellular structures, such as neuronal synapses.

In a second part, I will present recent results on the use of magnetic nanoparticles to manipulate intracellular signaling. After injection in live cells, the nanoparticles can be displaced using magnetic gradients. Once functionalized with constitutively active rho-GTPase (such as Cdc42 or Rac), these nanoparticles can be used to trigger local signaling events, such as the recruitment of actin, the growth of lamellipodia and possibly cell migration. These experiments are a first step towards the magnetic control of cell polarity.

FLUORESCENCE CORRELATION SPECTROSCOPY: AN ANALYTICAL TOOL FOR SYSTEMS BIOLOGY

Petra Schwill

Biotechnologisches Zentrum der TU Dresden, Germany

Cell and developmental biology are immensely complex and rapidly growing fields that are particularly in need of quantitative methods to determine their key processes. With all the data known about protein interactions and interaction networks from biochemical analysis, there still remains the important task of in situ biochemistry, i.e. determining the thermodynamic and kinetic parameters of certain reactions in the cellular environment. E.g., to understand how cells polarize and develop into organisms, we need quantitative methods to determine concentration gradients and diffusion coefficients of key factors such as morphogens. In conjunction with two-photon excitation and spectrally resolved detection, Fluorescence Correlation Spectroscopy (FCS) is a powerful means for the study of concentrations, translocation processes, molecular association or enzymatic turnovers. It is fair to state that this technique raises strong hopes for quantitative systems biology. During the past years, we applied FCS to a variety of cell-associated phenomena, among them protein-protein binding, protein-membrane interactions enzymatic reactions, endocytosis, and gene silencing. Recently, we established the possibility of determining how morphogen gradients form and maintain in living embryos, thus opening up a manifold of attractive applications in developmental biology.

DUAL MODALITY MRI/NIRF IN-VIVO IMAGING OF MULTIFUNCTIONAL NANOMEDICAL SYSTEMS

James Leary

Purdue University, United States of America

MR (Magnetic Resonance) imaging is too slow for real-time guided-surgery. NIRF (Near Infrared Fluorescence) imaging can be used to delineate tumor margins during surgery, but current NIRF imaging cannot provide enough penetration depth to detect early-stage cancer deep inside body. To overcome these restrictions we have developed dual-modality in-vivo imaging for MRI detection of tumors and NIRF-guided surgery using multi-component nanoparticle nanomedical systems. NIRF dye, Cy5.5, conjugated hydrophobically-modified glycol chitosan nanoparticles (HGC) exhibit excellent tumor targeting ability with NIRF imaging due to an enhanced permeability and retention (EPR) effect and prolonged circulation times. Superparamagnetic iron oxide (SPIO) nanoparticles, as MR contrast agents, were loaded into the nanoparticles, resulting in HGC-SPIO nanoparticles. HGC-SPIO nanoparticles were characterized and are being evaluated in mice by both NIRF and MR imaging. Our results indicate HGC-SPIO nanoparticles have the potential for dual-modality in-vivo imaging with MRI detection of tumors and NIRF-guided surgery.

Thursday, 14/Oct/2010

2:00pm - 3:30pm

IMMUNOLOGY: AUTOIMMUNE DISEASES

Chair: Alexander Scheffold

MOLECULAR AND CELLULAR PATHWAYS OF FOXP3+ REGULATORY T CELL GENERATION

Karsten Kretschmer

Center for Regenerative Therapies Dresden, Germany

In T cell-mediated autoimmune diseases, self-reactive T cells with known antigen specificity appear to be particularly promising targets for antigen-specific induction of tolerance without compromising desired protective host immune responses. It is now firmly established that, in certain experimental settings, Foxp3-expressing CD4+CD25+ regulatory T cells (Treg) can be induced from conventional CD4+CD25-Foxp3- T cells. However, limited evidence for extrathymic Treg generation from self-reactive T cells exists under physiological conditions.

Here we report on our attempts to compare events early during agonist-driven generation of Foxp3+ T cell receptor transgenic T cells to polyclonal CD4+ T cell populations. This approach led to the identification of an IL-2- and phosphatidylinositol 3-kinase-dependent precommitted precursor population to Foxp3+ Treg in peripheral lymphoid organs of nonmanipulated mice. In addition, extrathymic Treg generation as an approach towards novel prevention and intervention therapies will be discussed in the context of autoimmune encephalomyelitis and diabetes.

PATHOGENIC AND PROTECTIVE IMMUNOLOGICAL MEMORY

Hyun-Dong Chang

Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), Germany;

Memory is a key characteristic of the immune system, yet it is poorly understood, on the molecular, cellular and systemic level. Immunological memory protects from and provides an enhanced and qualified reaction against recurrent pathogens, but it also can be inducer and motor of immunopathology. Recently, we have analysed the basic biology of memory T helper lymphocytes, to understand their contribution to the pathogenesis of chronic inflammatory diseases.

CD4⁺ T lymphocytes are a critical component of immunological memory, they control the quality and quantity of immune reactions. They are essential for the generation of high-affinity memory B cells and memory plasma cells. It has been assumed that the numbers of memory CD4⁺ T cells are maintained by homeostatic proliferation in response to the survival signal interleukin-7 (IL-7), in the absence of antigen. This view may have to be revised, since we could show for several immune responses, that following clearance of the antigen, the precursors of CD4⁺ memory Th lymphocytes migrated to the bone marrow, docked with dedicated stromal cells expressing IL-7, differentiated into memory cells, and were maintained as resting cells, in terms of proliferation and transcription. Memory CD4⁺ T cells from the bone marrow express the marker Ly6C, are quickly reactivated upon challenge with antigen, and provide efficient help to activated B lymphocytes for the production of high-affinity antibodies. Their resting state, fast reactivation and efficient helper function define memory CD4⁺ T cells of the bone marrow as professional protective memory CD4⁺ T cells of successful immune reactions in which the antigen has been eliminated. In contrast, CD4⁺ T cells of the pathogenic memory found in chronic rheumatic autoimmune inflammation are constantly confronted with their antigens and have a history of repeated restimulation. From the comparison of transcriptomes of once versus several times restimulated Th lymphocytes we have identified the transcription factor *twist1*, as a transcription factor which is specifically upregulated in repeatedly restimulated pro-inflammatory Th1 cells and is highly and specifically expressed in T cells isolated from inflamed tissue of patients suffering from chronic inflammatory diseases, like rheumatoid arthritis, reactive arthritis or colitis.

Thus, protective and pathogenic CD4⁺ memory Th cells are distinct in location, bone marrow versus inflamed tissue, and gene expression signatures, e.g. *twist1* expression marking CD4⁺ T cells of pathogenic immunological memory Th1 cells. These differences define new options to target pathogenic memory Th cells selectively.

GLOBAL CYTOMETRIC PROFILING AS A NEW TOOL FOR DISEASE AND THERAPY MONITORING AND CLINICAL BIOMARKER DISCOVERY

Andreas Grützkau¹, Joachim Grün¹, Ursula Schulte-Wrede¹,
Marta Steinbrich-Zöllner², Toralf Kaiser¹, Till Sörensen², Thomas Häupl²,
Joachim Sieper², Andreas Radbruch¹

¹DRFZ Berlin, Ein Leibniz Institut, Germany; ²Abt. für Rheumatologie, Charité, Berlin, Germany

Early diagnosis and an improved disease and therapy stratification are fundamental challenges in multifactorial diseases such as cancer and autoimmune disorders. Here we present a new concept using global multiparametric, flow cytometric analyses in a combined hypothesis-based and hypothesis-generating manner. We developed multicolour staining panels including up to 50 different monoclonal antibodies directed against surface molecules that allowed the assessment of several hundreds of phenotypical parameters in a few milliliters of peripheral blood. Up to ten different surface antigens were measured simultaneously by the combination of seven different fluorescence colours. For straight-forward data analysis, a suitable bioinformatic strategy for data storage, data normalization and retrieval of differentially expressed parameters has been established. In a proof-of-principle study we used peripheral blood from active Ankylosing Spondylitis patients (AS), a chronic-inflammatory rheumatic disease, for disease classification and therapy monitoring. We could identify an immunophenotypical pattern that allowed a classification of AS if compared to healthy donors and other chronic rheumatic diseases. Additionally, a cytometric pattern of differentially regulated surface molecules has been identified that was sensitive to change during anti-TNF-alpha treatment of AS patients within the first 3 months of treatment. In conclusion, this study looks promising to apply this global cytometric approach for an extensive monitoring of dysregulated immune responses and their treatments and to integrate it in biomarker discovery workflows.

HIGH-SENSITIVITY DETECTION AND ANALYSIS OF RARE CELLS BY INTEGRATED MAGNETIC ENRICHMENT AND FLOW-CYTOMETRIC ANALYSIS USING THE MACSQUANT® ANALYZER

Alexander Scheffold, Mario Assenmacher,
Manuela Herber, Dorothee Köhler, Petra Bacher Bacher

Miltenyi Biotec GmbH, Germany

The limit of detection of rare cell types, such as antigen-specific lymphocytes, in standard flow-cytometric assays is restricted by the total number of cells acquired during a single measurement. For example, for 10^6 acquired cells the detection limit is typically between 10^{-4} - 10^{-5} depending on the quality of the reagents and the number of discriminative parameters. Pre-enrichment of the rare target population, e.g. by magnetic cell separation which allows rapid processing of large cell numbers, dramatically increases the sensitivity of the subsequent flow-cytometric analysis. Using combined MACS® and flow-cytometric analysis, sensitivities down to 10^{-7} have been described, which allowed even the detection of specific T cells within the naïve repertoire or rare circulating tumor cells. However so far the method is time consuming and requires multiple processing steps increasing variability and the risk of cell loss.

To overcome these limitations, the recently introduced MACSQuant® Analyzer, which contains a MACS® Cell Enrichment Unit, integrates magnetic pre-enrichment and flow-cytometric analysis in a single automated workflow. Simultaneous quantification of absolute target cell numbers is achieved by volumetric measurement in the MACSQuant® Analyzer.

In spiking experiments the detection and quantification of as few as 10 tumor cells (SK-BR3) out of 10^8 PBMC could be shown with a recovery of at least 70%. Furthermore we used the MACSQuant® Analyzer to quantify rare antigen specific T cells. HLA-A2 CMVpp65495-503 Tetramers were used to identify as little as 10 specific CD8⁺ T cells out of 10^8 PBMC. In addition we used CD154 expression by antigen-activated T cells to quantify and characterize rare Aspergillus reactive CD4⁺ T helper cells from healthy donors.

Taken together, the MACSQuant® Analyzer simplifies the combined enrichment and analysis of rare target cells for rapid and reproducible identification, quantification and characterization. This approach will be an invaluable tool for the routine analysis of rare cells, such as circulating tumor cells or antigen-specific lymphocytes.

Chair: Susann Müller

FROM CYTO- TO ARCHAEOMETRY: STUDIES ON ROMAN COINS IN THEIR ARCHAEOLOGICAL CONTEXT

Michael Nüsse

Johann-Wolfgang-Goethe-Universität Frankfurt am Main, Germany

Coin finds are an important part of archaeological records. By studying coins in their archaeological contexts information can be obtained on how coins functioned in past societies and to understand the use, loss or deposition of coins and other coin related problems. In addition, coins are the focus of interest of many archaeometric projects designed to deduce the origin of the ores used for minting coins, the mining activities, the reconstruction of smelting and refining processes, and the trade routes of the raw materials. Some examples of archaeometric and statistical analysis of Roman coins found at the Martberg, a Gallo-Roman sanctuary located on a hilltop above the Moselle near Pommern, are presented. Here, excavations and stray finds have yielded nearly 10.000 coins from Celtic times (200 BC) until the end of the 4th century AD.

1. The spatial and temporal distributions of the well dateable coins and other, not as well dateable artifacts were analyzed in the sanctuary, whose various temples were reconstructed several times during the lifetime of the sanctuary. Using cluster analysis combining such data from coins and artifacts, the temporal and spatial changes in the history of the sanctuary could be demonstrated and dated.

2. Coins found in ritual and non-ritual contexts largely reflect the development of the coinage and its circulation. Distinct differences, however, between coin finds from both kind of context can be observed because coins deposited in sacral contexts are supposed to underlie different selection principles compared to coins found in secular contexts. The relative frequencies of reverse types of coins (antoniniani from the 3rd century AD) deposited intentionally in sanctuaries were compared with those of coins lost accidentally in secular contexts and of coins deposited in hoards in order to discuss the question of whether coins dedicated in sanctuaries are, at least in part, deposited preferentially according to their reverse designs. The results of the analyses using hierarchical cluster analysis show that a number of factors can influence the frequency of coin types, the most prominent are the presence of regular/irregular emissions and chronological aspects; they are, however, difficult to quantify. In sanctuaries, evidence is provided for a negative selection of certain coin types with military symbols (Fides, Jupiter, Mars, Sol, Victoria).

3. During the reign of the Roman emperor Claudius and due to the fact that no copper money was minted in Rome after 41 AD, the demand for money apparently far exceeded the official supply. Local representatives began minting imitations of copper prototypes of Claudian and other available coins. These imitations must be regarded as "money of necessity" rather than as imitations for profit. To study which metal sources were used to mint these local "Claudian" imitations, more than 100 such coins

found at the Martberg were analyzed for their trace element distributions using electron microbeam technique, 30 coins were analyzed in addition for their lead isotope ratios which are commonly used to trace raw material sources of metals. The combined results were compared with similar data from earlier measurements of official copper coins from Augustus to Claudius, and show that the large majority of the imitations were reminted at a lower weight using copper from the available official coins.

References: B. Kaczynski and M. Nüsse, Reverse type selection in sanctuaries? A study of antoniniani found in various contexts. In H.-M. von Kaenel and F. Kemmers (Hrsg.) *Coins in Context I: New Perspectives for the Interpretation of Coin Finds*. Studien zu Fundmünzen der Antike 23, Mainz 2009, 93-108.

Friday, 15/10/2010:

9:00am - 10:30am

OMICS ON SORTED CELLS AND ORGANELLES

Chair: Dirk Bumann

Chair: Susann Müller

**"STUDYING PATHOGEN PROPERTIES IN INFECTED TISSUES USING
FLOW CYTOMETRY"**

Dirk Bumann

Biozentrum, University Basel, Switzerland

Flow cytometry offers unique possibilities for sensitive large-scale analysis of individual pathogen cells. In particular, inducible expression of fluorescent proteins enables high-content read-outs on pathogen microenvironments and pathogen responses. However, infected host tissues usually have a high autofluorescence background interfering with detection of weakly fluorescent pathogen cells. Here, I will discuss approaches to spectrally separate Salmonella cells from host tissue fragments for sensitive quantification of individual GFP expression levels, detection of Salmonella subpopulations in tissue homogenates, parallel tracking of clones expressing different fluorescent proteins during infection, and large-scale purification of Salmonella from infected host tissues for subsequent proteome analysis.

PROFILING OF THE ADAPTATION OF STAPHYLOCOCCUS AUREUS TO INTERNALIZATION BY HUMAN EPITHELIAL CELLS: A FUNCTIONAL GENOMICS APPROACH

Frank Schmidt¹, Sandra Scharf¹, Maren Depke¹, Henrike Pfortner¹,
Petra Hildebrandt¹, Melanie Gutjahr¹, Vishnu Dhople¹, Jörg Bernhardt²,
Michael Hecker², Ulrike Mäder¹, Uwe Völker¹

¹Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Germany; ²Institute for Microbiology, University of Greifswald, Germany

S. aureus is a versatile Gram-positive pathogen which gains increasing importance due to the rapid spreading of resistances. *S. aureus* is the cause of a wide spectrum of severe community-acquired and nosocomial infections. Functional genomics technologies can provide new insights into the adaptation network of this bacterium and its response to environmental challenges. However, particularly for proteomics studies the major bottleneck is the lack of sufficient proteomic coverage for low numbers of cells. In this study we introduce a workflow which combines a pulse-chase SILAC approach with high capacity cell sorting, on-membrane digestion, and high-sensitivity mass spectrometry to detect and quantitatively monitor several hundred *S. aureus* proteins from a few million internalized bacteria. In addition to proteomics data, complementary tiling arrays were used to gain a maximum of functional genomics information. In the presentation we will discuss the proteomic adaptation and the transcriptional response of the *S. aureus* wildtype strain RN1HG to internalization by human epithelial cells.

DESCRIBING MICROBIAL POPULATION DYNAMICS IN BIOGAS REACTORS USING FLOW CYTOMETRY

Christin Koch¹, Thomas Hübschmann², Susann Müller², Hauke Harms²

¹Helmholtz Centre for Environmental Research - UFZ, Department Bioenergy, Germany; ²Helmholtz Centre for Environmental Research - UFZ, Department Environmental Microbiology, Germany

The use of biomass as a renewable energy resource is becoming increasingly important in the substitution of fossil fuels. Over 5000 biogas plants of different scales are already installed in Germany and cover already about 1.5 % of our total energy demand. An upward trend is found worldwide.

Biogas reactors contain a highly diverse microbial community comprising of Bacteria and Archaea. They are able to degrade complex substrates, ranging from energy crops to organic wastes and distillers grains, to biogas mainly composed of methane and carbon dioxide.

Substrate choice, temperature, retention time, pH and the presence of trace metals or disturbing substances are just some factors influencing the community composition and the total reactor performance. Most studies only focused on the microbial composition at certain points of time so far using fingerprinting techniques. Therefore, only little is known about the dynamics within these biogas reactor communities.

The microbial community in a biogas reactor at the German Biomass Research Centre (DBFZ) was investigated with the aim of understanding the dynamics behind functional stability. The sample treatment and fixation procedure are described and first results on population dynamics presented.

CHANGES IN CELL CYCLE AND γ -H2AX PHOSPHORYLATION KINETICS AFTER IRRADIATION WITH 20 MEV PROTONS

Thomas Ernst Schmid¹, Günther Dollinger², Christoph Greubel²,
Volker Hable², Olga Zlobinskaya¹, Dörte Michalski¹, Attila Tarnok³,
Anja Mittag³, Michael Molls¹

¹Klinikum rechts der Isar, Germany; ²Universität der Bundeswehr München, Germany; ³Herzentrum der Universität Leipzig, Germany

In order to analyse the underlying mechanisms of the radiation damage in cells and tumors, an increasing number of charged-particle microbeams have been designed which deliver defined numbers of ions with high accuracy to single living cells at sub-millimeter dimensions.

The aim is to simulate the increased RBE (relative biological effectiveness) of heavy ions using spot application of a bunch of protons at two different application modes.

Irradiation containers for biological tissue samples had been developed specifically for use at the ion microprobe SNAKE of the 14 MV Munich tandem accelerator, which allows for irradiation of defined cell nuclei with single or counted protons. Counted protons were applied either in a focused mode flowing 5x5 μm^2 matrix with 100 protons per point or a nearly homogenous mode with 2 protons per point in a 0.5x1 μm^2 matrix (total area 50 mm^2). Thus in both cases the same dose of 1.7 Gy was applied.

A laser scanning cytometer, iCys (CompuCyte, Westwood, MA, USA), was used for analysis of cell cycle dependent CENP-F expression. For identification of cells DAPI (405 excitation) was used. Intensity of DAPI signal also served for cell cycle analysis. Additionally, for clear separation of cells in G2 and S-phase information of CENP-F-Alexa633 (633nm excitation) expression was used. Laser-scanning Cytometry was also performed to measure the integrated γ -H2AX fluorescence intensity in at least 100 cells. Intensities of integrated fluorescence of each of the dyes were measured and recorded for each cell. Fluorescence signal from cells irradiated 15 minutes before fixation were used for intensity calibration, which minimizes errors caused by variation between different dishes.

Our findings indicate significant differences in the cell cycle based on G2+S phase staining between both application modes in HeLa cells. After 2 hours cell cycle arrest in G2 phase was about 5% higher ($p \leq 0.05$) in the 5x5 μm application group than in the 0.5x1 μm application group.

Measurements of the γ -H2AX-signalling from 15 min to 60 min resulted in a 35% decrease for the 5x5 μm application group and in a 44% decrease ($p \leq 0.05$) for the 0.5x1 μm application group.

Further studies are in progress to analyze cell cycle changes and γ -H2AX phosphorylation kinetics after 12 and 24 hours.

Supported by the DFG Cluster of Excellence: Munich-Centre for Advanced Photonics, by the EU-project EuroDyna and by the Maier Leibnitz Laboratory Munich.

Friday, 15/10/2010: 11:00am - 12:30pm

NANOBIOTECHNOLOGY

Chair: Lars M. Blank

**SINGLE CELL ANALYSIS ON A CHIP: FROM PROTEIN EXPRESSION TO
CELL HETEROGENEITY**

Jan Regtmeier

Universität Bielefeld, Germany

Nanobiotechnology is an interdisciplinary research area and offers novel approaches for understanding biological processes at the level of single cells. Nanobiotechnology allows identification and quantification of cellular properties that can statistically be related to the heterogeneous environment of a single cell, individual heterogeneous cell-cell variations as well as to cellular subpopulations. Therefore, new insights from protein organisational structure (cellular toponome), biomolecular reaction kinetics, and spatio-temporal protein dynamics (systems nanobiology) can be accessed. A selection of micro- and nanofluidic techniques is presented that address different aspects of the analysis of single cells and their content, partly even with spatial and temporal resolution. The label-free protein fingerprinting of single cells is demonstrated allowing the full analysis on a chip, from cell positioning, over lysis to protein separation. A novel nanofluidic device exploits the physical properties of DNA and DNA protein complexes for the continuous separation that allows an immediate post-processing and further analysis. Despite the tiny dimensions of single bacterial cells, a space- and time resolved analysis of the protein dynamics in viable cells will be shown over many hours. This method provides insights in the highly organized protein dynamics during cell division. This technique can already be used to study and visualize cell-to-cell variations and – in the future – to study the response of single cells to externally added molecular cues.

A NEW REACTOR CONCEPT - THE ENVIROSTAT

Lars M. Blank¹, Hendrik Kortmann², Frederik Fritsch²,
Christian Dusny², Andreas Schmid^{1,2}

¹Laboratory of Chemical Biotechnology, TU Dortmund, Germany; ²Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.

One major goal of systems biology is to provide a quantitative description of cellular physiology. This task is complicated by population effects, which perturb culture conditions and mask the behavior of the individual cell. To overcome these limitations, we propose the use of a microfluidic bioreactor, of which the construction and operation is presented. The new reactor concept guarantees constant environmental conditions with single cell resolution, thus it was named Envirostat (environment, constant). In the Envirostat, cells are contactless trapped by negative dielectrophoresis (nDEP) and cultivated in a constant medium flow. For single cell cultivation of *Saccharomyces cerevisiae*, medium composition changes below 0.001% were estimated by computational fluid dynamic simulation. These changes were considered not to influence cell physiology. Single *S. cerevisiae* and *Schizosaccharomyces pombe* cells were cultivated in the Envirostat, confirming the applicability of the new reactor concept. The results are discussed in the context of single cell analysis with spatio-temporal resolution. The Envirostat facilitates single cell research and might simplify the investigation of hitherto difficult to access biological phenomena such as the true regulatory and physiological response to genetic and environmental perturbations.

PRECISION BIOLOGY WITH YEAST

Ali Kinkhabwala

Max Planck Institute of Molecular Physiology, Germany

To better understand the intricate and dynamic spatial orchestration of intracellular protein interaction networks, precise measurement and manipulation of important biochemical parameters (concentrations, localizations, interactions) across live cells is necessary ("precision biology"). Yeast provide an ideal platform for such precise biological investigations due to their facile and pliable genetics and their clearly resolvable subcellular structures. I will discuss our recent work on the further optimization of genetic tools for the chromosomal tagging of endogenous proteins with genetically-encoded fluorophores and for the precise transcriptional control of gene expression. I will also discuss our improved analytic approaches that enable truly quantitative determination of endogenous protein interactions using correlation- and lifetime-based microscopy approaches. The combination of precision microscopy techniques with the chromosomal labeling of endogenous proteins in yeast allows for unparalleled, physiologically-proper measurement and control of the biochemical parameters that underlie the functioning of various protein networks in this model organism.

SINGLE CELL ANALYSIS ON MICROFLUIDIC PLATFORMS

Petra S. Dittrich

ETH Zurich, Switzerland

Microfluidic platforms with integrated micro-sized structures are versatile tools for cell biological applications. The dimensions of such systems fabricated by means of microsystems technology are in the range of submicrometers up to a few micrometers and hence, are matching biological relevant length scales. In the recent past, numerous valuable microfluidic platforms for single cell analysis and cultivation have been designed, and completely novel methods without macroscopic equivalent have been developed that have led to novel insights into cell functioning.

In this presentation, approaches towards living cell analysis in microfluidic chips are presented, which are designed to systematically study the cellular response to the application of soluble factors and mechanical forces. Various microfluidic-based tools to immobilize or to cage cells individually have been developed. Furthermore, while the majority of microfluidic chips has a planar design, i. e. a channel system is fabricated into a substrate and is closed by a cover plate or foil so that processes are performed in a downstream mode, the benefits of bilayer microfluidic chips will be emphasized. The introduction of a second fluidic layer enables a further level of control, e.g. concerning cell handling and analyte supply. Additionally, the concept of multi-laminar streams facilitates the precise control of the chemical microenvironments of cells, and greatly supports systematic studies of cellular response. The first example focuses on the investigation of gene expression in mammalian cells (HEK 293). An inducible gene expression system (T-REx, Invitrogen) is used that can be controlled by tetracycline as soluble effector. The gene of interest in this proof-of-concept study is a destabilized mutant of the Green Fluorescent Protein (GFP). The gene expression is monitored by time-lapse fluorescence microscopy over 15 hours, and the fluorescence intensity profiles of individual cells, immobilized in micro-sized cages, reveal cell-to-cell differences. In the second example, a two-layer microfluidic platform is presented that facilitates planar voltage-clamp measurements on an individual oocyte trapped on a micropore. The investigations target the epithelial sodium channel (ENaC) that is investigated under normal conditions and addition of the blocking agent amiloride. The semi-automated operation of the device is very robust and optimized for measurements on parabolic flights of airplanes to study the influence of microgravity and zero gravity on individual cells. Finally, in the third example, the use of liposomes as simple cell models to investigate processes at or across the membrane is discussed.

Friday, 15/10/2010: 1:30pm - 3:00pm

WATER

Chair: Thomas Egli

Chair: Christian Wilhelm

SODIS: SUNLIGHT FOR DRINKING WATER DISINFECTION. FLOW CYTOMETRY AND OTHER METHODS REVEAL THE RESPIRATORY CHAIN AS THE MAIN TARGET IN ENTERIC BACTERIA

Thomas Egli

Swiss Federal Institute of Aquatic Science and Technology (eawag),
Switzerland

Availability of safe drinking water is a key health issue in developing countries; according to the UN World Water Development Report 3 over 1.2 billion people are at risk because they lack access to safe drinking water. In many parts of the world sunlight can be employed for disinfection purposes: 6 hours of exposure to the sun of hygienically unsafe drinking water in PET bottles results in a several log-fold inactivation of diarrhoea-causing bacteria, protozoa and viruses. Solar disinfection (SODIS) is a simple and cheap method that is based on easily available and low-cost tools (www.sodis.ch). Over 2 million people are already using SODIS it is on the WHO/UNICEF list of recommended methods for household drinking water disinfection. However, the exact inactivation mechanism(s) in microbial pathogens is not known and, therefore, this method meets much scepticism and is often rated as being too simple a technique to work safely. In the past years we have studied the mechanisms of inactivation by sunlight in enteric bacteria to provide a basis for broad acceptance of this elegant method.

A first insight into the processes of cell injury in *E. coli* during SODIS was obtained using flow cytometric methods for viability/activity determination combined with cultivation assays and ATP levels [1]. A UVA light dose of <math><500 \text{ kJ/m}^2</math> (ca. 2 hours of sunlight) was already sufficient to lower the proton motive force, such that efflux pump activity and ATP synthesis decreased significantly. Cells exposed to >1500 kJ/m² solar UVA radiation were no longer able to repair the damage and recover. Similar results were obtained with *S. typhimurium* and *S. flexneri* cells [2]. Recently, we have identified proteins as the early (and probably most important) targets of inactivation of sunlight at the molecular level [3,4]. Damaged (carbonylated) proteins were found by an immunological method. Tandem mass spectrometry (LC-MS/MS) allowed to identify damaged proteins. This information was complemented with activity measurements of selected enzymes. We observed an increase in protein carbonylation reproducibly at the very early state of irradiation (250 kJ/m²; i.e., one hour of sunlight). The first targets seemed to be proteins in the cytoplasmic membrane. A massive accumulation of protein aggregates was found in irradiated cells after about 1000 kJ/m². Some 73 out of 200 of the most abundant proteins of *E. coli* aggregated in the light. Target proteins included enzymes involved in translation, TCA cycle, transport, transcription, glycolysis, DNA-repair, protein folding, respiration and ATP synthesis. Enzyme assays after irradiation experiments confirmed these results.

We conclude that the primary reason for pathogen inactivation by sunlight during SODIS is caused by damage of various proteins (and not DNA), presumably largely by free radical formation in oxidative stress that is closely associated with the respiratory chain. Central metabolic pathways are then blocked by this protein damage which leads to cell death. Recovery from such very diverse damage seems very unlikely because the damaged proteins are prone to degradation and have to be re-synthesized, which requires much energy and building blocks. Therefore, SODIS is a save way to improve microbial water quality.

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**PHYTOPLANKTON ANALYSIS BY MEASURING FLUORESCENCE
ACTION SPECTRA IN FLOW CYTOMETRY**

Gerrit Jan van den Engh

BD Bioscience/Cytopeia, United States of America

RESOLUTION OF NATURAL MICROBIAL COMMUNITY DYNAMICS BY COMMUNITY FINGERPRINTING, FLOW CYTOMETRY AND TREND INTERPRETATION ANALYSIS

Sabine Kleinsteuber, Petra Bombach, Thomas Hübschmann,
Ingo Fetzer, Susann Müller

Helmholtz Centre for Environmental Research - UFZ, Germany

Natural microbial communities have generally an unknown structure and composition due to their still not yet cultivable members. Therefore, understanding the relationships between the bacterial members, prediction of their behaviour and controlling their functions is a difficult and often only partly successful endeavour to date. This study aims to test a new idea which allows following community dynamics on the basis of a simple concept. Terminal restriction fragment length polymorphisms (T-RFLP) analysis of bacterial 16S ribosomal RNA genes was used to describe a community profile which we define as composition of a community. Flow cytometry and analysis of DNA contents and forward scatter characteristics of the single cells were used to describe a community profile which we define as structure of a community. Both approaches were brought together by a non-metric multidimensional scaling for trend interpretation of changes in the complex community data sets. This was done on the basis of a graphical evaluation of the cytometric data, leading to the newly developed Dalmatian-plot tool, which gave an unexpected insight into the dynamics of the unknown bacterial members of the investigated natural microbial community. The approach presented here was compared with other techniques described in the literature.

The microbial community investigated in this study was obtained from a BTEX contaminated anoxic aquifer. The indigenous bacteria were allowed to colonize in situ microcosms consisting of activated carbon. These microcosms were amended with benzene and either of the electron acceptors nitrate, sulfate or ferric iron to stimulate microbial growth. The data obtained in this study indicated that the composition (via T-RFLP) and structure (via flow cytometry) of the natural bacterial community were influenced by the hydro-geochemical conditions at the test site but also by the supplied electron acceptors which led to distinct shifts in relative abundances of specific community members.

It was concluded that engineered environments can be successfully monitored by single cell analytics in combination with established molecular tools and sophisticated statistical analyses, a promising approach for studying and monitoring natural microbial community behaviour.

FLOW CYTOMETRY AS A TOOL TO MEASURE PHYTOPLANKTON DIVERSITY AND PHYSIOLOGICAL ACTIVITY WITH TAXONOMIC RESOLUTION

Susanne Dunker,
Zhixin Liu, Tobias Heinze, Christian Wilhelm

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Monitoring the phytoplankton community structure is an essential tool to control water quality in freshwater environments. Especially in eutrophic lakes prediction of harmful algal blooms is very important because accumulated biomass which has to be degraded via bacterial activity can have a great significance in the oxygen budget in the water body.

Modelling of the primary production is mostly done on the basis of the whole phytoplankton community and taxon-specific growth rates have not been measured in routine analysis. However, during the vegetation period phytoplankton structure varies drastically due to changes in many biotic and abiotic factors, like grazing, temperature or nutrient availability. The analysis of taxon-specific primary production needs the information both of the quantitative changes in the phytoplankton community structure as well as the physiological activities of the subpopulations. Therefore, flow cytometry can be used as a tool to observe changes in phytoplankton structure and to fractionate the algal subpopulations for further physiological analysis. For fractionating taxonomic groups phytoplankton samples of a hypertrophic lake were concentrated via membrane filters. Afterwards flow cytometric analyse and sorting of dominant taxa could be performed with the flow cytometer FacsAria TM II (BD). The physiological characterization of sorted probes has been done in form of absorption spectra, photosynthetic activity measurements and the analysis of macromolecular composition of cells. With the flow cytometer we could analyse the phytoplankton succession weekly over the whole vegetation period and by the use of sorting phytoplankton samples it was possible to get physiological data of the main algal taxa. The established methodology allows taxon-specific estimation of primary production with high resolution and can be applied in freshwater as well as in marine environments.

Friday, 15/10/2010: 3:30pm - 5:05pm

THE KLAUS GOERTTLER SESSION

Chair: Schildberg Frank Alexander

Chair: Thomas Kroneis

Chair: Stephan Schmid

MATERNAL MICROCHIMERISM: THE IMPORTANCE OF SINGLE CELLS IN HEALTH AND DISEASE

Kathleen M. Gillespie

University of Bristol, Clinical Science, United Kingdom

Maternal microchimerism (MMc, the harboring of a small number of maternal cells by her offspring) was recognized in children with severe combined immunodeficiency more than 20 years ago. Evidence of maternal cells in foetal tissue has been demonstrated as early as 6 weeks gestation and has been found to persist into adult life in healthy subjects. Some cells that transfer across the placenta in pregnancy must therefore be adult stem cells. Most studies of maternal microchimerism have focused on analysis of haematopoietic cells but it is increasingly clear from human and animal studies that microchimeric cells also exist in other tissues including the liver, heart and brain.

The persistence of maternal cells implies that her offspring is tolerant to a low level of genetically distinct antigen but some studies suggest that levels of MMc are higher in individuals with some autoimmune disease leading to the hypothesis that autoimmunity may result from loss of tolerance to MMc.

We recently identified female cells (presumed maternal) in autopsy pancreas from young males with, and without type 1 diabetes, that produce insulin. There was, however, no evidence that these microchimeric islet beta cells were the target of autoimmune attack. Preliminary data suggest that levels of MMc in T1D pancreas are higher in T1D islets than controls. DNA analysis of single microchimeric cells is vital to confirm that these female cells are indeed maternal and to allow phenotyping by mRNA analysis. These studies will determine whether MMc contribute to the disease process or as is increasingly hypothesized, to the regenerative process.

"CLINICAL POTENTIAL OF MESENCHYMAL STEM CELLS IN IMMUNE MODULATION AND REGENERATIVE MEDICINE"

Ingo Müller

Universitätsklinikum Hamburg-Eppendorf, Abteilung für Pädiatrische Hämatologie und Onkologie, Germany

Mesenchymal stem cells (MSC, multipotent mesenchymal stromal cells) are fibroblast-like cells which can be easily differentiated into osteoblasts, adipocytes and chondrocytes. We have used the osteogenic differentiation for treatment of steroid-induced avascular osteonecrosis in children who had undergone chemotherapy. In these cases, MSC contributed to the regeneration of bone in the affected areas. Moreover, MSC exhibit certain immunomodulatory effects, which we analysed in vitro and identified molecular mechanisms involving several signaling pathways. Based on these findings we applied MSC in pediatric patients who developed graft-versus-host disease after allogeneic bone marrow transplantation.

"REGULATORY T CELLS: MAJOR PLAYERS IN THE TUMOR MICROENVIRONMENT"

Marc Beyer

Universitäten Bonn, LIMES Institute, Germany

Immune inhibitory circuits are essential for the dampened immune response in the tumor microenvironment. Over the last years there is increasing evidence that elevated numbers of regulatory T (Treg) cells can be found in the microenvironment of tumors, but also in peripheral blood of patients with solid tumors and hematologic malignancies. Treg cells can migrate into tumors and suppress effective anti-tumor responses, thus contributing to the prosperity and growth of human tumors. In addition, several mechanisms have been described how conversion of conventional CD4⁺ T cells into Treg cells can occur in the context of human tumors, yet little is known about the molecular and cellular features responsible for the increase and maintenance of elevated levels of Treg cells in cancer. Recent studies now have elucidated how Treg cells mediate regulatory activity in the tumor microenvironment and enhanced our understanding of the underlying molecular mechanisms. The reversal of a non-responsive to a permissive situation will be critical for future therapeutic interventions. One option would be to switch the central players from suppressive to effector cells, e.g. Treg cells into effector T cells. We have identified SATB1 (special AT-rich binding protein 1) as such a central switch in T-cell differentiation. Overexpression of SATB1 in Treg cells significantly reprograms the molecular Treg-cell machinery towards an effector T-cell phenotype while loss of SATB1 in effector T cells results in a loss of effector molecules like TH1/TH2-cytokines. This is independent of epigenetic modifications such as CpG-methylation or histone-modifications but critically linked to the expression of the Treg-cell specific transcription factor FOXP3 as well as post-transcriptional control by several Treg-cell specific miRNAs. Central switches like SATB1 will be attractive new targets for future therapeutic strategies reversing immunosuppression in the tumor microenvironment.

FIBER-OPTIC CONFOCAL LIVE-IMAGING TO MONITOR VASCULAR HYPERPERMEABILITY IN TUMOR AND NORMAL TISSUE

Antje Dietrich, Melanie Huether, Leoni A. Kunz-Schughart

Tumor Pathophysiology, OncoRay - Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden, Germany

Background

Vascular permeability is essential for the supply and clearing of tissues but is dramatically increased under pathological conditions. The basal permeability of capillaries is characterized by the physiological exchange of a plasma filtrate consisting mainly of gases, small molecules and only few plasma proteins. Acute vascular hyperpermeability (AVH) is a rapid but self limited influx of an exudate into the tissue caused by a particular event, e.g. a lesion. In tumors, angiogenic factors lead to vascularization of the neoplasm. The chronic exposure to such factors, however, causes profound changes in vessel structure and function resulting in chaotic morphology, abnormal perfusion and chronic vascular hyperpermeability (CVH).

Aim and Methodology

The visualization and characterization of tumor vasculature *in vivo* is often attended with high invasiveness (e.g. window chambers) or low resolution (e.g. MRI). In this study we applied the Cellvizio®-technology - a fibered confocal microscopy method for imaging fluorescence signals with 8-12 frames/second which can be administered non or minimal invasive. Fiber-optic microprobes with small diameters (0.3-1.8 mm) can be positioned directly on the tissue surface requiring only a small skin incision in case of subcutaneous tissues.

Results and Conclusions

By i.v. injection of a large, 500 kDa FITC-dextran we realized live-imaging of vascular morphology and microcirculation in normal tissue and subcutaneous xenograft tumors of NMRI (nu/nu) mice. The comparison of growth curves revealed no influence on xenograft growth after repeated applications of the methodology. A 150 kDa FITC-dextran was injected to visualize and analyze hyperpermeability by monitoring and quantifying the increase in fluorescence intensity in the tissue at defined time points after injection (1 min intervals). The vascular surface area and the corresponding signal strength were measured using a segmentation algorithm and comprised to calculate the increasing signal of the tissue/background. The 150 kDa tracer did not extravasate under normal conditions but acute stimuli (e.g. skin incision) induced an influx in muscles and tongues. This form of hyperpermeability was shown to be transient and independent from the time point of tracer injection thus attributing to AVH. In contrast, the tracer extravasation in xenografts can be assessed as CVH because it started directly after tracer injection and was not influenced by the incision. The increase of fluorescence intensity was more pronounced in small as opposed to large tumors indicating a reduction of vascular hyperpermeability throughout growth which will be now studied in more detail.

(supported by BMBF and SMWK)

TARGET-SPECIFIC THERAPY TESTING IN SPHEROIDS TO EXAMINE NEW CETUXIMAB-BASED RADIOTHERAPEUTIC APPROACHES

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Background and Aim

Epidermal growth factor receptor (EGFR) overexpression is frequent in many epithelial tumors. Inhibition of EGFR activation through the therapeutic antibody Cetuximab is a target-specific strategy for the treatment of such tumors combined with radiotherapy. As multicellular tumor spheroids are a well-established 3-D in vitro culture model for experimental therapy testing, we intend to adapt our Spheroid-Based Drug Screen to a new Cetuximab-based radiotherapeutic approach. This, however, requires knowledge about the EGFR expression in the chosen 3-D model, its sensitivity to external irradiation as well as on the penetration kinetics and effect of the antibody alone. Various human squamous cell carcinoma cell lines were tested for implementation in the Spheroid-Based Screen and FaDu spheroids were further characterized for the specific purpose.

Materials and Methods

FaDu spheroids were initiated using our standardized semi-automated setup. Spheroid treatment at a size of 370-400 μm included external single dose irradiation from 0-20 Gy and/or expose to Cetuximab at different concentrations and incubation times. After irradiation, spheroids were analyzed for spheroid integrity and volume growth using phase contrast imaging. Penetrated Cetuximab was detected in median spheroid cryosections by immunofluorescence staining. EGFR expression was examined by western blot analysis and immunostaining.

Results and Conclusions

The Spheroid-Based Drug Screen could be easily adapted to monitor irradiation efficacy in FaDu spheroids by analyzing volume growth and regrowth characteristics including the analytical endpoint “spheroid control dose”. We further demonstrate that these spheroids are well suitable to monitor the penetration and impact of the therapeutic antibody Cetuximab. The target molecule EGFR can be detected in spheroid sections in parallel to the penetrated Cetuximab. The Cetuximab penetration kinetic is both time and concentration-dependent. Only a few cell layers in the spheroid periphery have bound Cetuximab after 1 h of exposure to 10 $\mu\text{g}/\text{ml}$ antibody. Spheroids with a mean diameter of 370-400 μm are entirely penetrated within 24 h when exposed to this antibody concentration while lower concentrations of 0.1 and 1 $\mu\text{g}/\text{ml}$ Cetuximab do not result in similar uniform distributions within this time period. The expression of EGFR appears homogeneous in spheroid sections of untreated spheroids but seems to be higher in Cetuximab-penetrated areas. This tendency was revealed by western blot analyses; the phenomenon is thus under further investigation. The model is now suitable to test innovative combination treatment regimes including radionuclide-conjugated Cetuximab.

(supported by BMBF grant 03NUK006)

ABSTRACTS
(POSTER PRESENTATIONS)

**ANALYSIS OF THE REPLICATIVE AGING OF BREWING YEAST CELLS
(*SACCHAROMYCES CARLSBERGENSIS* HEBRU) AFTER FIVE
REPITCHES**

Franziska Bühligen¹, Isabelle Giemulla¹, Frank Kinnigkeit²,
André Pflüger², Hauke Harms¹, Susann Müller¹

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Brewing yeast cells are repitched several times in a brewery. So time, labour and also money could be saved. But it depends on the yeast strain and the brewery how often they could be reused for an additional fermentation before the yeast cells lose their good fermentation abilities. So the question was if the replicative age has an influence on the brewing abilities of yeast cells and whether the replicative age and so the number of bud scars on each cell accelerates with the number of fermentations.

When yeast cells were harvested after fermentation from the fermentation vessel every 10 min samples were taken and fixed with 70% ethanol. These cells were stained with Calcoflour White® and the bud scars of 100 cells per sample were counted microscopically. Size of these cells was also measured.

Even after 5 repitches more than 50% of all cells in the conus of a fermentation vessel were virgin cells with just their birth scar. Only about 15% of the cells had more than 4 bud scars. So the whole population of yeast cells after 5 repitches was still young. Replicative aging seems to have no impact on fermentation performance.

Due to the results of bud scar counting of samples from all layers of the cylindroconical fermentation vessel no advanced replicative aging could be detected after five repitches. Therefore more repitches might be possible without any difficulties encouraging the breweries to reuse the yeast cells more frequently.

THE EFFECT OF INHIBITORS OF OXIDATIVE PHOSPHORYLATION ON EUKARYOTIC CELL ORGANELLES

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Eukaryotic cells possess several organelles, e.g. mitochondria and peroxisomes. Mitochondria are DNA containing organelles (16569 bp in *Homo sapiens*). Less than 5 % of all mitochondrial proteins are encoded by the genome of these organelles, more than 95 % are encoded by the nuclear genome and have to be transported into the mitochondria. Mitochondrial DNA contains 37 genes that are important for normal mitochondrial function. Thirteen of these genes are coding for essential enzymatic subunits involved in oxidative phosphorylation. Mitochondria contain the primary energy-generating system (ATP). Furthermore they play essential roles in processes such as intermediary metabolism, proliferation, apoptosis and ageing of the cell.

Peroxisomes are vesicle-like packets bounded by a single membrane that surrounds a compartment termed the matrix. They are crucial for lipid metabolism and free radical detoxification. Peroxisomes are able to respond to environmental changes and extracellular stimuli by changing their enzyme content, morphology and abundance. Both organelles, peroxisomes and mitochondria, are metabolically linked and are cooperating and cross-talking.

We studied the influence of inhibitors of respiratory chain on the morphology of mitochondria and peroxisomes. By transient transfection with subcellular localisation vectors these organelles can be visualised by fluorescence microscopy.

INVESTIGATION OF THE CYTOTOXIC EFFECTS OF SELECTED ANTIMICROBIAL PEPTIDES ON IMMUNE CELLS

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Antimicrobial peptides (AMPs) are an essential part of the innate immune system that serves as a first line of defense against invading pathogens. Recently, immunomodulatory activities of AMPs have begun to be appreciated. Therefore, more detailed understanding of how AMPs could affect host cells is needed. We tested five analogs of AMPs of natural origin and five AMPs with modified structure and one synthetic AMP for their activity on isolated leukocytes by FACSCalibur flow cytometer. The blood was collected from healthy volunteers. Cell viability was determined by propidium iodide exclusion after different times of incubation (5, 30, 60 minutes) with AMPs at concentrations: 1; 10; 100 µg/ml. During analysis we applied gating strategy based on FSC versus SSC to select for neutrophils, monocytes and lymphocytes to investigate the cytotoxic effect of AMPs on these subpopulations. For selected AMPs we performed cytotoxicity assay continued with antibody staining (CD8 FITC, CD19 FITC, CD3 APC, CD16 PE, CD56 PE; Beckman-Coulter, CA, USA) to test for differences in cytotoxic effects of AMPs on different leukocytes subpopulations. All compounds, at concentrations, at which they indicate antimicrobial activity (~1-10 µg/ml) didn't show significant cytotoxicity. However, at a concentration 100 µg/ml five peptides showed cytotoxic effect ($p < 0.05$). One peptide from the group of AMPs of natural origin after 30-minute incubation occurred to be cytotoxic to monocytes and neutrophils. After 60-minutes it reduced also the viability of lymphocytes. Experiments with antibody-staining proved that this AMP is especially toxic to B and NK cells. In case of group of synthetic AMPs or AMPs with modified structure we observed that one peptide was especially cytotoxic to lymphocytes. After 5-minute treatment with this AMP only ~8% lymphocytes survived. Two AMPs showed cytotoxic effect only on neutrophils. Another peptide exhibited no selectivity in cytotoxic effect on leukocytes. 30-minute exposure to this peptide caused significant decrease in cell viability of all subsets of leukocytes. Our data demonstrate that some of AMPs were cytotoxic to defined subpopulations of leukocytes (e.g. towards lymphocytes, neutrophils or specific to B and NK cells) indicating selective cytotoxicity. This fact implies usefulness of AMPs in new applications where depletion of certain cell types is needed. Furthermore, it was proved that many endogenous AMPs influence cell-mediated immunity by stimulation of chemokine production and enhance leukocyte recruitment. Therefore, screening for similar properties of exogenous AMPs remains a crucial aspect.

THE GLYCOGEN CONTENT IN HEPATOCYTES AND THEIR SIZE IN NORMAL AND CIRRHOTIC RAT LIVER

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It was shown that the glycogen content in hepatocytes of cirrhotic liver rises 2-4-times. The glycogen content in hepatocytes are determined, to a great extent, by the position of the liver lobule, degree of the ploidy, and also the phase of the cell cycle in which the cells reside. The effect of the hepatocytes size on the glycogen content in is not clear.

The aim of the present work was to study the interconnection between the glycogen content in hepatocytes and their size in normal and cirrhotic rat liver in different nutritional status.

The smears of isolated hepatocytes were obtained from normal and cirrhotic liver of 48-hours fasted rats and after 10 and 60 minutes after glucose administration to fasting animals. The cytophotometric method allowing the determination of the glycogen content and DNA in the same cell and also the measurement of the cell dry mass have been employed.

The dry mass of the hepatocytes was measured using a MBIN-4 (LOMO, St. Petersburg) interference microscope. The glycogen and DNA contents were measured using a cytofluorimeter LUMAM IUF-3 (LOMO, St. Petersburg).

The dry mass of a hepatocyte of the normal liver makes, on average, 685 ± 2 pg, meanwhile in cirrhosis that mass increases by 39.9% ($P < 0.01$). The mean ploidy of the hepatocytes in cirrhosis made 5.06c what is by 6.53% more than in norm ($P < 0.05$). In norm the variation coefficient of the 4c-hepatocytes dry mass has made 29.4%, meanwhile in cirrhosis it has made 35.5% ($P < 0.001$). The variation coefficient of glycogen in the normal and cirrhotic liver has made 34.7% and 45.8% respectively ($P < 0.001$). The clear correlation in normal liver between the hepatocytes size and the glycogen content in them is observed in every class of the cell ploidy in fasting ($r = 0.30 - 0.86$) as well in 10 ($r = 0.33 - 0.77$) and 60 minutes ($r = 0.42 - 0.70$) after the glucose load. In the cirrhotic liver similar relationship is absent.

Thus: the dry mass and glycogen content in hepatocytes of cirrhotic liver are proportional of ploidy level; the glycogen content of the normal liver hepatocytes depends on their size; the absence a similar correlation in cirrhosis it has testified about increase of the cell microenvironment heterogeneity and disturbance of the organization of the glycogen metabolism in pathology.

The work was supported by Russian Foundation for Basic Research (RFBR No. 08-04-00971-a).

CELLULAR EFFECTS OF TiO₂ AND OF FERROMAGNETIC NANOPARTICLES ON HUMAN MONO MAC 6 MONOCYTES AND MOUSE J774A.1 MACROPHAGES

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Magnetic nanoparticles are widely explored because of their superparamagnetic properties and they have numerous applications in biomedicine such as in hyperthermia, magnetic resonance imaging, drug delivery and cell separation (Wang et al, 2009; Pankhurst et al., 2003). They adjust their magnetization to the changes of the external field orientation by physical rotation. To understand their complexity and to improve their application we need to understand not only their physical behavior (particles relaxation phenomena) but also their cellular effects. The aim of the present study was to investigate and compare the cellular effects of TiO₂ nanoparticles (20 nm Ø) and of dextran-coated ferromagnetic nanoparticles (Fe₂O₃, 50 and 100 nm Ø, Micromod Partikeltechnologie GmbH, Rostock). Methods and experimental design: Human Mono Mac 6 and mouse J774A.1 immune-relevant cells were exposed to nanoparticles (0.5, 5, 10, 40, 60, 120 µg/cm²) or co-exposed with chemicals used as positive controls for 45 min or 24h. Cellular distribution and nanoparticle (NP) uptake was analysed by transmission (TEM) and raster electron microscopy (REM). Measurement of reactive oxygen species (ROS) species was carried out by flow cytometry using the fluorescent dyes dihydrorhodamine 123 and dichlorodihydrofluorescein diacetate. Cellular growth was monitored by cell counting and cellular viability was determined by Trypan Blue exclusion assay. For a better understanding of the heat transfer between the suspension of the nanoparticles and the cancer tissue we employ full 3D electromagnetic simulations using finite integration technique. The magnetic properties of the nanoparticles were numerical studied with the finite element method micromagnetics. Statistical differences were analysed with Student's t-test and one-way ANOVA. Results and Conclusion: TEM analysis of NP uptake showed their cytosolic and mitochondrial localisation. REM analysis showed the characteristic phagocytotic protrusions of macrophages and no NP could be detected at the cell surface. TiO₂ and PMA co-exposure of cells induced a significant increase of ROS level, whereas this was not found using the ferromagnetic nanoparticles. Mitochondrial activity, cellular growth and viability did not show any changes after NP exposure. We compared the experimental results of heating with nanoparticles and simulation results. The results of experiments and simulations were in good agreement with existing theories of the behavior of the magnetic nanoparticles in alternating magnetic field. Taken together, these results show that a short term exposure to the nanoparticles used causes a cellular uptake and a dose-dependent induction of free radicals without further effects on cellular growth, vitality and mitochondrial activity.

APTAMER BASED ANALYSIS OF INDOOR MOULDS AND THEIR ALLERGENS

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Aptamers are powerful nanotools, usable in different applications i.a. for single cell analytics. They can be used as molecular recognition elements, analogue to antibodies. Furnished with detection markers, aptamers are able to identify, characterise and quantify their targets optically or chemically. But they are also usable in label-free measurement systems. Accordingly, new detection technologies based on aptamers are now under development.

Aptamers are single stranded DNA (ssDNA) or RNA oligonucleotides with very high affinity to their target. The highly selective and specific binding abilities result from their specific three-dimensional shape. They can be generated in an in vitro selection process (SELEX – Systematic Evolution of Ligands by EXponential enrichment) without the need to use animals or cell cultures. Once selected, aptamers are producible by chemical synthesis at any time, in high amounts and reproducibility. Aptamers can be developed for a wide variety of targets ranging from small organic molecules to proteins to even whole cells.

Here we describe the selection of ssDNA aptamers for compounds of the soluble extract of mould spores. Applied in various measurement systems, the selected aptamers have the potential to detect invisible indoor mould contaminations.

Beside acarians, moulds represent the most important indoor allergens. Numerous studies document the relationship between respiratory symptoms respectively allergies and the presence of mould infestation.

The mould specific aptamers were developed in a FluMag-SELEX process, a modified variant of the established SELEX method. After eight rounds of selection, we obtained an aptamer pool which shows a relative specificity to *Penicillium expansum* spores, the selection target. The analysis of the enriched oligonucleotide pool revealed seven aptamer groups that bind to mould contents. We ascertained different binding characteristics of these groups to different mould species. Thereby, a differentiation between various fungi species via pattern recognition can be expected by using these aptamers.

Additionally we were able to show the ability of two groups of the selected aptamers to bind directly to an allergenic component of moulds. This offers further possibilities for applications in diagnostic platforms.

The binding suitability of the selected oligonucleotides was tested using an ELONA array (Enzyme Linked Oligonucleotide Assay), an aptamer based form of the ELISA technique.

The presented aptamers are good examples to show the feasibility to develop such nucleotide binders also to other targets for their use in clinical diagnostics, in environmental analytics, for medical or other applications, and for the development of nano-structured sensor surfaces and measuring tools.

ARE BACILLUS SPORES AND LATEX BEADS SUITABLE TRACERS FOR STUDIES ON RETENTION TIME IN BIOGAS PLANTS ?

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Spores of *Bacillus atrophaeus* are used as biological tracers for the analysis of the retention time properties in biogas plants. Conventionally, the qualitative detection of the spores in an environmental sample is done on nutrient agar. The quantification of the spores via this conventional method can be error-prone. Therefore, the suitability of flow cytometry as an alternative method for spore detection in a biogas fermenter was verified. Spores were mixed with different concentrations of an anaerobic digestion substrate (stillage) and detected by flow cytometry. The weak UV-induced autofluorescence of the spores was found unsuitable to serve as a unique marker in a substrate with a high diversity of autofluorescent events. As a result, spores of *B. atrophaeus* were reliably detected only at a concentration above 10^9 per ml in the stillage. To improve the detection of tracer particles, green fluorescent latex particles were also tested as tracer and followed using flow cytometry. The latex beads were accurately differentiated from other events in the stillage at a concentration of 10^8 per ml. To summarize, flow cytometry does not serve as an appropriate tool to detect *B. atrophaeus* at lower concentrations in environmental samples. In contrast, the beads are a tracer whose particle size is comparable to that of microorganisms. Regarding its suitability and practical application in biogas plants, additional studies are needed.

**CHARACTERIZATION OF HUMAN BLOOD CELLS BY ARESKA,
ANGULAR RESOLVED DETECTION OF LIGHT SCATTER**

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Denny Ragusch, Andreas Kummrow, Jörg Neukammer

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Measurement of scattered light provides an access for a wide range of parameters characterizing single cells. The intensity and angular distribution of light is influenced by the particle's shape, volume and optical properties, by sub-cellular components (e.g. granularity) as well as rheological parameters like cell deformation by hydrodynamic focusing. Light scattering is routinely used in flow cytometry, generally for preselecting cell subpopulations in combination with specific fluorescent labelling. For this application, in routine flow cytometers light scattering intensities are detected, integrated over the respective solid angle for forward and orthogonal (at 90° to the incident laser beam) light scatter.

To significantly extend the application of light scatter for cell characterisation, we incorporated cameras in a flow cytometer to allow simultaneous detection of angular distributions in forward and orthogonal direction. To derive specific parameters of individual cells and to improve cell classification, image analysis and pattern recognition is being applied.

A flow cytometer developed in our laboratory [1] was complemented by two CCD-camera systems detecting forward and sideward scattered light at a wavelength of 488 nm with angular resolution of approximately 0.5°. The opening angle amount to 35° and 54°, derived from the numerical apertures of NA=0.4 and NA=0.6 of the microscope objectives used. An image acquisition system was employed to record the patterns of light scattered by single cells synchronously to their integrated light scatter at the wavelengths of 413 nm and 640 nm. Besides angular distributions of light the camera system was also used for direct imaging of single cells. The experimental setup will be presented in detail and the first results obtained on human blood cells will be discussed.

[1] V. Ost, J. Neukammer, and H. Rinneberg, *Cytometry* 32, 191–197, 1998.

PRINCIPLE COMPONENT ANALYSIS (PCA) -BASED ANALYSIS OF DISCONTINUOUS EMISSION SPECTRA IN MULTICHROMATIC FLOW CYTOMETRY: LIFT OFF IN HIGHER-DIMENSIONAL DATA SPACES

Toralf Kaiser,

Jenny Kirsch, Ursula Schulte-Wrede, Andreas Grützkau
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During the last decade, flow cytometry has been rapidly developed from a three color- to a multichromatic-based technology. Actually, cytometers have been launched that are equipped with up to 7 lasers and 30 fluorescence detectors accompanied by a fast digital data processing system. Independently from these impressive hardware developments, the detection of fluorochromes is still based and therefore, restricted by the need of optical bandpass filters collecting only a small, but characteristic part of a particular emission spectrum. As a consequence, limitations arise in the sensitivity and resolution of partially overlapping fluorescence signals. Moreover, analysis of those high-dimensional data sets, especially in a non-hypothesis driven manner, will be a challenge of future software developments.

In this study, we introduce an experimental strategy, in which discontinuous emission spectra will be acquired and analysed by principal component analysis (PCA). In a proof-of-principle study we could show that the simultaneous detection of up to 4 fluorochromes, which were all excited by a 488 nm Argon-laser could be resolved by the detection of a discontinuous emission spectrum divided in 6 commensurate intervals that covered a wavelength range from 500 – 750 nm. We stained whole blood samples for lineage-specific antigens and analyzed the data without color compensation by PCA (Unscrambler software package) that allowed an automated and unbiased detection and quantification of leukocyte populations. A precise immunophenotypic description of cell populations identified by PCA was achieved by comparative data sets generated as FMO (fluorescence minus one) controls.

Altogether this study demonstrates for the first time that the alternate way of fluorescence detection described in this study offers new possibilities in combining fluorochromes with similar emission spectra and that these multi-dimensional data sets can be successfully dissected and displayed by PCA. For a complete unsupervised analysis of multichromatic flow cytometry data, additional tools, such as the Probability Binning algorithm have to be implemented, which finally will help to exploit new dimensions in multiparametric flow cytometry.

IMPEDANCE CYTOMETRY FOR LABEL-FREE CELL IDENTIFICATION AND CELL QUALITY CONTROL FOR REGENERATIVE MEDICINE

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Identification and profound characterization of cells is a necessity for their isolation and application in cell therapy for regenerative medicine. Present approaches for isolation of stem and progenitor cells rely on specific labelling, analysis by flow cytometry (FCM) and cell sorting. However, labelling may change the cells' characteristics or even induce genetic modifications, depending on the dyes used. Impedance flow cytometry (IFC) may be a promising label-free alternative to FCM. We tested if cell physiological changes can be monitored by IFC (Leister/Axetris) as a new label-free way to identify and finally isolate cells of interest.

IFC measures single cells at various frequencies simultaneously, which range from 0.1 to 20 MHz. Cells should be smaller than 40 µm in diameter. The impedance signal provides information about cell volume (<1 MHz), membrane capacitance (~1-4 MHz) and cytoplasmic conductivity (4-10 MHz), parameters directly related to the physiological conditions of single cells.

Hybridoma cells were incubated at various densities to induce cell death. Samples were analyzed by IFC at various frequencies to detect the most discriminative frequencies telling dead from alive. Buffers of different ionic strength were used to improve discrimination between dead and alive cells. FCM was used as gold standard after 7-AAD (necrosis) and AnnexinV (apoptosis) staining.

Genes@Work cluster analysis revealed parameters showing maximal discrimination between viable and dead cells. Angle shift for the highest frequencies turned out to be most discriminative. Cell death estimation was in agreement with results obtained by FCM.

In addition, neurospheres obtained from postnatal gut of transgenic Wnt1-YFP mice (reporter mouse strain labelling neural crest-derived cells including all cells of the enteric nervous system) were isolated, in vitro propagated and then upon cell dispersion analyzed by IFC. As control Wnt1-YFP sorted cells were taken. Percentage of YFP-positive cells was estimated by FCM and this was used as reference for IFC measurements. The results showed the ability to detect two distinct populations present within neurospheres namely – non-neural cells including smooth muscle-like cells and cells of neural origin. The data correlated well with FCM results.

IFC could become a valuable alternative to conventional FCM. In combination with cell sorting IFC may gain additional relevance in label-free stem cell isolation for therapeutic use. IFC could be applied in the future for quality control of precious samples such as isolated stem cells and biomedical cell analysis for regenerative therapy.

ANTI-TNF-ALPHA THERAPY MONITORING IN CHRONIC RHEUMATIC DISEASES: GLOBAL CYTOMETRIC PROFILING OF HUNDREDS OF IMMUNOPHENOTYPICAL PARAMETERS IN PERIPHERAL BLOOD CELLS

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Ankylosing spondylitis (AS) is a chronic inflammatory disorder mainly affecting the axial skeleton and belongs to a group of rheumatic diseases known as spondyloarthritides. Anti-TNF-alpha (tumor necrosis factor) biologicals, which aim to block the disease-associated pro-inflammatory activity of this cytokine, have been successfully applied for treatment of rheumatoid arthritis (RA) and AS as well. Unfortunately, only a subgroup of around 60% of patients is successfully responding to this treatment and up to now, there are no useful biomarkers available for predicting a beneficial clinical response to the cost-intensive TNF-alpha therapies. The aim of the present study was to monitor treatment of AS patients over time with a multiparametric approach, called cytometric profiling, which allows immunoscopying of hundreds of immunophenotypic parameters at the single cell level. 50 different monoclonal antibodies were combined to ten different staining cocktails, which allowed the detection of all major leukocyte populations and their activation state in a few millilitres of peripheral blood. Protocols applied include seven color stainings that will allow the detection of up to 12 parameters. This strategy combines the generation of hypothesis-based and hypothesis-generating knowledge. The huge amount of data generated per measurement made it necessary to develop appropriate bioinformatic solutions for data storage, data normalization and retrieval of differentially expressed parameters.

Here we present first data generated in a cohort of 10 AS patients 4, 8 and 12 weeks after starting treatment. Patterns of immunophenotypic parameters could be determined by bioinformatic tools originally applied to array-based transcriptome studies. Altogether, this study demonstrates the value of our cytometric profiling approach to reveal patterns of parameters that generally possess higher predictive and diagnostic power than solitary ones. Moreover, this approach will help to disclose hitherto unknown disease-associated parameters, which may be helpful to identify new pathophysiology- and therapy-related mechanisms.

EFFECTS OF 50 HZ EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMF) ON THE REDOX STATUS OF HUMAN MONO MAC 6 AND K562 CELLS

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Introduction

Previous work by our group showed that exposure to 50 Hz, 1.0 mT extremely low-frequency magnetic fields (ELF-MF) for a few hours induces an increase in free radical levels (Simkó, et al., 2001, 2007; Frahm et al., 2006, 2009; Lupke et al., 2004). In this study the effects of low flux density ELF EMF (200 μ T) on the redox status were evaluated. The explored parameters were levels of free radicals (reactive oxygen species (ROS) and superoxide radical anion), level of glutathione and of superoxide dismutase and mitochondrial dehydrogenase activity.

Methods and experimental design

Human Mono Mac 6 and K562 immune relevant cells were cultured at 37 C with 5 % CO₂ in air and seeded out 24 h prior to exposure. Cells were exposed within a double Helmholtz coil located in a CO₂-incubator (Mannerling et al., 2007) to horizontal 200 μ T (50 Hz) ELF EMF for 45 min or 12 h. All experiments were performed using both incubator and sham exposure as controls. The background flux densities in each incubator were 1-6 μ T. For comparison, cells were treated with different chemicals used as positive controls or in co-exposures with ELF MF. Measurement of ROS was carried out by flow cytometry and Live Cell Imaging immediately after 45 min exposure of cells using the fluorescent dye dihydrorhodamine 123. The detection of superoxide anion, glutathione level, superoxide dismutase and mitochondrial dehydrogenase activity were measured by photometry.

Results and Conclusion

After 45 min and 12 h MF exposure changes of redox status were detected. Mono Mac 6 cells showed increased superoxide anion and ROS levels, whereas this was not found in K562 cells. Mitochondrial activity was significantly increased in both cell lines after 12 h exposure. Mono Mac 6 cells showed no effect on glutathione level and we noted an increase in superoxide dismutase activity post ELF-EMF exposure. Regarding K562 cells no changes were found in glutathione levels or activity of superoxide dismutase.

This study shows that exposure to 50 Hz, 200 μ T ELF MF for 45 min or 12 h induces changes in the redox status such as increase of free radicals levels and mitochondrial dehydrogenase and superoxide dismutase activity. Therefore we conclude that ELF EMF can influence the redox status of immune relevant cells. It appears that at the time points studied, the cellular enzymatic and non-enzymatic antioxidant systems could not compensate MF induced increase of free radical levels.

TREG FREQUENCIES IN CORD BLOOD PREDICT THE DEVELOPMENT OF ALLERGIC SENSITISATION DURING THE FIRST YEAR OF LIFE

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Background : Within a prospective birth-cohort study we previously demonstrated that reduced numbers of CD4+CD25high regulatory T cells (Treg) in maternal blood during pregnancy are related to elevated cord blood IgE levels in the offspring. The aim of the present follow-up analysis was to assess the relevance of Treg frequencies in cord blood for the development of allergic sensitisations during the first year of age.

Methods : Within the mother-child study LINA (Lifestyle and Environmental factors and their Influence on Newborns Allergy risk) we determined the frequency and function of Treg and the concentration of total and specific IgE (food mix; Fx5) antibodies in children's blood at birth and first year of age (n = 23) as well as in maternal blood samples during and one year after pregnancy. Furthermore, we assessed the concentration of mitogen-induced Th1/Th2 and inflammatory cytokines in children's blood at age one.

Results : Reduced numbers of CD4+CD25high T cells in cord blood were found to be associated with the occurrence of food allergen specific IgE antibodies at age one (r = -0.43, P < 0.03). No relation was observable between Treg frequencies in cord blood and in blood samples at age one as well as between Treg numbers in maternal blood during pregnancy and one year after pregnancy. However, at age one (as already observed at birth), Treg frequencies in maternal and childrens' blood were significantly correlated (r = 0,49, P = 0.008). Cord blood IgE levels were associated to high levels of total IgE in blood samples of one year old children. Furthermore, Treg numbers were also associated with Th2 cytokine blood concentrations (IL-4, IL-13 and IL-10) at the age of one.

Conclusions : Our findings suggest that Treg numbers at birth may have a predictive value for atopic sensitisation later in life. The strong association between maternal and childrens' Treg numbers possibly originate from the genetic background or shared lifestyle and environmental factors.

COMBINATION OF MICROBIAL CYTOMICS AND PROTEOMICS APPROACHES FOR STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF BACTERIAL COMMUNITIES

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Knowledge of the structure and function of microbial communities is needed to improve our understanding of physiology and ecology. The dynamic in microbial communities in terms of the relative abundances of strains, migration, and the heterogeneity of microbial cell states presents a key challenge in cytomics and proteomics studies. Thus, the precise quantification of bacterial cellular constituents like the DNA contents performed by flow cytometrics with subsequent cell sorting of subpopulations in combination with proteomics, provide accurate data for comprehensive functional proteomic analysis. Since large amount of microbial cells were needed, due to their small size and low protein amount, in this study, we applied a newly developed filter system workflow with a high recovery rate of proteins without the need of centrifugation steps after cell sorting. We therefore applied an artificial community consisting of *E. coli* K-12 and *P. putida* KT2440 and identified in total 903 for *E. coli* K-12 and 867 for *P. putida* KT2440 mostly cytoplasmic proteins after cell sorting and LTQ-FT-ICR mass spectrometry from 5×10^6 cells of each. The proteome coverages of identified proteins were approx. 30-40% of the predicted number accessible by mass spectrometry. The high proteome coverage after elaborated wash, transfer and digest procedures shows the usefulness of the developed protocol. Furthermore, the bias of the M_r , the pI and the hydrophobicity was further investigated. As a result, the data demonstrated that the influence of cell sorting by flow cytometry is marginal and in combination with consecutive proteomic investigations and this workflow was useful for comprehensive analysis of all aspects of physiology.

COMPARATIVE IN VIVO PROTEOME ANALYSIS OF THE PROTEIN PROFILE OF INTERNALIZED STAPHYLOCOCCUS AUREUS RN1HG AND AN ISOGENIC SIGB MUTANT

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Staphylococcus aureus the cause of a wide spectrum of severe community-acquired and nosocomial infections is traditionally not considered as an intracellular pathogen. Even if there is evidence that *S. aureus* can be internalized and persist in non-professional phagocytic cells *in vitro*, little is known about the adaptation of *S. aureus* to internalization on the proteome level. However, we were recently able to analyze internalized *S. aureus* RN1HG wild type cells with our newly developed workflow, which combines pulse-chase labeling with cell sorting and sensitive gel-free proteomics.

The alternative sigma factor SigB regulates expression of a large set of *S. aureus* genes including the virulence regulator *agr*. However, its precise involvement in pathogenicity of *S. aureus* is still a matter of debate. Therefore, we started to comparatively explore the adaptational response of *S. aureus* RN1HG and its isogenic *sigB* mutant (Δ *rsbu*, *rsbv*, *rsbw*, *sigB*) to internalization by S9 human bronchial epithelial cells. *S. aureus* cells carrying plasmid pMV158GFP with a *mobM-gfp* reporter gene fusion were labeled with heavy amino acids using the SILAC method (stable isotope labeling by amino acids in cell culture) to saturation and then co-cultivated with S9 cells allowing internalization into S9 cells. Due to the continuous GFP expression by *S. aureus* cells, bacteria could be enriched by fluorescence assisted cell sorting (FACS) at different time points up to 6.5 hours post internalization. Peptides generated by tryptic digestion of *S. aureus* cells were measured by ESI-LC-MS/MS. With about $1-3 \cdot 10^6$ cells we were able to identify and quantify between 300 and 600 *S. aureus* proteins and comparatively profile differences in the adaptation of the *sigB* mutant and the wild type strain to internalization.

QUANTITATIVE IN-VIVO, EX-VIVO, AND IN-VITRO CELL ANALYSES

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Characterization of cells for clinical purposes such as determination of differential blood count is common standard. For some time now, harvested stem cells from different sources are used for cell therapy. But how pure are they or do they need to be respectively? What cell types at which differentiation status are they actually and what functions do they exhibit? This information is important to have and might be inevitable with upcoming regulations in cell therapy. Beside viability tests, cells' identity has to be checked in order to meet criteria for medicinal products (e.g. EMEA/CHMP/410869/2006). Detailed cell characterization can be done with different technologies. Flow cytometry (FCM) is well known, can be highly multiplexed and is able to analyze thousands to millions of cells in a short period of time. This technology however, is restricted to cells in suspension. For the analysis of cells in culture or tissue sections Image cytometry is more appropriate. It generates the same quantitative data as FCM and is capable of multiplexing as well. With both methods cells can be investigated on a single cell level. Subsequent in-vivo imaging allows for identification of cell clusters in live animals. Administered cells can be monitored over time enabling investigation of biodistribution, retention time, and biological effects. Another, relatively new technology is impedance flow cytometry (IFC). While most cell analysis techniques rely on fluorescence detection, IFC do not need any label at all. Dielectric properties such as membrane capacitance and cytoplasm conductivity are analyzed and reflect membrane morphology and cell function, which in turn correlate with physiological differences between cells or pathological changes in cells over time. Irrespective of the used technology, detailed cell characterization is of particular importance for cell therapy.

BIOMECHANICAL ANALYSIS OF PRIMARY BREAST CANCER CELLS

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Malignant tumors are not aggregations of homogeneous cells but rather complex formations of diverse cell types and pathological cells in different stages of aggressiveness. Recent investigations show that the biomechanical properties of benign cells differ from those of cancerous and metastatic cells. In order to characterise the biomechanical properties, selected regions of similar cellular composition of primary mamma carcinoma are used for measurements. Seven tissue samples, obtained from the pathology Niendorf-Hamper, Hamburg, were used for the measurements and compared to non-malignant primary breast epithelia cells. Single cells obtained by enzymatic dissociation of the homogeneous sample are analysed using the Optical Stretcher, a two beam laser trap enabling contact-free, whole cell elasticity measurements. Fluorescence imaging was used to demonstrate that cultured samples were not contaminated by fibroblasts. Differences in deformation at low optical forces were observed. At higher forces, these differences vanished and cells showed a uniform behavior suggesting that a different system of the cytoskeleton is tested at higher applied optical forces.

ANALYSIS OF PROLIFERATION PATTERNS OF THREE MARINE FLAVOBACTERIA OF THE GENUS MURICAUDA

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„A bacterial cell grows and separates into two equal daughter cells“ (Madigan/Martinko 2006). That the bacterial cell cycle is not just that easy as described above, was shown by studying the proliferation patterns of a marine *Flavobacterium* of the genus *Muricauda*. In detail the cell cycle of three *Muricauda* species was analysed via flowcytometry in combination with light-microscopy: *M. ruestringensis* B1T (DSM 13258) - isolated from the German North Sea - *M. aquimarina* (KCCM 41646) and *M. flavescens* (KCCM 41645) – isolates from Korean East Sea - were used.

All three organisms are pleomorph. At different growth-stages seven morphological subtypes could be identified by microscopical studies. The distribution of these subtypes during a 72 hour incubation at room temperature in complex- und minimal-media was estimated. Samples taken at different times of the cultivation and fixed by sodium azide were analysed not only by microscopy, but also the proliferation patterns were measured with the use of flowcytometry: Under nutrient-rich conditions rod-shaped cells were predominantly found [$> 60\%$ of all morphological subtypes], showing symmetric cell division. Under limited nutrient conditions the rod-shaped cells produced rounded appendages by asymmetric cell division. With a distribution of $> 70\%$ spherical cells built the main population reaching death-phase and while longterm storage at 4°C , indicating a temperature and starvation resistance.

Flowcytometry on the other hand revealed at least 5 subgroups: cells with C1n, C2n, C3n, C4n and $> C4n$; supporting the theory of a combined symmetrical and asymmetrical cell cycle. Subsequent cellsorting had verified the previous results. Proteomic analysis of the sorted cells will be investigated in detail in future studies.

DETECTION AND TRACKING OF VITAL BACTERIAL CELLS IN COMPLEX ENVIRONMENTS USING FLUORESCENT DYES AND THE CYTOSENSE FLOW CYTOMETER

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Bacterial strains that play key roles during the degradation of contaminants in wastewater treatment processes are of special interest. A promising approach is the bioaugmentation of vital key role-bacteria to verify their influence on biodegradation. The tracking of them within natural microbial communities as can be found in tannery wastewater of Modjo river in Ethiopia through flow cytometry can give important information about their abundance and population dynamics in these complex systems. Through marking the cells they can be quantitative and qualitative tracked only, if a specific (and expensive) labeling procedure is available. Commercial DNA/RNA dyes, however, seem to be well suited due to the fact that these dyes incorporate in the nucleic acids of the vital cells while the metabolic activities of the cells keep unaffected and should still proceed. They are also relatively cheap and easy to handle in their application. For our approach high fluorescence intensity of targeted cells, distinct clustering and a high stability of the staining was aimed for following bacterial cells over short time periods (few hours up to several days). On the basis of these assumptions several experiments were undertaken in flask-scale with *E.coli* K12 as a substitute strain and the fluorescent dyes SYTO 9, SYTO 16, SYBR®Green I (all Invitrogen Inc.). Exceptional challenges in handling the fluorescent dyes and measuring the labeled bacterial cells with the CytoSense flow cytometer are discussed. All investigated fluorescent dyes showed good results for the detection of fixed bacteria cells with CytoSense while only SYTO 9 was found to stain vital cells with sufficient high fluorescence intensity. Low amounts of augmented cells could than be detected in the natural community by the CytoSense. However, the dye, originally bounded to the substitute cells or unbounded excess dye, leaked also into those of the natural community. As a result different cell types were labeled after a short time period. Washing steps after staining significantly decreased the fluorescence intensity. Stained and washed vital bacteria cells lost their fluorescence rapidly within 2-4 h after transferring them into synthetic wastewater media or complex tannery wastewater from Modjo river, Ethiopia. Therefore, the tracking of stained *E. coli* cells over a long period and the determination of their increase in cell number or probably cell multiplication was not possible in this way. Other techniques must be developed if stability of bioaugmentation will be ensured and controlled.

THE CORE UNIT FLUORESCENCE-TECHNOLOGIES IN THE IZKF LEIPZIG

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The Core Unit Fluorescence-Technologies in the Interdisciplinary Centre for Clinical Research (IZKF) Leipzig provides access to high quality, state of the art flow and slide-based cytometry and high-speed cell sorting as well as the scientific expertise necessary to effectively integrate this technologies into research projects. The Unit is open to all scientists from the Faculty of Medicine and other faculties of the University of Leipzig as well as to external researchers from other institutions.

Currently the Core Unit houses a BD LSR II digital benchtop analyser, a Laser Scanning Cytometer and a FACSVantage SE high-speed cell sorter.

The analytical flow cytometer is equipped with four lasers (355 nm, 405 nm, 488 nm, and 633 nm) and up to 12 parameters (10 fluorescence and 2 scatter parameters, upgradable) can be measured at the same time.

The sorter has three lasers for the excitation (ML UV, 488 nm, 633 nm) and up to 8 parameters are measurable simultaneously. With a special soft- and hardware it is possible to deposit a predefined number of cells onto slides, filters or in individual wells of microtiter plates.

One of the major missions of the Core Unit is to train investigators, their students and staff to use analytical flow cytometry to its fullest advantage in their research. Rigorous quality control is performed daily on this instruments and users are provided with validate settings for common fluorochromes used in multiparameter cytometry. The high-speed cell sorter FACSVantage SE is solely staff operated to provide high-quality viable cell sorting experiments for all researchers.

Another mission is the further training of graduate students, postdoctoral fellows, residents and other colleagues. So workshops, hands-on seminars and user seminars are offered.

The goal of the facility is to introduce cytometric methods into new research areas while supporting and extending current research.

IMPACT OF DIFFERENT OPTICAL FILTER SETS AND QUALITY CONTROL BEADS ON MULTI-LASER FLOW CYTOMETER PERFORMANCE

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The use of optical filters and quality control beads are essential in flow cytometry. We show that the use of different filter sets in multi-laser flow cytometers for detecting APC (670/14), PE (586/15) and FITC/AmCyan (532nm notch) provide higher resolution and more distinct separation of populations as seen using stained cells and control beads. Also, we show that the use of BD Calibrite beads is comparable to and may even afford more benefits over using 8-peak beads in visualizing individual laser performance. The use of the new filter sets together with the Calibrite beads facilitates a faster, reliable and more intuitive optimization and evaluation of multi-color flow cytometers.

HER4 - A DIAGNOSTIC MARKER AND THERAPEUTIC TARGET IN BREAST CANCER?

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Triple-negative breast cancer (TNBC) is characterized by the absence of expression of human epithelial growth factor receptor 2 (Her2) as well as estrogen receptor (ER) and progesterone receptor. Thus lacking the classical proteins for target specific therapy the only therapeutic option for this aggressive tumor phenotype in the adjuvant or metastatic setting remains surgery, radiation- and chemotherapy which gains only partial response.

In previous data we showed for a collective of Herceptin (Trastuzumab) treated Her2 overexpressing breast cancer patients that both Her4 expression and Her4 gene amplification emerged as independent prognostic markers. Responsiveness to Herceptin turned out to be more efficient if tumor cells coexpress Her4 (1). This remarkable impact of Her4 in this collective of patients suggests the deduction of its potential as a new diagnostic marker and therapeutic target not only in Her2 overexpressing breast cancer but also in TNBC.

Using qPCR we retrospectively investigated primary breast cancer tissues from TNBC and Herceptin treated patients for expression of different Her4 splice variants. In addition we analyzed cell proliferation and apoptosis of breast cancer cell lines after treatment with Her4 targeting antibody Ab1479 (2) and combined it with Herceptin treatment in breast cancer cells with Her2 overexpression. Preliminary data show that Her4 is expressed in a subgroup of TNBC, however only the Her4 JM-a splice variant and not Her4 JM-b is present in tumor samples. Complementing functional studies will elucidate the special role of Her4 in the context of TNBC and Her2 overexpression and will advance the design of highly efficient and individualized receptor targeting.

(1) Sassen A, Diermeier-Daucher S, Sieben M, Ortmann O, Hofstaedter F, Schwarz S, Brockhoff G. Presence of HER4 associates with increased sensitivity to Herceptin in patients with metastatic breast cancer. *Breast Cancer Res.* 2009;11(4):R50.

(2) Hollmén M, Määttä JA, Bald L, Sliwkowski MX, Elenius K. Suppression of breast cancer cell growth by a monoclonal antibody targeting cleavable ErbB4 isoforms. *Oncogene.* 2009;28(10):1309-19.

INFLUENCE OF PHYTOHORMONES ON THE PLOIDY LEVELS OF *SALVIA OFFICINALIS* CALLUS CULTURES

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Salvia sp. is a producer of substances with interesting biological activities like ursolic acid and oleanolic acid. Due to their hepatoprotective, anti-inflammatory, antihyperlipidemic, natural anti-aging effects and cancer chemo-preventative functions these substances are of significant interest in the pharmaceutical and cosmetic industries.

In vitro plant cell cultures are suitable systems for the production of these secondary metabolites, since they allow the cultivation under defined conditions guaranteeing high and reproducible product quality. Here especially calli are in the focus of research as they can be cultivated in conventional bioreactors up to industrial scale.

In order to design an efficient production process the optimization of both the media composition and the culture is mandatory. To maintain calli in an undifferentiated status it is necessary to add phytohormones to the media. It is known that the composition and concentrations of these hormones influences the ploidy of the cells. Furthermore the ploidy of the culture affects the profile and concentration of the produced secondary metabolites. Therefore the combination and concentration of phytohormones in the media is an important parameter for the optimization of the production process.

Calli from *Salvia officinalis* were induced and subcultivated using 29 different combinations and concentrations of hormones (6-Benzylaminopurine; 2,4-Dichlorophenoxyacetic acid; Kinetin; 1-Naphthaleneacetic acid; Picloram; Zeatin) under identical conditions to assess the influence of these hormones on the ploidy. Subsequently the DNA contents of the resulting calli were measured by Flow Cytometry. It could be shown that the phytohormones strongly influence the number of endoreduplication cycles the cells undergo. Hence cultures from the same species cultivated under the identical conditions varying only in the phytohormones may have just 2 Cn nuclei or possess nuclei with ploidy levels ranging from 2 Cn up to 16 Cn.

FLOW CYTOMETRIC ANALYSIS OF *STAPHYLOCOCCUS AUREUS* IN A MEDICALLY RELEVANT MIXED CULTURE

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Lectins bind specifically to glycan structures and can therefore be applied as fluorescent conjugates for detection of bacterial species in mixed cultures by flow cytometry. In this work an assay using fluorescently labelled wheat germ agglutinin (WGA) was successfully established to detect *Staphylococcus aureus* in mixed cultures relevant to cystic fibrosis pulmonary infection. WGA binds to N-acetylglucosamine and N-acetylneuraminic acid residues in the peptidoglycan layer of the cell wall of gram positive bacteria. In contrast WGA does not bind to gram negative bacteria since the peptidoglycan layer is covered by an outer membrane.

Here, WGA was used to differentiate *S. aureus* from *Burkholderia cepacia* and *Pseudomonas aeruginosa*, respectively. In comparison to Holm et al. [1] no KCL had to be added to the staining buffer to achieve a distinct positive fluorescence signal, which could be discriminated clearly from the background as well as both unstained, gram negative bacteria. In addition, samples were stained with 4',6-Diamidin-2-phenylindol (DAPI) to detect populations of *B. cepacia* and *P. aeruginosa*.

For prospective studies concerning mixed culture dynamics, e.g. monitoring of the impact of antibiotics, WGA staining was combined with a membrane integrity based viability assay using SYBR® green I and propidium iodide. This protocol was applied for the analysis of *S. aureus* in pure and defined binary cultures.

Overall, WGA is a suitable specific marker for gram positive bacteria in mixed cultures. The presented WGA staining protocol can be combined with other specific fluorescent markers like monoclonal antibodies and / or fluorescence viability assays in the flow cytometric analysis of mixed cultures to resolve the dynamics of microbial communities.

[1] Holm, C. and Jespersen, L. (2003): A flow-cytometric gram-staining technique for milk associated bacteria. Applied and Environmental Microbiology, 69 (5), p. 2857-2863.

THE INVOLVEMENT OF REACTIVE OXYGEN SPECIES IN THE REGULATION OF WNT SIGNALING PATHWAYS DURING NEURAL DIFFERENTIATION

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Introduction: Dishevelled (Dvl) is a key protein involved in the activation of Wnt signaling pathways. Recently it has been shown that nucleoredoxin (NRX), a member of the thioredoxin antioxidant family, interacts with Dvl and leads to inhibition of Wnt-mediated cell proliferation. On the other hand, an increase of reactive oxygen species (ROS) level (i.e. induction of oxidative stress) stimulates the cell proliferation [Y. Funato et al, *Nature Cell Biol*, 8:501-508 (2006)]. In our study we investigated the role of ROS in NRX-Dvl interaction during the early steps of differentiation of ReNcell VM197 human neural progenitor cells.

Materials and Methods: Intracellular ROS level was evaluated using 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (Carboxy-H2DCFDA) through confocal laser scanning microscopy. Living cells were also stained with Hoechst 33258 and Nile Red for obtaining cell discrimination and cell contour, respectively. Additional experiments were performed with flow cytometry using dihydrorhodamine 123 (DHR) and Carboxy-H2DCFDA dyes. The cytosolic Ca²⁺ level was evaluated using the high-affinity fluorescent Ca²⁺ indicator Fura Red. Fluorescent images were recorded every 5 min during cell differentiation (i.e. removal of EGF and bFGF growth factors). Data were compared with those obtained after treatment with 3 mM H₂O₂ solution (i.e. induction of oxidative stress) and 30 mM caffeine (i.e. induction of Ca²⁺ release from intracellular stores) used as positive controls. Dvl-NRX co-localization was evaluated through immunostaining and confocal image analyses, and their expression was quantified through Western blotting. Furthermore, the binding complex of both proteins was studied by structural modeling of Dvl and NRX active domains by means of Brownian Dynamics Simulations.

Results: We showed that the induction of differentiation led first to an increase of the cytosolic Ca²⁺ level after 15 min, which subsequently triggered endogenous ROS production after 50 min. Interestingly these events were followed by an increase of both Dvl and NRX expression after 2h of differentiation. Furthermore, in silico experiments showed a binding ability between NRX and Dvl, and image analyses of immunolabeled cells confirmed their co-localization within the cytoplasmic area. Currently we are performing further experiments on the functional role of the ROS and NRX-Dvl interplay in Wnt-mediated cell differentiation.

GROWTH PATTERNS OF DIFFERENT PLANT IN VITRO SYSTEMS OF DEVILS CLAW IN BATCH CULTURES.

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Plant in vitro systems have the potential to synthesize a variety of useful low molecular weight molecules. Further plant cells were successfully established as expression systems for recombinant protein expression. In order to utilize the potential of plant in vitro systems it is necessary to optimize both the plant expression systems and the cultivation process.

For plant in vitro systems many different types of cultures can be obtained. Generally hairy root cultures, with their differentiated tissue, possess higher growth and genetic and biochemical stability than suspension cultures. However, suspension cultures are favoured due to the fact that they can be cultivated in established cultivation systems with defined engineering parameters.

Growth and hence the evolution of the concentration of the biomass is one of the most important parameters in process optimization. Therefore we investigated the accumulation of biomass of both hairy root and suspension cultures in batch cultures. The observation of the biomass concentration was flanked by the measurement of the cell cycle distribution (DNA content). Here it was observed that the proportion of the cells in the particular cycle phases do not exhibit major changes in the course of a batch cultivation. For hairy roots no G2/M cells were observed, probably due to large proportion of non merismatic cells in the differentiated transformed roots. For suspensions cells from all phases of the cell cycle were detected. The changes of the respective cycle phase correspond with the course of the biomass concentration. However the rather small variations of the fractions in the cycle phase during the course of the batch cultivation suggest that only a portion of the cells contribute to the increase in biomass by division. To verify this a two parametric cell cycle analysis, detecting DNA content and BrdU incorporation would be desirable.