

American Electrophoresis Society

Poster Abstracts

2007 Annual Meeting

Salt Lake City, UT

November 5-9, 2007

Abstract	Poster Title	Page
AES-1	Dynamical Analysis of Silver-Staining by <i>Y. Tatsumi, Y. Kumada & M. Kishimoto</i>	3
AES-2	Quantification And Control Of Passive Flows To Minimize Sample Losses At Channel Junctions by <i>H. Xu, J. Paschkewitz, C. Park, & R. Bharadwaj</i>	3
AES-3	Nanoparticle-Composite Gels for Protein Separation: Characterization Based on Acoustic Methods by <i>H. Sedrick, J. Bollig, N. Burns, H. Stretz, and P. Arce</i>	4
AES-4	Novel Biased Ac Electroosmosis Micropump Using Symmetrical Array By <i>N. Islam</i>	5
AES-5	Rapid Cell Rupture in A Dielectrophoretic Field by <i>K. Leonard, S. Reeves, A. Pate, S. Thompson, and A. Minerick</i>	6
AES-6	Role Of Electro-Osmosis Based Flows On The Effective Transport In A Rectangular Geometrical Electrophoretic Cell by <i>J. God, M. Oyanader, and P. Arce</i>	6
AES-7	Modeling Of High Frequency Diamond Layered Saw Devices For Potential Microfluidic Applications by <i>S. Sankaranarayanan, S. Cular and V. Bhethanabotla</i>	7
AES-8	Optimal Separation Times for Biomacromolecules in A Couette-Based/ electrophoretic Separation Device: by <i>JA Pascal, M. Oyanader, and P. Arce</i>	8
AES-9	A Simplified Mathematical Model Of An Ek Cell To Assess The Influence Of Buoyancy Driven Flows by <i>C. Torres, M. Oyanader, and P. Arce</i>	9
AES-10	Comparison Of Convective-Dispersive Aspects In Electrokinetic Cells Of Rectangular And Cylindrical Geometry by <i>A. Martinez, M. Oyanader, and P. Arce</i>	10
AES-11	Effect Of Dispersion Of Ions On The Performance Of An Electrophoretic Cell With Orthogonal Electrical Field by <i>P Vergara, M. Oyanader, and P. Arce</i>	11
AES-12	In-Line Sample Pre-Concentration Employing Octadecylsilane by <i>D. Morris & K. Sorvig</i>	11
AES-13	Identification And Antibody-Therapeutic Targeting Of Antibiotic-Resistant Outer Membrane Proteins In E. Coli by <i>H. Li, X. Lin, C. Huang, S. Wang, and X. Peng</i>	12
AES-14	Rapid Screen Of Highly Efficient Vaccine Candidates By Immuno-proteomics And Cross Immunoproteomics by <i>X. Peng and S. Wang</i>	13

AES-1 (333a on website)

Dynamical Analysis of Silver-Staining Gel for 2DE

By Yusuke Tatsumi¹, Yoichi Kumada², and Michimasa Kishimoto². (1) Kyoto institute of technology, Kyoto, Japan, (2) Department of chemistry and materials technology, Kyoto institute of technology, Sakyouku Matsugasaki Hashigamicho, Kyoto, 606-8585, Japan

Abstract: The time courses of 2DE gel images were analyzed dynamically by our new system, which consist of the intermittent scanner system and the software for the dynamic analysis of the intermittent gel images. The system can recognize protein spots more elaborately compared with the conventional image analyzing system using a single gel image because our system can recognize the overlapped protein spots by use of sequential gel images that were scanned intermittently in the color developing reaction.

Our software can adjust the gel positions on the sequential images by recognition of artificial color points that were inserted on the 2DE gel just before developing process. Furthermore, the time course of each spot image could be displayed and the detailed information were obtained in addition to the spot volume and/or location. Our noble method can analyze 2DE-gel without the harmful influence of the addition time of stopper on the measurement result, and we can detect the difference of the spot of the cancer and non-cancer tissue protein more precisely than those with the normal approach with the image of single developing time.

AES-2 (333b)

Quantification And Control Of Passive Flows To Minimize Sample Losses At Channel Junctions

By Hui Xu, John S. Paschkewitz, C. Charles Park, and Rajiv Bharadwaj. Microfluidics, Caliper Life Sciences, 605 Fairchild Drive, Mountain View, CA 94043

Abstract: In the absence of valves, sample material in microfluidic channels can be lost in channel junctions. These losses are strongly affected by “passive” flows that result from an imbalance in the Laplace pressure due to liquid surface curvature in the sample reservoirs, or wells, and hydrostatic pressure resulting from differences in fluid level between wells. We present a systematic study of the behavior of these passive flows using a model microfluidic system and then demonstrate a control strategy to minimize their impact on sample loss. Based on direct observations of liquid menisci in the wells, we found that the surface curvature of the liquid in the well was strongly affected by the contact angle hysteresis and whether or not an electrode pin was inserted. Flow measurements indicated that the passive flow has a time-dependent behavior that may be of importance in microfluidic chip operation. The passive flow effects can be controlled using a pressure compensation strategy that balances the hydrostatic and Laplace pressures. We demonstrate this concept by controlling the passive flow at a channel junction in an isotachopheresis (ITP) chip. The loss of the stacked sample band can be significantly reduced when the passive flow is largely suppressed or directed out of the channel junction and is quantified by computer simulation.

AES-3 (333e)

Nanoparticle-Composite Gels for Protein Separation: Characterization Based on Acoustic Methods

By Hope E. Sedrick¹, Jennifer R. Bollig¹, Nancy A. Burns¹, Holly A. Stretz¹, and Pedro E. Arce². (1) Department of Chemical Engineering, Tennessee Technological University, Cookeville, TN 38501, (2) Chemical Engineering, Tennessee Technological University, Department of Chemical Engineering, P.O. Box 5013, Cookeville, TN 38505

Abstract: Currently, there is an interest in novel drug delivery systems, diagnostic capabilities and improving separation of biomacromolecules such as DNA and proteins. After the successful modification of the gel morphology by using DNA, Xanthan, and SDS (1) as templating agents, electrophoresis efficiency for protein separation increases. Motivated by this success, we are currently using charged nanoparticles to modify the internal gel architecture as well as manipulate the electrostatics properties of the gels. Recently (2), the addition of nanoparticles has shown a modification of the electroosmotic flow. We believe that both the electroosmosis and the gel morphology will affect tremendously the separation efficiency. In this project, polyacrylamide gels were successfully cast and crosslinked with well dispersed, charged nanoparticles of varying diameters (Southern Clay Laponite RD and an experimental Laponite) at a concentration of approximately 1% (w/w). In this presentation, the dispersion of the nanoparticles, or filler, will be characterized by acoustical testing. Acoustical testing measures the speed of sound through a material. The methodology is based on the fact that the speed of sound through a material is related to its density and the density of the nanocomposite gels differ from the standard gels used in current electrophoresis separations. Several preliminary results based on the principle described will be presented and analyzed. Other microscopic characterization techniques which ensure the well dispersion of the nanoparticles may also be presented for comparison purposes. Future work could include, for example, modifying the gel morphology and the electrostatics characteristics to tailor the rate of the macromolecule in applications such as drug delivery systems, bioseparation protocols and other related pharmaceutical applications to optimize the performance of the material.

1. Rill RL, Locke, BR, Liu, Y, Dharia, J, Van Winkle, D, "Protein electrophoresis in polyacrylamide gels with templated pores," *Electrophoresis* 17 (1996) 1304-1312
2. Matos, MA, White LR, Tilton RD, "Electroosmotically enhanced mass transfer through polyacrylamide gels," *Journal of Colloid and Interface Science* 2006 (300): 429-436.

Novel Biased Ac Electroosmosis Micropump Using Symmetrical Electrode Array

By Nazmul Islam, ECE Dept., Northern Arizona University, Flagstaff, AZ 86011 and Jie Wu, Dept. of Electrical and Computer Engineering., The University of Tennessee, Knoxville, 420 Ferris Hall, 1508 Middle Drive, Knoxville, TN TN 37996.

Abstract: The interaction of AC electric field with the double layer surface charge creates uni-directional force, which in turn produce fluid flow for AC electroosmosis (ACEO). In the last decade ACEO has received increasing interest due to its important applications in micro total analysis systems and miniaturized biomedical devices. With the application of very low voltage ($\sim 1V_{rms}$), ACEO can be utilized to control and manipulate particles and fluids at the micro/nano scale, which are very difficult to achieve with existing techniques, such as pressure driven flow.

A novel micropump is presented in this research for lab-on-a-chip applications that can be fabricated with MEMS-compatible semiconductor micro-fabrication. The proposed AC electroosmotic micropump is based on biased ACEO technique for symmetrical electrode array. Biased ACEO is realized by applying biased AC signals over electrode pairs, leaving the electrolyte floating; therefore, two electrodes have different electrical potentials relative to the electrolyte. With a biased AC signal, $V_{applied} = V_0(1 + \cos\omega t)$ over the electrodes, the left electrode is always positive and more prone to Faradaic charging, while the other is always negative and subject to capacitive charging.

The main concept of getting the pumping action is to get the uni-directional flow by breaking the reflection-symmetry in the geometry or applied signal. In our research we have broken the symmetry by applying asymmetric voltage on the symmetric electrode pattern, which eventually breaks the symmetry of the pattern. The corresponding numerical simulations using the finite element software Comsol multiphysics (FEMLab) are also conducted to verify the concept. The good agreement between the simulations and the experimental data regarding the uni-directional flow is also demonstrated. Our analysis also has shown that by decreasing the micro-channel height, the higher surface-to-volume ratio can be achieved, that eventually increases the micro-pumping velocity. At the end of the paper feedback control of micropump is also presented.

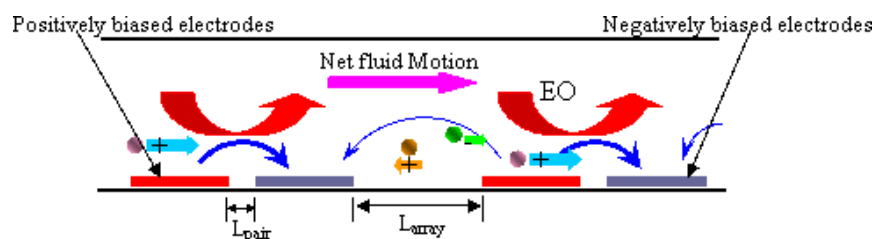


Figure: Biased ACEO produce uni-direction fluid motion, which also imparts differential velocities to particles with various charge/mass ratio.

AES-5 (333g)

Rapid Cell Rupture in A Dielectrophoretic Field

By Kaela M. Leonard, Sheena Reeves, Ashley Pate, Sarah Thompson, and Adrienne R. Minerick. Dave C. Swalm School of Chemical Engineering, Mississippi State University, Box 9595, Mississippi State, MS 39762

Abstract: Laboratories-on-a-chip and micro Total Analytical Systems (microTAS) are seen as one of the key growth industries for the 21st century. These systems are attractive due to the promise of raw unprocessed samples entering the device with quantitative analysis results as an output from the device. Because of their small size, these microdevices require small sample volumes and conceivably can return quantitative results within a matter of minutes. Such devices also have the potential to decrease the cost of analysis for applications such as medical testing, do not require technician operation, and can be adapted as point of care devices to diagnose and monitor diseases.

Electrokinetic tools show promise in the manipulation and subsequent quantification of analytes within microdevices. This work examines the use of special electric fields to reliably and rapidly rupture erythrocytes, which can be useful for subsequent subcellular analysis. Rupturing frequencies around 1 kHz were utilized and the field intensity for a series of dependence experiments was varied from 1V_{pp}/200 μ m to 10V_{pp}/200 μ m. All eight blood types were screened first as fresh samples, then as a function of age. Cell counts were tabulated as a function of time, treatment frequency, amplitude, and age. Results from this process show that there exists a relationship between the age of the blood sample and the applied field.

AES-6 (333m)

Role Of Electro-Osmosis Based Flows On The Effective Transport In A Rectangular Geometrical Electrophoretic Cell

By Jasna Godoy, University Catolica del Norte, Avda Angamos 0610, Antofagasta, Chile, Mario A. Oyanader, Universidad Catolica del Norte, Avenida Angamos, Antofagasta, 0610, Chile, and Pedro E. Arce, Chemical Engineering, Tennessee Technological University, Department of Chemical Engineering, P.O. Box 5013, Cookeville, TN 38505.

Abstract: Separation of biomacromolecules embraces a significant number of applications related to, for example, electro-assisted drug delivery, micro-electrophoretic separations, soil remediation processes, and material processing, just to name a few. Although, the influence on the flow regimes on effective transport has been identified in the literature, the specific role of electroosmotic type forces has not been reported. This contribution focuses on the understanding of the role of electro-advective, i.e. electro-osmosis driven flows on dispersion of solutes. Furthermore, this contribution builds the baseline for comparison with different capillary geometries and how separation or mixing may be controlled. Using the spatial averaging method in combination with the solute species continuity equation, the authors have derived analytical expressions for effective parameters, i.e., effective dispersion coefficient and effective convective velocity. Illustrations are presented to demonstrate the effect of different parameters in a capillary channel of rectangular geometry. The influence of applied electric field, surface potential and Debye length on the velocity profiles are examined.

Modeling Of High Frequency Diamond Layered Saw Devices For Potential Microfluidic Applications

Subramanian Sankaranarayanan¹, Stefan Cular², and Venkat R. Bhethanabotla². (1) University of South Florida, Dept. of Chemical Engg., ENB 118, 4202 E Fowler Avenue, Tampa, FL 33620, (2) Department of Chemical Engineering, University of South Florida, 4202 East Fowler Ave., ENB 118, Tampa, FL 33620

Abstract: Integration of microelectronics and biotechnology requires developing interfaces that are compatible with microelectronics processing methods, and also provide the requisite selectivity and stability when exposed to biological environments. Diamond is especially attractive because, in addition to having good electrical and chemical properties, it is widely considered to be biocompatible, and can be used as a waveguide in layered SAW devices. Additionally, the high density of diamond layer helps achieve higher acoustic wave propagation velocity and realize GHz frequency devices with transducer finger spacing achievable by optical lithography. In the present work, we use 3-D structural and 3-D/2-D fluid solid interaction finite element models to study the wave propagation characteristics in diamond layered piezoelectric surface acoustic wave devices under the influence of a fluid loading.

The structural model is first utilized to study the wave propagation in diamond layered SAW devices. The 3-D FE model describes two-port structures on the surface of LiNbO₃ and LiTaO₃ substrates. The simulated device consists of three finger pairs in each port. The fingers are considered as mass-less electrodes to ignore the second-order effects arising from electrode mass, thereby simplifying computation. The periodicity of the finger pairs is 40 microns and the aperture width is 200 microns. The transmitting and receiving IDT's are spaced 130 microns or 3.25λ apart. The simulated SAW device dimensions are 800 microns in propagation length, 500 microns wide and 400 microns deep. The simulated models have a total of approx. 250000 nodes and are solved for four degrees of freedom (three displacements and voltage). The model was created to have the highest densities throughout the surface and middle of the substrate. The simulated voltage and displacement profiles obtained at the output IDT's are used to measure wave attenuation and velocity changes and thereby optimize the thickness of the waveguide, i.e. the diamond layer. The optimized waveguide thickness is then used in the fluid-solid interaction FE model to investigate the influence of fluid loading on Rayleigh and SH wave propagation in diamond layered SAW devices and evaluate their use as potential biosensors.

Optimal Separation Times for Biomacromolecules in A Couette-Based/ electrophoretic Separation Device: Effect of Capillary Wall Velocities

By Jennifer Anne Pascal, Chemical Engineering, Tennessee Tech University, PH-214, Cookeville, TN 38505, Mario A. Oyanader, Universidad Catolica del Norte, Avenida Angamos, Antofagasta, 0610, Chile, and Pedro E. Arce, Chemical Engineering, Tennessee Technological University, Department of Chemical Engineering, P.O. Box 5013, Cookeville, TN 38505.

Abstract: The ratio of capillary wall velocities in an electrophoretic/Couette-based separation device is examined in this contribution. The flow within the device is modeled by the coupling electrostatics and hydrodynamics. In addition, the convective-diffusive differential model with the spatial averaging technique is used to predict the behavior of the system by obtaining analytical expressions for effective parameters (i.e. the convective velocity and universal dispersion) (Zanotti and Carbonell, 1984a, 1984b, 1984c). Previous analyses have yielded numerical solutions for a Poiseuille type of flow in a rectangular capillary channel (Sauer et. al., 1995). Furthermore, analytical solutions for a Poiseuille flow regime in a rectangular capillary channel were obtained by Oyanader and Arce (2005); it was also found in this contribution that the orthogonal field controlled the efficiency of separation in the device. Recently, Pascal et. al. (2007) applied the same approach to a Couette type of flow in a rectangular capillary channel with the top wall of the channel moving at velocity, v_p , to study the effects of the cation valence, z , of two biomacromolecules. In this contribution, both walls of the capillary channel have velocities different from zero, and move in opposite directions to produce a Couette type of flow. By using the Peclet number as a parameter, the optimal time of separation has been computed for a range of values of the ratio of the channel wall velocities, R .

1. Oyanader, M. A. and P. Arce, *Electrophoresis*, 26, 2857 (2005).
2. Pascal, J.A., R. O'Hara, M. Oyanader, and P. Arce, *Electrophoresis*. Submitted.
3. Sauer, S. G., Locke, B. R., Arce, P., *I&EC Research*, 1995, 34, 886.
4. Zanotti, F.; Carbonell, R. G., *Chem. Eng. Sci.* 1984a, 39, 263.
5. Zanotti, F.; Carbonell, R. G., *Chem. Eng. Sci.* 1984b, 39, 279.
6. Zanotti, F.; Carbonell, R. G., *Chem. Eng. Sci.* 1984c, 39,299.

AES-9 (333j)

A Simplified Mathematical Model Of An Ek Cell To Assess The Influence Of Buoyancy Driven Flows On Removal Efficiency

By Cynthia Torres, Chemical Engineering, Tennessee Tech University, PH-214-Chemical Engineering, Cookeville, TN 38501, Mario A. Oyanader, Universidad Catolica del Norte, Avenida Angamos, Antofagasta, 0610, Chile, and Pedro E. Arce, Chemical Engineering, Tennessee Technological University, Department of Chemical Engineering, P.O. Box 5013, Cookeville, TN 38505.

Abstract: Soil clean up embraces a large family of approaches based on the fundamental principles applied in the operation. For example, EK soil remediation, identified as a versatile technology that can be applied to heavy metal as well as hydrocarbons, has a family of at least seven sub-technologies (i.e. Cation Selective Membrane, Ceramic Casting, Lasagna, Electrochemical ion exchange, etc). Although temperature development on the soil matrix has been observed, little effort has been dedicated to demonstrate or document how temperature triggered mechanisms may be used to overcome soil remediation pitfalls. This contribution focuses on the analysis of results obtained by simulation with a simplified mathematical model. The general hypothesis is that there exist an effect of buoyancy driven flows on the over all removal efficiency of contaminant and that the inclination a EK cell may be used to enhance solute transport. To validate the findings, an experimental approach will be outlined. On the overall, the results and conclusions derived from this study are the baseline for the EK cell system modeling. Comprehensive details of key factors are highlighted to illustrate the modeling technique as well as the system representation.

AES-10 (333k)

Comparison Of Convective-Dispersive Aspects In Electrokinetic Cells Of Rectangular And Cylindrical Geometry

By Antonio Martinez¹, Mario A. Oyanader¹, and Pedro E. Arce². (1) Universidad Catolica del Norte, Avenida Angamos, Antofagasta, 0610, Chile, (2) Chemical Engineering, Tennessee Technological University, Department of Chemical Engineering, P.O. Box 5013, Cookeville, TN 38505

Abstract: The occurrences of flow reversals during EK processes has been explained by Oyanader et al (2003); Oyanader et al. (2005a&b) using different geometrical models for the soil porous matrix. The conjunction of three competing driving forces (pressure, buoyancy and electrostatic) have an affect on the motion of the solute species that need further understanding. The different driving forces need to be studied in term of how they promote mixing or separation of solute species in an EK cell. Most EK technologies, i.e. Cation Selective Membrane, Ceramic Casting, Lasagna, Electrochemical ion exchange, etc., have reported low removal efficiency. In this contribution, the main focus is on unveiling the possible reasons for EK pitfalls from a fundametal point of view. The approach is based on modeling and fundamental principles applied, first, to the hydrodynamic part of the system and, later, to the species/analytes transport. An area averaging technique has been used to capture the main parameters of the system such as effective mixing, and diffusivity. Furthermore, an approach has been applied to describe the capillary aspect of the soil matrix. This approach involves the characterization of the capillary channels as of rectangular and/or cylindrical aspect to present and discuss several limiting cases related to the influence of flow regimes on the transport of solute in an EK for removal purposes. Optimal operating conditions are highlighted to introduce design criteria and protocols.

Oyanader, M., P. Arce, and A. Dzurik, "Avoiding Pitfalls In Electrokinetic Remediation: Robust Design And Operation Criteria Based On First Principles For Maximizing Performance In A Rectangular Geometry", Electrophoresis, 2003. Oyanader, M., P. Arce, And A. Dzurik, "Design Criteria For Soil Cleaning Operations In Electrokinetic Remediation. Hydrodynamic Aspects In A Cylindrical Geometry", Electrophoresis, 2005a. Oyanader, M., P. Arce and A. Dzurik "Design Criteria For Soil Cleaning Operations In Electrokinetic Remediation. Hydrodynamic Aspects In An Annular Geometry", I&ECh Research, 2005b.

AES-11 (3331)

Effect Of Dispersion Of Ions On The Performance Of An Electrophoretic Cell With Orthogonal Electrical Field

By Pablo Vergara, Chemical Engineering and Material Sciences, University Catolica del Norte, Avenida Angamos 0610, Antofagasta, Chile, Mario A. Oyanader, Universidad Catolica del Norte, Avenida Angamos, Antofagasta, 0610, Chile, and Pedro E. Arce, Chemical Engineering, Tennessee Technological University, Department of Chemical Engineering, P.O. Box 5013, Cookeville, TN 38505.

Abstract: The hydrodynamic of a system, as it has been demonstrated, has an effect on the transport of solute in an electrophoretic cell. The role of an orthogonal field to flow direction on the hydrodynamic of a system has been studied by the authors using a cell of rectangular geometry*. The study has been extended to include an annular system where the role of the radial transport may be different to that in rectangular systems. Furthermore, the role of the cross sectional area size in an annular cell may have a different effect on the movement of ions of different oxidation state. The method of spatial averaging is used in combination with the solute species continuity equation to determine the effect of the dispersion of the charged ions on device performance in a Poiseuille type flows, under the stress of electric fields. Explicit analytical expressions are derived for the effective parameters as a function of the applied electric field, capillary porosity and the flow regime. The result of the analysis can be used in the design of separation processes as well as environmental applications such as soil remediation and wastewater treatment.

*Mario A. Oyanader and Pedro Arce, Role of Geometrical Dimensions in Electrophoresis Applications with Orthogonal Fields, *Electrophoresis* 2005, 26, 2857–2866.

AES-12 (333p)

In-Line Sample Pre-Concentration Employing Octadecylsilane

By Daniel L. Morris and Katherine A. Sorvig, Department of Chemistry, Rose-Hulman Institute of Technology, 5500 Wabash Avenue, Terre Haute, IN 47803

Abstract: We report an in-line, solid-phase extraction based method for pre-concentrating samples using a previously reported photoinduced polymerization technique for trapping octadecylsilane (ODS) spheres in capillaries. Previous workers have used this method of particle entrapment for capillary electrochromatography and the fabrication of spray tips for capillary ESI-MS. The method allows for entrapment of ODS spheres to produce varying lengths of stationary phase without the use of retaining frits and their associated problems (bubble formation and void spaces). We entrapped a 1-2 cm length of 4 μm ODS spheres at the end of a capillary using this method and demonstrate its ability to pre-concentrate samples for detecting the oxidative DNA damage marker 8-hydroxy-2'-deoxyguanosine (8-OH-dG) using micellar electrokinetic capillary chromatography (MEKC) with UV absorption detection.

AES-13 (333n)

Identification And Antibody-Therapeutic Targeting Of Antibiotic-Resistant Outer Membrane Proteins In E. Coli

By Hui Li¹, Xiangmin Lin¹, Chuangzhong Huang¹, Sanying Wang², and Xuanxian Peng¹. (1) State Key Laboratory of Biocontrol, School of Life Sciences, Zhongshan University, Guangzhou, 510275, China, (2) Dept of Biology, Xiamen University, Xiamen, 361005, China

Abstract: The elucidation of the molecular details of antibiotic resistance will lead to improvements in extending the efficacy of current antimicrobials. Our research focuses on Om proteins in response to antibiotic resistance. This investigation was performed by proteomic methodologies for the altered Om proteins from survived bacteria in a suddenly strong antibiotic or in sub-MIC of the drug because bacteria may be exposed in the two treatments in their becoming resistance to the antibiotic. Their shared proteins were further confirmed by Western blotting and gene mutant methods as potential targets for designation of new drugs to inhibit the growth of the antibiotic-resistant bacteria. Moreover, a novel method of specific antibody combating bacterial growth was developed based on these altered Om proteins. Our results suggest that combination therapy involving antibiotics that enhance the expression of an antibody target could be far more effective than either drug alone. Our study gives a novel insight into therapy to infection by antibiotic-resistant bacteria. Our study has been extended into a variety of antibiotics including tetracycline, ampicillin, chloramphenicol, chlortetracycline, Streptomycin, kanamycin, nalidixic acid, ceftazidime, ciprofloxacin, balofloxacin, ceftriaxone sodium. *Corresponding author: Dr. Xuanxian Peng This work was sponsored by grants from Guangzhou Key Project 2006Z3-E0251

AES-14 (333o)

Rapid Screen Of Highly Efficient Vaccine Candidates By Immunoproteomics And Cross Immunoproteomics

By Xuanxian Peng, State Key Laboratory of Biocontrol, School of Life Sciences, Zhongshan University, Guangzhou, 510275, China and Sanying Wang, Department of Biology, Xiamen University, Xiamen, 361005, China.

Diseases caused by microorganisms can be controlled by vaccines, which require neutralizing antigens. Therefore, it is very important to identify highly efficient immunogens for immunoprevention. By combining immunoproteomics and bacterial challenge after immunization, we developed a rapid method for screening protected antigens of pathogenic bacteria in aquaculture. Our approach may be divided into three consecutive steps. First, dominant immunogens of outer membrane proteins are screened by immunoproteomics. Second, proteins with the ability to induce production of neutralizing antibodies are identified from the immunogens by virulent bacterium challenge following vaccination. Third, vaccine candidates are determined by evaluation of neutralizing abilities. Information on the candidates has been obtained for further gene cloning by mass spectrometry. Our results indicate that highly efficient protected antigens were identified from the outer membrane proteome of *Aeromonas hydrophila*, in which an immunogen showed 71.4% protective ability with multivalent functions to *A. hydrophila* and *Aeromonas sobria*.

Moreover, a cross immunoproteomics was developed with the use of homogeneous and heterogeneous antibodies as the primary ones, which were prepared from bacterium as an antigen in Western blotting and other bacteria, respectively. These spots recognized by both homogeneous and heterogeneous antibodies were cross-protective antigens and identified with mass spectrometry. Their genes were cloned and expressed. The purified recombinant proteins were applied for investigation of cross-protective immunity in fish and mice. Two highly efficient vaccine candidates determined in *V. parahaemolyticus* outer membrane proteins could be against infections by not *V. parahaemolyticus*, but also *V. alginolyticus*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa*, which indicates that two outer membrane proteins show universal function against bacteria.

In summary, the methodologies developed in the current study is high throughout and hence approached rapid, highly efficient and accuracy aims, which will play active role in immune prevention for microbiological diseases. This work was sponsored by grants from National Basic Research Program of China (2006CB101807) and “863” project (2006AA09Z43Z).

Many Thanks to the AES
Poster Reception Sponsor!

