*J. Iran. Chem. Soc.*, Vol. 6, Suppl., November 2009, pp. S8-S19.

JOURNAL OF THE
Iranian
Chemical Society

# Nanobiotechnology; Application in Clinical Diagnosis and Treatment

**P-10-70-2**
**Facile interfacial electron transfer of hemoglobin mediated by nafion-riboflavin nano-composition**

*Saeed Rezaei-Zarchi*

Payame Noor University, Yazd, Iran

We herein describe a method to stabilize hemoglobin (Hb) on gold electrodes by using nafion-riboflavin film that facilitates interfacial protein electron transfer. Well-defined, reproducible, chemically-reversible peaks of Hb could be observed by this method. The formal potential of Hb was 180 mV (versus Ag/AgCl) in 20mM MOPS buffer solution, pH 7.0 at 25°C. The cathodic transfer coefficient was 0.70 and electron transfer rate constant was evaluated to be $1.5s^{-1}$. We also observed peroxidase activity of Hb stabilized on nafion-riboflavin film that can be useful as one biosensor for determination of H2O2 with detection limit of 5µM. These results clearly showed that nafion-riboflavin film worked as molecular wires and effectively exchanged electrons between Hb and electrodes.

**Keywords**: hemoglobin, nafion-riboflavin film, biosensors, bio-electrochemistry

**P-10-210-1**
**Development and characterization of chitosan/poly (vinyl alcohol) conjugated nerve growth factor scaffolds for peripheral nerve regeneration**

*Fatemeh Mottaghitalab [1]*, Nasim Amiralyan [2], Sadegh Babashah [2], Ali Eslamifar [3], Mohammad Ali Shokrgozar [4], Vahid Mottaghitalab [4]*

1- Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, 2- Guilan Science and Technology Park (GSTP), 3- Clinical Research Group, Pasteur Institute of Iran, 4- National Cell Bank, Pasteur Institute of Iran

The complexity of the nervous system allows in which the information to be received and transmitted through the body. As a result, peripheral nerve tissue poses many challenges when designing scaffolds to serve as unique replacements for injured or diseased tissue. Sustained release of proteins from aligned polymeric fibers hold great potential in tissue-engineering applications. One of the most important challenges in the peripheral nerve tissue engineering is production of an implantable scaffold, capable of bridging long gaps, which will produce results similar to an autograft without requiring the harvest of autologus donor tissue. A possible improvement of the electrospun scaffold system could be including biochemical cues by encapsulating proteins into the fibers to form a protein-polymer composite system. These composite fibers will not only posses a large network of interconnected pores that is conductive to tissue in-growth. However, a high surface area provides local and sustained delivery of biochemical signals to the site of injury. This study was conducted to evaluate the potential of such protein-polymer composite fibers which could be useful as a tissue-engineering platform, aligned electrospun protein-encapsulated fibers for the treatment of peripheral nerve injury. Results showed that biodegradable chitosan/poly (vinyl alcohol) conjugated NGF scaffolds could integrate with the damaged tissue to promote consistent functional recovery but additional investigation is required for this to be confirmed.

**Keywords**: peripheral nerve tissue engineering, electrospun, biodegradable, chitosan, poly (vinyl alcohol)

**P-10-210-2**
**Electrospun chitosan/poly (vinyl alcohol) fibrous scaffolds for nerve tissue regeneration**

*Fatemeh Mottaghitalab [1]*, Nasim Amiralyan [2], Sadegh Babashah [2], Ali Eslamifar [3], Mohammad Ali Shokrgozar [4], Vahid Mottaghitalab [4]*

1- Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, 2- Guilan Science and Technology Park (GSTP), Faculty of Engineering, Guilan University, 3- Clinical Research Group, Pasteur Institute of Iran, 4- National Cell Bank, Pasteur Institute of Iran

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to develop the biological subunits that restore, maintain and improve tissue formation. Such tissue regenerations are achieved with the aid of polymeric scaffolds which serves as matrixes for cellular in-growth, proliferation and new tissue formation in three-dimensions. These scaffolds have the ability to form a sub-micron sized fibrous structure with interconnected pores mimicking the extracellular matrix (ECM) for tissue formation. Electrospinning was used to fabricate chitosan solution mixed with poly (vinyl alcohol) (PVA). In the present study, the in vitro biocompatibility of the electrospun fibers was evaluated. The obtained results showed the potential for use of the electrospun chitosan/PVA scaffolds for nerve tissue regeneration.

**Keywords**: tissue engineering, chitosan, PVA, electrospinning, nerve tissue regeneration

**O-10-214-1**

**Evaluation of two strategies to increase the DNA vaccine potency using HPV16 E7 as an antigenic model**

*Azam Bolhassani [1]*, Nahid Ghasemi [1], Mohammad Taghikhani [2], Sima Rafati [2]*

1- Molecular Immunology and Vaccine Research Laboratory, Pasteur Institute of Iran, Tehran, Iran, 2- Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran

High-risk human papillomavirus types, such as HPV16 and HPV18 are the most frequent HPV type associated with cervical cancer. DNA vaccines have emerged as an attractive approach for antigen-specific T-cell mediated immunotherapy to combat cancers. The utilization of adjuvant such as heat shock proteins and different non-viral delivery systems such as polymers and cationic peptides have been demonstrated as the efficient approaches for increasing the potency of DNA vaccines. In current study, at first two delivery systems including polymer PEI 25kDa and polymer-peptide hybrid as PEI600-Tat conjugate were used to compare their efficiency for HPV16 E7 DNA transfection in vitro. Our data indicated that both delivery systems including PEI 25kDa and PEI600-Tat conjugate are efficient tools for E7 gene transfection. In fact, PEI potency for E7 gene transfection is higher than PEI600-Tat in vitro, but its toxicity is an obstacle in vivo. Then, we evaluated the effects of PEI600-Tat conjugate as a novel delivery system and Gp96 as an adjuvant on the potency of antigen-specific immunity in C57BL/6 mice model, using HPV16 E7 as an antigenic model. Assessment of cellular immune response against recombinant E7 protein showed that co-delivery of naked E7+Gp96 DNA plasmid induces Th1 response. A similar result was obtained by using E7DNA/PEI600-Tat complex at ratio of 50:10 (w/w). Our study indicated that DNA vaccinations including E7+Gp96 and/or E7DNA/PEI600-Tat complex were immunologically more effective than E7DNA alone. Further studies are needed to demonstrate the co-delivering effect of E7 and GP96 by using PEI600-Tat after challenging the mice with cell lines such as TC1.

**Keywords**: adjuvant, delivery system, DNA vaccine, heat shock protein, human papillomavirus

---

**P-10-132-2**

**Higher serum phosphorus and calcium levels are associated with an increased lipid profile in reproductive age women**

*Mahdieh Abbasalizad Farhangi [1]*, Alireza Ostadrahimi [2], Soltanali Mahboob [2]*

1- Faculty of Public Health, Tehran University of Medical Sciences, 2- Nutritional Research Center, Tabriz University of Medical Sciences

Some researches have reported that people with high levels of serum phosphorus and calcium seem to be at increased risk for developing heart disease. Since lipid profile is a good marker of cardiovascular disease, the aim of this cross sectional study was to investigate the relationship between serum phosphorus and calcium levels with serum triglyceride (TG), total cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein Cholesterol (HDL-C) in a group of women. This study was performed on 82 reproductive age (17-50 years) women. Serum magnesium, calcium and phosphorous were measured colorimetrically and serum alkaline phosphatase and lipid profile by enzymatic methods except for LDL which was calculated from serum TC, TG and HDL using the Friedwald formula. Pearson correlation coefficient was used to investigate association between the variables. There was a significant positive relationship between serum phosphorous and TC (r=0.31, P=0.002) and LDL-C (r=0.214, P=0.004). Serum calcium also had a significant positive relationship with TG (r=0.301, P=0.005), TC (r=0.267, P=0.01) and LDL-C (r=0.027, P=0.241). According to our results lipid profile with a predictive potency of cardiovascular disease was significantly correlated with serum calcium and phosphorous concentration. Therefore serum calcium and phosphorous can be a good markers of CVD.

**Keywords**: phosphorus, calcium, lipid profile, reproductive age women

---

**P-10-304-2**

**Bactericidal activity of carbenicillin in free and liposomal form against resistant strain of Pseudomonas aeruginosa**

*Manizheh Kadkhodaei Elyaderani [1], Ahmad Faraj Zadeh Sheikh [2]*, Amir Gharib [3], Maryam Kochak [4], Efat Abas Montazeri [5], Zohreh Faezizadeh [6], Hosein Hanifi [7]*

1- Department of Biochemistry & Thalassemia Research Center, Faculty of Medicine, Ahwaz Jundishapur University of Medical Sciences, 2- Department of Microbiology, Faculty of Medicine, Ahwaz, 3- Ahwaz Jundishapur University of Medical Sciences, 4- Faculty of Pharmacy, Ahwaz Jundishapur University of Medical Sciences, 5- Department of Microbiology, Faculty of Medicine, Ahwaz Jundishapur University of Medical Sciences, 6- Department of Laboratory science, Para-Medical school, Islamic Azad University, Branch of Brojerd, Iran, 7- Department of Biochemistry, Faculty of Medicine, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

Pseudomonas aeruginosa is resistant to most conventional antibiotics. The mechanism of resistance of this bacterium is mainly associated with the low permeability of its outer membrane. A delivery system that reduces the drugs resistance while increasing their therapeutic index is of great interest. The present in vitro study was designed to evaluate the antimicrobial activities of free and liposomal carbenicillin on P. aeruginosa. Liposomes encapsulated ciprofloxacin with three different surface charges: positive, negative and neutral with using lecithin, cholesterol, stearyl amine, cetyl diphosphate were prepared by reverse phase evaporation method. The drug contained in the liposomes was measured by spectrophotometer technique after lyses of the vesicles by 0.2% Triton X-100. The comparative encapsulation efficiency of three antibiotic preparations was assessed. The minimal inhibitory concentrations (MICs) were determined and compared to those of respective free drugs. The carbenicillin encapsulation efficiencies for cationic, anionic and neutral liposome forms were 76%, 61% and 70%, significantly higher than those of anionic liposomal forms ($P \leq 0.05$). The MIC of cationic and anionic liposome forms of carbenicillin against this strains were lower compared to those of free antibiotics; the MIC for cationic form was at least 10 times lower than free carbenicillin ($P \leq 0.05$) . Our data suggest that probably electrostatic interaction between the outer membrane lipopolysaccharides of P. aeruginosa and cationic liposome-entrapped carbenicillin could enhanced mechanism of drug entry into this microorganism cells.

**Keywords**: carbenicillin, liposome, bactericidal activity, Pseudomonas aeruginosa

---

**P-10-327-2**

**High level expression of functional recombinant human coagulation Factor VII**

*Mehryar Habibi Roudkenar*, Raheleh Halabian, Nasser Massrori, Mohammad Ali Shokrgozar*

Research Center, IBTO

Factor VII is a vitamin K-dependent coagulation factor. According to previous studies, Sf9 insect cells lack endogenous vitamin k-dependent carboxylase. In this study, unraveling the defect and high level expression of functional human rFVII using Baculovirus expression vector system (BEVS) and Gateway technology were the main aims. Therefore, we assumed that co-transfection of human gamma carboxylase with human FVII would lead to produce functional hFVII. Human gamma-carboxylase and h FVII cDNA were isolated and cloned to appropriate vectors. Recombinant baculoviruses carrying hFVII and hGC were generated and transfected to SF9 cells. The expression of recombinant FVII was confirmed by RT-PCR and ELISA. Culture medium containing his-FVII was added to the nickel-nitrilotriacetic acid resin column and bound protein was eluted. The purified protein was detected by SDS-PAGE and western blot analysis. Biological activity of the recombinant factor VII was determined by prothrombin time assay using factor FVII-depleted plasma. The results showed that human recombinant FVII successfully was cloned. A protein of about 55KD was detected in SDS-PAGE and was further confirmed by western blot analysis. About four fold decreases in clotting time by a commercially available FVIIa preparation, NovoSeven, was also observed which confirms biological activity of rFVII produced in the present study. It is noteworthy that there was no decrease in prothrombin time when only the FVII prepared in SF9 cells was used. Together, this indicates exogenous expression of hGC confer functional activity of recombinant FVII.

**Keywords**: vitamin K-dependent coagulation factor, gamma carboxylase, Factor VII

---

**P-10-286-1**

**Investigation on acid hydrolysis of Inulin: A response surface methodology approach**

*Ebrahim Eskandari*, Mehran Habibi-Rezaei, Afshin Khaki, Mohammad Balvardi*

School of Biology, College of Science, University of Tehran, Tehran, Iran

Inulin as a fructan-type carbohydrate has prebiotic properties such as the ability to stimulate probiotic bacteria without adversely affecting flavor and has been recognized as an anti-cancer material. In this study, the acid hydrolysis of inulin was investigated as a function of three variables: pH, temperature and time. Inulin hydrolysis detected by measurement of reducing sugars, using Dinitro Salicylic acid (DNS) method. The central composite rotatable design (CCRD) used to design an experimental program to model the effects of acidic and alkaline hydrolysis of the inulin biopolymer. However, response surface methodology (RSM) used for data analysis. The statistical analysis of the results showed that pH, temperature and time are significant variables at acidic pH, while none of these variables is significant at alkaline pH.

**Keywords**: Inulin, acid hydrolysis, response surface methodology

---

**P-10-367-1**

**Capacity examination of phage lambda derived nanobioparticles as a new generation of eukaryotic gene delivery vehicles**

*Mohammad Khalaj-kondori*, Pooria Gill, Mehrdad Behmanesh, Majid Sadeghizadeh*

Genetics Department, School of Basic Sciences, Tarbiat Modares University, Tehran, Iran

Attempts to treatment of diseases caused by genetic deficiencies – gene therapy- have ever faced with various challenges. One of the major challenges in this regard, is selection of a safe and proper carrier. Viral vectors are efficient gene carriers to eukaryotic cells, which suffer from frailties such as stimulation of the immune system, likelihood of transformation of the host cells, in spite of their high gene delivery efficiency. Bacteriophages have recently been proposed as safe gene delivery vehicles to eukaryotic cells especially to that of human. As phages reside every where including the human body, they have developed significant adaptation to the immune system. Moreover, phages possess various capabilities e.g. potential of genetic manipulation and targeting which are the most important features of a proper delivery vehicle. In the present study, initially, the GFP gene was PCR-amplified using the pEGFP-C1 vector as the template and further cloned into the Lambda ZAP –CMV vector, under CMV promoter. The plaques were verified and tittered following in vitro packaging and propagation of the resultant phages. Different dilutions of the phages were used for transfection of the human AGS cell line. GFP delivery and expression was assessed using fluorescent microscopy. Our finding reveal the applicability of the nanobioparticles derived from Lambda phage for gene delivery into eukaryotic cells in gene therapy trails, as in addition to their capability in delivery of genes, this efficacy is correlated with the number of phages. So, it would be possible to enhance the transfection efficacy by optimization and targeting of phages.

**Keywords**: bacteriophage, gene delivery, nanobioparticle

---

**P-11-278-2**

**Gastrointestinal tumor therapy usage of surgical and molecular continuously**

*Mohamad Rahmati [1]*, Davood Fazli [2], Farnaz Tahmasebzade [2], Naiyereh Mohamadzadeh [2]*

1- Department of Clinical Chemistry, Faculty of Medicine, Tabriz University of Medical Science, Tabriz, Iran, 2- Payame Noor University, Marand, Iran

Gastrointestinal tumors (GIST) are the most common tumor in gastrointestinal tract. The routine methods for treatment of GIST have poor results and are not satisfactory. Therefore the usage of surgical and molecular therapy together is emphasized recently. Previously the surgical method was used for GIST therapy alone, but we have to monitor the patients after surgical procedure in order to prevent recurrence. The discovery of the role of KIT proto-oncogene mutations in the pathogenesis of this tumor, and the development of imatinib mesylate as a specific inhibitor of KIT function has caused a revolution in the treatment of GIST and the use of molecular methods for cure have become prevalent. The use of surgical and molecular therapy, continuously, for GIST treatment has had the best effects and in the

future it will likely serve as a new treatment method for other solid tumors as well.

**Keywords**: surgical therapy, molecular therapy and GIST

---

**P-10-512-1**

**The influence of human intron insertion on eukaryotic vector expression**

*Sadegh Hasannia [1]*, Nazanin Pirooznia [1], M.A Karimi [1], M. Eskandani [1], F. Rahbarizadeh [2]*

1- Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran, 2- Department of Medical Biotechnology, Tarbiat Modares University, Tehran, Iran

The rising rate of cancer-related diseases underscores the need for new approaches to fight cancer. Cancer immunotherapy is the use of the immune system to reject cancer. The specific activation of the immune system to control cancer growth has been long-lasting goal in cancer immunotherapy. T cells expressing chimeric receptors are able to discriminate between antigen-expressing and normal cells. T cells are well suited to penetrate and destroy solid tumors. In this study, we use chimeric T Cell Receptor (CR) with four proteins fused at the DNA level in such a way that, once expressed, they result in a single TCR polypeptide chain consisting of four moieties: Camelid heavy chain antibody moiety (VHH), a spacer (FcgRIIa), A transmembrane or signaling moiety (CD3Zeta) and co-stimulating moiety (CD28). Various methods, such as using various promoters, selection of specific untranslated region (UTRs), various signal sequences, appropriate inducer and other biotechnological procedures are being employed to increase expression in eukaryotic vectors. In this study we use human intron integration to study its effect on gene expression. After intron insertion in vector containing chimeric T- cell receptor cassette, this new construct has been transform into the eukaryotic cell line, jurkat E6.1. Expression analysis through RT-PCR method and their function using bioassay have been examined. The results showed more expression and better function of constructed chimeric receptor. Therefore, we can use intron sequence to increase expression in eukaryotic vectors in immunotherapy. Base of previous studies, this high expression effect is related to the increase of mRNA stability.

**Keywords**: chimeric receptor, immunotherapy, intron, T cell therapy, VHH

---

**P-11-525-3**

**Survey effect of in-vivo and in-vitro condition on expression of surface layer genes in bacteria**

*Shilla Jalalpoor [1]*, Rooha Kasra Kermanshahi [2], Hamid Zarkesh [3], Hamid Abousaidi [4]*

1- Departments of Microbiology, Shahreza and Falavarjan University, 2- Departments of Science, Alzahra and Isfahan University, 3- Azad University of Tehran, 4- Department of Science, Isfahan University, Isfahan, Iran

Over the past 3 decades of research, it has become apparent that one of the most common surface structures on Archaea and Bacteria are monomolecular crystalline arrays of proteinaceous subunits termed surface layer or S-layer. S-layer is the outer protein layer in Bacteria and is a monomolecular protein or glycoprotein subunits. S-layer protects Bacteria against phagocytosis and prohibits the entry of some Biomolecules and adhesion to matrix proteins. It is the virulence agent in bacteria. Expression of S-layer genes is under the influence of environmental conditions and despite of widespread occurrence on prokaryotic organisms it has not always been appreciated. In this research that was performed in 84/85 years in ALZAHRA Hospital in Isfahan, we study 26 Bacillus cereus strain, 13 clinical strains (in-vivo) and 13 environmental strains (in-vitro). Identification of Bacteria, were performed with microbiological methods including staining, chemical test and use of differential and selective media. For preparation samples, Bacteria were cultured in TSA, for 16h, then surface proteins were separated and finally, specimen's electrophoresis was performed. The molecular weight of the S-layer in Bacillus cereus was estimated to be 97KD. From among 13 isolated of Bacillus cereus from clinical samples, 11 strain (84.6%), and from 13 isolated Bacillus cereus from environmental samples 1 strain (7.7%) produced S-layer. On the basis of the results, we can say that the expression of S-layer genes in Bacillus cereus strains in-vivo condition, the best condition for grow human pathogen Bacteria, is better compared to in-vitro condition. We offer for determine S-layer in Bacteria, instead use environmental and sub cultural Bacteria, use fresh isolated Bacteria.

**Keywords**: surface layer, in-vitro condition, in vivo condition, Bacillus cereus, 10X SDS-PAG

---

**O-10-572-1**

**Production and characterization of camel recombinant anti HER-2 nanobody**

*Farzane Sheikholeslami [1]*, Mohammad Javad Rasaee [2], Mohammad Ali Shokrgozar [3], F. Rahbarizadeh [3], M. Navari [4], M. Nasri [4]*

1- Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, 2- Department of Clinical Biochemistry, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 3- The Cell Bank of Institute Pasteur of Iran, Tehran, 4- Department of Medical Biotechnology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Anti-HER-2 antibody is used for the clinical treatment of HER-2 positive cancerous tissue such as breast cancer. Today FDA approved recombinant antibodies of mouse origin humanized with human FC region are available for the treatment. Camel nanobodies are found to be useful replacement since they are single chain as well as highly soluble with lower molecular weight (15KD), hence high penetration rate .We prepares an anti-HER-2 recombinant antibody by injecting the homogenate of cancerous tissues to two camels of old world. The reactive immunoglobulin was selected following phage display technique and was transferred to high expression host system. The soluble antibody produced was purified following IMAC and characterized by SDS-PAGE, Western blotting and ELISA methods. The results indicated that the antibody produced reacted towards HER2 with high affinity.

**Keywords**: breast cancer, HER-2, recombinant nanobody, VHH

---

**P-10-579-1**

**Cytotoxicity in HepG2 cells after extended exposure to subtoxic levels of silver nanoparticles**

*Khadije Meghrazi [1]*, Azin Nowrouzi [1], Taghi Golmohammadi [1], Abolfazl Golestani [1], Zahra Shajary [1], Shahin Ahmadian [2], Mahshid Shfiezadeh [2]*

1- Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, 2- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

The present study was conducted to assess the toxicity of low concentrations of silver nanoparticles (SNP) (5-10 nm) in HepG2 cell line for a period of 4 consecutive generations. In toxicity evaluation, the TC50 value of the SNP on HepG2 cells was revealed to be around 2.75-3.0 mg/l using MTT and XTT assays. Then the cells were exposed to concentrations of 1%, 4% and 8% of TC50 for four generations. The cells were compared to the control group with respect to morphology and proliferation at the end of the period which showed a significant decrease in cell proliferation after exposure to 8% of TC50 concentration of SNP as compared to the control group. Biochemical studies revealed a significant increase of NO, LDH and ALT in the 8% of TC50 in the cell media. Also, at 8% of TC50 value, in the cell extracts, a decrease of GSH content and an increase of lipid peroxidation were remarkable, while for lower concentrations the changes were insignificant. The decrease in the activity of SOD enzyme in the cells exposed to 4% and 8% of TC50 concentration proved to be notable, but GPx activity and total protein content did not change during the testing period. The released cytochrome c from the mitochondria was significant at 8% of TC50. The results indicate that silver nanoparticles act by destroying cellular antioxidant defense mechanisms and meanwhile exert severe oxidative stress upon cells. The results may be used to specify a safer amount of SNP used for different applications.

**Keywords**: silver nanoparticles, HepG2, cytotoxicity, liver

---

**P-10-775-1**

**A new radiopharmaceutical compound (131I-PR81) for radioimmunotherapy of breast cancer: Labeling of antibody and its quality control**

*Javad Mohammadnejad [1]* , Mohammad Javad Rasaee [1] , Mohammad Hossein Babaee [2] , Malihe Paknejad [3] , Zahir Mohammad Hasan [4] , Mojtaba Salouti [5] , Ahmad Bitarafan Rajabi [6]*

1- Department of Clinical Biochemistry, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 2- Department of Radioisotope, Nuclear Research Center, Atomic Energy Organization of Iran, Tehran, Iran, 3- Department of Biochemistry, School of Medicine, Medical Sciences University of Tehran, Tehran, Iran, 4- Department of Immunology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 5- Department of Biology, School of Sciences, Islamic Azad University of Zanjan, Zanjan, Iran, 6- Cardiovascular Research Center, Shahid Rajaee hospital, Tehran, Iran

PR81 is a monoclonal antibody that binds with high affinity to MUC1, which is over expressed on breast and other tumors. The objective of this study was to evaluate the application of this antibody against MUC1 as a radioimmunotherapeutical agent. Monoclonal antibody (PR81) against the tandem repeat of the core protein (MUC1) was prepared, characterized, purified, and labeled with 131I using the chloramin-T method. The immunoreactivity of radiolabeled mAb PR81with MUC1 (the native protein), BSA-P20 (a 20 amino acid corresponding the tandem repeat of MUC1) and MCF7 cell line were performed by RIA. In vitro stability of radiolabeled mAb in human serum was determined by thin layer chromatography (TLC). Cell toxicity and in vitro internalization studies were performed with the MCF7 cell line, and the tissue biodistribution of the radioiodinated PR81 was evaluated in normal BALB/c mice at 4, 24 and 48 hrs. The tumor imaging was performed in BALB/c mice with breast xenograft tumors at 24 and 72 hr after the complex injection. The labeling efficiency was determined by measuring the percentage recovery of radioactivity in the final product relative to the initial activity in the shipment vial, was found to be 59.9% ±7.9%. MAb-131I conjugates showed high immunoreactivity towards MUC1 protein, BSA-P20 and MCF7 cell line. In vitro stability of the labeled product in human serum which was measured by thin layer chromatography (TLC) was found to be more than %50 over 24 hr. Cell toxicity and in vitro internalization studies showed that the mAb-131I conjugate inhibited 80% growth of the MCF7 cultured cell lines in vitro in a high concentration and up to %60 of the conjugate internalized after 24 h. Biodistribution studies were performed in normal BALB/c mice at 4, 24 and 48 hrs post-injection and no important accumulation was observed in vital organs. The tumors were visualized with high sensitivity after 24 and 72 hr in radioimmunoscintographical studies. These results show that the new radiopharmaceutical may be considered as a promising candidate for therapy of breast cancer.

**Keywords**: breast cancer, radioimmunotherapy, antibody

---

**P-10-612-2**

**Evaluation of the ability of luciferase gene transfer to Eukaryotic cell line by cationic liposome**

*Asia Majidi [1]*, Saman Hosseinkhani [1], Mahmoud Reza Jafari [2]*

1- Department of Biochemistry, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran, 2- Department of Medical Science, Faculty of Pharmacy, University of Medical Science, Mashhad, Iran

Cationic liposomes are composed of phospholipids bilayer. One of the most important applications of these particles is applying them as delivery systems to deliver genes into cells. Luciferase gene is one of popular reporter genes which are often used to measure the efficiency of gene transfer in vivo and in vitro. In previous studies, different formulations of liposome are used to delivery of different kinds of gene reporters. This study–for the first time– evaluated the usefulness of two formulations of liposome to deliver pGl3 –the plasmid which has luciferase gene as reporter– into CHO cell line. The cationic liposomes were composed of different molar ratio of DPPC/Chol/DOAB and DPPC/DOAB by FDEL method. CHO cells were transfected by pGl3 using specific concentration of cationic liposome. Transfection efficiency and gene expression was evaluated by luciferase assay system. The high gene Transfection efficiency of plasmid by these formulations was obtained.

**Keywords**: cationic liposomes, gene delivery, luciferase, transfection

---

**P-10-618-1**

**Preparation of the nanogold-based immunochromatography test strip for detecting digoxin toxicity**

*Solmaz Kia [1], Kobra Omidfar [2]*, Soheila Kashanian [3], Susan Kashanian [3], Bagher Larijani [4]*

1- Department of Biology, School of Sciences, Razi University, Kermanshah, Iran, 2- Laboratory of Biochemistry, Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran, 3- Department of Chemistry, Sensor and Biosensor Research Center (SBRC) and Nanoscience & Nanotechnology Research Center (NNRC), Faculty of Science, Razi University, Kermanshah, Iran, 4- Endocrinology and Metabolism Research Center, Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran

Digoxin has been used as a cardiac glycoside drug in the treatment of various heart conditions. Because it is a toxic drug, it should be regularly monitored in the serum of patients under treatment. In this study, colloidal nanogold is synthesized and also mouse monoclonal anti-digoxin antibody (clone DI-22) with high affinity constant and low cross reactivity is used for the preparation of nanogold–labeled monoclonal antibody probe to digoxin under optimal conditions. Combination of antibody with nanogold particles was also characterized by UV-visible (UV-vis) light absorption spectra, transmission electron microscopy (TEM), and enzyme-linked immunosorbent assay (ELISA) and stability measurements. In addition, an immunochromatographic (IC) method for digoxin analysis employing nanogold-labeled probe is developed. With this technique, it requires only 10 min to complete the quantitative detection of digoxin. The sensitivity to digoxin was about 2ng/ml by naked eyes, which is within the therapeutic and toxic ranges of digoxin. The results of serum samples obtained by IC strip were in agreement with those obtained by RIA. The IC strip was sufficiently sensitive and accurate to be used for the rapid detection of digoxin in serum samples.

**Keywords**: digoxin, immunochromatography, colloidal nanogold, monoclonal antibody

---

**P-10-646-1**

**Production and purification of lentiviral vectors containing HER-2 nonobody gene**

*Masoud Nasri [1]*, Mohammad Javad Rasaee [1], M. Rajabi Bazl [2], M. Navari [2], F. Sheikholeslami [2]*

1- Department of Medical Biotechnology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 2- Department of Clinical Biochemistry, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Viral vectors are valuable tool to deliver genetic materials into cells. Vectors derived from Human Immunodeficiency Virus type 1 (HIV-1) are being widely used for gene delivery, principally because they are able to transduce both dividing and non-dividing cells and results in stable and long term gene expression. In addition these types of vectors are safe, has low toxicity, high stability and cell type specificity. In order to produce lentiviral vectors we used three plasmids system in which the gene of interest (HER-2 nanobody) was cloned in vector plasmid pWPXLd. This vector plasmid along with packaging plasmids, psPAX2 and envelope plasmid pMD2.G, was co-transfected into packaging cell line (293T) using optimized calcium phosphate method. After 36-48 h post transfection the supernatant of 293T cells containing viral vectors were harvested and stored at - 80 degree for further analysis and experiments. Titration was performed by P24 ELISA on supernatant and the product was concentrated by polyethylene glycol 6000, the efficiency of transduction was determined using quantitative real-time PCR and the presence of target gene in transformed cells were confirmed by PCR.

**Keywords**: lentiviral vectors, 293T, calcium phosphate trasfection, gene delivery, HER-2 nanobody

---

**P-11-656-1**

**Curcumin release in alginate/pectin beads for stomach, intestine and colon buffers**

*Parviz Bazzi [1], Samineh Sadat Pourtakdoost [1]*, Naghmeh Sattarahmady [2], Ali Akbar Moosavi-Movahedi [2]*

1- Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran, 2- Department of Biochemistry, Shiraz University of Medical Sciences, Shiraz, Iran

Curcumin, the newest nutraceutical agent for treatment of colon cancer, is isolated from the rhizome of curcuma longa. Unfortunately, its usage has been limited, because of water insolubility and low bioavailability. Through this study, polysaccharide beads were used as carrier and entrapping curcumin to development usage of curcumin as a drug of colon cancer. These beads were prepared by dispersing curcumin in a mix solution of sodium alginate/pectin (100/0, 75/25 and 50/50 %), then dropping the dispersion into calcium chloride solution and using glutaraldehyde as hardening agent. Releasing curcumin from these beads under condition mimicking stomach to colon transit was evaluated in the presence of pectinolytic enzymes. Enzymes within the human gastrointestinal tract do not have effect on digestion of pectin; digestion of it needs intestinal flora including pectinolytic enzymes. The results show that bead with 100 % alginate composition and without pectin has the highest release in all buffer solutions (stomach, intestine and colon buffers). But a bead with 50/50 % alginate/pectin composition has little curcumin release in stomach and intestine buffer, but has releasing of curcumin in colon buffer. Therefore, beads with 50/50 % alginate/pectin composition could be a useful carrier for controlled release of curcumin for colon cancer treatment.

**Keywords**: curcumin, bead, alginate/pectin, nanobiotechnology

---

**O-11-680-1**

**Spectroscopic studies on the interaction of human hemoglobin and copper nanoparticles**

*Samaneh Zolghadri* , Ali Akbar Saboury*

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

The interaction between human hemoglobin (Hb) and copper nanoparticles (CuNP) was investigated by UV/visible and fluorescence spectroscopies under physiological pH (7.43) and room temperature (25°C). UV/visible spectroscopy shows a change in the absorbtion spectrum of Hb with increasing concentrations of CuNP. As the concentration of CuNP increased the maximum absorption of the soret band decreased and the maximum absorption wavelength of the soret band shifted to a higher wavelength. The UV/visible spectra show a clear isosbestic point at 366nm upon addition of the CuNP. This indicates the formation of a ground stated complex between Hb and

CuNP. Fluorescence data revealed that the fluorescence quenching of Hb by CuNP was the result of CuNP-Hb complex formation.

**Keywords**: human hemoglobin, copper nanoparticles, spectroscopy

---

**P-11-609-1**

**Uptake and cytotoxicity of gold nanoparticles synthesized in photochemical method on human neural cells and fibroblasts**

*Tahereh Javdani Khalife [1]*, Gholam Hossein Riazi [1], Shahin Ahmadian [1], Mohammad Ali Shokrghozar [2]*

1- Institute of Biochemistry and Biophysics (IBB), Tehran University, Tehran, Iran, 2- National cell Bank of Iran (NCBI), Pasteur institute, Tehran Iran

Gold nanoparticles (GNPs) nowadays are widely used in the emerging and highly interdisciplinary field of nanotechnology and it is high controversial in the field of diagnosis. Nanoparticles of gold, which are in the size range 10–100 nm, undergo a plasmon resonance with light which is one of the reasons of its usage. Biocompatibility, toxicity and the ability of gold nanoparticle to penetrate cells are three critical factors that will determine the utility of them in clinical applications. In this study the ability of cell uptake and cytotoxicity of GNPs have been studied. At the first step GNPs in small size and in biological PH with non intrusive agents were synthesized in photochemical synthesis way. The GNPs uptake studies on human neural cells (SK-NM-C and SK-BE (2)) and fibroblasts (L929). Uptake of nanogold particles was followed by Transmission Electron Microscopy (TEM). The MTT assay was used to assess cell viability after 24 and 48 h of incubation with nanoparticles at concentrations that ranged from 50 to 500 nm. Also the morphology of the cells during the incubation was followed too. As a result the GNPs in the above concentration ranges showed significant toxicity in both neural cell lines and fibroblast. In addition the morphology of human neuroblastoma cells changed and showed completely different shape from the normal ones. According to our cell culture results nanogold particles have a potential to be an anti cancer drug and more studies in this GNP is still needed.

**Keywords**: cytotoxicity, gold nanaoparticle (GNP), MTT assay, uptake

---

**P-10-741-1**

**A novel metal-enzyme nanobioconjugate for application in biosensors, medicine, and biological systems**

*Afshin Mohsenifar [1]*, Nasrin Hashemifard [2], Bijan Ranjbar [2], Abdolamir Allameh [2], Abbas Lotfi [2], Batool Etemadikia [2]*

1- Tarbiat Modares University, Tehran, Iran, 2- Sciences-Research Branch of Azad University, Tehran, Iran

Aim of the Study was immobilization of Glucose Oxidase by silver nanoparticles for application in biosensors, medicine and biological systems. Kinetic and stability of the immobilized enzyme in different pH range and temperatures were investigated. In this study, silver nanoparticles were synthesized by reduction of silver nitrate by NaBH4. Carbohydrate moieties of the enzyme were oxidized with periodic acid and then dialyzed. Oxidized enzyme was reacted with 6-aminohexanoic acid. Then Ag nanoparticles were added to GOX-6-aminohexanoic acid and stirred gently until enzyme immobilized on the Ag nanoparticles. Transmission electron microscope examination showed that the average size of nanoparticles is about 10-20 nm. Enzymatic activity of free, oxidized and immobilized GOX decreased similarly, by increasing temperature. No enzymatic activity was shown after 15min incubation in more than 90 degree centigrade in all three samples. Samples showed the lowest enzyme activity at very acidic pH because partially denaturation of the enzyme or coenzyme extraction from the enzyme in acidic pH. The storage stability of free, oxidized and immobilized GOX showed that AgNPs-GOX nanobioconjugate was very stable comparing with free and oxidized GOX. The kinetic studies indicated that catalytic activity of immobilized enzyme increased considerably, relative to free and oxidized GOX (km of free, oxidized and immobilized GOX: 5.31, 6.64 and 4.16 respectively). This nanobioconjugate showed remarkably storage stability and good resistance against a wide range temperature and pH in comparison with free and oxidized forms. The main goal of this study was immobilization of GOX on silver nanoparticles as a novel nanobioconjugate for application in biosensors, medicine, and biological systems.

**Keywords**: silver nanoparticles, nanobioconjugate, biosensors, immobilized GOX

---

**O-10-623-1**

**Inhibition of cornea angiogenesis by small interference RNA targeting vascular endothelial growth factor receptors**

*Moslem Jafari Sani [1]*, Ali Zaree Mahmoudabady [2]*

1- Biochemistry Department Faculty of Medicine, Baqiyatallah University, Tehran, Iran, 2- Biochemical Department and Molecular Biology Research Center, Baqiyatallah University, Tehran, Iran

Cornea neovascularization (NV) occurs in various ocular disorders including proliferate diabetic retinopathy, retinopathy of prematurity and secondary neovascular glaucoma and chemical injuries which often result in blindness. Frequently vascular endothelial cell growth factors (VEGFs) are mainly responsible for the pathological neovascularization. Therefore, VEGF & VEGF Receptors (VEGFRs) could provide targets for therapeutic intervention on Cornea NV. SiRNAs targeting VEGFA, VEGFR1 and VEGFR2 were shown to significantly inhibit neovascularization when given locally or systemically. In this study, we inspected the inhibitory effects of the specific small interfering RNAs (siRNA) targeting VEGFRs (VEGFR1-2) mixed together on the expression of VEGFRs in human umbilical vein endothelial cells (HUVEC) in vitro. Three different SiRNAs against the VEGFR1 and VEGFR2 were designed and chemically synthesized. Using GFP expression vector the specificity of SiRNAs were investigated in HeLa cell and the suitable SiRNA which significantly silenced the target mRNA were transfected HUVEC cell using lipfecthaminth 2000. 24h after transfection the cells were harvested ant total RNA purified. RT-PCR and PCR Using VEGFR1 and VEGFR2 specific primers were done and PCR products were electrophoreses. The result has shown significantly decreased in VEGFR1 and VEGFR2 mRNA. The clinical examination shown significantly reduce in corneal neovascularization these results indicate that VEGFRs-specific siRNAs together can be use as a suitable therapy against corneal neovascularization in eye diseases.

**Keywords**: angiogenesis, cornea, siRNA, VEGFR, HUVEC

---

**P-10-767-1**
**Fabrication of cobalt nanoparticles in the cage-shaped protein, apoferritin**

*Soheila Kashanian [1]*, Ronak Rafipour [2], Fereshteh Abasi Tarighat [3]*

1- Department of Chemistry & Sensor and Biosensor Research Center (SBRC), Faculty of Science, Razi University, Kermanshah, Iran, 2- Department of Chemistry, Faculty of Science, Razi University, Kermanshah, Iran, 3- Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

Nanoparticles (NPs) are attracting researchers' interest as the key material of nanotechnology. There were reports of artificial synthesis of metal oxide, metal alloy, or inorganic NPs using the protein cage, apoferritin, as bio-template. In this method NPs are both size and shape constrained by the inner volume of the protein cage. Horse spleen apoferritin (HsAFr) is a semispherical protein shell with an outer diameter of 12 nm and inner cavity of 8 nm. In this report, we synthesized cobalt NPs in horse spleen apoferritin (HsAFr) using H2O2 as the oxidant agent at pH 7.5 and 8.5. The products of mineralization were characterized by transmission electron microscopy (TEM), UV/Vis spectroscopy, and native polyacrylamide gel electrophoresis (PAGE). The UV-Visible absorption spectra showed well defined peak at around 350nm (corresponding to Co3+). Electrophoresis data supports our assertion that mineralization occurred in a spatially selective manner and that insignificant amount of cobalt deposited on the outer surface of the protein. NP-ferritin sample were stained with uranyl acetate to show the presence of the intact protein surrounding the mineral. Precipitate formation in ferritin containing solutions at pH 7.5 was more than that at pH 8.5. Many amino acid residues at pH 8.5 possess a negative charge and are exposed on the inside surface of the apoferritin, thus Co2+ ions are led to the apoferritin. Therefore, cobalt NPs can be easily formed in the apoferritin at this pH.

**Keywords**: nanotechnology, nanoparticle, apoferritin, cage

---

**P-11-772-1**
**Cytotoxicity and spectroscopic studies of silver nanoparticle: A biophysical approach to nanaotoxicology**

*Mahdie Rahban [1]*, Adeleh Divsalar [2], Ali Akbar Saboury [2]*

1- Institute of Biochemistry and Biophysics, University of Tehran, 2- Department of Biological Sciences, Tarbiat Moallem University, Tehran, Iran

Silver nanoparticles are being used increasingly in wound dressings, catheters, and various household products due to their antimicrobial activity. Particle size may be a critical parameter for nanomaterial bioactivity, but it is difficult to ascertain which parameter plays an essential role in the biological effects when concerning various types of nanoparticles with different shapes and composition. In present study, we have investigated the biological effects of silver nanoparticle against informational molecule of calf thymus DNA, and model cancer cell line of K562. Thermal denaturation studies of DNA in the presence of different concentrations of silver nanoparticle represented that silver nanoparticle could increase the thermal stability of DNA. Also, intercalation studies of silver nanoparticle were measured by ethidium bromide. Fluorescence intensity of DNA intercalated ethidium reveled that; ethidium is removed from the duplexes of DNA by the action of silver nanoparticle. Cytotoxicity of this nanoparticle was determined using MTT assay against K562 cell line after 24 h incubation time. The 50% cytotoxic concentration of silver nanoparticle (Cc50) was determined. This data have shown that cell growing after 24 h was significantly reduced in the presence of different concentration of nanoparticle. From these results, it can be concluded that silver nanoparticles could able change DNA structure and inhibited growth of K562 cell line.

**Keywords**: silver nanoparticle, cytotoxic, DNA, intercalation

---

**P-11-764-1**
**Chitosan nanoparticles containing salicylic acid as a model for an oral drug delivery system**

*Raheleh Safaeijavan [1], Mojtaba Taghizadeh [2]*, Ali Akbar Saboury [3], Adeleh Divsalar [4]*

1- Science and Research Branch, Islamic Azad University, 2- Novel Drug Delivery Systems Department, Science Faculty, Iran Polymer & Petrochemical Institute, 3- Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran, 4- Department of Biological Sciences, Tarbiat Moallem University, Tehran, Iran

Nanoparticulate delivery systems have the potential to improve drug stability, increase the duration of the therapeutic effect and permit administration through non-parental routes. In the present study, we have investigated the biocompatible and biodegradable nanoparticulate drug delivery system to increase the efficiency and bioavailability of oral drugs. Nanoparticles containing Salicylic acid were prepared by emulsion solvent diffusion "in oil" technique. Particle size distribution was examined by laser light scattering. Scanning electron microscope (SEM) was utilized to observe the surface characteristics and the morphology of nanoparticles. The drug released and content was examined by UV spectroscopy. The effects of different initial drug loading on the drug content and released and swelling behavior were investigated. The laser light scattering studies demonstrated when the stirrer sped up and the aqueous phase added in smaller droplets into oil phase could affect on particles size distribution, such away the peak of distribution was moved from 700 nm to 300 nm. SEM observations represented that the particles morphology was spherical and the surfaces were smooth. The drug content was in the range of 20-35 %. All the nanoparticles had a burst release initially and the burst release behavior of them was related to the initial drug loadings. From a above results, it can be concluded that we have successfully prepared chitosan nanoparticles containing maximum 35% (w/w) of SA with emulsion solvent diffusion "in oil" method and evaluated their morphology, drug content and drug release.

**Keywords**: chitosan, nanoparticles, oral drug delivery, salicylic acid

---

**O-10-806-1**
**Anti-MUC1 nanobody targeted PEI nanoparticles for suicide gene delivery**

*Elham Sadeqzadeh [1]*, Fatemeh Rahbarizadeh [1], Mohammad Javad Rasaee [1], Moein Moghimi [2]*

1- Department of Medical Biotechnology, Tarbiat Modares University, Tehran, Iran, 2- Universitatsparken 2 DK2100 Cpenhagen, Denmark

One of the big challenges in gene delivery treatments is finding a good vector for gene delivery. Poly-ehtylenimine (PEI), a molecule having the highest positive surface charge, has been used in a large number of studies for this purpose. Its high positive charge results in good condensation of the DNA to be delivered to cells, and also easy entry

inside cells. The matter of gene delivery to target cells, without affecting non-target cells is also of utmost importance resulting in a lower toxicity for normal cells. Some antibodies have been used to this end, but as they are immunogenic, single chain domain antibodies in camelidae, which consist of only one chain of the variable heavy chain (VHH) can be an alternative. In this study, for the first time, we attached an anti-MUC1 VHH, isolated from a phagemeid library, on PEI nanoparticles to deliver a suicide gene construct consisting of the gene coding tBid and some regulating structures inside the cell. The polyplex was tested on 6 different cell lines, with different expression levels of MUC1. Results showed that the presence of the ant-MUC1 nanobody on the surface of PEI polyplexes had been effective in delivering the gene construct mostly to MUC1 expressing cell lines checked. It can be deducted that this targeted PEI nanoparticle can be used as a means of targeted gene delivery to cells expressing MUC1.

**Keywords**: nanoparticle, PEI, nanobody, targeted delivery, gene delivery

---

**P-10-815-1**

**Cell selection and characterization of a novel human endothelial cell specific nanobody**

*Davoud Ahmadvand [1]*, Mohamad Javad Rasaee [1], Fatemeh Rahbarizadeh [2], Roland E. Kontermann [3]*

1- Department of Clinical Biochemistry, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 2- Department of Medical Biotechnology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 3- Institut für Zellbiologie und Immunologie, Universität Stuttgart, Allmandring, Germany

Antibody-based targeting of angiogenesis and vascular targeting therapy of cancer are extremely attractive conceptually and open new important diagnostic and therapeutic opportunities. Compelling evidence suggests that CD105 represents an ideal target for antiangiogenic therapy and its presence in solid tumor vasculature has prognostic value. Camelids produce functional antibodies devoid of light chains and constant heavy chain domain (CH1). Nanobodies, the antigen binding fragments of such heavy chain antibodies, are therefore comprised in one single domain. The aim of this study was to explore the possibilities of using anti-endoglin nanobody as an angiogenesis inhibitor. The anti-CD105 nanobody (AR-86a) was isolated from immune library by selections on purified antigens and target cells. Immunocytochemistry and FACS analysis showed that the purified nanobody reacted specifically with human umbilical vein endothelial cells (HUVECs) but not with other cell lines such as MDA-MB-453, Mel III, T-47D, MCF-7, AGO and HT 29. Further, selected nanobody potently inhibited proliferation of human endothelial cells and formation of capillary-like structures. This selected high affinity anti-endoglin nanobody may offer high specificity towards tumors with reduced side effects, and may be less likely to elicit drug resistance compared to conventional therapy.

**Keywords**: nanobody, endoglin (CD105), angiogenesis, cancer, camelid

---

**P-10-812-1**

**Enrichment of two-humped camel nanobody gene library by panning against human ErbB2/Fc chimera and breast cancer cells**

*Fatemeh Rahimi [1]*, Fatemeh Rahbarizadeh [2], Mohammad Ali Shokrgozar [2], Fereidoun Mahboudi [2], Davoud Ahmadvand [3]*

1- Pasteur Institute of Iran, Tehran, Iran,
2- Tarbiat Modares University, Tehran, Iran,
3- Tarbiat Modares University, Tehran, Iran

HER2 is a tumor associated antigen, which can be a potential target on the surface of breast cancer cells with diagnostic and therapeutic usage. This study carried out to enrich a phagmid library aiming at finding clones with a relatively high affinity for HER2. Polyclonal library phagmids were multiplied in TG1 bacteria. Panning and screening were carried out by means of recombinant human ErbB2/Fc chimera which was coated on ELISA wells. Parallel selection was done by breast cancer cells (whole cell panning) which used to separate polyclonal phagmids with the highest affinity for the mentioned antigen. Concordance of binding specificity to antigen and tumor cells between phage clones and parent antibodies was analyzed. The phage of positive clones was identified with competitive ELISA, and transferred to E. coli TG1 to express nanobody (VHH). The increase in the number of output clones from the first till the fifth pannings and the rise in the difference of OD450 compared to negative control (BSA and NIH3T3 for antigen and whole cell panning, respectively) in phage ELISA and the results of monoclonal phage ELISA confirmed that the correctness of the process enrichment of the specific phagemids.

**Keywords**: nanobody, panning, HER2, two-hamped camel

---

**P-10-244-1**

**Development of a colloidal gold-based immunochromatographic test strip for the screening of microalbuminuria**

*Kobra Omidfar [1]*, Solmaz Kia [2], Susan Kashanian [2], Maliihe Paknejad [3], Bagher Larijani [3]*

1- Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, 2- Department of Biology, School of Sciences, Razie University, Kermanshah, Iran, 3- Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

A rapid immunochromatography assay based on antibody-colloidal gold nanoparticles specific to human serum albumin was developed and evaluated its applications for primary screening of HSA in the urine. A monoclonal antibody specific to HSA was produced from the cloned hybridoma cell (EMRC3) and used to develop an ICG strip. The nanocolloidal gold, with an average particle diameter of 20 nm was synthesized and labeled to mAb as the detection reagent. Antibody colloidal gold probe was applied on the conjugate pad and HSA antigen was immobilized to a nitrocellulose membrane as the capture reagent to prepare the ICG strip test. This test required only ten min to accomplish semiquantitative detection of the albumin. The sensitivity to urinary albumin was found to be around 20µg/ml. In order to examine the reliability of the testing procedures, we carried out the ICG strip test with 40 urine sample and compared the results of these test with those obtained by immunoturbidimetry. The ICG strip was

adequately sensitive and accurate for rapid screening of HSA in the urine.

**Keywords**: Human serum albumin, gold nanoparticles, immunochromatographic assay, microalbuminuria

---

**P-10-740-2**

**Agents generated analysis in sterilization of the conical reactor atmospheric plasma by Optical Emission Spectroscopy**

*Farshad Sohbatzadeh [1]*, Abasalt Hosseinzadeh Colagar [2], Saeid Mirzanejhad [2], Somayeh Mahmodi [3]*

1- Physics Department, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran, 2- Biology Department, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran, 3- Nano and Biotechnology Research Group, University of Mazandaran, Babolsar, Iran

The use of non-thermal plasmas for sterilization is a relatively new method. In pervious study, we have designed conical reactor as a plasma nozzle and generated atmospheric pressure dielectric barrier discharge in Oxygen excited with AC power at 50 HZ and 5400 volt (rms value). Non-thermal plasma afterglow were used to sterilize of Luria Bertini broth media suspensions from gram-negative (e.g: Escherichia coli and Pseudomonas aeruginosa) and gram-positive (e.g.: Bacillus cereus) bacteria in final concentrations of OD600nm=0.25 McFarland standard. Minimum time of afterglow for complete sterilization of P. aeruginosa, E. coli and B.cereus were 10, 15 and 15 min., respectively. For analysis of the relationship between sterilization results and chemical species generated in the discharge, a diagnostic technique based on optical emission spectroscopy has been applied to the discharge gas in afterglow region. Emission spectra checked and showed that Ozone molecules and atomic Oxygen were the dominant germicidal species, and germicidal effect of UV radiation in afterglow Oxygen plasma is feebleness. We recommended that afterglow non-thermal plasma is very effective for killing microbial strains and reactive species including Ozone and Oxygen radical play the major role in bactericidal.

**Keywords**: atmospheric plasma, emission spectra, ozone, sterilization

---

**P-10-863-1**

**Selection of anti-TAG72 single-domain antibodies from a Camelus Dromedarius nanobody gene library**

*Zahra Sharifzadeh [1]*, Fatemeh Rabarizadeh [2], Mohammad Ali Shokrgozar [1], Fereidoun Mahboudi [1], Davoood Ahmadvand [1]*

1- Pasteur Institute of Iran, Tehran, Iran, 2- Tarbiat Modares University, Tehran ,Iran

The tumor-associated glycoprotein (TAG)-72 is expressed on the cells of a majority of human carcinomas, including adenocarcinoma, colorectal, gastric, pancreatic, breast, lung and ovarian carcinomas and can be a potential target on the surface of these cells. The aim of this study was the enrichment of a Camelus dromedarius nanobody gene library by biopanning and cell-panning to find some clones with a relatively high affinity for TAG-72 antigen. Phagmids, from a polyclonal library, were amplified in E. coli. TAG-72 antigen which was coated on ELISA wells and LS-174T cells (TAG-72 expressing cells) were used to the enrichment of the phagmids that include anti-TAG72 nanobody. In order to make sure of the reliability of the results, a comparison between the titer of input and output phagmids and also phage ELISA was carried out. An increase in the number of output clones from the first till the fifth panning and the rise in the difference of OD450 compared to negative control (BSA) in phage ELISA shows the accuracy of the enrichment process of the specific phagmids. These anti-TAG72 nanobodies may be useful in diagnostic and therapeutic applications.

**Keywords**: nanobody, panning, TAG-72

---

**P-11-887-1**

**Laser based EMFA Technique as a tool for blood pressure simulation in vitro: Determination of blood pressure relaxing mechanisms in human endothelial cells**

*Shamci Monajembashi*, Karl Otto Greulich, Gabi Günther, Silke Schulz*

Leibniz Institute for Age Research, Fritz-Lipmann Institute, Beutenbergstraße 11, 07745 Jena, Germany

Erythrocyte Mediated Force Application technique (EMFA), a variant of optical tweezers, is used to exert a vertical pressure on cells using erythrocytes as pure force transducers. This simulates pressure on blood vessel cells much better than techniques available so far. High chronic blood pressure (Hypertension) is worldwide the endemic disease No.1, and a major cause of human deaths. In response to hypertension, vascular endothelial cells which act as primary sensors of the blood pressure and blood flow, produce vasoactive substances such as nitric oxide (NO) in order to induce the relaxation of muscle cells of the vessel wall. Understanding signal transduction in blood pressure research is the basis for new drugs. We demonstrate that the pressure-induced morphological response of endothelial cells was dependent on external Ca2+. Calcium antagonists (CAs) are important drugs for the treatment of hypertension. They induce vascular relaxation of smooth muscle cells via inhibition of the calcium influx. It has become evident that parts of these cardiovascular protective actions of some CAs are dependent on the vascular endothelium. Using EMFA provides evidence that different classic CAs (Verapamil, Nifedipine, and Amlidipine) have different effects concerning NO bioavailability on endothelial cells.

**Keywords**: hypertension, optical tweezers, nitric oxide, calcium antagonists

---

**P-10-1000-2**

**Assessing the dynamic range and peak capacity of nanoflow LC-FAIMS-MS on an ion trap mass spectrometer for proteomics**

*Saeed Yari [1]* , Maryam Avar [1] , Ali Majdi [2] , Seyed Majid Madani [2]*

1- IAU of Tonekabon-Cellular Group, 2- IAU of Tonekabon-Microbiology Group, Tonekabon, Iran

Proteomics experiments on complex mixtures have benefited greatly from the advent of fast-scanning ion trap mass spectrometers. However, the complexity and dynamic range of mixtures analyzed using shotgun proteomics is still beyond what can be sampled by data-dependent acquisition. Furthermore, the total liquid chromatography-mass spectrometry (LC-MS) peak capacity is not sufficient to resolve the precursors within these mixtures, let alone acquire tandem mass spectra on all of them. The research describes the application of a

high-field asymmetric waveform ion mobility spectrometry (FAIMS) device as an interface to an ion trap mass spectrometer. The dynamic range and peak capacity of the nanoflow LC-FAIMS-MS analysis was assessed using a complex tryptic digest of S. cerevisiae proteins. By adding this relatively simple device to the front of the mass spectrometer, an increase in peak capacity >8-fold and an increase in dynamic range of >5-fold, without increasing the length of the LC-MS analysis was obtained. Thus, the addition of FAIMS to the front of a table-top mass spectrometer can obtain the peak capacity of multidimensional protein identification technology (MudPIT) while increasing the throughput by a factor of 12.

**Keywords**: proteomics, spectrometry, nanoflow

---

**P-10-1011-1**

**Production and epitope mapping of Anti-MUC1 single domain heavy chain recombinant nanobody VHH (MR86)**

*Mohammad Mohammadi [1]*, Mohammad Javad Rasaee [2], Sara Mohammadzadeh [2], Masoumeh Rajabi Bazl [2]*

1- Department of Biology, Faculty of Sciences, Shahid Chamran University Ahvaz, Iran, 2- Department of Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

By using phage display technology we generated anti-MUC1 single domain heavy chain recombinant nanobody VHH (MR86) against VNTR of MUC1. The library was generated from a repertoire of the single domain heavy chain gene antibody from immunized camel (camelus dromedaries) and pComb3x phagemid. Here, we characterized the binding site of this antibody on MUC1 by two different eppitope mapping techniques, PEPSCAN and Phage-display peptide library. MR86 showed an activity with synthetic 20 amino acid peptide of MUC1 and purified MUC1 from the cancerous tissue. Epitope mapping of this antibody by PEPSCAN revealed a minimal consensus binding sequence, PPAH, which is found on MUC1 peptide as the most important epitope. Results of phage display peptide library showed that PXH was the important motif for MR86 binding which X is the small amino acid and motif PGRGPPSHLPIL is the mimotope. With regard to the results of these two methods motifs GPAH, GPPSH, PPHA and PCHI probably had major roles in binding of MR86 antibody and proline and histidine were the key amino acids.

**Keywords**: epitope mapping, MUC1, PEPSCAN, phage display, VHH

---

**O-10-1031-1**

**Preparation and characterization of surface - modified solid lipid nanoparticles as a drug carrier system**

*Roghayeh Abbasalipourkabir [2]*, Mazna Ismail [1], Shaidan Radmin [2], Arifah Abdulkadir [2], Rasedee Abdullah [2]*

1- UPM-Malaysia, 2- UKM-Malaysia

Recently it has become more evident that the development of new drug alone is not sufficient to ensure progress in drug therapy. Solid lipid nanoparticles (SLNs) as particulate systems for parenteral drug administration with mean particle diameters ranging from 50 up to 1000 nm introduced in 1990. SLNs have been proposed as an alternative colloidal drug delivery system to polymer nanoparticles, emulsions and liposomes. The aim of this study was development and characterization of surface-modified SLN using varieties of emulsifier for encapsulation of the drug with poor water solubility. In this study variation of surface properties of solid lipid nanoparticles were prepared based on palm oil by high pressure homogenization method. Then SLNs were characterized and the optimum stability factors for one year stability were determined. Variations in SLN composition resulted in particle size between 168nm and 322nm and zeta potentials between -10.4mV and -26.3mV. Thermoanalysis and X-ray diffraction revealed crystalline particles. Electron microscopy showed that particles have round and uniform shapes. The SLN dimensional data obtained by different techniques, suggest the surface –modified SLN bearing high quality physicochemical characteristics with property of lipophilic drugs loading. The results of this study showed that SLN may improve formulation of poorly soluble drugs.

**Keywords**: colloidal system, drug delivery, physicochemical characterization, solid lipid nanoparticles

---

**O-10-1057-1**

**Development of transgenic chicken containing fluonanobody**

*Masoumeh Rajabi Bazl [1]* , Mohammad Javad Rasaee [2] , Mehdi Forouzandeh [2] , Mohammad Mohammadi [3] , Azam Rahimpour [3] , Mohsen Navari [3] , Mahmoud Naderi [3]*

1- Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 2- Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, 3- Department of Biology, Faculty of Basic Sciences, Chamran University, Ahwaz, Iran

Transgenic animals are potentially a source of producing economical therapeutic proteins. Germ cells are used in gene transfer technology by applying biological vectors such as viruses. VHH small part of camel heavy chain antibody is considered as a magic bullet in cancer therapy. Our goal in this study is to transfer VHH gene into the egg cells in the germinal region. In order to make a secreted protein, lysozomal signal was added to VHH gene using SOE-PCR technique. For in vivo imaging VHH along with Lysozyme signal were fused to GFP reporter gene. Viral vectors such as retro and lentiviral containing Fluonanobody (Lysozyme signal–VHH –GFP) were used to transfer VHH gene into the cells. To introduce the transgene, viral packaging with specific packaging cell lines and helper vectors were performed. Obtained viral particles were injected to laid embryos at the blastodermal X stage, and the embryos were hatched in vitro to generate G0 birds. The VHH-GFP sequence was detected in the blood and different tissues of all G0 birds. VHH gene was detected in some tissues of manipulated chickens by real time PCR. In conclusion; we report here the possibility of production of transgenic chicken by using recombinant virus technology.

**Keywords**: VHH, GFP, recombinant virus, transgenic animal

---

**P-10-1002-1**

**Introductory studies on organophosphate hydrolase enzyme acquired from species of bacteria present in agricultural soils in North of Iran**

*Mehdi Yazdani [1]*, Abbas Lotfi [1], Morteza Sattari [2], Afshin Mohsenifar [3]*

1- Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, 2- Department of Microbiology Tarbiat Modares University, Tehran, 3- Department of Toxicology, Tarbiat Modares University, Tehran, Iran

The aim of this study is the acquisition of Organophosphate Hydrolase enzyme from bacteria present in farms treated with organophosphate toxins specially Diazinon. 35 samples of soil and water were collected from farms of North of Iran. Soil samples were taken into mineral base medium containing Diazinon. After a week, by passaging on nutrient agar culture medium, 53 colonies of bacteria were isolated and purified. The purified bacteria were passaged as single colonies on mineral base plate containing Diazinon. Observation of a clear hydrolyzed bond around the colony indicates the presence of OPH enzyme in the bacteria. 13 colonies had hydrolyzed bonds with diameters of 0.9 to 2cm after 5 days, and the rest lacked a hydrolyzed bond. Two bacteria which had the highest hydrolyzed bonds were passaged on a mineral base medium containing Diazinon of one liter. After one week, the culture medium and the primary extraction of protein from bacteria were used for studying the activity of OPH enzyme. In order to study the activity of enzyme, Methyl-Parathion substrate in 50 mmol/L Tris-CL (pH=8) buffer was used. The hydrolysis of Methyl-Parathion by the enzyme caused the freeing of p-itrophenol, which could be measured at 410 nm.The activity of the enzyme was 100 IU/L. Considering the good activity of the enzyme which was found in Iran, it is suggested that the present species and the enzyme, be used in systems measuring organophosphate toxins, in design of biosensors, and reactors of sewage refinery, and environment cleaning.

**Keywords**: Diazinon, methyl parathion, organophosphate hydrolase

---

**P-10-1135-1**

**DFT study of CNT interaction by DNA simulated at polar solvents**

*Fatemeh Mollaamin [1]*, Behafarid Ghalandari [2]*

1- Department of Chemistry, Qom Branch, Islamic Azad University, Qom, Iran, 2- Science and Research Branch, Islamic Azad University, Tehran, Iran

The interaction between single-strand DNA (ssDNA) and single-walled carbon nanotubes (SWCNTs) is a subject of intense current interest. In this work, we have investigated interaction of ssDNA with open-end of SWCNT; using AMBER, MM+ and OPLS force fields in molecular mechanic (MM) method. We studied the effects of water and ethanol on ssDNA with SWCNTs, using these force fields too. By density functional theory (DFT) at the theoretical level of B3LYP/6-31G (d, p), we calculated interaction of ssDNA with open-end of SWCNT in vacuum, water and ethanol, then we made a comparison between dipole moment; energy and atomic charges in various environments. The quantum mechanics (QM) calculations were carried out by GAUSSIAN 98 program. Our results from density functional theory (DFT) and force fields calculations showed that interaction of ssDNA with open-end of SWCNT could be optimum in polar solvent albeit the changes obtained are very small. Our results have shown that the optimum level calculated this interaction in water and ethanol is near to vacuum value.

**Keywords**: DNA, SWCNTs, interaction, solvent effect

---