nonradioactive and can be quantified in a noninvasive manner. Recently, Renilla luciferase has been interested in clinical diagnosis based on in vivo and cell imaging. Here we report a new generation of IgGs targeting nanobioreporter which is containingRenilla luciferase and Fc binding peptide. Materials and Methods: DNA sequence encoding the luciferase-based nanobioreporter was amplified using specific primers. The PCR reaction was carried out by 32 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min and the final extension was performed at 72 °C for 10 min. Amplified products were separated on 1% agarose gel, and ~1kb long fragment were purified from the gel using the gel extraction kit (vivantis). cDNA fragments were then digested by Nhe I/HindIII and inserted into the digested pET21a. Competent cells of *E. coli* XL1-Blue were transformed by pET21-nanoRLuc construct using chemical method. Positive colonies were screened for Nono-probe, by PCR and sequencing. Expression by IPTG and purification by Ni-NTA Sepharose column was designed to achieve according to standard protocols and the purified probe was designed for binding to Fc IgGs. Finally, Bioluminescence signals of the nanoprobe from renilla luciferase were planned to measure with a luminometer apparatus. Results: Sequence encoding the RLuciferase-based nanobioreporter including the binding peptide for Fc reign of IgGs was amplified by PCR. From the sequence designed for the new probe, the nanobioreporter amplicon is about 1000 bp long and has an open reading frame of 330 amino acid residues. The construct obtained from the manipulated sequence is able to express an IgG-binding nonoprobe after expression in *E.coli* BL21. Moreover the probe, which can purify by IMAC method, shows the ability to bind the Fc region of IgG and produce a detectable signal in luminometer device. Conclusion: In this research a new reporter based on the Nanolight-technology was designed, assembled by PCR and developed for binding to IgGs. It seems that it is a useful tool for clinical diagnosis approaches based on IgG-technology. Moreover it seems that the new probe is a useful tool for future analysis in the field of in vivo imaging.

Keyword: IgG, Renilla luciferase, nanobioreporter, Imaging

**98. Production of ScFv Antibody Fragments Specific to CCK-BR/gastrin Receptor from a Semi-Synthetic Phage Antibody Library**

*Tohidkia M.R[1,4], Farashi Bonab S[2,4], Malekzadeh R[3], Asadi F[1*], Omidi Y[4*]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [2]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [3]Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran, [4]Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Background: It has been found that phage display technology is a powerful and widespread approach for production of monoclonal antibodies (mAbs) with therapeutic, diagnostic and targeting purposes. Cholecystokinin-B receptor (CCK-BR)/gastrin receptor, a member of GPCR superfamily, plays a crucial role in pathogenesis of several malignancies particularly gastric cancers and it has been confirmed that targeting CCK-BR with monoclonal antibody (mAb) seems to be a promising modality for gastric cancer therapy. The aim of this study was production and characterization of CCK-BR specific scFv (single-chain fragment variable) antibodies using a phage antibody library and solution-phase biopanning. Materials and Methods: A semi-synthetic phage scFv library was panned against a biotinylated peptide (BP) equal to the second extracellular loop of CCK-BR, subsequently phage-antibody binders were captured by streptavidin-coated magnetic beads. After four rounds of selection with 100 nM (rounds 1&2) and 50 nM (rounds 3&4) concentrations of BP, polyclonal phage ELISA was used for monitoring the progress of biopanning. Individual soluble antibody producing clones were obtained from the fourth round of selection and screened for their peptide-binding activity by ELISA. The ELISA-positive clones were sequenced and then analyzed for binding to native CCK-BR protein by Western blotting. Results: Polyclonal phage ELISA confirmed enrichment of peptide-specific phages with significant increasing signal after four rounds of selection (14 folds greater than round 1). Screening of 196 soluble antibody clones by ELISA revealed nine antibody fragments (4.68%) with binding activity to the peptide. Interestingly, sequencing of these soluble antibody fragments showed eight different scFv and one $V_L$ single domain. Five out of eight scFvs are able to detect a 80 kDa band protein corresponding to recombinant CCK-BR in Western blot analysis. Conclusion: The results of this study showed that phage display technology together with solution-phase biopanning can be a useful method for production of scFv antibody fragments specific to CCK-BR protein.

Keywords: ScFv, CCK-BR/gastrin Receptor, Phage Antibody Library

**99. Production of Nanobody (VHH) Derived Camelid Heavy Chain Antibody against PSMA Antigen in Prostate Cancer by Phage Dislay Technique**

Zare H[1*], Mousavi Gargari S.L[1], Rajabi BazlM[3], Bakherad H[1], Safaee Ardakani L[1]

[1]Biology Department, Basic Science Faculty, Shahed University, [2]Department of Clinical Biochemistry, Faculty of Medicine, Shaheed Beheshti University Of Medical Sciences

Background: Prostate cancer is the most cancer in men. Antibody therapy offers promise for cancer treatment. Antibody therapy depends on the identification of molecular targets (antigen). Prostate-specific membrane antigen (PSMA) is a potential molecular target in prostate cancer that abundantly expressed on prostate cancer epithelial cells. The interesting type antibody fragments find in *Camelidae* are VHHs. Antibody fragments could be displayed on the surface of filamentous phages, called phage-display. After displaying an antibody fragment on the protein surface of the phage, antigen specific phages can be selected and enriched by multiple rounds of affinity panning. Finally, monoclonal nanobody against PSMA was produced. Design and production of an epitope of PSMA antigen, production of immune camel nanobody phage library, screening of library against PSMA and finally production of monoclonal nanobody (VHH) against PSMA was performed in this study.
Materials and Methods: A DNA fragment encoding dominant epitope of PSMA was synthesized and expressed on *E.coli*. Camel was immunized with purified rPSMA. Following mRNA isolation and CH2 gene specific reverse transcription, two successive PCRs are performed. VHH fragments are cloned and displayed on phage for selection. Bound phages can be reinfected into bacteria and re-grown for further enrichment and eventually for analysis of binding. After phage Elisa and selection some clony, production of soluble nanobody was performed. Result and Conclusion: Purified rPSMA protein was confirmed by Western blot and SDS-PAGE. Amplification of VHH gene was confirmed with electrophoresis. Confirmation of VHH library was done with colony PCR. Increased affinity of library was confirmed with Elisa. Production of recombinant monoclonal nanobody (VHH) was confirmed by Western blot and SDS-PAGE. Affinity of nanobody against rPSMA was $5.7 \times 10^{-7}$. Respect to this point that this nanobody has high level of specifity and affinity, so it can be an effective tool for curing and diagnosing prostate cancer.

Keyword: Nanobody, VHH, Phage display, PSMA, Prostate cancer

**0. Alginate Nanoparticles: Noval Vaccine Delivery Systems for Infection Diseases**

*Saraei F[1], Mohamadpour Dounighi N[3], Zolfagharian H[3], Khaki P[1,2]

[1]Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran, [2]Department of Microbiology, Razi Vaccine &Serum Research Institute, Karaj, Iran, [3]Department of Venom and Human Sera, Razi Vaccine &Serum Research Institute, Karaj, Iran

Background: Today, nanotechnology as a multidisciplinary field covers sciences especially medicine sciences. Nanoparticles (NPs) as engineered structures with diameters of <100 nm produced by physicochemical processes. Among polymers, sodium alginate due to its unique properties (adjuvant, biodegradable and mucoadhesive polymer) has been used as carrier for different biological agents such as genes, drugs and antigens that cause to sustain release in the human body. The aims of the present study were to synthesis of DT-loaded nanoparticles as novel vaccine delivery system. Materials and Mathods: Alginate nanoparticles were prepared by ionic gelation technique. In order to manufacture of NPs, CaCl2 solution was added to sodium alginate solution dropwisly under homogenization. In different steps, influence of physicochemical factors such as different concentrations of polymer and CaCl2 solutions, homogenization time and stirring speed were studied. The NPs were characterized for their morphology and size distribution by SEM and DLS, respectively. Diphtheria toxoid (DT) was loaded in optimum NPs and loading efficiency and loading capacity were assessed. Invitro release profile of DT were investigated in (PBS, pH7.4, 37°C). The antigen activity was evaluated by double immunodiffusion test Results and Conclusion: Results showed that, concentration of 0.3%w/v polymer and 0.1%w/v CaCl2, stirring speed 1300 rpm and homogenization time 45min were obtained as optimum conditions that redounded to desirable size and