increase the absorption and dispersion of injected drugs. Hyaluronidase triggers the re-myelination of the affected axons through degradation of hyaluronan accumulated in inflammatory demyelinating lesions in the CNS. Accumulation of hyaluronan has been found to prevent the maturation of oligodendrocyte progenitors into myelinating cells in demyelinating lesions in MS. Materials and Methods: At first step, total mRNA was extracted from testis tissue and cDNA was synthesized. Ph20 coding sequence deleted GPI anchor was amplified by means of specific primers designed based on ph20 special CDS and also contained additional regionsencoding His tag and distinctive sequence recognized by entrokinase enzyme. Then, an amplified fragment was inserted into pTZ57R and treated by appropriate restriction enzyme to sub clone into pBudCE4.1. In this recombinant expression vector, attB region was added for insertion of this construct into genome by phiC31 integrase produced by another vector termed as pCMV-Int. Results: The constructed expression vector was confirmed successfully as verified by sequence analysis. After transfection, culture media was extracted and tested on Granulosa cells. The cell mass was separated effectively that indicate this protein is active. Conclusion: In this study, we produced an appropriate vehicle encoding recombinant hyaluronidase for therapeutic approach and recombinant protein for MS and infertility therapy.
Keywords: Hyaluronidase Type PH20, MS compound therapy

294. Engineering of Recombinant Nanobody against *Clostridium botulinum* Neurotoxin Type E by Error-prone PCR

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Background: Botulinum is one of the most toxic substrate acting on the peripheral nerve system causing acute flaccid paralysis. Rapid diagnosis and treatment of botulinum neurotoxins are crucial. Antibody products are being used for diagnosis as well as for the treatment of adults.VHHs are a new class of single-domain antigen binding fragments derived from heavy chain antibodies, found within sera of the camelidae. These antibodies have several characteristics like persistent to acid and alkaline pH, temperature, high solubility and ability to cross the stomach without loss of biological activity. In the present research in order to enrich the VHH library against binding domain of BONT/E, we introduced random mutations to the previously constructed gene using error- prone PCR. Materials and Methods: Error-prone PCR technique was applied with taq DNA polymerase which have no detectable 3′ to 5′ exonuclease proofreading activity and low-fidelity reaction. In order to increase the frequencies of mutation, non-standard PCR reaction conditions such as addition of Mn^{2+}, introduction of dITP, unbalanced dNTP levels, raising Mg^{2+} and taq DNA polymerase concentration and reduced template concentration were implemented. Results: DNA fragments obtained from PCR products with different reaction conditions were analyzed for their nucleotide sequences . The results showed varied rates of mutation (3%-48%). Mutagenic effect of Mn^{2+}, dITP and high concentration ofMg^{2+} on different PCR products were significant. Conclusion: In this study, we employed error-prone PCR to generate broad diversity in anti-toxin E nanobody gene. Our aim is to construct various random libraries for this nanobody gene. These libraries could be valuable sources for selection of high affinity anti-toxin E nanobody.
Keywords: Recombinant Nanobody, *Clostridium botulinum*, Neurotoxin Type E, Error-prone PCR

295. Preparation of Infusible Platelet Membrane Microvesicles and Evaluation of Sonication Effects on it

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Background: Platelets are the components of blood necessary for hemostasis and wound healing. At the most of blood transfusion centers, the shelf-life of platelet units are only 5 days. Due to short half-life of platelets, there have been a number of attempts to develop substitutes for platelets. One of the strategies for extending the storage time of platelets is preparation of platelet fragments to synthesizing artificial platelets from purified phospholipids and proteins. The aim of this study is to prepare Infusible Platelet Membrane (IPM) microvesicles and evaluation of sonication effects on it by measuring its particles size. Materials and Methods: Eight units of outdated platelets are collected from Tehran Blood Transfusion Center and the units were pooled and centrifuged at 1000 RPM, 15 min for removing WBC and RBC cells. Supernatant was centrifuged at 2500 RPM, 30 min to discard platelet poor plasma. For fragmentation of platelets, precipitate was resuspended in 25 ml of normal saline and freeze-thaw procedure was repeated three times at -80°C and 22°C respectively. The product was centrifuged at 2500 RPM, 30 min and precipitate was resuspended in 45 ml of normal saline and for viral inactivation, pasteurization method for 20 h at 60°C was applied. Particles size was measured before and after sonication by using Zetasizer, Malvern instrument. Results: The results showed that, before sonication of IPM, %66.5 of particles had 231 diameter (nm) and %33.5 of particles 1020 diameter (nm), meanwhile after sonication of IPM, %94.4 of particles had 232 diameter (nm) and %5.3 of particles 37.3 diameter (nm). Conclusion: With regard to previous studies, Cyplex company have been produced IPM microvesicles with average size of 700 nm. The reason of this selection of particle size was pharmaceutical effectiveness of IPM which is used in the treatment of bleeding due to thrombocytopenia. The results of this study shows that method of preparation of IPM without sonication is closer to 700 nm , in other words our method indicates that sonication causes more fragmentation of IPM microvesicles and so application of freeze-thaw procedure is sufficient to achieve optimal size particles.
Keywords: Infusible Platelet, Microvesicles, Sonication Effects

296. Homology Modelling and Comparison of A Recombinant Anti-Human CD4 Single-Chain Variable-Fragment Antibody with Native Antibody

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Background: Antibodies have diagnostics and therapeutics applications. Full size antibodies are more immunogenic and more difficult to manipulate compared to Single-chain variable fragments (scFv) of antibodies. scFv is a fusion protein of the variable regions of the heavy (VH) and light (VL) chains of immunoglobulin, connected with a short linker peptide. Materials and Methods: The scFv of a monoclonal mouse antibody against human CD4 was cloned from hybridoma cells using the phage display technique and was produced in recombinant form in *Escherichia coli*. Expression, production, and purification of anti-CD4 scFv was tested using SDS-PAGE, Western blotting, and the specificity of scFv for human CD4 molecule was examined using ELISA and flow cytometry. Results: A 31 kDa recombinant anti-CD4 scFv was expressed and produced successfully in bacteria. Sequence analysis proved the scFv structure of the construct. Recombinant scFv t was able to bind to CD4 with the affinity comparable to native original hybridomal anti-CD4 antibody as assessed by ELISA. The binding site of the antibody was domain 3 of the human CD4 molecule. The canonical structure of anti-CD4 scFv antibody was obtained using the SWISS_MODEL bioinformatics tool and compared to scFv general structure and native hybridomal anti-CD4 antibody. Conclusion: Recombinant scFv is able to bind to the CD4 molecule with affinity comparable to native antibody. Engineered anti-CD4 scFv could be used in immunological studies, including fluorochrome conjugation, bispecific antibody production, bifunctional protein synthesis, and other genetic engineering manipulations. Since the binding site of our product is domain 3 (D3) of the CD4 molecule which is different from the HIV binding domain of CD4 molecule (D1), further studies are needed to evaluate the anti-CD4 scFv potential for diagnostic and therapeutic applications.
Keywords: Recombinant Anti-Human CD4, Native Antibody, scFv

297. Luciferase-based Nanobioreporter, a Useful Tool for Targeting IgGs

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Background: Renilla luciferase, a monomeric 36kDa protein from Renilla reniformis, catalyzes coelenterazine oxidation to produce light. Post-translational modification is not required for its activity and the enzyme expresses functionally both in prokaryotic and eukaryotic cells. Renilla Luciferase is widely used to determine biological process, because the enzymatic luminescence assay is highly sensitive, rapid, and