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Fabrication of Electrospun Green Fluorescent Protein Nano-Fibers for Biomedical Applications

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Abstract : GFP discovered in the mid-1970s, has been used as a marker after replicated genetic study by scientists. In biotechnology, cell, molecular biology, the GFP gene is frequently used as a reporter of expression. In modified forms, it has been used to make biosensors. Many animals have been created that express GFP as an evidence that a gene can be expressed throughout a given organism. Proteins labeled with GFP identified locations are determined. And so, cell connections can be monitored, gene expression can be reported, protein-protein interactions can be observed and signals that create events can be detected. Additionally, monitoring GFP is noninvasive; it can be detected by under UV-light because of simply generating fluorescence. Moreover, GFP is a relatively small and inert molecule, that does not seem to treat any biological processes of interest. The synthesis of GFP has some steps like, to construct the plasmid system, transformation in E. coli, production and purification of protein. GFP carrying plasmid vector pBAD–GFPuv was digested using two different restriction endonuclease enzymes (NheI and Eco RI) and DNA fragment of GFP was gel purified before cloning. The GFP-encoding DNA fragment was ligated into pET28a plasmid using NheI and Eco RI restriction sites. The final plasmid was named pETGFP and DNA sequencing of this plasmid indicated that the hexa histidine-tagged GFP was correctly inserted. Histidine-tagged GFP was expressed in an Escherichia coli BL21 DE3 (pLysE) strain. The strain was transformed with pETGFP plasmid and grown on LuiraBertoni (LB) plates with kanamycin and chloramphenicol selection. E. coli cells were grown up to an optical density (OD 600) of 0.8 and induced by the addition of a final concentration of 1mM isopropyl-thiogalactopyranoside (IPTG) and then grown for additional 4 h. The amino-terminal hexa-histidine-tag facilitated purification of the GFP by using a His Bind affinity chromatography resin (Novagen). Purity of GFP protein was analyzed by a 12 % sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of protein was determined by UV absorption at 280 nm (Varian Cary 50 Scan UV/VIS spectrophotometer). Synthesis of GFP-Polymer composite nanofibers was produced by using GFP solution (10mg/mL) and polymer precursor Polyvinylpyrrolidone, (PVP, Mw=1300000) as starting materials and template, respectively. For the fabrication of nanofibers with the different fiber diameter; a sol–gel solution comprising of 0.40, 0.60 and 0.80 g PVP (depending upon the desired fiber diameter) and 100 mg GFP in 10 mL water: ethanol (3:2) mixtures were prepared and then the solution was covered on collecting plate via electro spinning at 10 kV with a feed-rate of 0.25 mL h-1 using Spellman electro spinning system. Results show that GFP-based nano-fiber can be used plenty of biomedical applications such as bio-imaging, bio-mechanic, bio-material and tissue engineering.

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