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IN-VITRO RELEASE KINETICS OF RECOMBINANT FUSION PROTEIN OF NEWCASTLE DISEASE VIRUS COUPLED WITH POLY (LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES

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ABSTRACT

Newcastle disease is the most important disease next to avian influenza which causes high morbidity and mortality resulting in huge economic loss to poultry industry. Vaccination is the best way to control many infectious diseases. Currently available live attenuated and killed vaccines for Newcastle disease require cold chain maintenance. In the third world countries cold chain maintenance is cumbersome. To overcome this problem, recombinant protein vaccine delivered through biodegradable polylactic-co-glycolic acid (PLGA) nanoparticles offers a promising opportunity in vaccine technology.

Poly (lactic-co-glycolic acid) (PLGA) is a biocompatible member of the aliphatic polyester family of biodegradable polymers. PLGA has long been a popular choice for drug delivery applications, particularly; it is already FDA-approved for use in humans in the form of resorbable sutures.

Polylactide-co-glycolic acid (PLGA) nanoparticles were synthesized by Double emulsion solvent evaporation method. The size of the PLGA nanoparticles measured in the scanning electron microscopy (SEM) was 280- 320 nm. The polydispersity index (PDI) of PLGA nanoparticles was 0.192. Zeta potential of the PLGA nanoparticles was -2.61 mV. The protein release was determined by in vitro using Bradford assay. It showed consistent protein release was observed every 24 hrs interval.

Keywords: PLGA nanoparticles, Biodegradability, Zeta potential

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease of poultry that is characterized by respiratory, nervous, enteric, and reproductive infections. The causative organism of the infectious disease is the virulent ND virus (NDV), which belongs to the genus Avulavirus within the family Paramyxoviridae (Sinkovics and Horvath, (2000). Current vaccination strategy for ND includes the use of lentogenic live-virus vaccines or inactivated

Vol. 2, No. 02; 2017

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vaccines to induce protective immunity while producing minimal adverse effects in chickens (Tseng *et al*. (2009).Although the inactivated and attenuated live NDV vaccines have been playing an important role in prevention and control of ND in practice, these conventional vaccines have some disadvantages including partial virus toxicity reservation. Nanoparticles often present significant adjuvant effects in parenteral vaccine delivery due to their effective uptake by antigen presenting cells. The nanoscaled size allows nanoparticles to be taken up by M-cells in mucosa-associated lymphoid tissue (MALT), i.e., gut-associated, nasal-associated and bronchus-associated lymphoid tissues to initiate vigorous immunological responses (Praveen*et al. (*2004).

To meet the need for effective vaccine delivery systems for mass vaccination, particulate delivery system like PLGA nanoparticles offer promising opportunity to deliver the variety of biomolecules like DNA, protein, peptides. Polylactic-co-glycoloic acid (PLGA) nanoparticles are synthetic polymer prepared from lactic acid and glycolic acid. PLGA is non-toxic, biodegradable and biocompatible.

Polylactic-co-glycolic acid (PLGA) is the most popular among the various available biodegradable polymers because of its biodegradability, biocompatibility and sustained drug delivery (Hans and Lowman (2002). Polylactic-co-glycolic acid polymers were authorized by Food and Drug Administration (FDA) for drug delivery. Present study focused on *in-vitro* release kinetics of recombinant fusion protein coupled with PLGA nanoparticles.

Materials and Methods

Polylactic-co-glycolic acid (PLGA) nanoparticles synthesis

Polylactic-co-glycolic acid (PLGA) nanoparticles were prepared by double emulsion solvent evaporation methodas described (Praveen*et al*., 2004) with some modifications. 100 mg of PLGA dissolved in 4 ml of dimethyl chloromethane (DCM). To this organic phase, 0.5 ml aqueoussolution was added slowly while sonication at 25 KHz for 5 min. This primary emulsion was added gradually to 40 ml of 2.5% polyvinyl alcohol (PVA).

This secondary emulsion was kept in a magnetic stirrer overnight at room temperature. PLGA NPs were harvested by high speed centrifugation at 12,000 rpm for 20 min at 4^{o}C. The pellet was washed with triple distilled water. The final pellet was resuspended in sterile PBS (pH 7.2).

Quantification of PLGA nanoparticles

Nanoparticles were lyophilized using 5% trehalose as lyoprotectant. Lyophilized nanoparticles were scrapped aseptically, quantified, labeled and stored at -20^{o}C for further analysis.

Vol. 2, No. 02; 2017

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$$\text{Yield of PLGA nanoparticles} = \frac{\text{Final yield of PLGA nanoparticles x 100}}{\text{Initial amount of PLGA used for synthesis}}$$

Determination of loading capacity of fusion (F) protein coupled nanoparticles

The ability of PLGA nanoparticles to entrap fusion protein was directly determined by centrifuging synthesized nanoparticles at 16,000 rpm for 30 min at 4^0C. The loading capacity of nanoparticles was determined by quantifying the unbound fusion protein fraction in the supernatant by Bradford assay. Loading efficiency (LE) were determined as follows

$$\text{Loading efficiency (LE)} = \frac{\text{Total amount of protein – free protein}}{\text{Total amount of protein}}$$

Morphology of PLGA nanoparticles

The surface morphology of the PLGA nanoparticles was determined by scanning electron microscopy (JEOL, JSM5200, TOKYO, Japan). The lyophilized samples were spread on metal stubs and gold coating was done using an ion-sputtering device. The gold-coated samples were vacuum dried and then examined. Polydispersibility was performed by the instrument.

Zeta potential of PLGA nanoparticles

Zetasizer nano ZS with DTS software (Malvern Instrument Limited, UK). NIBS® (noninvasive backscatter optics) technology was used for the size measurement and net charge of the nanoparticles.

***In - vitro* release study of fusion (F) protein coupled PLGA nanoparticles**

In - vitro release studies of the antigen from PLGA-based nanoparticles were performed with the goal of investigating the ability of these delivery systems to release the entrapped recombinant fusion protein in its active form. Lyophilized fusion protein coupled PLGA nanoparticles were dissolved in 5 ml of phosphate buffered saline (PBS, pH 7.2) and suspension

Vol. 2, No. 02; 2017

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was incubated at 37 ^{0}C in a shaking incubator. One milliliter of samples was taken at 24 hrs interval and centrifuged at 12,000 rpm for 10 min. One ml of fresh PBS was added to remaining 4 ml of suspension to continue the incubation. Supernatant was checked for release of fusion protein by Bradford assay.

Results

Polylactide-co-glycolic acid nanoparticles synthesis,yieldand loading efficiency

Polylactide-co-glycolic acid nanoparticles (PLGA) were synthesized by double emulsion solvent evaporation method and the resulting PLGA nanoparticles were lyophilized and quantified. The yield of PLGA nanoparticles synthesized by double emulsion solvent evaporation was 19.2 %.

Morphology, zeta potential of PLGA nanoparticles

Freeze dried polylactic-co-glycolic acid (PLGA) nanoparticles prepared by double emulsion solvent evaporation method was analyzed by SEM. The observed particles were smooth, spherical in shape with an average size of 280-320 nm under 10.0 KV at 9000 magnification (Fig. 5).

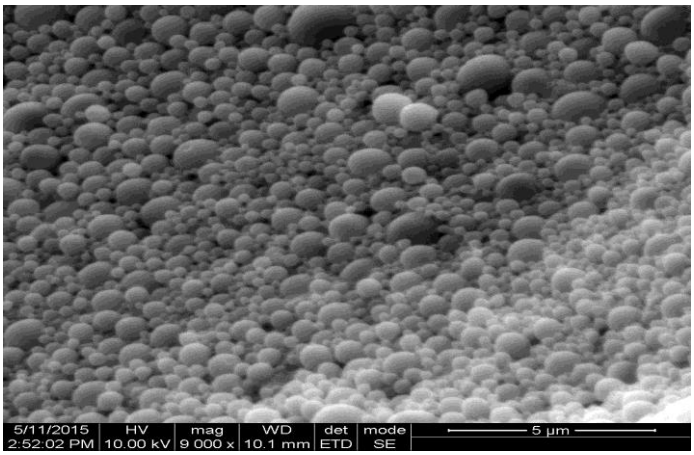

Fig 1: **Morphology of PLGA nanoparticles visualized by scanning Electronmicroscope - Magnification - 9000X**

Vol. 2, No. 02; 2017

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Zeta potential and polydispersity index of PLGA nanoparticles

Blank PLGA nanoparticles were subjected to zetasizer and the size of the PLGA nanoparticles were 312.4 nm and the polydispersity index (PDI) was 0.192. Zeta potential of the blank PLGA nanoparticles was -2.61 mV.

Protein release kinetics and structural integrity analysis fusion protein of PLGA nanoparticles

Lyophilized NDV recombinant fusion protein coupled PLGA nanoparticles were analyzed for the release of NDV fusion protein in the releasing medium. The results indicated that the protein was released from the PLGA nanoparticles from 24 hrs onwards. The release was consistent and gradual from 48 hrs onwards (Figure. 2)

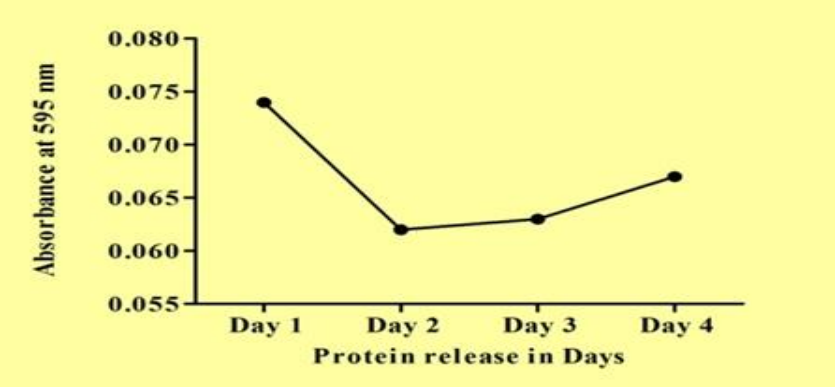

Figure 2: In vitro release of NDV fusion (F) protein from PLGA nanoparticles showing initial burst release followed by gradual increase in release of fusion protein

Discussion

Nanoparticle delivery system offers promising opportunity to deliver variety of bioactive compounds such as DNA, proteins, peptides and pharmaceutical drugs to the target site for a long period of time at consistent releasing pattern. Coupling of protein with nanoparticles delivery system improves the efficacy of the immunogenic protein. PLGA is biodegradable and is widely used for vaccine and drug delivery (McFarren and McCracken, 1988). Hence, in the present study, a biodegradable PLGA nanoparticle is used as antigen delivery systems to improve the immunogenicity of protein antigen.

PLGA nanoparticles could be synthesized by double emulsion solvent evaporation method. Scanning electron microscopy (SEM) is being used to study the three dimensional

Vol. 2, No. 02; 2017

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structural analysis. SEM image of PLGA nanoparticles showed smooth individual particles with an average size of 286.5 to 320 nm.

Zeta potential represents the net charge of the synthesized PLGA nanoparticles. Zeta potential of the PLGA with fusion protein was +2.61 mV, which indicates that net charge of the PLGA nanoparticles is negatively charged. Negative charge of PLGA nanoparticles is due to presence of lactic acid and glycolic acid moiety present in the PLGA nanoparticles. Zeta potential could combine fusion protein better and slow down the burst release process (Basarkar*et al*., 2007).

Another critical feature is the release kinetics of fusion protein from PLGA nanoparticles. PLGA nanoparticles havereleased the fusion protein, initial burst followed by slow and consistent release pattern. PLGA nanoparticles is desorbed locally by aqueous environment and release the lactic acid and glycolic acid which lower the pH of the micro-environment, in the acidic condition PLGA nanoparticles degradation is further catalyzed and ensures the slow and progress release of protein (Panyam and Labhasetwar, 2012). In this study, the pFNDV-PLGA-NPs slowly released the loaded protein continuously for up to 7 days from the in vitro release analysis that was conducted under the physiological pH conditions. There was a burst release of the plasmid protein between 0 h and 24 h due to detachment of the protein that was adhered to the nanoparticles surface. From days 2 to 7, the protein was continuously released.

Conclusion

This study concluded that PLGA nanoparticles synthesized by double emulsion solvent evaporation method has revealed the smooth and spherical appearance of PLGA nanoparticles as viewed in the scanning electron microscopy. This method has also increased the relative homogeneity of PLGA nanoparticles morphology. The yield of PLGA nanoparticles can be further improved by optimizing the parameters like centrifugation speed use of surfactant and ratio of glycolic acid and lacticacid used for the synthesis of PLGA nanoparticles

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Vol. 2, No. 02; 2017

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