Preparation and in vitro Evaluation of Pullulan Nanoparticles as Gene Transfection System

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INTRODUCTION: Over the past few decades, there has been considerable interest in developing biodegradable nanoparticles as effective delivery vehicle of drugs, proteins, peptides and nucleotides [1]. Pullulan is a water soluble, neutral linear polysaccharide consisting of α-1,4 and α-1,6 glycosidic linkages [2]. The aim of this study was to prepare hydrogel nanoparticles of pullulan that can encapsulate water-soluble materials for intracellular delivery and targeting.

METHODS : In this study, pullulan nanoparticles in narrow size range encapsulating DNA (pBUDLacZ) have been prepared inside the aqueous core of the reverse micelles formed by AOT/n-hexane (w/o microemulsion). The effect of pullulan-DNA nanoparticles on cell viability has been illustrated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) on HEK293 and COS-7 cells with transfection potential of these nanoparticles evaluated using LipofectAMINE2000 (Invitrogen Ltd.) as a positive control.

RESULTS: Pullulan nanoparticles encapsulating plasmid pBUDLACZ were prepared using the highly monodispersed aqueous core of AOT/n-hexane reverse micellar droplets with the size in the range of 35-40nm in diameter with narrow size distribution. The integrity of the plasmid encapsulated inside the pullulan nanoparticles was illustrated by agarose gel electrophoresis. The nanoparticles encapsulating plasmid treated with DNase showed band of similar intensity as the nanoparticles-DNA without treatment (figure-1).

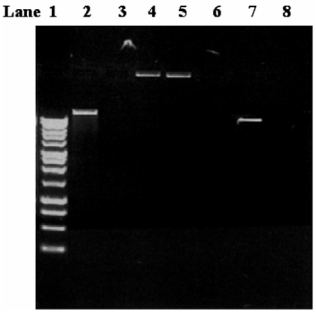

Fig.-1: Agarose Gel Electrphoresis. Lane1: Molecular weight marker; Lane2: Free pBUDLacZ DNA; Lane3: Free pBUDLacZ DNA treated with Dnase; Lane4: Pullulan nanoparticles encapsulating pBUDLacZ DNA; Lane5: Pullulan nanoparticles encapsulating pBUDLacZ DNA treated with Dnase; Lane6: Void pullulan nanoparticles; Lane7: Void pullulan nanoparticles with adsorbed DNA; Lane8: Void pullulan nanoparticles with adsorbed DNA treated with DNase

For eventual applicability in vivo, a required characteristic of gene delivery system is absence of cytotoxicity. The viability of Cos7 and Hek cells after incubation was measured by MTT assay after culturing for 24 hours. As it is evident from figure, the cytotoxicity of the nanoparticles was found to increase in relation to increased concentration of pullulan. The pullulan nanoparticles were found to be more than 100% viable relative to control at the concentration as high as 1mg/ml (fig.2). The transfection efficiency

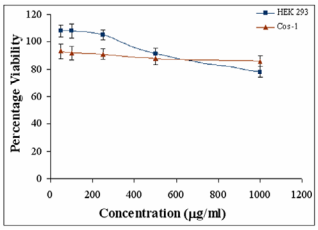

Figure-2: Cytotoxicity Profile of pullulan nanoparticles in HEK293 and COS-7 Cells as Determined by MTT Assay

of the pullulan nanoparticles was evaluated in Cos-7 and Hek 293 cells and compared with commercial reagent-LipofectAMINE2000 (fig.-3). The efficacy of transfection in vitro on HEK293 and COS-7 cells demonstrated cell type dependence with Cos-7 cells found to have a higher gene expression as compared to HEK293. The β-gal expression in Cos-7 cells by pullulan nanoparticle was comparable to commercially available LipofectAMINE2000.

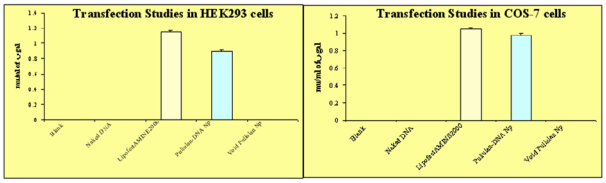

Figure-3: Comparison of Transfection Efficiency of Pullulan Nanoparticles with LipofectAMINE2000 incubated with HEK293 and Cos-7 cells

DISCUSSION & CONCLUSIONS: The nanoparticles of pullulan were prepared in the aqueous core of the reverse micelles formed by dissolving a surfactant AOT in n-Hexane with the size in the range of 35-40nm in diameter with narrow size distribution. Cell viability studies following incubation with the nanoparticles showed the lack of toxicity of pullulan. The efficacy of transfection in vitro on HEK293 and COS-7 cells demonstrated cell type dependence, with COS cells having a higher gene expression. The results of this study are very encouraging for the development of pullulan nanoparticles as an intracellular delivery system for drugs and genes. Further investigations on using these nanoparticles as a receptor-mediated drug carrier can be achieved by the use of cell specific ligands on the surface of these nanoparticles.

REFERENCES: [1] S.M. Moghimi et al., *Pharm. Rev.* (2001) 53, 283-318.; [2] K.Na and Y.H. Bae; *Pharm. Res.* (2002) 19 681-688.