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Preparation Characterization and Evaluation of Gel Cored Liposomes for Delivery of Recombinant Antigen Vaccine

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INTRODUCTION AND OBJECTIVES

Liposomes have been widely studied as novel vaccine adjuvant for delivery of immunogens due to their safety, biocompatibility, biodegradability and their ability to target immunogens to antigen presenting cells, but their use is largely limited because of instability and hasty release of antigen content from liposomes. The present work has combined the properties of two different delivery systems, hydrogels and liposomes, by incorporating a core of polymer inside the phospholipids vesicles, which serve the function of skeleton and provide mechanical support to vesicle. In the present investigation, biocompatible polymer poly acrylic acid (PAA) was selected as a polymer for coring, which shows short gelling time on varying pH (5–6.5). BSA (Bovine serum albumin) loaded gel core liposomes and conventional liposomes were prepared by reverse phase evaporation method and optimized for size, shape, entrapment efficiency and *in vitro* release and compared with conventional liposomes for stability studies. *In vivo* antigen presentation ability of gel core liposomes was evaluated by measuring systemic IgG immune response after intramuscular administration of gel core liposomes and compared with that of conventional liposomes alum adsorbed antigen and plain antigen.

MATERIALS AND METHODS

Gel core Liposomes were prepared by reverse phase evaporation technique. The prepared vesicular systems were characterized with following parameter -
{1}.Morphology & shape -using a transmission electron microscopy.
{2}. Entrapment efficiency- The formulation was pelleted by centrifugation (50,000 g) and suspended in PBS buffer (pH 4.2). The fraction was finally collected and mixed with minimum amount of triton X-100 (0.5%, w/v) to disrupt the vesicles and liberated antigen was estimated using a BCA (bicinchoninic acid) protein assay and percentage antigen entrapment was calculated.
{3}.Confirmation of gelling in the inner compartment of vesicle- observed by treating the formulation with Triton X-100 and evaluated for morphological characterization by a light microscope (Nikon, Japan).
{4}.In process stability-was carried out by SDS-PAGE of encapsulated protein and compared with native protein.
{5} *In Vitro* release- was done by dialysis method.
{6} Storage physical stability-The formulation was stored at 4°C and 25°C, the samples were withdrawn at different time interval and analyzed for residual antigen content and antigen integrity by SDS-PAGE analysis.
{7} *Ex-vivo* cellular uptake studies-florescent dye (FITC) loaded vesicles were incubated with cultured macrophage cells. After 30min. cells were washed and observed under fluorescent microscope (Nikon, japan)
{8} Immunization of animal and measurement of serum IgG- Balb/C mice, 4–6 weeks old were used and serum antibody titer was accessed by ELISA.

RESULTS AND DISCUSSION

Prepared liposomes were optimized by incorporating different concentrations of polymer in the core of vesicle. The result indicate that formulation containing 0.3% w/v polymer in the core of vesicle was optimum and hence selected for further studies. A very low polydispersity index of less than 0.2 obtained for the formulations indicate narrow size distribution of the vesicles (Table I).

Table I. Characterization of BSA-loaded optimized liposomal formulation and its comparison with conventional liposome.

S. no	Parameter	Gel-core liposomes	Conventional liposomes
1	Mean vesicle size	1.52 ± 0.39 µm	1.18 ± 0.4 µm
2	% Entrapment efficiency	37.8 ± 2.4	28.3 ± 3.6
3	Zeta potential	−38.32 ± 2.34 mV	−9.64 ± 1.53 mV
4	Polydispersity index	0.142	0.092
5	Cumulative release in 24 h	10.32 ± 2.4%	25.5 ± 3.1%

Values represent mean ± SD ($n = 3$).

Gel cored and conventional liposomes were unilamellar and spherical in shape (Fig-1). Confirmation of gelling in the core of vesicle was done by treating with triton X-100, no change in the shape and size of the vesicle following treatment with triton after gelling of polymer in the core of vesicle (coring) was observed (Fig-2).However on treatment with triton X100 before polymer coring the spherical structure of vesicles disappears since the sol form of polymer cannot retain spherical structure after removal of phospholipid bilayer. The in vitro release pattern of gel core liposome (Fig-3) depicts gradual release of protein in the first 24 h during which only 10.32±2.4% of the total protein was released while 25.5±3.1% protein was released from conventional liposomes.

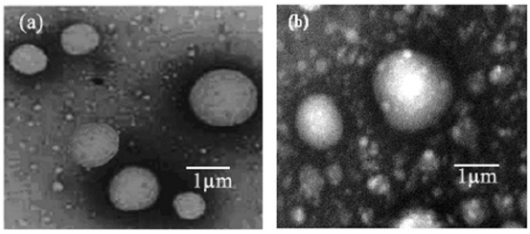

Fig-1:Transmission electron photomicrograph of (a)Conventional liposomes,(b) Gel cored liposomes.

A very weak fluorescence was observed in the cells incubated with conventional liposomes alone (Fig-4a) whereas a strong fluorescence was observed when the cells were incubated with gel core liposomes (Fig-4b), clearly indicating that the adsorbed poly acrylic acid on to the surface of gel core liposome enhances the uptake of vesicle by macrophages (confirmed by measuring zeta potential). In SDS-PAGE, the visible band of pure as well as antigen extracted from conventional liposomes and gel core liposomes (Fig-5) clearly indicate that the method of preparation does not have any deleterious effect on protein integrity.

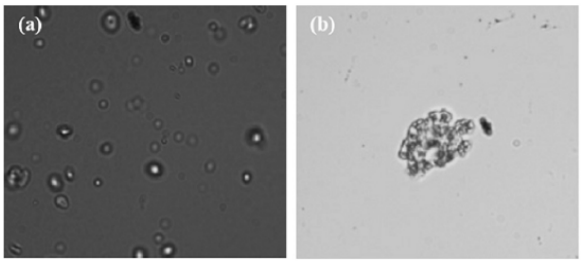

Fig-2: Photomicrograph of liposomal formulation after treatment with Triton X-100 (a) after gelling in the core (b) before gelling in the core

The antigen presentation ability of gel core liposomes was evaluated by measuring serum IgG titer following intramuscular administration by ELISA. A significantly high ($P<0.001$) systemic immune response was observed with gel core liposomes as compared to conventional liposomes and plain antigen formulations (Fig-6). However the immune response elicited by conventional liposomes is comparable to alum adsorbed formulations.

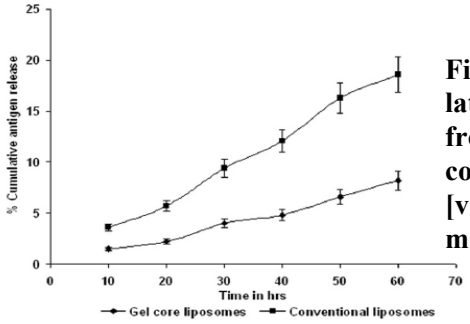

Fig-3: *In vitro* cumulative release of BSA from optimized gel core liposomes [value represents as mean±SD(n=3)]

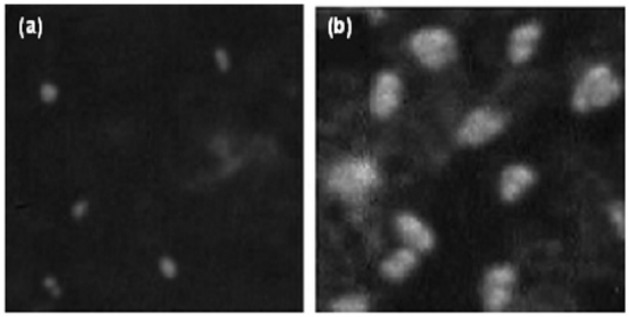

Fig-4:Fluorescent microscopy of gel core liposomes mediated effective delivery of FITC labeled vesicular carriers(A) cells treated with conventional liposomes (b) cells treated with gel core liposomes.

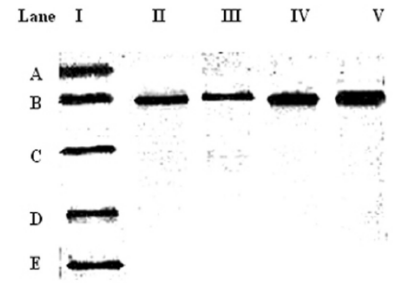

Fig 5. SDS PAGE of different liposomal formulations, Lane I=molecular weight marker (A=94, B=67, C=43, D=30, E¼21), Lane II=BSA (Plain), Lane III=BSA in conventional liposome, Lane IV=BSA in Alum, Lane V=BSA in gel core liposome.

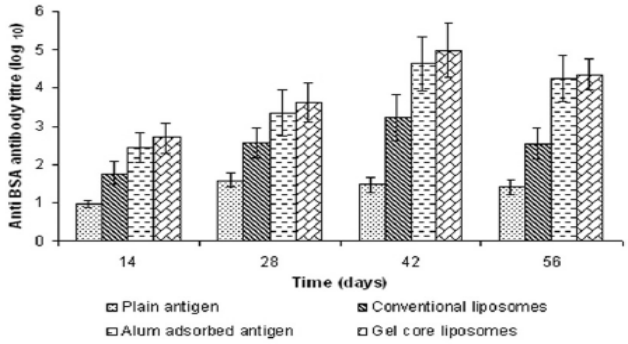

Fig6. Serum IgG titer of different formulation after intramuscular immunization. Values are expressed as mean ±SD (n=5).

CONCLUSION

This study examined the application novel carrier gel core liposomes for intramuscular vaccine delivery using BSA as model antigen and the observation on the basis of measurement of serum IgG titer by ELISA indicate that gel core liposomes may greatly improve the efficacy of antigenic formulations. Their enhanced stability, better antigen presentation, controlled release, immunoadjuvant property and significant entrapment profile could be utilized as effective tools for immunization.

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