Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization

V. Sihorkar, S.P. Vyas
Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar, M.P., India

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ABSTRACT **Purpose**: Recently the emphasis has been laid upon the carbohydrate mediated liposomal interactions with the target cells. Among the various carbohydrate ligands, such as glycoproteins, glycolipids, viral proteins, polysaccharides, lipo-polysaccharides and other oligosaccharides, this review deals with the polysaccharide anchored liposomal system for their potential in drug delivery, targeting and immunization. Over the years, various strategies have been developed which include coating of the liposomal surface with natural or hydrophobized polysaccharides, namely mannan, pullulan, amylopectin, dextran etc., or their palmitoyl or cholesteroyl derivatives. The polysaccharide(s) coat tends vesicular constructs physicochemically stable in bio-environments and site-specific. The aim of improving the physical and biochemical stability of liposomes and the ability to target liposomes to specific organs and cells, were the major attributes of the polysaccharide anchored liposomes. In this review the authors attempted to overview various applications of polysaccharide bearing liposomes, including lung therapeutics, targeted chemotherapy, cellular targeting, cellular or mucosal immunity and macrophage activation. Future prospects of the delivery module are also discussed. The review in general explores the concepts, options and opportunities of polysaccharide anchored liposomes with newer perspectives.

Drug delivery with liposomes as carrier systems provide options and opportunities for designing bio-stable and/or site specific drug therapy. Liposomal systems have been optimistically considered as "magic bullets" for more than 3 decades. The engineered or tailored versions of liposomes offer potentials of exquisite levels of specificity and drug targetability (1). The structural versatility of liposomal systems in terms of vesicle size, shape, surface morphology, composition, surface charge and bilayer fluidity; their ability to incorporate a wide spectrum of drugs; or to carry cell-specific ligands render them clinically and therapeutically viable and clinically versatile.

Corresponding Author: S.P. Vyas, Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar, M.P., India.
spvyas@bom6.vsnl.net.in

The incorporation of various explicit and site directing bio-molecules (ligands) on colloidal carriers make them suitable either for stability in bio-fluids or site specificity towards receptors or antigenic determinants expressed on target sites. Paul Ehrlich (6) in his pioneering 'magic bullet' concept proposed and realized that the drug could be targeted with the help of groups/ligands having well defined affinity for specific cells (or receptors/antigenic determinants expressed on target cells). The ligands can either covalently or non-covalently be attached to the surface of liposomes and could direct liposomes and encapsulated contents *en route* to predefined accessible cells. Liposomes as a drug carrier system has been utilized as circulating units for therapeutic delivery of various bio-sensitive and bio-active ligands including antibodies (2), glycopeptides (3), oligo-saccharides (4), viral proteins and fusogenic residues (5). The ligands confer target specificity and recognition ability to the drug-carrier system. However, in some cases, ligands only confer stability and better structure integrity against harsh bio-environments encountered after oral or parenteral administration.

Liposomes based target recognition is a critical prerequisite for ligand mediated targeting and customarily selected ligands should have a well defined propensity, avidity and specificity towards receptor portals expressed selectively on the selected target cell(s). Various ligands have been investigated for their bio-signaling and bio-sensing potential. Some of them are, anti-target monoclonal antibodies or haptens (7), sialic acid

(3), lectins (8), polysaccharides (9), glyco-conjugates like glycoproteins (10), glycolipids (11) and sialo-glyco-conjugates (12).

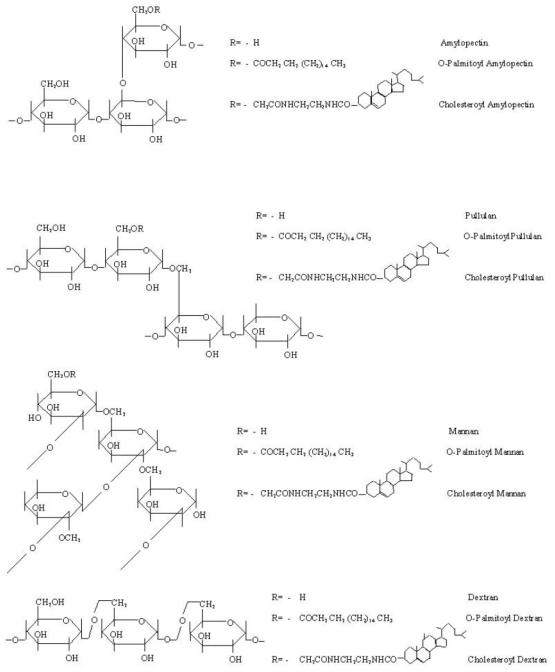
The objective of this article is to provide an overview on possibilities and potentials of polysaccharide(s) as ligands for liposomes. The major focus of the previous reviews being the stability and physicochemical characterization of the polysaccharide coated liposomes and their utility to serve as models for cell-cell adhesion or interaction studies. However, in the present review their potential, as drug delivery systems and drug targeting systems, will be revealed.

POLYSACCHARIDES AS DELIVERY PORTALS

The role of cell surface oligo-saccharide(s) and their functional attributes are pivotal in order to rationalize and realize their significance in targeted drug delivery. It is a well-known fact that the surface of the mammalian or microbial cells contains carbohydrate moieties in abundance mainly oligosaccharides associated with membrane lipids, proteins or peptide glycans. This membrane associated carbohydrate-rich material referred to as "glycocalyx" and is the focal and prime locus around which research over last few years has been revolving and proliferating. The glycocalyx is specially involved in cell processes such as cell-cell recognition and adhesion, the binding of pathogens, bacteria and virus to their target tissue, sperm-egg binding and lymphocyte-endothelium recognition. These oligosaccharides (saccharide determinants) of the cell surface glycocalyx play a central role in cellular adhesions and biological recognition processes and constitute potential recognition sites for carbohydrate-mediated interactions between cells and drug carriers bearing suitable site directing molecules (13). The glycocalyx of cells usually contains high proportions of polysaccharides therefore, it has been thought appropriate to explore and investigate the utility of polysaccharides in targeted drug delivery. Some of the naturally occurring polysaccharides anchored to artificial cell walls can potentially become a target cell-sensing device imparting specificity, avidity and targetability to the carrier. Moreover, the polysaccharide appended drug carriers can circumvent bio-environment-derived stresses and could effectively deter bio-sensitive contents from biodegradation (14-16).

Polysaccharides from higher plants and algae have already been used intensively on the technical scale for a long time. Recently, more attention is being paid towards polysaccharides from microorganisms and yeast. In addition to the well-established polysaccharides containing pharmaceutical materials, considerable interest has been generated in a number of polysaccharides with intrinsic pharmacological activities. These include immuno-modulation, anti-tumor, anti-inflammatory, anti-coagulant, hypoglycemic and antiviral activities. However more recently, these polysaccharide modules have been exploited to navigate the carrier to its destined site of action (14-23).

Among the macromolecular polysaccharides reported as molecular carriers are, mannan, amylopectin, pullulan and dextran, either in their native form or as carrier-conjugates. Almost all naturally occurring polysaccharides mentioned here are known to protect cell plasma membranes against physicochemical stimuli, such as osmotic pressure and ionic stress. However, on adsorption to lipid carriers, the peptization or coagulation of the system may occur, and probably due to this reason partially hydrophobized polysaccharides are recommended and used in drug delivery system (scheme 1).



Scheme 1 Naturally occurring polysaccharides used for the surface anchoring of liposomes with their hydrophobized derivatives

Table 1: Various polysaccharide-based systems and their therapeutic significance reported in the literature

Polysaccharide-based systems	Purpose	Reference
Pullulan-interferon conjugate	Liver targeting	(24)
Carboxymethylpullulan-Sialyl Lewis X (sialyl-N-acetyl-lactosamine)	Targeting to microvessels expressing E-selectin in inflammatory sites	(25)
Mannosylated dendrimers (glyco-dendrimers)	Macro-molecular recognition and bio-chromatography	(26,27)
Polysaccharide (mannan, amylopectin and pullulan) coated multiple emulsion	Macrophage targeting	(28)
O/W emulsion stabilized with hydrophobized polysaccharide and trioctanoylglyceride	Cytotoxicitiy of entrapped α-linolenic acid against human colon cancer cells	(29)
Hydrogel nanoparticles of self-aggregated hydrophobized polysaccharides	Intravenous injectable and biocompatible drug targeting carriers	(30-35)
Hydrophobized pullulan/mannan-oncoprotein complex	Potentitation of humoral and cellular immune response	(36, 40)
Mannan-bovine serum albumin conjugates	Augmentation of humoral and cellular immune response	(37, 38)
Mannan-linear/multiple antigen peptide conjugate	Augmentation of humoral and cellular immune response	(39)
Mannan-MUC1 fusion protein conjugates	Augmentation of humoral and cellular immune response	(41)

Some of them have intrinsic biological responses however others show their targetability when they are administered in conjugated macromolecular form and some are used when they are anchored on an appropriate delivery system (24-42) (Table 1).

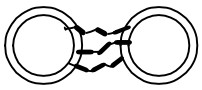
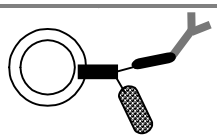
POLYSACCHARIDE ANCHORED LIPOSOMES

In the development of polysaccharide anchored liposomes for therapeutic purposes, it is important to consider the mechanisms and methodologies of the polysaccharide association with the bilayer membrane and resultant effect on the bilayer permeability, fluidity, and integrity. The affinity and selectivity of the anchored polysaccharide towards its complementary ligand(s) is a desirable prerequisite that makes the system site specific and target oriented. A great deal of work has been reported on liposomal systems involving polysaccharide-mediated interactions however, the realistic use of polysaccharide(s) for the drug delivery to the desired site(s) has not been realized clinically. Nevertheless, the significance of carbohydrate and polysaccharide specific recognition domains on the cell surface has stimulated the research quantitatively towards exploitation of technology to develop systems for drug(s) and/or antigen(s) (43-45).

Polysaccharide-anchored liposomes are well documented and mostly studied as a model to study cell-cell adhesion (reviewed by Jones, 13) but recently they are also studied for varied therapeutic potentials. Considering the potential of natural or hydrophobized polysaccharides, methods have been developed to link polysaccharides to the surface of liposomes (43). Earlier methods were attempted to anchor polysaccharides on the surface of the liposomes through adsorption, however recently spacer activated covalent coupling or hydrophobic anchoring have been appreciated as methods of anchoring (Table 2).

Earlier methods of anchoring exploited possible interaction of liposomes and polysaccharides. Sunamoto and co-workers (46,47,49) investigated interactions of simple polysaccharides and liposomal membranes. These workers revealed that simple and naturally occurring polysaccharides, such as dextran, chitosan, pullulan, mannan, or amylopectin, strongly adhere on to the liposomal surface mostly via hydrophobic interactions inducing subsequent aggregation and fusion of liposomes. Under specific conditions however, which do not allow for aggregation or fusion, the adsorption of polysaccharides over liposomal membranes may be due to diffusion controlled mechanism of constitutive components and coat, followed by lateral diffusion and subsequent inter-digitization of adsorbed polysaccharide molecules into bilayers (48). This hypothesis was later confirmed and substantiated by fluorescence depolarization technique using ^{FITC}-dextran as marker probe (49).

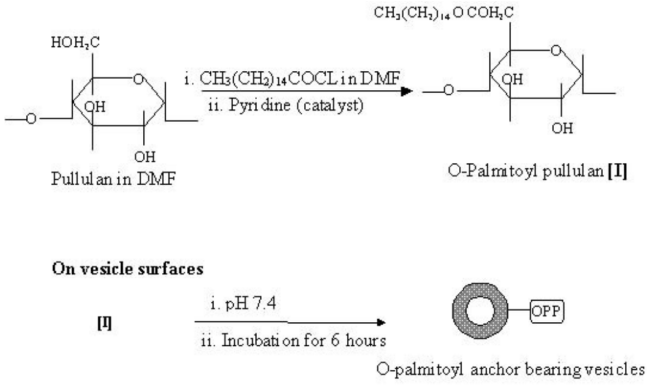
Table 2: Various polysaccharides capped liposomes with their formulation strategies and aims

Schematic diagram				
Method of anchoring	Polysaccharide induced-aggregation/fusion	Adsorption of poly-saccharide	Covalent anchoring	Covalent anchoring with sensors
Poly-saccharide type	Pullulan, amylopectin and mannan	Pullulan, amylopectin, dextran, mannan and chitosan	Pullulan, amylopectin and mannan (Palmitoyl/ (Cholesteroyl-derivative)	Palmitoyl/Chol conjugates with MoAb /sialic acid)
Purpose(s)	Model to study protein-lipid adhesion	Model for protein- lipid adhesion	Bio-stability and targeting	Targeting through attached sensory device
Ref	46,47,49	48, 49	44, 45,51,52	14, 15, 53-55

Polysaccharide anchoring by adsorption was found to be thermodynamically unstable and pharmaceutically unacceptable due to following reasons:

- The polysaccharides adsorbed on the liposomal surfaces easily desorb/delodge on dilution or on mechanical agitation.
- Peptization or coagulation of the polysaccharides could lead to subsequent destabilization of the liposomal bilayer
- Stoichiometric ligand density is often non-reproducible.

In order to obviate adsorptive coating related limitations, Sunamoto and Iwamoto (50) employed chemically modified polysaccharides, i.e., palmitoylated polysaccharides, to coat the liposomes. These partially hydrophobized polysaccharides were allowed to react covalently and subsequently integrate with the lipid constituents of liposomal membranes.



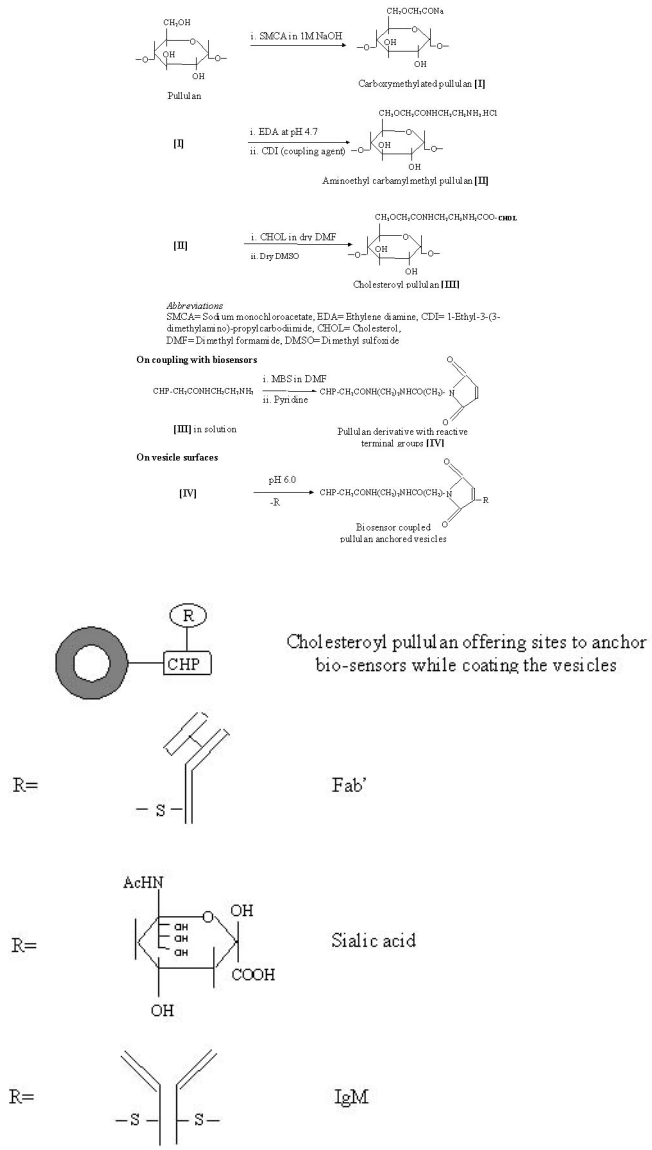
Scheme 2 Preparation of O-palmitoyl poly-saccharides (pullulan is being used as an example) anchored vesicles

The surface modification of liposomes is mediated through the hydrophobic legs (chemically conjugated or palmitoylated) of pullulan or amylopectin derivatives that digitize into the liposomal bilayer. Palmitoyl conjugates of pullulan, pullulan phosphate, amylopectin, amylopectin phosphate, mannan and dextrans have been employed intensively to coat the liposomes (10). When added to liposomes the hydrophobic anchors interact with the outer half of the bilayer orienting and projecting hydrophilic portion towards the aqueous bulk. This architect a polysaccharide based artificial cell wall on the outermost surface of the liposomes (Scheme 2).

An improved methodology in order to prepare both bio-stable and targetable drug carrier systems, has been developed (15). In place of palmitoyl anchor, cholesteroyl anchor has been employed for polysaccharide inter-digitization. In place of palmitoyl anchor, cholesteroyl anchor has been employed for polysaccharide inter-digitization. Cholesterol moiety can be introduced in to the (Scheme 3).

Coating of liposomes with these hydrophobized polysaccharides can be performed by incubation of aqueous solutions of polysaccharide derivatives with preformed liposomal dispersion. In some cases, Chol substituted polysaccharide was used to conjugate sensory devices like sialic acid derivative (51) or an IgM fragment (52). The sialic acid conjugated Chol substituted polysaccharide or immuno-polysaccharide derivatives were subsequently anchored over liposomes by dispersion-incubation technique under optimized standard conditions. The method has been used in the preparation of newly designed immunoliposomes where PC based large oligolamellar vesicles were anchored to the polysaccharide pullulan (54). The system has been modified to carry both, i.e., cholesterol as a hydrophobic anchor, and monoclonal antibody frag-

ment (anti-sialosyl Lewis^x IgMs) as a sensory device (Scheme 3).



Scheme 3 Preparation of cholesteroyl polysaccharide-anchored vesicles with the possibilities of anchoring of biosensors

Various studies moreover reflect that polysaccharides anchored on liposomes using above-mentioned methods retained their ligand affinity and specificity (29,60).

Stability Of Polysaccharide Anchored Liposomes And Their Oral Delivery Potential

Polysaccharide capped liposomes have been appreciated to be physically and chemically stable systems against biochemical and physicochemical stresses encountered in bio-fluids specially after oral administration. Sunamoto and Iwamaoto (50) categorically reviewed some characteristics of polysaccharide capped liposomes which rationalize their use in drug delivery:

- Reduced permeability to water-soluble encapsulated materials in the presence of blood plasma/serum or its components.
- Increased stability against enzymatic attack and protection of phospholipids from lipases and lipoxygenases.
- Mechanical and biochemical stability towards bio-stimuli such as pH, osmotic pressure, ionic strength, temperature and dynamic challenges of bio-fluids.

Unfortunately, most of the studies to date cover *in vitro* stability aspects of these liposomes and lack parallel *in vivo* studies to correlate the results.

Structural stability of polysaccharide-anchored liposomes is examined by Moellerfeld and co-workers (53) employing both hydrophilic and lipophilic markers. O-palmitoyl amylopectin anchored liposomes labeled with [^{3}H] inulin in the internal aqueous phase and [^{14}C] coenzyme Q10 in the lipid bilayer were fairly stable in the blood circulation as well as in tissues as evident from radio-isotope analysis. Increased long-term stability and membrane integrity was also recorded in black lipid membranes anchored with polysaccharide derivatives bearing hydrophobic (mainly palmitoyl or cholesteroyl) anchor groups (53). In another study, Sunamoto and co-workers (15) developed site-specific and target oriented liposomes anchored with immuno-polysaccharide conjugates and demonstrated their structural stability in the presence of 18% (v/v) human serum *in vitro*.

Coating using hydrophobized polysaccharide not only stabilizes liposomes but also stabilizes proteo-liposomes in bio-environment (54). The stability of proteo-liposomes prepared from Escherichia coli phospholipids and anchored with hydrophobized dextran was evaluated (54). A high concentration of hydrophobized dextran protected the liposomes against detergent degradation, decreased the fluidity of the membrane, prevented fusion of the liposomes and enhanced their biochemical stability. Reduced fusion, protection against the loss of membrane by freezing/thawing, and reduced permeability of the water soluble marker 6-carboxyfluorescein (6-CF) were the substantial evidences towards better stability (mechanical and chemical) of the proteo-liposomes. In the latest developments, Sehgal and Rogers (55) reported poly-

mer-anchored liposomes containing cytosine-arabinoside (Ara-C) anchored with OPP. These liposomes were challenged with sodium cholate (SC) concentrations at varied pH conditions. Stability of OPP anchored liposomes in sodium cholate (SC) concentrations up to 16 mM at pH 5.6 or in SC solutions at pH 7.4 was appreciably improved. Further, at pH 2.0 and 37×C, the ratio of Ara-C released from uncoated versus coated liposomes (kuo/kco) was found to be 1.9 and 5.7 for the liposomes constructed of DMPC and DPPC respectively. At pH 7.4 and 37°C in the presence of 10 mM SC, the ratio kuo/kco was 5.1 and 1.4 respectively. These studies suggest that polysaccharide anchored liposomes are relatively stable systems and hence could be exploited for the delivery of drugs to harsh bio-environments such as those encountered after oral administration.

Moreira *et al.* (56) prepared fluorescent probe bearing liposomes anchored with partially hydrophobized O-palmitoyl pullulan at an OPP/PC weight ratio of 3. The improved stability probably imparted by decreased permeability and the fluidity of the outer region of the liposomal membranes. Furthermore, the same group encapsulated carboplatin in OPP anchored liposomes and studied platinum latency in liposomes to relate liposome stability on shelf and long term storage (57). The polysaccharide/lipid weight ratio has been reported to be critical and necessitates optimization. The higher ratio presumably causes dislocations or defects in the bilayer probably due to interference of the derivatized or palmitoyl anchored lipid molecules in membrane packing characteristics. The derivatized lipids create locus of defects allowing free volume with passage of encapsulated content.

In different studies however, the oral delivery potential of polysaccharide and polymer coated liposomes have been addressed. These studies suggest the role of transport via paracellular and transcellular routes from normal epithelial tissue or Peyer's patches, leading to different outcomes of drug delivery (Rogers and Anderson, 58) and immunization (Aramaki et al., 59).

Recently, Mumper and Hoffman (61) reported stabilization of hirudin (a potent and specific inhibitor of thrombin) entrapped liposomes upon coating with palmitoyl dextran. These workers correlated the effect of hirudin stability provided by the coated liposomes with the sustained release and resultant higher inhibition of thrombin formation, using an *in vitro* thrombin chromogenic substrate assay. Palmitoyl dextran– coated-liposomes showed a burst of 30% hirudin released in 5 hours with an additional 10% to 35% released over the next 600 hours. The released hirudin retained only 33% of its ability to inhibit thrombin when released from uncoated liposomes. However, hirudin retained 95% of its thrombin inhibitory activity when released from palmitoyl dextran– coated liposomes. Coated liposomes were found to stabilize hirudin and result in greater retention of hirudin's ability to inhibit thrombin's enzymatic activity.

Sihorkar and Vyas (62) investigated the oral delivery potential of palmitoyl pullulan (OPPu) and cholesteroyl pullulan (CHPu) coated liposomes against the challenges of detergent and bile (bile salts and fresh-pooled rat bile), freeze-thaw cycling and long-term storage. The stability of liposomes was tested by incubating them with different molar concentrations of bile salt, taurocholate (below, above and near CMC) at 37°C for a period of 2 hours. No significant changes in the vesicle size, integrity and drug content were observed and both OPPu and CHPu coated liposomes exhibited exceptional stability as compared against plain formulations. (Fig 1).

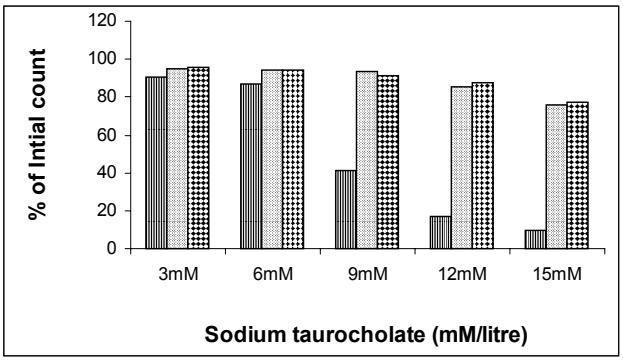


Figure 1: Interaction of different polysaccharide anchored and plain liposomal formulations with sodium taurocholate. The experiment was conducted with sodium taurocholate dissolved in phosphate buffered saline (pH 7.4) to concentrations below, at and above critical micelle concentrations. At an incubation time of 2 h at 37°C aliquots of plain liposomes (▥), OPPu anchored liposomes (□), and CHPu anchored liposomes (▦), were removed, centrifuged and supernatant analyzed for the drug content and from the data so obtained % of initial contents were calculated. Reproduced from (62), with permission.

Table 3: Stability studies of various anchored and plain liposomal systems[1]. Adapted and reproduced from (62) with permission.

Liposomal composition [2]	Shelf stability in terms of % drug latency							
	1 week		1 month		3 month		7 month	
	37±1°C	4±1°C	37±1°C	4±1°C	37±1°C	4±1°C	37±1°C	4±1°C
PC: CH (7:3)	–	±	– –	–	– – – –	– – –	– – – –	– – – –
PC: CH: DCP (7:3:0.5)	±	+	– –	±	– – –	– –	– – – –	– – –
(PC:CH:DCP): CHPu ({7:3:0.5}: 1)	+	+	±	+	±	+	±	+
(PC:CH:DCP): OPPu ({7:3:0.5}:1)	+	+	±	+	±	+	±	+

[1] All results are recorded in terms of % drug leaching values.
** Leaching criteria are related to the amount of liposome bound Propranolol HCl at day zero or at the start of the incubation, (+) leaching within accepted limits, 0-10% (+); (±) 10-25%; (−) 25-50%; (− −) 50-75%; (− − −) above 75% (− − − −) vesicles disrupted and released their contents.*
Abbreviations: OPPu, O-palmitoyl pullulan; CHPu, cholesteroyl pullulan
[2] Liposome construction is given in terms of molar ratio of constitutive components. However the ratio of coating material (hydrophobized polysaccharide) and that of total lipid is on a weight basis.

Double diffusion barrier encountered by diffusing drug(s) in case of anchored liposome could be a determinant in offering exceptional stability profile. Similarly, freeze-thaw cycling could not bring any fusion or collapse of the liposomal membrane (unlike unanchored ones). Furthermore, an appreciable shelf stability of the anchored vesicles both at 37±1°C and at 4±1°C (Table 3) was recorded. These results establish the potential of polysaccharide coated liposomes as a stabilized delivery system for oral administration of water-soluble agents. However, these *in vitro* stability studies need a better correlation with the *in vivo* performances of these stabilized systems.

Therapeutic And Clinical Applications

Polysaccharides have been utilized in recent years for various delivery and targeting strategies, either for they provided stabilization and formed a skeleton on which suitable sensory molecules were appended or otherwise they themselves behave as sensory devices to bring out the resultant targeted therapeutic effects (Figure 2). Some of the therapeutic benefits that polysaccharide anchored vesicles offer is discussed.

Lung Therapeutics

Increased lung accumulation of polysaccharides anchored liposome promises for their selectivity and potential as drug delivery system for the therapy of lung diseases (63). The alveolar macrophages selectively sequester the O-palmitoyl amylopectin anchored liposomes (64). The macrophagic uptake has been confirmed with the help of fluorescent probe marker, as marker was traced mainly in macrophages after IV injection (65). In experiments based on fluorescent probe-loaded and ^{14}C-labelled liposomes, it has been observed that both human monocytes and alveolar macrophages of guinea pig can internalize the O-palmitoyl amylopectin anchored liposomes at higher accumulation levels than conventional liposomes. Moreover, OPA anchored liposomes are reported to be sequestered and retained selectively in lungs by anionic-scavenging receptors. Liposomes appended with O-palmitoyl pullulan (OPP) and O-palmitoyl amylopectin (OPA) are rapidly cleared from the blood as compared to 'naked' liposomes. Though they have a relatively wide tissue distribution including liver and spleen, it is found that OPA anchored liposomes are selectively intercepted sequestered and internalized by the lung macrophages and monocytes. Subsequent to this observation, investigations were made on OPA anchored liposomes to explore their potential as a delivery system for sisomycin treatment of lung diseases in guinea pigs infected with *Legionella pneumophila* (49,66). The therapeutically beneficial results of these studies subsequently promoted further investigations, where OPA anchored liposomes were tested for targeted delivery of antimicrobial agents against intrac-

ytoplasmic pathogens and fungus (67). Specifically, amylopectin anchored liposomes were found to be effective for the delivery of ceftazidine to *L pneumophila* infected guinea pigs where relative to treatment with free drug the survival rate achieved following the liposome treatment was 30%.

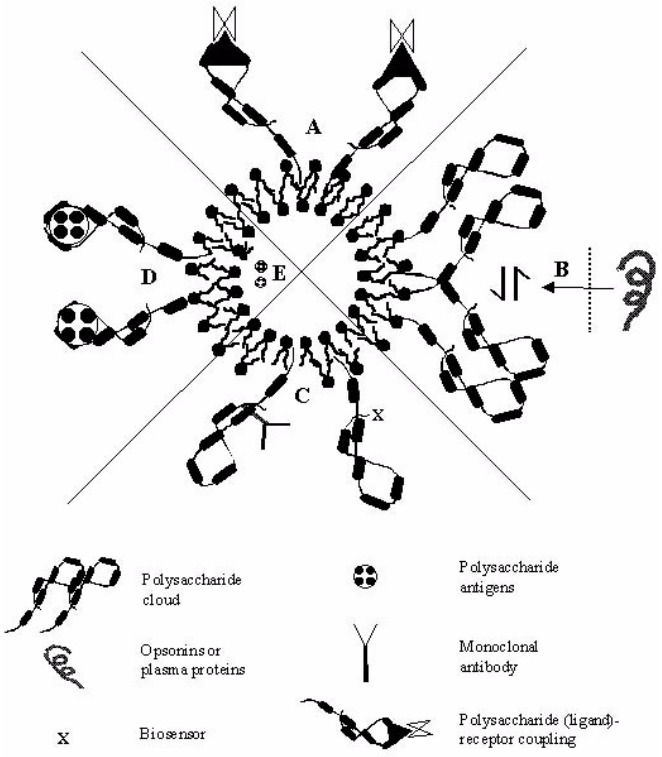


Figure 2: Various drug delivery, targeting and biotechnological areas where polysaccharide -bearing liposomes could be exploited as tools for future therapy. (A) Macrophage targeting based upon the ligand-receptor interaction. (B) Stealth behavior of the polysaccharide cloud, which further provides a hydrophilic environment to deter them from the opsonins and plasma proteins. (C) Polysaccharides as bio-sensors (targeting ligands) and offering bio-protection. (D) Vaccination potential of anchored bacterial polysaccharides or lipo-polysaccharides. (E) Encapsulating polysaccharide antigens for vaccination.

The liposomally encapsulated drug accumulation in the lung was two-fold higher compared to lung drug concentration following free drug administration. In the treatment of *Listeria monocytogenes* infection in mice with ampicillin or minocycline bearing surface modified liposomes (anchored with cholesteroylated amylopectin), the survival rate recorded was 100% as compared against 70% recorded for free ampicillin, and 80% as compared against 20% for free minocycline. The toxicity of amphotericin B was found to be substantially reduced in the case of amylopectin anchored liposomes when administered in mice infected with pulmonary candidiasis. The LD_{50} for free amphotericin B in mice was 1.2 mg kg^{-1}. It was increased to 12 mg kg^{-1} when administered encapsulated in polysaccharide-anchored liposomes, suggesting increased therapeutic benefits with greater safety index. Miyazaki and coworkers (68) revealed that coating liposomes with amylopectin negotiates targeting of the incorporated amphotericin B to the lungs. The LD_{50} of amylopectin-anchored liposomal amphotericin B in normal mice was more than 10.0 mg/kg, whilst for conventional amphotericin B, LD_{50} recorded to be 1.2 mg/kg. Amylopectin-anchored liposomes showed two-fold higher accumulation in the lungs as compared to conventional liposomes. These workers further studied *in vivo* efficacy of the system using murine model of pulmonary candidiasis. *Candida albicans* was inoculated intratracheally into BALB/C mice and the number of Candida in the lungs of mice treated with amylopectin-anchored liposomes and conventional liposomes were compared. The amylopectin-anchored liposomes improved the survival rate of inoculated mice.

Recently, Poiani and co-workers (69) encapsulated the copolymer of cis-4-hydroxy-L-proline (an anti-fibrotic agent) and PEG in liposomes. These liposomes were anchored with amylopectin and their efficacy was compared with liposomes conjugated with PEG in terms of improved lung uptake after intravenous infusion. The proline analogue cis-4-hydroxy-L-proline (cHyp) inhibits collagen accumulation and diffuses out of tissues. The encapsulation in amylopectin anchored liposomes prolonged the antifibrotic effect of the polymer. Amylopectin anchored liposomes had approximately 3-fold greater uptake in cultured endothelial cells compared with PEG-liposomes with greater lung retention as estimated 1 week after infusion ($5.2 \pm 0.8\%$ vs. $2.7 \pm 0.2\%$, $p < 0.05$). Sustained antifibrotic activity was assessed by recording the inhibition of collagen accumulation in pulmonary arteries of hypoxic (10% O_2) rats and by growth inhibition of cultured endothelial cells and fibroblasts. The activity was greater for amylopectin anchored liposomes/copolymer system than PEG-liposomes/copolymer system. Amylopectin-liposomes/copolymer attenuated increased right ventricular pressure by approximately 50% and it was inferred

that antifibrotic polymer could totally prevent collagen accumulation for nearly 1 week, which in fact is desirable for vascular remodeling in pulmonary arteries. Very recently, Cansell and co-workers (70,71) reported phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cholesterol (70:10:20 mol%) liposomes anchored with dextran (Dx) or functionalized dextran (FDx), both hydrophobized using a cholesterol anchor (CholDx or CholFDx) that penetrates the lipid bilayer during the vesicle formation. The study was performed using radiolabeled markers and fluorescent probes and revealed that coating of liposomes with FDx enables specific interactions with human endothelial cells in culture. Conclusively, bioactive polymer anchored liposomes hold promise as an attractive approach for vascular cell targeting.

Deol and Khullar (72) who developed polysaccharide-anchored liposomes for long circulation and/or lung localization experimented with *in vitro* and *in vivo* models and suggestively reported similar findings. The anchored modules were reported effective in chemotherapy against tuberculosis. Modification of surface of stealth (pegylated) liposomes by tagging O-stearoylamylopectin (O-SAP) resulted in an increased affinity and hence a higher accumulation in lungs than RES predominant organs in normal and tubercular mice (Table 4). Isoniazid and Rifampicin encapsulated in stealth liposomes designed with predominant lung specificity demonstrated reduced *in vivo* toxicity (72). These observations suggest that lung specific stealth liposomes will certainly improve the chemotherapy against human pulmonary tuberculosis and related pulmonary disorders.

Thus it may be realized that alveolar macrophages constitute ligand responsive cellular species which could operate bio-mechanically for drug-carrier uptake, especially those which are anchored with hydrophobized polysaccharides. The uptake may be either by a receptor mediated or receptor independent affinity mechanisms. Furthermore, the colloidal carriers with neutral surface charge are taken up more slowly by macrophages as compared to those, which bear charged surfaces (74,75). The negative charged surfaces are rapidly and excessively taken up probably via charged scavenger receptors. The role of anionic ligands and their subsequent receptor mediated uptake was further evidenced by coating amphotericin bearing liposomes using O-palmitoylated mannan (OPM) and p-aminophenyl-mannopyranoside (PAM) as specific ligand modules and assessing their selective role in receptor-mediated endocytosis (73). Comparative *in vivo* distributions and targeting profiles of O-palmitoylated mannan and P-aminophenyl-mannopyranoside anchored liposomes as compared to plain liposomes were studied in terms of% drug localization indices (Figure 3).

Table 4: Distribution of different labeled liposome formulations after 1 h of intravenous injection in tuberculous mice[1]. Adapted and reproduced from (72) with permission

	% Dose recovered in each organ/tissue after 1 h injection		
Formulation	**Liver**	**Spleen**	**Lung**
PC/Chol	60.0±1.73	3.70±1.62	4.70±1.06
PC/Chol/O-SAP	53.6±3.1	4.0±0.5	19.0±2.0
PC/Chol/O-SAP/DCP	47.1±1.8	2.56±0.5	23.0±2.0
PC/Chol/O-SAP/DCP/GM_1	30.0±1.7	1.7±0.5	29.7±1.5
PC/Chol/O-SAP/DCP/DSPE-PEG	27.1±0.4	3.2±0.9	28.0±0.4
PC/Chol/+PC/Chol/O-SAP/DCP/DSPE-PEG	17.3±0.5	1.0±0.05	37.3±0.5

[1] Tuberculos infection was established in mice with LD_{25} dose (1.5×10^4 CFU/mouse). Different formulations of liposomes were injected intravenously and the animals were sacrificed 1h after injection. Organs were removed, weighed, oxidized and % dose recovered calculated.

Abbreviations: PC, phosphatidylcholine; Chol, cholesterol; O-Sap, O-stearoyl amylopectin; DCP, dicetyl phosphate; DSPE-PEG, disteroylphosphatidylethanolamin-polyethylene glycol.

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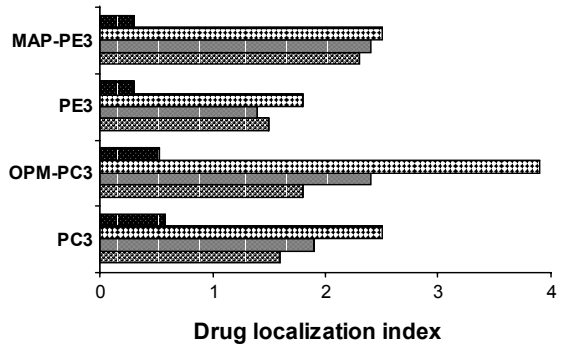


Figure 3: Drug localization indices for different organs recorded with different formulations, i.e., OSAP anchored liposomes (OPM-PC3) and their plain version (PC3) and PAM anchored liposomes (PAM-CE3) and their plain versions (CE3). Drug localization indices for liver (▨), spleen (▩), lung (▦) and kidney (■) were calculated using the data from organ distribution studies after 1 hr. Data points are means ± S.D., n=3.

The comparison of bio-distribution patterns of ligand anchored MLVs revealed that PAM linked liposomes are subjected to higher hepato-splenic accumulation. The drug accumulation in lungs was maximum in the case of OPM anchored liposomes. Thus, mannopyranoside is a specific ligand for targeting bioactives to the macrophages of liver and spleen while OPM could preferentially negotiate selective uptake of bioactives by alveolar macrophages. In an attempt to combine the concept of nebulization and ligand mediated targeting to alveolar macrophages, Vyas and co-workers (unpublished data) used macrophages specific ligands as sensing module appended to liposomes. The study deals with the oral nebulization of rifampicin entrapped ligand appended liposomes as a possible means for direct targeting to the infected alveolar macrophages. The ligands chosen for the study were maleylated bovine serum albumin (MBSA) and O-stearyl amylopectin (O-SAP). The quantitative *in vitro* phagocytic activity and therapeutic index were determined and the degree of (alveolar) macrophage uptake of negatively charged and ligand anchored liposomes, which are comparable to plain liposomes at 1 h interval, nearly doubled at 6-h interval, suggesting that these formulations were avidly phagocytosed. The developed system being a ligand-anchored system is selective for alveolar macrophages because the abundance and exclusive expression of the specific (scavenger) or non-specific (receptors for amylopectin) receptors on the surface of the mature alveolar macrophages.

Targeted chemotherapy

The polysaccharide-anchored liposomes have been studied as stable and targetable drug carriers adaptable in effective chemotherapy, particularly for introducing chemo-therapeutics into target cells or tumor cell lines. These systems possess a unique targetability to specific tissues such as alveolar macrophages and other macrophages of RES. Polysaccharide-anchored liposomes could be employed as carrier constructs on to which an site-specific sensing molecule(s) like MoAb against the tumor surface antigens could be physically or chemically attached (15). The system being site-specific can transport a sufficient quantity of an anti-tumor drug releasing it within or in the vicinity of the target. Polysaccharide-anchored liposomes directed by monoclonal antibody fragment (anti-sialosyl Lewis^x, IgMs) as the sensory device were investigated for their tumor cell binding specificity against human stomach cancer cell line (KATO -III), human lung cancer cell line (PC-9) and the mouse Lewis lung carcinoma cell line (3LL). These ligand directed liposomes showed relatively high specificity towards tumor cell lines *in-vitro* as estimated from radioisotope and fluorescence microscopic techniques. Adriamycin bearing liposomes anchored with immuno-polysaccharide derivative demonstrated *in-vivo* targetability against human lung cancer (PC-9 grafted) in experimental athymic mice (15, 76). Yagi and co-workers (77,78) in different studies have developed and engineered liposomal constructs for brain targeting at human glioma. They employed sulfatides and MoAb as site directing devices to endow targetability to the liposomes. Targeted chemotherapy of brain tumor using polysaccharide-anchored liposomes loaded with antitumor drug cisplatin has been attempted by Ochi *et al.* (79). Survival of 9L-glioma implanted rats with CHP based liposomes loaded with cis-platinum diamino-dichloride (cisplatin) was significantly higher as compared to average survival recorded for untreated groups. Targeted chemotherapy to colon cancer cell lines was performed using polysaccharide-anchored immunoliposomes bearing anti-CEA Fab'(IgG) fragments. The system was able to induce specific binding to carcinoembryonic antigen producing BM314 cell lines, indicating that anti-CEA IgG is an excellent site-specific ligand for CEA producing cells, where encap-

sulated chemotherapeutic agents in polysaccharide-anchored liposomes could be delivered selectively to the site (80).

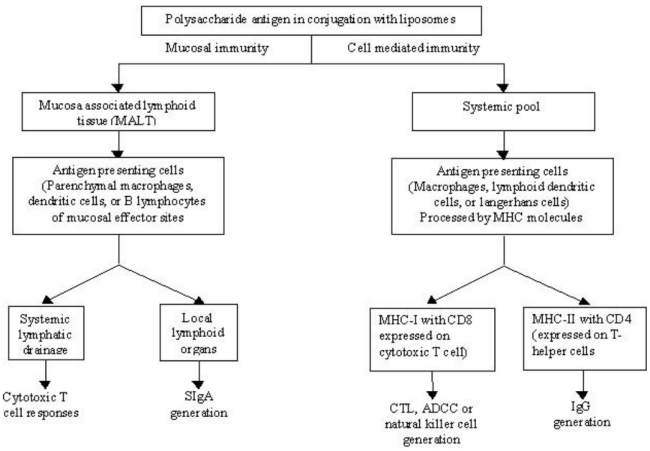
Recently, the polysaccharide-anchored liposomes have been exploited as carrier cargo equipped with a targeting ligand, where the polysaccharide coating stabilizes the system both *in vivo* and *in vitro* while the anchored recognition ligand confer the system a target selectivity. Shinkai and co-workers (81) prepared magnetoliposomes for hyperthermia based treatment of cancer. The liposomes were anchored with hydrazide pullulan to stabilize the phospholipid capsules and to provide an anchor for the immobilization of antibodies. By this method, 90-180 molecules of a specific monoclonal antibody were immobilized on to magnetoliposomes. When the antibody-conjugated and polysaccharide stabilized magnetoliposomes were incubated with cancer cells, they bound to the cell surface and were taken up by cells in about 12 times higher magnitude than the control formulations.

Ichinose and co-workers (82,83) evaluated anti-tumor effects of polysaccharide-stabilized and ligand anchored liposomal Adriamycin on A66 hepatoma transplanted in nude mice. Tumor recognition ligand, 1-amino lactose (1-AL) was appended on the surface of cholesteroyl amylopectin (CHP)-anchored liposomes. The study was aimed at evaluating a role of polysaccharide coating on stability and tumor recognition ligand as a target site ligand. The uptake of these liposomes by AH66 rat hepatoma cells was estimated to be higher than liposomes without 1-aminolactose *in vitro*. Furthermore, 1-AL/CHP liposomal Adriamycin showed a stronger antitumor effect compared to other types of liposomal Adriamycin *in vitro*. When *in vivo* tumor-targeting efficacy was investigated in AH66 tumor transplanted mice using 3H-liposome, the tumor/serum radioactivity ratio in mice injected with 1-AL/CHP liposome was higher than with mice injected with other liposomes. These observations suggest that on anchoring cell recognition elements along with polysaccharide anchored liposomes, anticancer drug carriers can be engineered for the active targeting to tumor cells.

Systemic and mucosal vaccination

Polysaccharides from the natural or bacterial origin exhibit excellent immune responses in association with protein carriers. However, their conjugation with liposomes for provoking desired systemic or mucosal immune responses has not been widely investigated. Immunization potential of the bacterial polysaccharides encapsulated within liposomes is frequently suggested in the literature (85-87). Recently, the potential of natural polysaccharide-anchored liposomes as an adjuvant for cell mediated immunity has also been explored (88-90). Wachsmann and co-workers (85) reported serum and salivary antibody responses in rats orally immunized with *Streptococous mutans* carbohydrate protein conjugate associated with liposomes as vaccine adjuvant. The purified polysaccharide antigen of *S. mutans* was coupled through reductive amination to a cell wall protein of molecular weight 74000. The liposomes bearing the conjugate on intra-gastric administration to rats produced a local immunoglbulin A response (secretory IgA). The conjugate of cell wall protein and polysaccharide incorporated in liposomal system may thus be presented as a potential adjuvant for oral vaccination against *S. mutans* vis a vis dental caries.

The intracellular components or antigens are processed through MHC class-I thus restricted to CD8+ T cells, whereas MHC class-II and CD4+ T lymphocytes are involved in the processing of exogenous components, pathogens or antigens (Scheme 4).



Scheme 4 Proposed mechanisms for systemic or mucosal immunization by polysaccharide-anchored liposomes

It has also been demonstrated in several cases that MHC class I restricted CD8+ cytotoxic T lymphocytes are responsible for providing immuno-protection (or rejection) against grafted tumors and viral infections. For instance, cytotoxic T lymphocytes (CTL) were induced in WKA/H rats against syngeneic human T-lymphotropic virus-I positive (HTLV-I) cell lines

Cellular immunity was examined after immunizing rats with a truncated hybrid protein (228 amino acids) of gag and env of HTLV-1 produced by *Escherichia coli*. It was found that CTL recognizes *gag-env* coded antigens more explicitly than *env*-coded antigen (86). On the basis of these findings, *in vivo* immunization of WKA/H rats was attempted by Noguchi and co-workers (87) with the use of cholesteroyl-mannan (CHM) anchored liposomes bearing a HTLV-I related antigen (*gag-env* hybrid protein). The *gag-env* hybrid protein-reconstituted liposomes (gag-env-lipo) with or without polysaccharide coat were used to immunize WKA/H rats subcutaneously twice, with one- week intervals. One week after the last immunization, HTLV-I^+ tumor cells (TARS-I) were implanted intradermally and subsequently splenic cells were isolated for assessing CTL responses. Isolated splenic cells were sensitized *in vivo* with mitomycin-C treated TARS-cells. Killer cell activity against TARS-I was observed only in the case of immunization with CHM anchored *gag env*-reconstituted liposomes. Rats immunized with gag-env-lipo displayed accelerated rejection of TARS-1 but not of other HTLV-1-negative tumor lines. Injection of carrageenan into animals strongly inhibited generation of killer cells, which indicates for the need of macrophages for priming of CD8+ T cells with gag-env-lipo. No killer activity was recorded when spleen cells were obtained from animals immunized with the hybrid protein alone, the liposome alone, or the hybrid protein reconstituted into conventional liposomes without any polysaccharide coating. (87). The induced killer cells were MHC class-I restricted CD8+/CD3+ CTL (not CD4+ cells) and were completely specific to syngeneic HTLV-I^+ cells. The effective tumor rejection was assumed to involve a macrophagic phagocytic process for CHM anchored liposomes. The generation of MHC class-I restricted CD8+CTL could be due to the cytosolic processing of *gag-env* hybrid protein after it releases from the endosomal apparatus.

The antigen presenting cells taking part in the immunological consequences in case of viral infections express mannose receptors on the cell surface and hence mannose-terminated polysaccharide ligand could be appreciated as a targeting ligand in viral infections. Ohishi and co-workers (91) developed a peptide-based vaccine that induced cell-mediated immunity. 20-mer synthetic peptide, spanning the 98-117 amino acids of bovine leukaemia virus (BLV) envelope glycoprotein (Env) gp51 was encapsulated in mannan-anchored liposomes. The liposomes induced specific delayed-type hypersensitivity, lymphocyte proliferative responses with a weak cytotoxic lymphocyte response in mice. The spleen cells from the immunized mice secreted a large amount of IFN-gamma and IL-2, indicating the induction of Th-1 type immunity in mice elicited through T-cell epitope on synthetic peptide-liposomes.

Promising results were recorded when cDNA of HIV-1 was incorporated into mannan-anchored liposomes (90,92). These workers studied the adjuvanticity of mannan-anchored liposomes for human immunodeficiency virus type-1 (HIV-1) DNA vaccine and the mechanism involved in the immunogenicity enhancement. Coating of cationic liposomes with mannan significantly potentiated the vaccine and induced an HIV-specific delayed-type hypersensitivity (DTH) response. HIV-specific cytotoxic T-cell (CTL) activity elicited by DNA vaccination was also significantly potentiated on co-administration with mannan-liposome in the form of a therapeutic cocktail. This mannan-liposome-mediated activity was inhibited noticeably by pre-injection of anti-interferon (IFN)- γ antibody suggesting an important role of IFN-γ in HIV-specific immune response. The results of both isotype-specific antibody and cytokine analysis revealed that mannan-anchored liposome-based DNA vaccination could prove to be a valuable tool for the enhancement of HIV-1 specific cell-mediated immune response (Table 5). A predominance of Th1 cells through use of mannan-anchored liposomes was evident from cytokine assay data, wherein high IFN-γ production and moderate IL-4 levels were estimated on administration of liposomal adjuvant based DNA vaccine.

The finding that mannan abundant in mannose residues is critical in eliciting cytotoxic T lymphocyte (CTL) response substantiates and supports the findings of Fukusawa and co-workers (89,93).

Table 5: Cytokine profile of supernatants of lymphoid cell cultures of mice immunized with IIIB/REV plus mannan and/or liposomes[1]. Reproduced from (90) with permission

	Amount of cytokines					
	IFN-γ (ng/ml)			IL-4 (pg/ml)		
Immunogens	Ag (-)	Con A	IIIB peptide	Ag (-)	Con A	III B peptide
Non-immunogenic	UD	13.8±5.1	UD	35±6	260±56	39±4
IIIB/REV	UD	20.2±10.3	4.6±2	31±8	383±51	42±6
IIIB/REV+liposomes	UD	30.7±8.6	5.4±1	42±4	302±73	40±12
IIIB/REV+liposomes+ mannan	5.2±4	63.9±9.5*	10.2±3	40±11	311±42	35±6
IIIB/REV+liposomes+ mannan+anti-TFN-γ Ab	UD	UD	UD	76±24*	485±35*	NT
IIIB/REV+liposomes+ mannan+anti-IL-12 Ab	UD	UD	UD	86±34*	512±42*	NT

[1] Mice were immunized with a dose of 0.1 or 0.2μg of HIV-1 DNA vaccines (IIIB/REV) and with or without liposomes and mannan or mannan anchored liposomes, which were administered intramuscularly on days 0,7 and 21. The immune lymphoid cells were activated with Con A and HIV-IIIB-V3 peptide. Mean levels ±SE of cytokines were determined.

UD, undetectable; NT, not tested;

** Stastistically significant difference ($p<0.05$) between non-immune controls and experimental groups*

Reproduced from (90) with permission

The coating of liposomes with various oligosaccharides and yeast derived mannan drastically enhanced the induction of ovalbumin-specific delayed-type footpad swelling response in Balb/c mice with a peak at 24 to 48 host-challenge (93). The mannan- coated liposome was included in the study as a reference to compare the effects of various neoglycolipids for their augmentation of a delayed type of response. It is possible that the receptor-mannose interaction of oligomannose- or mannan-anchored liposomes might have augmented the processing of OVA reconstituted in these liposomes. In addition, the mannose residues may possess some other activity such as stimulation of IL-12 release culminating the activation of T-lymphocytes. Only those neoglycolipids (oligosaccharides) with mannose residues at non-reducing termini were found effective. However, these workers suggested that instead of using mannan, which can elicit antibody and B-cell mitosis (immunogenic) and toxic effects on iv administration, safer neoglycolipids like oligomannose, which are ubiquitously found in the body, should be used as an adjuvant for the induction of cell mediated immunity.

Recently, Venketesan and Vyas (94) rationalized the role of polysaccharide anchored liposomes in the enhancement of the immunogenicity of the model antigen after oral administration. The results were compared in terms of serum IgG and IgA titers against bovine serum albumin (BSA) administered as control, plain and anchored formulations (Figure 4).

The antibody titre (IgA) value recorded using ELISA with a microplate reader as optical density at 405 nm, was found to be 0.134 following oral administration and 0.139 after intraperitoneal administration for OPP-anchored liposomes. The results showed insignificant effect of the route of administration on secretion of IgA, whilst IgG levels were significantly different. Serum IgG levels on day 15, in the case of orally administered OPP-anchored liposomes were found to be 0.394 while those administered through intraperitoneal route recorded an optical density of 0.500. Antibody levels on administration of liposomes anchored with pullulan alone did elicit an immune response better compared to plain liposomes. This seemingly attributes to the intrinsic immuno-genicity provoked by pullulan. The pullulan borne antigenicity may contribute to an increase in serum IgG levels. However, the serum IgA level recorded was lower as compared to its palmitoyl derivative. The increased IgA level recorded following oral administration of OPP anchored liposomes as compared to plain liposomes or pullulan anchored liposomes suggests that the OPP anchored liposomes were stable in harsh bio-environment of stomach and might be taken up by the Peyer's patches. However, the role of polysaccharide anchored liposomes for mucosal immunization especially through the Peyer's patches and gut associated lymphoid tissue (GALT) is yet to be explored.

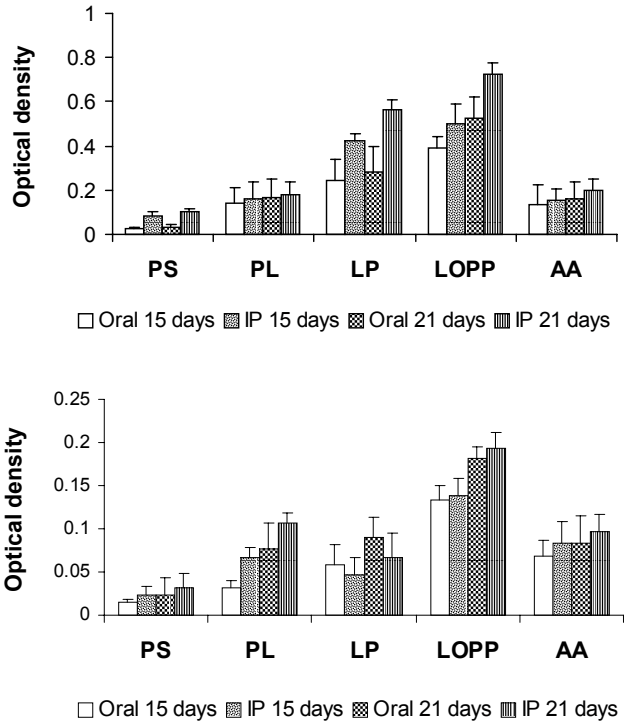


Figure 4: Serum IgA (Figure 4A) and IgG (Figure 4B) level in rats following oral and intra-peritoneal administration of various formulations in albino rats. PS, plain BSA solution; PL, plain BSA-liposome; LP, liposomes anchored with pullulan; LOPP, liposomes anchored with O-palmitoyl pullulan (OPP); AA, alum adsorbed. On day 1, groups of rats were administered orally with preparations containing BSA equivalent to 100μg. A similar group of mice was injected intraperitoneally. Secondary immunization was done on day 15 with plain and polysaccharide anchored liposomes containing BSA equivalent to 100 μg. Blood was collected from the orbital plexus on day 15 and 21. Specific anti-IgG and IgA antibody levels in the serum were determined. Adapted from 94 with permission.

Macrophage activation

Immunomodulators are biological response modifiers and defined as substances, which activate macrophages, e.g., muramyldipeptide and its derivative (95), polyanionic polymer (96) and polynucleotides (97). These immuno-modulators by themselves, however, do not usually have any specificity or affinity to activate macrophages. Liposomes interact efficiently with macrophages, thus macrophages may serve as antigen presenting cells for liposomal antigens/immuno-modulators. In order to modulate the *in-vivo* activity of several immunomodulators, they are generally administered encapsulated in polysaccharide-anchored liposomes. Polyanion polymers and synthetic polynucleotides were encapsulated into macrophage specific liposomes such as those anchored with mannan-Chol dervatives (97-99). Ottenbrite and coworkers (100) reported improvement in immuno-potentiation activity of poly-anionic polymers following encapsulation in polysaccharide-anchored liposomes. The effective activation of mouse peritoneal macrophages was observed by poly (maleic acid -alt-2-cyclohexyl-1,3-doxap-5ene) (MA-CDA), when administered encapsulated in CHM anchored liposomes. Macrophage activation was mediated through superoxide anion production and as a result tumoricidal activity was increased nearly 5 times over plain MA-CDP at three-day post administration interval. Akashi and co-workers (101) evaluated potential immuno-modulator activities of polysaccharide-anchored liposome bearing synthetic polynucleotide, polyvinyladenine and vinyladenine-alt-maleic acid (poly VA-MA). CHM anchored liposomes bearing poly VA-MA were more effective as compared against the free poly (VA-MA), when evaluated for superoxide anion production by mouse peritoneal macrophages. These studies signify measurable macrophage activation and increased immunopotentiation of liposome encapsulated contents on surface anchoring of polysaccharides.

Gastric mucoadhesion

The ability of polysaccharides to interact with liposomes has been reported to have three possible implications: The stabilization of the liposomes, the targetability of the appended polysaccharide ligand, and the possibility of targeting the vesicles to a specific site due to its bioadhesive and mucoadhesive properties. Among the polysaccharides used in drug delivery, chitosan has been widely employed for its bioadhesive properties. This is due in part to its characters like high molecular weight and degree of de-acetylation, its gel forming at low pH and also due to its poly-cationic character, which imparts it ability to bind strongly to mammalian cells.

Takeuchi and co-workers (102) studied polymer anchored muco-adhesive multilamellar liposomes consisting of dipalmitoyl phosphatidylcholine (DPPC) and dicetyl phosphate (DCP) (8:2 molar ratio) and anchored with three different types of polymers: chito-

san, polyvinyl alcohol having a long alkyl chain, and poly (acrylic acid) bearing cholesterol. The muco-adhesive function of the polymer-anchored liposomes was evaluated *in vitro* using rat intestine and % adhesion was estimated by a particle counting method. Chitosan anchored liposomes showed the highest% adhesion among the polymer-anchored liposomes tested while non-anchored liposomes exhibited negligible to no adhesion. The adhesion of chitosan-anchored liposomes to the intestine wall was further confirmed by fluorescence microscopy using pyrene-loaded liposomes. The muco-adhesiveness of chitosan-anchored liposomes was subsequently evaluated to develop a novel drug carrier system for oral administration of poorly absorbed drugs such as peptides (103). Takeuchi and co-workers (103) prepared muco-adhesive chitosan-anchored liposomes to improve oral absorption of insulin. After *in vivo* administration of the chitosan-anchored liposomes to male wister rats, the hypoglycemic response was prolonged over a period of up to 12 h. This sustained effect was attributed to the muco-adhesiveness of the system leading to an increased duration of contact with intestinal mucosa and hence an increased probability of insulin absorption. These studies explore the possibilities of polysaccharide anchored liposomes for the administration and muco-adhesion of poorly adsorbed drugs and macromolecules.

Conclusion And Future Prospects

Ligand mediated bio-disposition and cellular interaction of liposomes especially at the target sites would be a focal paradigm of the future research in the field of drug and antigen delivery. Polysaccharide anchored liposomes have paved the way for the bio-stable, site-specific and ligand directed delivery systems with desired therapeutic and immunological characteristics. Encapsulation of cytotoxic drugs, antimicrobial agents, immunomodulators and macrophage activators, and natural or bacterial polysaccharides into polysaccharide anchored liposome generates better pharmacological and immunological activity at the desired limits (Table 6). The delivery module appears conceivably promising for delivery of pharmacological and biologically active molecules to the specific cells or tissues. Furthermore, by keying a relevant ligand as polysaccharide-Chol derivatives, the system can be utilized either for cellular targeting or as a long circulatory stealth system, depending upon the nature of the conjugated bio-sensor molecules. Reports are quite vague and controversial as far as the priming of liposomes with polysaccharides for systemic long circulation is concerned (50,84). Amongst the abounding reports in the literature, the majority of the cases refer to the intrinsic or natural distribution pattern of the polysaccharide-anchored liposomes towards the alveolar macrophages and monocytes. Though they themselves seem not to offer any long circulation, the increased surface hydrophilicity due to inclusion of negatively charged sialic acid reported to provide a much better stealth character to the polysaccharide-anchored liposomes. Polysaccharide-anchored liposomes bearing a sialic acid moiety [sialosyl $\propto(2\rightarrow6)$ glycopyranose] have been reported to evade phagocytosis as a consequence of decreased RES interception (54). Conjugation of sialic acid derivatives to pullulan or amylopectin demonstrated their effective rejection by phagocytosis (and hence in accumulation/uptake in MPS) when compared for their internalization efficiencies in human blood monocytes *in vitro*. Hence, liposomes anchored with such modified polysaccharides promise enormous potential as long circulatory drug carrying vehicle. The role of the glycocalyx in regulating access of particles in to the apical plasma membranes of intestinal epithelial cells is mainly clearing up, the microbial attachment and holds for oral vaccination potential of polysaccharide bearing systems, which may become a future therapeutic tool. With the emergence of mannan binding lectins known to participate in complement activation, a new field of drug carrier investigation seems to open up (36-40). Recently, anti-tumor activity of mannan binding protein has been established hence mannan as epitope on liposomal surface can prove to be an ideal carrier for expressing cell specific cytotoxicity. Mannan-anchored liposomes were found to deliver the paramagnetic contrast agent (Gadolinium-diethylene-triamine-penta-acetic acid, Gd-DTPA), which is used in the magnetic resonance imaging (MRI) primarily of liver and lungs at a significantly lower dose than required by conventional means thus increasing MRI contrast precision (104). The transformation of cells in disease(s) results in to subsequent specific changes in carbohydrate recognition domains (lectins) of the cell surfaces thus offers a means of specific targeting to the diseased cells. Mannan-binding lectin deficiency was found to be associated with recurring cutaneous abscesses, prurigo and possibly atopic dermatitis and a plethora of pathological disorders and

immuno-defects. The findings suggest that liposomes appended with mannan could serve as handles to selectively deliver the drugs to the complementary lectin receptors, which are down regulated in these diseased states. The role of polysaccharide anchored liposomes in topical and cosmetic applications, non-viral gene vectorization, oligonucleotide delivery, oral, intragastric, nasal or other mucosal immunization and bio-film targeting is yet to be explored and realized. It is conceivably convincing that the polysaccharide(s) imparts specific functional characteristics to the liposomes and a simultaneous improvement *in vitro* and *in vivo* stability. They therefore have a distinctive role and potential in site-specific specialized drug delivery.

Table 6: Therapeutic applications of polysaccharide-anchored liposomes

Applications	System	Drug/probe	Status/comments	Ref
Microbial and lung therapeutics				
	Liposomes bearing amylopectin derivatized with cholesterol	Ampicillin/Minocycline	100% survival from Listeria monocyto-genes infection in mice	66
	O-palmitoyl pullulan and O-palmitoyl amylopectin bearing liposomes	Sisomycin	Therapy of Legionella pneumophila infected monocytes and guinea pigs	14
	Liposomes anchored with amylopectin and mannan	CeftazidineamphotericinB	Intracytoplasmic pathogens and fungus inhabitant to lung and liver macrophages	67
	Liposomes anchored with amylopectin	Amphotericin B	Therapy of murine model of pulmonary candidiasis	68
	Amylopectin anchored liposomes	Copolymers of cis-4-hydroxy-L-proline and PEG	Lung specific antifibrotic activity in pulmonary artery undergoing vascular remodelling	69
	O-Stearoyl amylopectin anchored liposomes	Rifampicin,Isoniazid	Improved chemotherapy against pulmonary tuberculosis in mice	72
	O-palmitoyl mannan (OPM) and P-aminophenol mannopyranoside (PAM) anchored liposomes	Amphotericin B	Targeting to alveolar macrophages	73
Targeting chemotherapy				
	Polysaccharide anchored immunoliposomes bearing an IgM fragment	Terbium tri-sacetyl acetonate as probe, Adriamycin	Cell specificity and targetability towards tumor cell lines; In-vivo targetability against human lung cancer (PC-9)grafted disease in athymic mice	14
	O-palmitoyl pullulan anchored liposomes	Cisplatin	Targeting to brain tumour	79
	Polysaccharide-anchored immuno-liposome bearing anti-CEA Fab' (IgG) fragment	Hydrophobic fluorescent probe	Targeting to colon cancer cells; specificity of MoAbs to CEA producing BM314 cells	80
	Cholesteroyl pullulan anchored liposomes bearing 1-amino lactose as targeting ligand	Adriamycin	Restrained tumor growth against A66 Hepatoma transplanted in mice	82,83
Cellular targeting				
	Phosphorylated CHM (Chol derivative of mannan) anchored liposomes	Water soluble nutrients	Targeting to fibroblasts Specificity for mouse fibroblasts (L-cells)	99
	Mannan or amylopectin anchored liposomes	Amphotericin B	Targeting to phagocytes mediated by alveolar macrophage/monocytes or neutrophiles	68
Vaccine adjuvant				
	Polysaccharide-anchored liposomes bearing an HTLV-I related protein, gag-env hybrid protein	antigen	Effective immunization with CTL CD3+/CD8+; generation of killer cells and rejection of grafted tumor	86,87
	Bacterial polysaccharide containing liposomes	antigen	Pulmonary secretory antibody response	88
	Mannan anchored liposomes	ovalbumin	Adjuvant for cell mediated immunity	89
	Mannan anchored cationic liposomes	antigen	Enhancement of HIV-1 DNA vaccine and cell mediated immunity	90
	O-palmitoyl pullulan anchored liposomes	Bovine serum albumin	Enhancement of IgA and IgG levels against model antigen	94
Macrophage activation				
	Polysaccharide (CHM)anchored liposomes encapsulating immuno-modulators	Polyanionic polymer, Poly MA-CDA)	Targeting to macrophages, macrophage activation, immunopotentiation	100
	Polysaccharide (CHM)anchored liposomes encapsulating immuno-modulators	Synthetic polynucleotide poly (VA-MA)	Targeting to macrophages, macrophage activation	101
Long term stability in-vivo				
	O-polysaccharide-anchored liposomes	Coenzyme Q10	In-vivo stability against serum, lipases and lipoxygenases	52
	Black lipid membrane anchored with pullulan/amylopectin (either palmitoylated /cholesteroylated)	Dipicrylamine, a lipophilic ion membrane probe	Stabilization due to combined effect of long term stability of black lipid membrane and polysaccharide capping	53
	Proteoliposomes mixed with dextran derivatives bearing hydrophobic anchor groups	Water soluble fluorescent probe, 6-CF	Reduced fusogenicity; protection against membrane disintegration on freezing/thawing	54
	O-palmitoyl pullulan anchored liposomes	6-CF	Improved stability, reduced permeability and fluidity	56
	O-palmitoyl pullulan anchored liposomes	Carboplatin	Improved stability, increased platinum latency and long term storage	57
	O-palmitoyl pullulan (OPPu) and cholesteroyl pullulan (CHPu) anchored liposomes	Propranolol HCl	Improved stability against detergent and bile salts, freeze-thaw cycling, osmotic stress and long-term storage	62

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