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ARCHAEOSOMES: INNOVATIVE APPROACH FOR MODERN DRUG DELIVERY PERSPECTIVES

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ABSTRACT

Archaeosomes, or liposomes made up of one or more ether lipids unique to the Archaeobacteria domain, are a novel kind of liposome found in Archaea. The fundamental structures of Achaean-type lipids are archaeol (diether) and caldarchaeol (tetraether). Traditional techniques (hydrated film sonicated, extrusion, and detergent dialysis) may be used to create archaeosomes at any temperature in the physiological range or lower, enabling thermally stable compounds to be encapsulated. A number of physiological and environmental factors have an impact on its stability. Archaeosomes are often used as drug delivery systems for cancer vaccines, Chagas disease, proteins and peptides, gene delivery, antigen delivery, and natural antioxidant compounds. The primary aim of this study was to examine the applications of this new carrier technology in the pharmaceutical industry.

KEYWORDS: Archaeosomes, Archaeobacteria, Drug Delivery, Cell Delivery, Applications, Formulations.

INTRODUCTION

Archaeosomes are liposomes containing one or more ether lipids unique to the Archaeobacteria domain, as well as vesicles made up of a range of lipids, including archaea bacterial ether lipids. Archaeosome lipid membranes can be bilayers (if made exclusively from monopole archaeol lipids or lipid mixtures containing archaeols and non-archaeobacterial monopolar lipids), monolayers (if made exclusively from bipolar caldarchaeol lipids), or a combination of mono and bilayers (if made exclusively from bipolar caldarchaeol lipids) (if made from caldarchaeol lipids and archaeols or other monopolar lipids). Archaeosomes are a novel kind of liposome formed from ether lipids found in Archaea. The basic structures of lipids of the Achaean type are archaeol (diether) and caldarchaeol (tetraether). Archaeosomes' design encourages the presence of both hydrophobic and hydrophilic domains, enabling them to entrap both hydrophilic and hydrophobic molecules, which is very helpful for drug encapsulation and administration. The idea of archaeosomes includes the use of synthetically generated lipids with archaeobacterial ether lipid structural characteristics including branching phytanyl chains connected via ether bonds at sn-2, 3 glycerol carbons. The lipid membrane of archaeosomes can be found as a bilayer when made entirely of monopolar archaeol (diether) lipids, a monolayer when made entirely of bipolar caldarchaeol (tetraether) lipids, or a mixture of monolayers and bilayers when made entirely of caldarchaeol lipids in addition to archaeol lipids or standard bilayer-forming lipids. The vast range of lipid structures reflects Archaea's need to change their fundamental lipid structures in order to sustain membrane function even under harsh conditions.[1]

A distinguishing feature should be especially helpful in the production of extremely stable archaeosomes.

- The branching methyl groups help to reduce both crystallisation and formation time (membrane lipids in the liquid crystalline state at ambient temperature, membrane permeability, membrane lipids in the liquid crystalline state at ambient temperature, and steric hindrance of the methyl side groups).
- The saturated alkyl chains would offer stability against oxidative degradation, and the glycerol backbone's distinctive stereochemistry (opposite to mesophilic species) would provide resistance to phospholipases generated by other organisms.
- Adding cyclic structures to the transmembrane area of the lipids seems to be a thermo-adaptive response, resulting in increased membrane packing and reduced membrane fluidity.[2]

Traditional techniques (hydrated film sonicated, extrusion, and detergent dialysis) may be used to make archaeosomes (**Figure 1**) at any temperature in the physiological range or lower, enabling thermally stable compounds to be encapsulated. They may be made and stored in the presence of air/oxygen without deteriorating. According to *in vitro* and *in vivo* studies, archaeosomes are safe and do not induce toxicity in mice.[3]

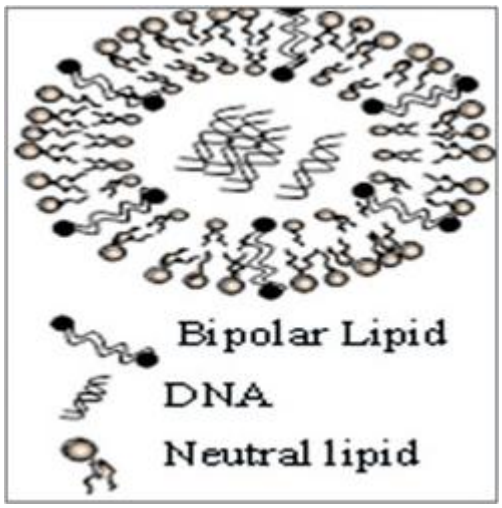

Figure 1: Structure of Archaeosomes.

SOURCE OF ARCHAEA

Three significant sources of archaea have been discovered, which will greatly aid in the availability of the same.[4]

- **Halophiles:** *Natronobacterium magadii*, *Halobacterium cutirubrum*.
- **Thermoacidophiles:** *Thermoplasma acidophilum.*
- **Methanogens:** *Methanococcus jannaschii*, *Methanococcus voltae*, *Methanosphaera stadtmanae*, *Methanosaeta concilii*, *Methanospirillum hungatei*, *Methanobacterium espanolae*, *Methanobrevibacter smithii*, *Methanosarcina mazei*.

ADVANTAGES OF ARCHAEAL LIPIDS

From the manufacture of pharmaceuticals through their stability maintenance, archaeal lipids play an essential role. The following are some of the most significant benefits of archaeal lipids.

- Archaeal lipids are more stable than phospholipids from other organisms.
- The hydrolysis stability of other enzymes is improved by the chemically modified archaeal lipid derivative.
- Adding cyclic structure to lipids seems to be a thermo-adaptive response, with increased membrane packing and reduced membrane fluidity as a consequence.
- Stability against oxidative degradation would be enhanced by saturated alkyl chains.
- Cholesterol is not required in the formulation.
- Resistance to phospholipases generated by other species is ensured by the stereochemistry of the glycerol backbone.
- It may be applied to specific organs.
- Because bipolar lipids are more stable, they can be manufactured and stored in the presence of air/oxygen without deteriorating.
- Due to their excellent thermostability, archaeosome formulations can be sterilised by autoclaving.
- Archaeal lipids have a higher thermo-labile in the environment.
- Phagocytic cell absorption is greater, resulting in good adjuvant activity.
- Archaeal lipids serve as self-adjuvanting drug delivery systems.[5]

TYPES OF ARCHAEA LIPIDS

The following are the three main types of Archaea lipids:

Natural lipids

The structures of archaeal lipids are quite similar to those of the species from which they were derived. Depending on their life circumstances, Archaea may be classified into a variety of species, each with its unique set of lipids. Monopolar dieter compounds are used to make halophilic polar lipids (archaeal), while bipolar tetraethers are used to make acidophilic polar lipids (caldarchaeol). However, most archaeal lipid membranes are made up of a mixture of lipids with various polar heads (phosphate, glycerophosphate, sugar, and so on); furthermore, some archaea species, such as methanogens, have developed polar lipid mixtures of diether and tetraether type. Natural archaeal lipids, which span the whole spectrum of archaeal lipid structures, are isolated from common Archaea species. Many research groups have extracted and purified total polar lipids (TPL) from methanogens, halophiles, and thermoacidophiles. Archaea were grown in perfect conditions before being removed in huge numbers using organic solvents. TPL was created by using acetone to precipitate a chloroform/methanol (2:1 v/v) solution. The extracted polar lipid components were identified using thin-layer chromatography (TLC) and mass spectrometry (MS), and the extracts were either used directly or processed further using preparative TLC to identify pure polar lipid components. Diether structures are built on the basis of a glycerol moiety with two phytanyl chains on the sn-2,3 positions. The main polar head groups of such archaeal derivatives are negative-charged phosphoethanolamine and phosphoglycerol. Tetraether components are defined by the presence of two diphytanyl chains linked at both ends to two glycerol residues in either an antiparallel (caldarchaeol) or parallel (isocaldarchaeol) manner, while the glycerol configuration stays unchanged. The polar lipid structure may be completed by sugar neutral head groups, negative phosphoinositol, or zwitter ionic groups.[6]

Chemically modified natural lipids

The polar head groups of natural polar lipids from archaea lipid extracts are usually sensitive to acidic hydrolysis. Matching dihydroxyl archaeal lipid cores are obtained as a result of this chemical procedure. The Syrinx Diagnostika Company patented many new archaeal lipid structures derived from *T. acidophilum* lipid extracts. Following the hydrolysis of the initial polar head groups, a few chemical synthesis steps such as oxidation of primary alcohols to carboxylic acids, activation, and coupling with suitable amines made the necessary archaeal lipids with aminated polar head groups readily available. The modified diether and tetraether lipid cores were from *Halobacterium salinarum* and *Tetrabacterium acidophilum*, respectively. Archaeols were then functionalized with sugar or phosphorylated head groups, whereas caldarchaeols were symmetrically or unsymmetrically functionalized with the same polar head groups. Phosphoserine, phosphoethanolamine, phosphoinositol, and phosphoglycerol were utilized to generate phosphorylated groups from glucose disaccharides, trisaccharides, and tetrasaccharides, galactose, and mannose di-, tri-, and tetrasaccharides.[7]

Totally synthetic lipids

Despite the fact that natural archaeal lipid extraction produced vast amounts of pure molecules with certain difficulties, several academic or business research teams investigated the full synthesis of archaeal lipid analogues. Archaeal lipid analogues are formed by combining a symmetrical 1,3-cyclopentane ring with two alkyl or alkoxy chains and two glycerol units. Finally, a phytanyl arm is attached to each glycerol residue, forming a quasimacrocyclic tetraether structure. The stereochemistry of the glycerol moieties was maintained when compared to natural archaeal lipids generated from isocaldarchaeol (parallel arrangement of the glycerol groups). Sugars, glycine betaine, phosphorylated groups, PEG chains, and ligands were introduced symmetrically or asymmetrically at both terminal ends of the tetraether core. In this instance as well, two phytanyl or linear C_{16} arms produced quasimacrocyclic backbones. Genuine archaeal lipids have the same stereochemistry. Caldarchaeol and isocaldarchaeol analogues were constructed and functionalized using several polar head groups, such as a PEG chain, aminated groups, or phosphorylated groups.[8]

PREPARATION TECHNIQUES

Lipid extraction from suitable archaeobacterial species has started. The entire natural archaeal lipid extract is made up of TPL, neutral lipids like Squalene, and other hydrocarbons (TLE). TLE is made by extracting freeze-thawed biomass from selected archaea species in a chloroform/methanol/water mixture. By precipitating TPL with acetone, neutral lipids and TPL may be separated from TLE. TPL made from isoprenoid ether lipids with opposing sn-2,3 stereochemistry may be stored in chloroform or chloroform/methanol (2:1) solutions without any extra conditions. The glycol lipid sulphate and phospatidylglycerophosphate fraction make up the whole of the vesicle. Pure archaeal lipids may be obtained via chromatography, either column or preparative thin-layer chromatography. To add certain head groups to archaeal lipids, chemical changes may be performed. One polar group is the archaeal lipid phosphatidylmyoinositol, while the other polar head group, glucopyranose or galactopyranosyl glucopyranose, is needed for structural stability. It's also difficult to hydrate these lipids. It was possible to construct archaeosome formulations and encapsulate/associate hydrophilic or hydrophobic molecules beginning with natural, chemically modified, or synthesized archaeal lipids using methods developed for the manufacture of conventional liposomes.[9]

Mechanical dispersion method

All methods that begin with a lipid solution in an organic solvent and end with a lipid dispersion in water fall under this category. Typically, the various components are combined by co-dissolving the lipid in an organic solvent, then extracting the organic solvent and hydrating the solid–lipid combination with an aqueous buffer. When lipids stretch and hydrate on their own, archaeosomes are produced. Procedures alter their final features at this step by including various extra processing variables. Some post-hydration treatments include vortexing, sonication, freezing and thawing, and high-pressure extrusion.[10]

Lipid hydration method

In a rotary evaporator flask, Archaea lipid is dissolved in a solvent mixture of chloroform-methanol (2:1), and a dried thin film of lipid is produced using the rotary evaporator. This process was performed for 15 mins at room temperature (30°C) and at 60 rpm. Adding 5 mL of saline phosphate buffer to the drug/antigen to be encapsulated achieves lipid hydration in this technique. A rotating evaporator was used to produce a uniform suspension. The liquid crystalline-like or fluid state of archaeobacterial polar ether lipids at room temperature allows them to be hydrated, thus it was kept at room temperature for 2 hrs to complete the swelling process. This technique may be used to make multilamellar vesicles (MLVs). Archaeosomes anneal even at subzero temperatures. For example, archaeal lipids generated from Archaea *H. salinarum* and improved soy phosphatidylcholine were utilized to produce BMD-loaded archaeosomes and conventional liposomes using thin-lipid film techniques. Ultra deformable archaeosomes are vesicles made out of soybean phosphatidylcholine (SPC), sodium cholate (NaChol), and polar lipids from *Halorubrum tebenquichense* (3:1:3 wt/wt) (UDA). UDA was created utilizing the lipid hydration method for topical applications.[11]

Membrane extrusion

The size of prepared archaeosomes can be reduced by gently passing them through a membrane filter with a

specified pore size, and this can be accomplished at a much lower pressure. During the breaking and resealing of polar phospholipids as they pass through the polycarbonate membrane, the vesicles content is extruded with the dispersion medium in order to achieve high entrapment. This method produces archaeosomes called large unilamellar vesicles through extrusion (LUVETs), which may encapsulate up to 30% of the lipid content. The polar lipid methanol fraction was used to produce archaeosomes. Before being vortexed to form multilamellar (ML) vesicles, the dry lipid film was hydrated using KPB buffer (250 nM sucrose, 10 mM phosphate ($K2HPO_4/KH2PO_4$), and 1 mM $MgCl_2$, pH 7.4. They become unilamellar (UL) vesicles after extrusion over a 400 nm membrane.[12]

Freeze-thaw method

The UL dispersion is frozen for 15 minutes before being thawed (melted) and subjected to a sonication cycle in this method. The archaeosomes themselves combine and expand in size considerably. Sonication then reduces the permeability of the archaeosomes membrane by speeding up the rate at which packing mistakes are eliminated. To generate giant vesicles with a diameter of 41 μm, the sonication phase may be replaced by dialysis against hypo-osmolar buffer. The salt solution is mixed with tiny unilamellar vesicles (SUVs), which are frozen and thawed before being used. The large vesicles generated by freeze-thawing grow and rupture during dialysis as a result of osmotic lysis, producing giant vesicles. For oral vaccination, archaeosomes made of polar lipid fraction E (PLFE) and conventional liposomes built of EPC/cholesterol (3:2 molar ratio) were created using the freeze-thawing method.[13]

Sonication

Without the need for external lipid replenishment, archaeosomes may be made from the polar lipid fraction "PLF" of *Sulfolobus solfataricus* by sonication at 60°C. At 0°C, polar lipids from *S. acidocaldarius* were sonicated to produce archaeosomes. Archaeal lipids extracted from Archaea *H. salinarum* and enriched with phosphatidylcholine, as well as BMD-loaded archaeosomes and standard liposomes, were made using sonication techniques. Using a Hielscher UP50H ultrasonic disintegrator, sonicated vesicles were created for topical delivery by sonicating MLV dispersions at 80% amplitude for 4 mins.[14]

French pressure cell extrusion

A liquid sample of prefabricated MLVs is injected into the sample cavity once the piston and pressure are set, the sample is filled in the exit hole, and the power is turned on. MLVs are extruded via a small hole at 40°C and 2000 psi, and then collected in an appropriate container. This method may be used to make uni- or oligo-lamellar archaeosomes. Extrusion 10 times at the proper temperature (65°C for archaeosomes) through two-layered polycarbonate membranes (pore size = 200 nm) under nitrogen gas pressure was used to make UL vesicles from ML vesicles.[15]

Solvent dispersion method

The lipids are dissolved in an organic solution, which is then combined with an aqueous phase containing the components to be entrapped within the archaeosomes. The lipids form a lipid monolayer at the organic-aqueous phase border, which comprises up half of the archaeosome bilayer. The miscibility of the organic solvent and the aqueous solution may be used to categorise the solvent dispersion techniques. The organic solvent is miscible with the aqueous phase; the organic solvent is in excess and immiscible with the aqueous phase; and the organic solvent is in excess and immiscible with the aqueous phase.[16]

Reverse phase evaporation

In this method, the solvent was removed from the emulsion via evaporation. Polar lipids are dissolved in organic solvents and sonicated in a bath to create an emulsion (w/o), which is then dried to a semisolid gel using a rotary evaporator at reduced pressure. Finally, LUVs may be made utilising a vortex mixer and intense mechanical shaking to generate a certain proportion of water droplets.[17]

Detergent dialysis method

Using detergents as a mediator, the ethereal phospholipids are brought into close contact with the aqueous phase in this method. Detergents establish bonds with phospholipid molecules and aid in the separation of the molecule's hydrophobic portions from water.[18] Detergent reduction may be accomplished in four ways.

Dialysis: Dialysis may be performed with either dialysis bags wrapped in large detergent-free buffers (equilibrium dialysis) or continuous flow cells (continuous flow dialysis).

Gel filtration: In this method, the detergent is washed away using size-exclusive chromatography. Any size filter (from Sephadex G-50 to Sephacryl S200–S1000) may be used for gel filtering. The pores of the beads packed in a column prevent archaeosomes from passing through.

Adsorption using bio-beads: Detergent adsorption is achieved by shaking a mixed micelle solution with beaded organic polystyrene absorbers, such as XAD-2 beads and Bio-beads SM2. Detergent absorbers have the advantage of being able to remove detergents with a low critical micelle concentration that aren't completely eliminated by dialysis or gel filtration.

Dilution: When an aqueous mixed micellar solution of detergent and phospholipids is diluted with buffer, the micellar size and polydispersity increase significantly, and as the system is diluted away from the mixed micellar phase boundary, an amorphous change from

polydisperse micelles to monodisperse vesicles occurs. The detergent/dialysis method may result in inadequate trapping due to the leakage of loaded molecules during the dialysis phase.

HALLMARKS OF AN IDEAL ADJUVANT

- Stability, bioavailability, and cost-effectiveness are all critical factors to consider.
- Antibodies against autoimmune responses are avoided.
- Inflammatory reactions that are not pathogenic are generated.
- Encouraging communication between the innate and acquired immune systems.
- A boost in antibody response to a specific (humoral) antigen.
- Induction of a cytotoxic T-cell response mediated by cells.
- T-helper cell activation.[19]

APPLICATIONS OF ARCHAEOSOME FORMULATIONS

Self-adjuvanting drug delivery systems for cancer vaccines

Archaeosome adjuvants, which are generated as archaeal ether glycolipid vesicles, stimulate CD4+ and CD8+ CTL responses to entrapped soluble antigens. CD8+ CTL responses in the host are needed for long-term tumor prevention. Mouse tumors develop spontaneously in the absence of CD8+ T-cell cytotoxicity. Human CD8+ CTL responses to tumor-associated antigens have been shown to be very helpful to cancer patients, particularly those with advanced disease. As a consequence, creating cancer immune therapies has piqued people's attention. Cancer vaccines' ability to elicit a robust and adequate immune response is based on two key factors: the identification of specific antigenic targets and the ability to elicit a robust and appropriate immune response. Archaeal ether glycerolipid vesicles (archaeosomes) efficiently transport foreign antigens for humoral and cell-mediated immune development. Since the activation of CD8+ cytotoxic T cells is important for protective immunization against malignancies, the ability of various archaeosome lipid compositions to induce a strong CD8+ CTL response to entrapped antigen has been assessed. When mice were vaccinated with ovalbumin (OVA) entrapped in all archaeosomes lipid compositions, a major (day 10) splenic CTL response was elicited, indicating MHC class-I presentation processing. The use of polar lipid compositions from *Halophilic archaea* as adjuvants for this early CTL response was very successful. The lytic units had reduced significantly by weeks 6–7. At 50 weeks, only *M. smithii* and *T. acidophilum*, which both had significant levels of bipolar membrane-spanning caldarchaeols, were shown to induce memory CTLs. Mice vaccinated with OVA encased in *M. smithii*, *H. salinarum*, and *T. acidophilum* vesicles are protected for 6 weeks against an OVA-expressing solid tumor challenge. In archaeosomes, even a 3mg OVA dose significantly inhibited tumor growth. Tumor prevention was achieved using a therapeutic strategy that included the administration of OVA-archaeosomes in conjunction with the tumor challenge. Antigen-free *T. acidophilum* archaeosomes engulfed antigen *H. salinarum* archaeosomes, providing innate therapeutic protection. Vaccination of archaeosomes with a CTL peptide epitope from the melanoma separation antigen, tyrosinase-related protein-2, resulted in a protective CD8+ response against B16OVA metastases, suggesting that self-tumor antigens may be targeted. The lipid structural properties of Archaea may influence primary, long-term, and/or innate immunity in a variety of ways, potentially affecting vaccine adjuvant selection.[20]

Immunoadjuvant for a vaccine against Chagas disease

American trypanosomiasis (Chagas disease) is a neglected tropical ailment caused by the protozoan parasite *Trypanosoma cruzi*. The WHO believes that approximately 15 million individuals are sick worldwide. *T. cruzi*-induced cardiac disease affects 50,000 children and adults each year, with many of them dying due to a lack of effective treatment. The disease has been discovered in non-endemic areas of the Americas and Europe as a result of large-scale migrations, presenting a substantial danger of transmission. It is essential to find new ways for preventing and controlling Chagas disease. There are currently no vaccines or immunological therapies for *T. cruzi* infection. Several adjuvants have been tried in combination with vaccine candidate identification to elicit protective immunity against *T. cruzi*, but none have been successful. Archaeosomes have been found to have substantial adjuvant effects in an increasing body of studies (ARC). Traditional liposomes are more actively absorbed by macrophages and antigen-presenting cells *in vitro* and *in vivo* than these vesicles enclosed by one or more bilayers made with TPL generated from Archaea-domain bacteria. They also differ from liposomes in that immune modulators aren't needed to boost adjuvancy beyond a basic depot effect, allowing for greater production scale. *T. cruzi* antigens can be successfully incorporated into ARC, and the resulting immunogen can trigger a protective response in mice following sc injection against an intracellular parasite infection. ARCs have shown promise as a safe and effective carrier adjuvant in the development of future vaccines against this human illness.[21]

As novel gene delivery systems

Novel cationic liposomes based on a mixture of neutral/cationic bilayer-forming lipids and archaeobacterial synthetic tetraether-type bipolar lipids have been shown to have *in vitro* gene transfection properties, and they represent a new approach for controlling the lipidic membrane fluidity of the complexes they form with DNA. Archaeobacterial lipids as cationic or co-lipids may be used to accomplish *in vitro* gene transfection. It has been found that combining

conventional bilayer-forming lipids with monolayer-forming lipids may change the membrane properties of CL–DNA complexes. The potential of novel archaeoplexes for *in vivo* gene transfection into the airway epithelium through nasal instillation or aerosolization in the hopes of developing lung-directed gene therapy for cystic fibrosis. Oral delivery systems for proteins and peptides Oral administration of peptide and protein medications is challenging due to the hostile GI environment. Lipid-based delivery techniques preserve peptides and proteins better. Archaeosomes are a novel lipid-based oral medicine delivery system derived from *S. acidocaldarius* PLFE. Archaeosomes exhibited greater stability in simulated GI fluids, enabling fluorescently tagged peptides to remain in the GI tract for longer after oral administration. *In vivo* experiments showed that archaeosomes carrying insulin produced lower blood glucose levels than a conventional liposome formulation, despite the fact that archaeosomes had minimal effect on insulin transport through Caco-2 cell monolayers. Archaeosomes produced from PLFE were rather stable under simulated GI tract conditions in vitro, and they facilitated the slow transit of fluorescently tagged peptides through the GI tract *in vivo*. Archaeosomes outperformed conventional liposomes as an oral insulin carrier in diabetic rats, reducing blood glucose levels. The formulation's poor permeability of the intestinal epithelium following oral administration may have contributed to the mild hypoglycemia effect.[22]

As novel antigen delivery systems

The humoral immune response produced in BALB/c mice against bovine serum albumin or cholera toxin B subunit was evaluated when the antigens were combined with liposomes containing either archaeal ether lipids or conventional lipids. Antibody titers in sera from mice vaccinated intraperitoneally were increased to levels comparable to those achieved with Freund's adjuvant by encapsulating bovine serum albumin in archaeal lipid vesicles (archaeosomes) of about 200 nm diameter. Six archaeosome formulations and three conventional liposome formulations were found to be comparable, indicating that archaeosomes are generally better at potentiating an immune response. Furthermore, archaeosomes composed of polar lipids from *M. smithii*, a human colon bacterium, only needed two injections to get close to the maximum antibody titer. After a positive response to the more immunogenic cholera B-component protein being presented to the immune system of mice, *M. smithii* archaeosomes were discovered. The antigen must be enclosed in archaeosomes to produce a full humoral response.[23]

Drug delivery systems for natural antioxidant compounds

Multilamellar (MLVs) and unilamellar (SUVs) liposomes made of archaeal polar lipids have been used as a topical delivery system for natural antioxidant compounds recovered from olive mill waste. SUVs were smaller than MLVs, with size values less than 200 nm, and this trend continued throughout the stability tests. Transmission electron microscopy revealed that traditional liposomes had spherical membranes, while archaeosomes had more uneven membranes. Both formulations exhibited a similar rate of vesicle encapsulation, which was adequate to provide strong antioxidant activity. Stability tests were performed one month after the formulations were produced, and they showed excellent stability with no change in the suspensions' early characteristics. It was also looked at the possibility of combining liposomal suspensions with different excipients (Carbopol-940 and Pluronic-127) for topical administration. Vertical diffusion Franz cells were used in *in vitro* diffusion studies to assess the release behavior of the different systems developed. The vesicles were placed in the gels and released 24 hrs later. Archaeosome gels generated the same amount of phenolic compounds regardless of the excipient. Although there were significant differences in release between carbopol and pluronic gels in liposomal gels, archaeosomes appeared to be a viable carrier for topical administration of antioxidant phenolic chemicals due to their stability, entrapment efficiency, and antioxidant activity, which were similar to those produced with traditional phosphatidylcholine liposomes. In addition, archaeosomes seemed to be more adaptable in terms of gel inclusion than regular liposomes. Thus, when employing archaeosomes, the release of carbopol or pluronic is quite similar, enabling both excipients to be utilized interchangeably, while this does not happen when using conventional liposomes, allowing the choice of one excipient to be based on the desired effect.[24]

New drug carrier for delivery of Paclitaxel to breast cancer

Archaeosomes containing paclitaxel reduce side effects and improve the drug's therapeutic index. Carriers have made major advancements in the treatment of a wide range of diseases. There are substantial individual variations in lipid carriers. Archaeosomes are a major lipid transporter. Despite its therapeutic advantages, the drug paclitaxel, which is used to treat breast cancer, has a number of negative side effects. Archaeosomes were generated in PBS with a particular ratio of Paclitaxel after methanogenic archi bacteria were employed to obtain them. Archaeosomes and medication delivery Paclitaxel was examined for 26 hrs and found that the most drug was released in the first 3 hrs. Archaeosomes have a high cytotoxicity. The MTT test was used to evaluate the cytotoxicity of archaeosomes Paclitaxel on a breast cancer cell line, and it showed that the cytotoxicity of archaeosomes Paclitaxel is greater than the standard Paclitaxel formulation. A novel medication delivery technique that utilizes archaeosomes has improved the therapeutic index of paclitaxel.[25]

A carrier for topical delivery of BMD

The features and potential of archaeosomes as novel colloidal carriers for efficient drug administration to the skin were evaluated using archaeosomes, lipid vesicles

generated from archaea polar lipids, and conventional phospholipid liposomes. Using archaeal lipids produced from Archaea *H. salinarum* and augmented with phosphatidylcholine, thin-lipid films and sonication techniques were utilized to generate BMD-loaded archaeosomes and conventional liposomes, respectively. Vesicular formulations were assessed in terms of size, zeta potential, entrapment efficiency, and shape. To investigate the impact of inclusion in two different colloidal carrier systems on the (trans) cutaneous delivery of medication, in vitro drug permeation experiments through full-thickness pig skin were conducted using Franz diffusion vertical cells, evaluating both archaeal and liposomal dispersions. The most effective drug transporters were archaeosomes, which had substantial drug penetration and accumulation in the skin stratum and epidermis. For present uses, archaeosomes have a lot of promise as a delivery system. Incorporating archaeosomes into an anti-inflammatory drug's efficacy in the treatment of skin disorders with a local effect may be a novel and promising strategy.[26]

Archaeosomes delivery to different types of cargo into epithelial cells grown *in vitro*

Archaeosomes are archaeobacterial polar lipid-based liposomes. These have distinct structural properties that improve lipid bilayer stability in the presence of high temperatures, low or high pH, phospholipases, and bile salts. As a consequence, they're excellent for creating new drug, gene, and vaccine delivery systems. *Aeropyrum pernix* K1 generated large UL archaeosomes (400 nm in size) that showed promise as a basis for developing an effective and universal technique for administering medicine or therapy to epithelial cells. These archaeosomes may transport tiny fluorescent chemicals (calcein), smaller proteins (60 kD like alisteriolysin), large protein aggregates (like keratin 14), and plasmid DNA to epithelial cells grown in vitro. At this early stage of research, small compounds have a high delivery effectiveness of approximately 40%. Prepared UL archaeosomes aren't toxic to keratinocytes even at high concentrations (500 μg/mL).[27]

UDA as topical adjuvant

The skin has a high number of potent antigen-presenting cells (APC) that are difficult to reach through parenteral vaccination unless done via a difficult intradermal route. Because it allows for much closer contact with the APC on the skin, the topical method is an attractive alternative. Topical vaccination has a number of advantages over injectable vaccination, including better patient compliance, a reduced risk of re-infection from contaminated material, and a reduced requirement for specially trained personnel, sterilized equipment, and cold chain management. The need for strong immunomodulators such bacterial ADP-ribosylating exotoxins, as well as dose variability, negates these advantages (cholera toxin, Escherichia coli, and their mutants). SPC, NaCl, and polar lipids from *H. tebenquichense* (3:1:3 w/w) are used to make UDA vesicles. UDL (made of SPC and NaChol at a 6:1 w/w ratio) and UDA were neither captured nor cytotoxic to keratinocytes; UDA was mostly captured by macrophages, with the potential to be reduced by 0.4–1.6 mg/mL phospholipids by 25–60%. UDL was difficult to get and posed little hazard. Balb/C mice immunized topically with four doses of Ovalbumin (OVA)-loaded UDA at 75 μg OVA/600 g phospholipids (125 nm mean size and 42 mV zeta potential) generate ten- to hundred-fold higher IgG titers than those inoculated with OVA-loaded UDL at the same dosage. Both matrices penetrate to a similar skin depth (nearly 10 μm after 1 hr on human skin) owing to UDA's higher topical adjutancy and superior phagocytic absorption due to its glycolipid content. In the traditional meaning, liposomes combine and do not penetrate the unbroken skin of a mouse beyond a depth of 1 mm. UDL's lipid matrix, on the other hand, penetrates as least as far as SC, delivering the aqueous content to the viable epidermis. Thanks to the presence of edge activators such as NaChol, polysorbate, or ethanol in the phospholipid matrix, UDL outperforms traditional liposomes as topical adjuvants due to its unique mechanical behavior or ultra-deformability. After SC penetration, living skin cells took up the UDL, which were lipids. After sc injection in mice, archaeosomes act as efficient adjuvant for the establishment of Th1, Th2, and CD8+ T cell responses to entrapped soluble Ag.[28]

CONCLUSION

Finally, the nano-carrier system described above offers a lot of promise for creating novel, low-dose, and effective treatments for a range of diseases. The overall number of clinical trials using archaeosomes and lipid-based products is a motivating figure. A number of companies are actively investing in the development and testing of archaeosome products for use in the treatment of a range of diseases. Archaeosomes have long been known as very efficient delivery vehicles for medications, genes, and cells. They seem to be promising and may be the subject of further research.

CONFLICTS OF INTEREST

No conflict of interest is declared.

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