Archaeosomes as means of nano-drug delivery

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Archaeosomes are a novel generation of liposomes that are made from polar ether lipids extracted from the Archaea. They have higher stabilities in acidic or alkaline pH, bile salts, high temperatures and against phospholipase, oxidation, chemical and enzymatic hydrolysis in comparison with conventional liposomes. Ether links are more stable than ester links. The ability of Archaea to adapt their membrane lipid compositions to harsh environments has resulted in archaeal lipids to be considered for the development of nano-drug delivery capable of overcoming the biophysical, biological and biomedical barriers that the body displays towards gene, drug and vaccine therapies. Archaeosomes are prepared from various type of Archaea which show high adjuvant activity and can promote humoral and cell-mediated immune responses. In-vitro and in-vivo studies indicate that archaeosomes are safe and can be used in biotechnology applications such as drug, gene and vaccine delivery.

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Introduction

Archaea comprise one class of microorganisms which live in unusual habitats including high salinity, high temperatures, high pressures, and low and high pH [1,2]. They are grouped on the basis of their living habitats: 'methanogens', which can grow under anaerobic conditions, producing methane from simple sources such as H_2 and CO_2; 'halophiles', which need high salt concentrations; 'thermophiles', which live high temperatures in the range of 50–110°C such as hot springs and submarine volcanoes; and 'psychrophiles', which can survive at low temperature [3]. Images of some Archaea are shown in Fig. 1 [4]. These microorganisms have many applications in the pharmaceutical industry. For example, halophilic Archaea produce halocin that has antimicrobial activity. It has been suggested that halocin H7 is useful for reducing infection during organ transplantation. Canthaxanthin isolated from *Haloferax alexandrines* shows antioxidant activity and can prevent cancer and heart disease. Poly (γ-D-glutamic acid) extracted from *Natrialba aegyptiaca* can be used as drug carrier. Exopolysaccharides separated from *Haloferax* and *Halobacterium* are employed as emulsifiers [5]. Archaea have numerous unknown potential applications.

Liposomes are spherical vesicles composed of concentric phospholipid bilayers that can entrap hydrophilic, hydrophobic and amphiphilic drugs. These vesicles can be prepared from natural phospholipids (e.g. egg or soya), synthetic lipids or bacterial lipids. Liposomes have several advantages such as biocompatibility and biodegradability, penetration enhancing and retardation of drug release, prolonging release of active pharmaceutical agents, non-toxicity, protecting encapsulated agents from metabolic processes and ability to entrap small molecules as well as macromolecules such as haemoglobin, superoxide dismutase, interleukin (IL)-2 and erythropoietin. They have many applications in the fields of antimicrobial therapy, gene therapy, immunology, tumour therapy, vaccine adjuvant, chelation therapy for treatment of heavy metal poisoning and delivery of radiopharmaceuticals for diagnostic imaging [6,7]. Despite these advances, the major limitations to the use of liposomes are their short half-lives, their instability and high cost of production, especially in large scales [8,9]. Archaeosomes are a new generation of liposomes which are made from one or more polar ether lipids that can be extracted from Archaea or synthetic archaeal lipids [10,11]. A schematic structure of an archaeosome is shown in Fig. 2 [12]. As shown in

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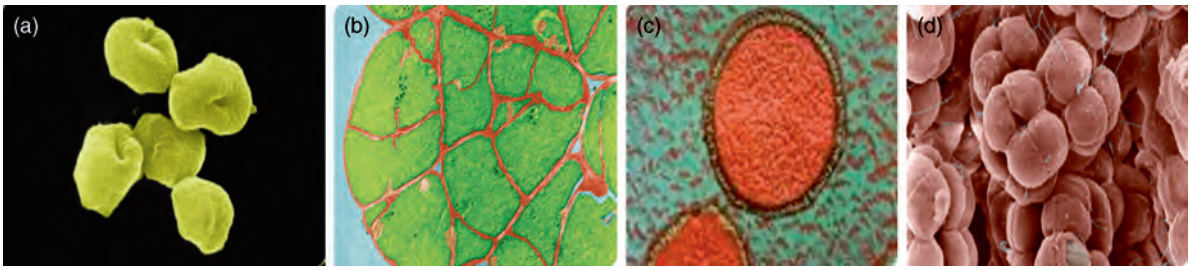

Fig. 1. Images of Archaea. (a) *Sulfolobus* is an extremophile that is found in hot springs and sulphur-rich environments. (b) *Methanoumensarcina rumen* is anaerobic and a methane-producing organism. It is found in the rumen of animals such as sheep and cattle. (c) *Staphylothermus marinus* is an extremophile that is found in deep oceans. (d) *Halococcus salifodinae* is found in water with a high concentration of salt [4].

the figure, these carriers are composed of conventional phospholipids and biopolar lipids that enclose an aqueous core. The term 'archaeosome' was introduced by Alqueres *et al*. [13]. Archaea are different from eukarya and bacteria due to their genetic, biochemical and structural features. For instance, Archaea possess unique flagellins, ether-linked lipids and lack murein in their cell walls [14]. Ether links are more stable against oxidation and high temperature than ester links [15]. Therefore, archaeosomes are more resistant to oxidation, action of phospholipases, chemical hydrolysis, bile salts, lipases, serum, alkaline or acidic pH, and high temperatures [1,2,11,16,17]. Archaeosomes are more stable and less permeable than conventional liposomes which frequently need up to 33% of cholesterol content to improve their stability [11]. Due to their extraordinary stability, which permits sterilization and filtration, archaeosomes have found many applications in vaccine, gene and drug delivery [18,19]. Furthermore, because of their thermostability, they are considered ideal candidates to protect antioxidants during food processing [20]. They can be administered by intravenous, intranasal, oral and subcutaneous routes [21]. Archaeosomes as drug delivery systems have several advantages including biocompatibility and biodegradability, lack of toxicity, stability during storage, capability to promote both Th1 and Th2 response with long memories and exhibit adjuvant properties [1,16,22]. Also, synthetic archaeal lipids are able to significantly increase the stability of drug delivery systems and are suitable helper lipids for in-vitro gene delivery in comparison with dioleoylphosphatidyl ethanolamine and cholesterol [11].

Biotechnological application of Archaea

The stability of Archaea in harsh environments led to their use in variety of biotechnological applications. DNA polymerases isolated from hyperthermophilic Archaea is used for elongation of the primer strand of a growing DNA molecule. Also, DNA polymerase I is separated from *Thermus aquaticus*, called *Taq* polymerase, and used in PCR. Xylanases extracted from *Thermofilum* strain and *Thermococcus zilligii* can be employed in the food and paper industries. Chitinases – enzymes involved in chitin degradation – were detected in the hyperthermophilic *Thermococcus chitonophagus*. Trehalose from *Sufolobales* is used in food preparations as a preservative as well as a moisture retainer. Exogluconases and β-glucosidase isolated from *Sulfolobales* sp. can degrade cellulose to its monomer, glucose. Lipases and esterases from *Sulfolobus shibatae* and *Archaeoglobus fulgidus* can be used in detergent formulations and the dairy industry [13,23–25].

Types of Archaea for preparation of archaeosomes

Archaeosomes are prepared from the total polar lipids extracted from various Archaea including *Sulfolobus*

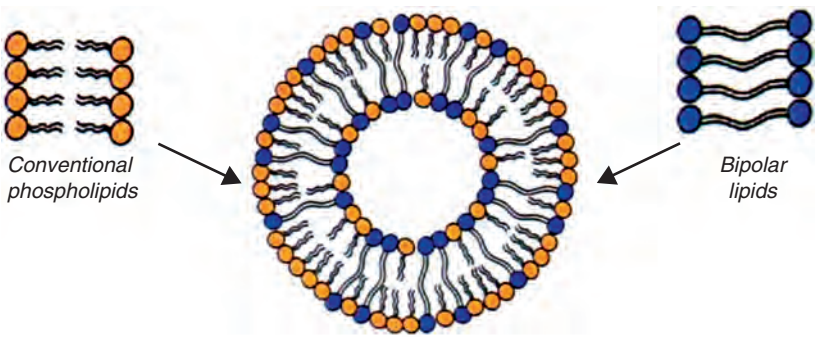

Fig. 2. Structure of archaeosome [12].

acidocaldarius [26,27], *Methanococus voltae*, *Methanosarcina mazel*, *Methanosaeta concilii*, *Mthanococcus jannaschii* [28], *Methanobrevibacter smithii* [22,29,30], *Halobacterium cutirubrum* [31], *Thermoplasma acidophilum* [32], *Halorubrum tebenquichense* [33], *Haloferax volcanii* [34], *Natronobacterium magadii* [35], and *Aeropyrum pernix* [36] and from conventional ester lipids such as cholesterol, dicetylphosphate, dipalmitoylphosphatidylcoline, dimyristoylphosphatidylcholine, disteraroyl-phosphatidylcholine and dimyristoylphosphatidylglycerol [19].

Lipid structures of archaeosomes

Many studies indicated that membrane lipid components are responsible for the survival and growth of Archaea in such harsh environments. The Archaea membrane core lipids are composed of saturated isoprenoid chains attached via ether bonds at the *sn*-2,3 position of the glycerol carbons. In contrast, the core lipids found in bacteria and eucarya consist of unbranched fatty acyl chains and often unsaturated, that attached via ester bonds to the *sn*-1,2 glycerol carbons. The lipid membrane of archaeosomes may be made from monopolar diether (archaeol) lipids or made from bipolar tetraether lipids, or a combination of monolayers and bilayers. Tetraether components are characterized by the presence of two diphytanyl chains linked at both ends to two glycerol residues in an antiparallel manner (caldarchaeol) or in a parallel manner (isocaldarchaeol) (Fig. 3). Moreover, the extensive hydrogen bonding networks between the polar glycolipid moieties further add to the stability of the membrane [2,15,16,19].

Methods of archaeosomes preparation

In the same way as liposomes, archaeosomes can be prepared using thin film, freeze/thawing and reverse-phase evaporation (M).

Thin film method

In this method, archaeosomes are prepared by hydrating the thin lipid film in an organic solvent and then the organic solvent is removed under vacuum. After completely removing the solvent, the solid lipid mixture is hydrated by aqueous buffer. The lipids spontaneously swell and hydrate to form archaeosomes [8].

Freeze–thaw method

Archaeosomes are primarily prepared by the thin film method and then is vortexed with the solute to be entrapped until the entire film is suspended. Then archaeosomes are frozen in dry ice–ethanol (−80°C) or in liquid nitrogen followed by thawing and vortexing again. The freezing and thawing cycles are repeated three to eight times to form archaeosomes [37,38].

Reverse-phase evaporation

In this method, the lipids are dissolved in chloroform and methanol and this mixture is evaporated in a rotary evaporator. Thereafter, the lipids are suspended in chloroform, methanol and water, and evaporated under vacuum again. To the dried lipids, buffer is added and vigorously vortexed and then incubated at 65°C to form archaeosomes [39].

Characterization of archaeosomes

Phase-contrast light microscopy, scanning electron microscopy and cryo-transmission electron microscopy (TEM) are used for observing the structure of archaeosomes. The size, polydispersity and zeta potential of the archaeosomes are determined using dynamic light scattering. Also, TEM, Cryo-TEM and fluorescence microscopy are employed to determine shape, diameter and structure of the archaeosomes. High-performance thin-layer chromatography (HPTLC) is used for the determination of lipid composition of archaeosomes. HPTLC is a qualitative and quantitative analytic method that gives reliable results [1,12,19]. It is believed that homogenization and sonication can significantly affect the particle size of vesicles [40,41]. It has been accepted that decreasing the particle size of vesicles may cause an increase in the penetration of encapsulated drugs into the deeper skin layers [42]. Accordingly, homogenization and sonication can be used to decrease the size of the archaeosome.

Application of archaeosomes

Antigens encapsulated in archaeosomes can be phagocytosed by antigen-presenting cells and target antigens for presentation onto major histocompatibility complex (MHC) molecules. Apart from this, recognition of the MHC–antigen complex by the T-cell receptor triggers T-cell activation [30]. Archaeosomes can be used for the entrapment of different antigens such as bovine serum albumin (BSA), hen egg lysozyme, cholera toxin subunit B and ovalbumin [43]. Gonzalez *et al.* [33] in 2009 prepared archaeosomes with total lipid extracted from *Halorubrum tebebquichense* and encapsulated BSA. Their results showed that with subcutaneous immunization of mice, BSA entrapped in archaeosomes elicited a strong and sustained antibody response and improved humoral immunity. According to their results, IgG1 and IgG2-enhanced antibody titres could be demonstrated as a result of the induction of a mixed Th1/Th2 response. So, these carriers could be used as a vaccine delivery system [33].

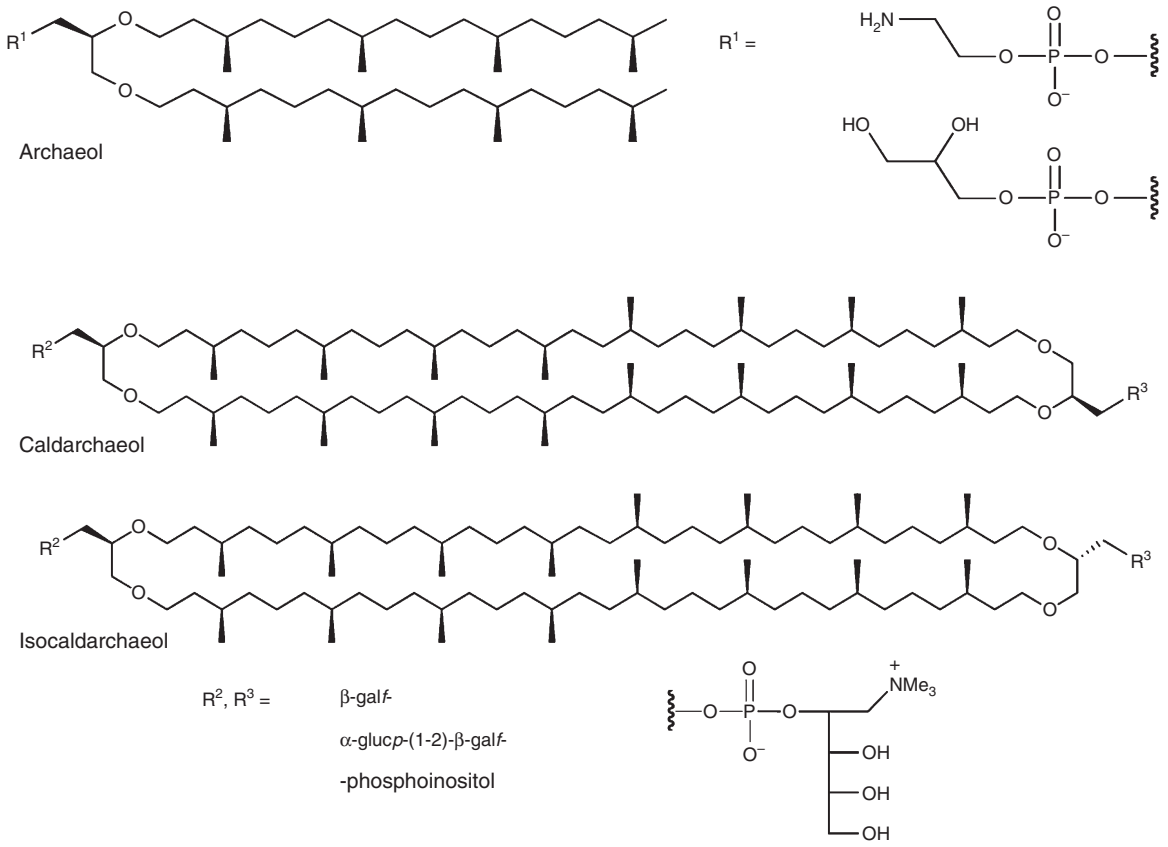

Fig. 3. Structure of Archaeal lipids [19].

Krishnan *et al.* [29] in 2010 indicated that archaeosomes composed of the lipids extracted from *M. smithii* are potent adjuvants for evoking a $CD8^+$ T-cell response. They showed that vaccination of mice with melanoma antigenic peptides transient receptor potential and Gp100 delivered in archaeosomes resulted in IFN-γ production and activation of specific $CD8^+$ T cells with strong cytolytic capability which resulted in a strong protection against melanoma. Therefore, they demonstrated that this system can be used to formulate cancer vaccines [29]. Several studies have indicated the potential of archaeosomes for development of vaccines against infections by *Listeria monocytogenes* and *Francisella tularensis* [19]. Wayne Conlan *et al.* [44] in 2001 immunized mice with lipopeptide antigens encapsulated in archaeosomes prepared from *M. smithii*, *Halobacterium salinarum* and *T. acidophilum* against infection with the facultative intracellular pathogen *L. monocytogenes.* The results of the study indicated that archaeosome-entrapped antigens had great potential as self-adjuvant delivery systems to elicit rapid and prolonged specific immunity against a prototypical intracellular pathogen. Mice vaccinated with lipopeptide containing archaeosome as antigen had 8–38-fold fewer *Listeria* in their livers and at least 380–2042-fold fewer *Listeria* in their spleens than was found in untreated mice [44]. Rethore *et al.* [45] in 2009 investigated the use of archaeosomes based on synthetic tetraether lipids as a new gene delivery system for plasmid DNA. The results showed these archaeosomes could mediate significant in-vitro gene transfection [45]. Archeosomes can be considered as suitable carriers for protein and peptide delivery due to higher stability. Li *et al.* [46] in 2010 prepared archeosomes from lipid extracted of *S. acidocaldarius,* as carriers for oral delivery of insulin as a model peptide. Their results indicated that archaeosomes were stable in simulated gastrointestinal fluids and in-vivo experiments showed that archaeosomes could control blood glucose levels better than conventional liposomes [46]. Patel *et al.* [47] in 2000 observed that pancreatic lipase only had a minor effect on the stability of archaeosomes made from *Methanosarcina mazei*, *Methanobacterium espanolae* and *T. acidophilum*, and caused the release of 12–27% of the encapsulated 5(6)-carboxyfluorescein from these archaeosome after 90 min. Thus, they demonstrated that archaeosomes can be employed as an oral delivery system [47].

Conclusion

Archaeosomes are more stable in oxidative conditions, against the action phospholipase, high pressure, high and low temperature and bile salts in comparison with

liposomes, because of their lipid structure. Therefore, they can be employed as carriers for drugs, genes and vaccines.

Acknowledgements

Conflicts of interest

We do not have a direct financial relation with the commercial identities mentioned in our study.

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