New Methods for Preparing Robust Functional Nano-biomaterials

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Statement of Purpose: We present methods for preserving the stability and function of phospholipid assemblies – including bilayers, multilamellae, liposomes, proteoliposomes, and microbubbles– as well as proteins and cells in silica-based nanocomposites. A number of synthetic methods have been developed and relationships between phospholipid molecular structure and nanostructure of hybrid materials have been explored. Methods for controlling the porosity of the silica matrix have also been developed. These hybrid nanomaterials retain the exquisite molecular function of biological membrane systems, while exhibiting excellent stability, a property that has thus far eluded traditional phospholipid membrane systems and has thus precluded their integration into a number of proposed devices and technological applications. In contrast to other methods for stabilizing phospholipid assemblies (e.g., lipid crosslinking) the methods described can be designed to preserve lipid mobility while allowing active transport, light harvesting and redox activity. These materials have potential for a broad range of applications including biosensing, drug-delivery, separations and imaging.

Methods:

Materials. All reagents were used without further purification. All lipids used in the study were purchased from Avanti polar lipids, Alabama. Tetra methyl ortho silicate (TMOS) and tetra ethyl ortho silicate (TEOS), were purchased from Sigma, Aldrich Chemical Co.

Ultra-thin films. Lipid solutions (20 mg/ml in chloroform) are spin-coated on clean silicon wafers at speed of 3000 RPM, which leads to the formation of a lamellar template. The template is exposed to vapors of TMOS for a desired amount of time.

Bulk gels. Aqueous solution with or without relevant biological species (such as proteoliposomes) is placed next to a vial containing silica precursor, TMOS in a closed container at 37^{o}C.

Preparation of Micro bubbles. A solution of lipids (5-10mg/ml in buffer) is sonicated for 1 min. with a horn sonicator at the liquid/gas interface. This results in formation of micro bubbles, which are further exposed to TMOS.

Result and Discussion:

Ultra-thin films. The spin-coated lipid samples are exposed to vapors of TMOS at 37^{o}C for varying periods of time. TMOS penetrates the multi-lamellar structure of lipids and arranges at the head groups of the lipids. Further, condensation leads to densification of the structure and results in formation of multi-lamellar hybrid systems. These homogeneous films retain mobility and are stable in air and water.

Bulk Gels. We report here a relatively simple one-step approach, which avoids the use of a co-solvent as well as the use of acids.

Due to the volatile nature of TMOS at low temperatures, TMOS evaporates and reacts at the aqueous solution-air interface and further hydrolyzes to silicon hydroxide and methanol. Upon further condensation Si-O-Si linkages are formed and leads to formation of gel.

Micro Bubbles. Micro bubbles are generally unstable and thus cannot with stand pressure or long term storage.[4] We have coated micro bubbles in silica shells using the vaporization process. (Fig. 1A) Pressure tests have been conducted with micro bubbles using a custom built apparatus.

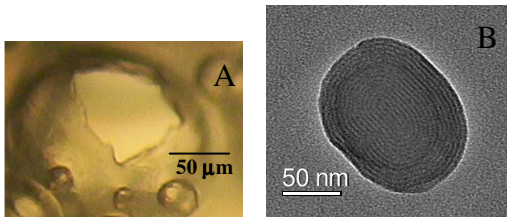

Fig1. A) An optical image of cracked silica shelled bubble synthesized using vaporization technique. B) A TEM micrograph of a multi-lamellar lipid silica liposome.

While control samples (without silica) lose all bubbles within hours, the silicified bubbles are stable in solution for months. Silicified bubbles can also withstand higher pressures.

Proteoliposomes. We have also encapsulated liposomes (uni-lamellar and multi-lamellar) in silica gel using the vaporization process. (Fig. 1B) Active transport is demonstrated by uni-lamellar proteo-liposomes containing bacteriorhodopsin encapsulated in these gels. A pH change is clearly observed in the gels with bacteriorhodopsin upon excitation by light. The process offers advantages including low temperature synthesis, short times for gelation, any pH and ionic strength, and a wide choice of precursors. These materials exhibit excellent mechanical stability, long shelf life, biocompatibility and efficient molecular transport.

Conclusions: We have successfully synthesized and characterized nano structured materials. The new synthetic method described is widely applicable and will have a significant impact on the ability to harness delicate biological attributes into a host of functional biomaterials.

References:

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