

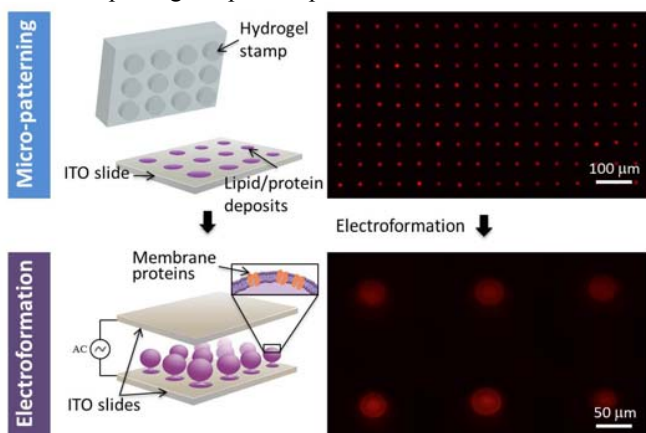
## Preparation of Cell Membrane-Mimicking Liposomes with Controlled Size and Composition

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**Statement of Purpose:** Liposomes that are tiny spherical capsules composed of a lipid bilayer shell and an aqueous core, have many applications including gene and drug delivery. When prepared in the size scale of cells and with the lipid and protein compositions similar to those in natural cell membranes, these liposomes are referred to as giant proteoliposomes and are excellent model systems for cellular membranes. These model systems are, hence, ideal for studying processes that happen at the surface of cells such as the molecular events during the entry of therapeutic molecules or pathogens into cells. Preparation of giant proteoliposomes typically requires sophisticated devices such as microfluidic-based platforms that are not readily accessible. The aim of this study was to develop a simple, readily-accessible, and versatile approach for producing large number of cell-sized liposomes with a range of lipid and protein compositions without the need for any specialized equipment.

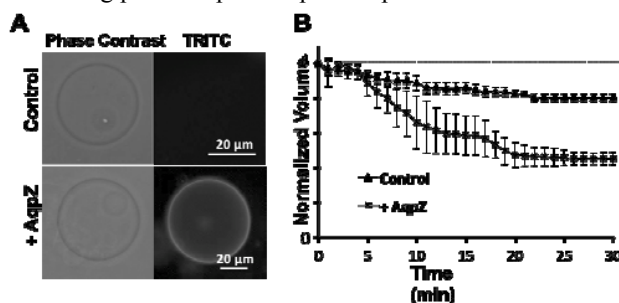
**Methods:** We first employed a topographically-patterned agarose stamp that was inked with an aqueous dispersion of small proteoliposomes, to pattern a lipid/protein array on an electrode surface, (e.g. indium tin oxide (ITO) slide). We then performed electroformation to form giant proteoliposomes from these patterned deposits. Upon rehydration and exposure to an AC electric field, each patch of lipid deposit generated multiple large vesicles. These large vesicles then gradually fused together forming a single unilamellar giant proteoliposome per deposit (**Figure 1**). In these arrays, the electroformed vesicles remained attached to the surface presumably through a lipid tether to the deposit on the ITO surface. These vesicles could also be easily detached from the surface within minutes by reducing the applied frequency of the applied AC field, producing a large number of monodisperse giant proteoliposomes.



**Figure 1.** Schematic representation (left) and fluorescent images (right) of hydrogel-stamped arrays of lipid/protein deposits (top) and electroformed array of giant proteoliposomes (bottom).

**Results:** The combined technique of electroformation and hydrogel stamping provided the following advantages: (i)

controlling the size of the resulting vesicles through adjusting the size of patterned lipid/protein patches and (ii) incorporating functional membrane proteins in the membrane of the giant vesicles. We confirmed the capability of the present method to produce giant vesicles with a relatively narrow size distribution ( $38.8 \pm 6.72$  (mean  $\pm$  S.D.)  $\mu\text{m}$ ). In addition, the use of adsorbent hydrogel stamps enabled rapid production of multiple copies ( $>30$ ) of a liposome array with minute amounts of inking solution. We applied this technique to prepare giant liposomes from a variety of lipid and protein compositions and further confirmed the bioactivity of the integral membrane proteins such as Aquaporin Z water channel in these vesicles (**Figure 2**). We also demonstrated the applicability of these liposomes for screening protein-lipid and protein-protein interactions.



**Figure 2.** Activity of Aquaporin Z (AqpZ) water channel protein in giant proteoliposomes. (A) Immunofluorescence assays confirm the presence of AqpZ in liposomes. (B) The bioactivity of AqpZ channel in the liposomes is demonstrated through a relatively fast (compared to control liposomes) rate of osmotic-induced changes change in liposomal volume.

**Conclusions:** In summary, we present a unique and versatile approach for producing uniformly sized giant proteoliposomes with various membrane compositions. This approach that combines the versatile technique of hydrogel-based microcontact printing and the commonly used technique of electroformation is efficient, low-cost, and does not require any specialized equipment. The resulting liposomes are initially attached to the surface in an array format and make it possible to collect the data for statistical analysis in a rapid and easy manner. Alternatively, the liposomes can be easily detached from the surface in order to produce a large number of giant liposomes with functional proteins. This method may further be applicable to produce giant polyerosomes that offer increased stability compared to the liposomes. Therefore, this technique of preparation of giant liposomes with proteins will be useful in the area of biomaterials and biotechnology, as well as in the biophysical and biochemical fundamental studies.

### Reference:

[1] Kang YJ, et al. *Advanced Materials*. (2013) *in press*.