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The Use of Giant Vesicles for Medical Applications: A Trend in the Last Decade

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A giant vesicle (GV) is composed of an amphiphile and a closed bilayer membrane with a diameter of 1 μm or more in water. GVs composed of phospholipids are attracting attention as cell models because their constituent molecules, structure, and size resemble those of cell membranes. In the last decade, with the development of GV preparation methods, functionalized GVs that sense a stimulus as an input and can give a corresponding output have been reported as new medical molecular devices. This technical report overviews the applicability of GVs.

1. Introduction

When an amphiphile, with both a hydrophilic part and a hydrophobic part at the ends, is dispersed in water, molecular self-assemblies such as micelles and vesicles autonomously appear [Fig. 1(a)] A vesicle is a closed bilayer membrane of amphiphiles with hydrophobic parts facing each other. Nanometer-size vesicles are characterized as either small vesicles (less than ca. 100 nm) or large vesicles (ca. 100 nm to 1 μm). Vesicles with a diameter of 1 μm or more are called giant vesicles (GVs).[(1)] GVs are the same size as cells and can be individually observed in real time under an optical microscope [Fig. 1(b)]. As a chemical model that mimics cells, GVs have recently attracted interest in supramolecular chemistry and a wide range of research fields such as physics, chemistry, life sciences, and bioengineering.

Small vesicles and large vesicles are relatively effortlessly homogenized in size, shape, and internal structure; hence, they are widely used in pure and applied sciences, such as in drug delivery systems.[(2)] For over 50 years, research on GVs has not been prevalent as they are considered difficult to handle quantitatively with good reproducibility. GVs prepared using conventional methods have significant variations in size, shape, and internal structure. In recent years, GV preparation methods have been greatly improved, and active research is

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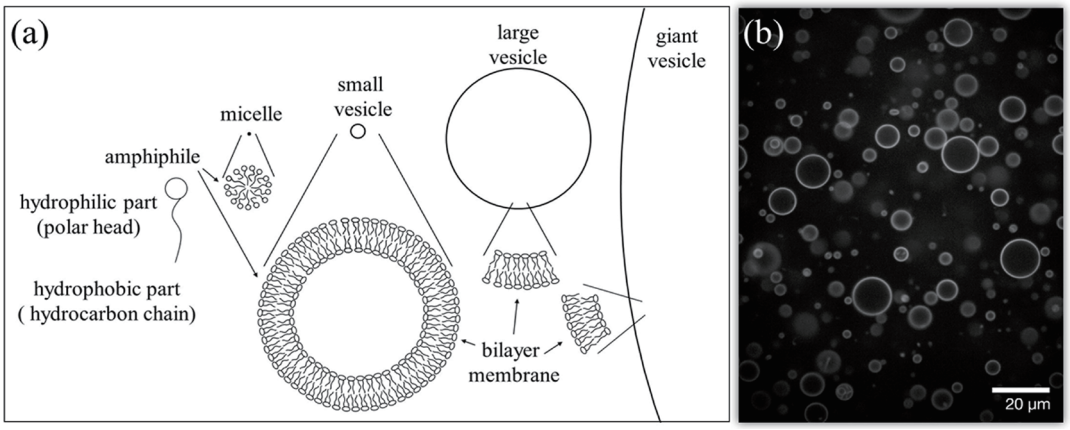

Fig. 1. (a) Schematic illustration of micelles and vesicles. (b) Confocal laser scanning fluorescence microscopy image of GVs stained by a synthesized fluorescent phospholipid.

being conducted on the application of GVs. Here, we introduce the recent improvements in GV preparation and provide an overview of the medical applications of GV at preclinical stages.

2. Preparation of GVs

GVs include giant unilamellar vesicles (GUVs) consisting of a single bilayer membrane and giant multilamellar vesicles (GMVs) that are closed by overlapping several bilayer membranes. GMVs have fewer inner water regions than GUVs of the same diameter, making it difficult to encapsulate water-soluble substances and water-dispersed particles with a high volume ratio. The multiple nested membrane structure of GMVs causes a fault in the control of the membrane dynamics of sensing and releasing. Therefore, the preparation methods for GUVs have primarily been developed in the last decade. GUV preparation is usually associated with one of four methods: the thin film swelling method, water-in-oil (W/O) emulsion template method, jetting method, and water-in-oil-in-water (W/O/W) double emulsion templating method (Fig. 2).[3]

In 1969, the first preparation method of GVs using a dry thin film of a phospholipid (a type of amphiphile, which is a primary component of the cell membrane) was reported and this preparation was named the thin film swelling method.[4,5] In this method, GUVs are formed by the swelling of bilayer membranes on a thin film after water (or a buffered solution) is added. A patterning technology that involves applying a dry thin film to a glass substrate with a microfabricated stamp affords size-controlled GUVs.[6,7]

The W/O emulsion template method involves wrapping W/O emulsion droplets (surrounded by an amphiphile monolayer) with an amphiphile monolayer formed at the interface between the emulsion and water phases.[8] The use of centrifugation for the wrapping is common. Several research groups have established new laboratory-built devices and novel methods, including the continuous droplet interface crossing encapsulation (cDICE) method[9] and droplet-shooting and size-filtration (DSSF) method,[10] for controlling the size of emulsion droplets.

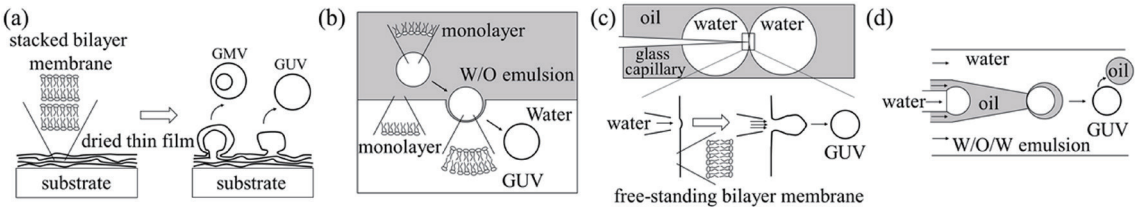

Fig. 2. Schematic illustrations of the thin film swelling method (a), W/O emulsion template method (b), jetting method (c), and W/O/W double emulsion templating method (d).

Along with the recent development of microfabrication techniques, the W/O emulsion template method was improved,[11] and new methods, including the jetting method and the W/O/W double emulsion templating method, were established. The jetting method is based on a free-standing bilayer membrane (called a black lipid membrane) and a glass capillary. GUVs are formed by a process resembling that of blowing soap bubbles.[12] The W/O/W double emulsion templating method involves producing uniform W/O/W double emulsion droplets in a microfabricated fluidic device and obtaining GUVs by utilizing the phase separation of oil from the droplets.[13,14] Although there are still some limitations to the versatility of these methods (Table 1), such as the use of reagents and obtaining appropriate sizes, GV preparation methods using microfabricated fluidic devices are expected to develop technologically and promote the application of GVs.

Table 1
Advantages and disadvantages of four preparation methods of GVs.[3]

	Thin film swelling method	W/O emulsion template method	Jetting method	W/O/W double emulsion templating method
Description	GVs formed by swelling of stacked bilayer membranes (thin film) after adding water (or buffered solution).	W/O emulsion droplets surrounded by amphiphile monolayer wrapped by another amphiphile monolayer formed at interface between emulsion and water phases.	Free-standing bilayer membrane extended and swollen to form GUVs by flow through glass capillary.	Uniform W/O/W double emulsion droplets produced in microfabricated fluidic device and transformed into GUVs via phase separation of oil from droplets.
Advantages	Simple procedure. Many types of amphiphiles in use. No oil remaining in membrane.	Simple procedure. GUVs mainly produced. High encapsulation efficiency.	GUVs mainly produced. High encapsulation efficiency. Many types of amphiphiles in use. Uniform size.	GUVs mainly produced. High encapsulation efficiency. Many types of amphiphiles in use. Uniform size. High throughput.
Disadvantages	Mixture of GUVs and GMVs. Difficult to achieve uniform size. Low encapsulation efficiency.	Difficult to achieve uniform size. Some amphiphiles are not applicable. Oil remaining in membrane.	Specific device needed. Oil remaining in membrane.	Specific device needed. Oil remaining in membrane.

3. GV-based Sensors

GVs have drawn considerable attention as new sensing devices. They provide a micrometer-size reaction field that is surrounded by a soft boundary, and are composed of biodegradable molecules (such as phospholipids), and possess membrane proteins anchored in the phospholipid bilayer membrane, which act as effective and specialized molecular sensors. Yanagisawa *et al.* reported that KcsA, a potassium ion channel, can change its function in the inward and outward directions when incorporated into GUVs produced using the W/O emulsion template method.[15] Dwidar *et al.* reported a GUV sensor for histamine, a membrane-permeant molecule, by constructing a cell-free protein synthesis system that contains RNA, which responds to histamine.[16] Hamada *et al.* synthesized an insect pheromone receptor, which was a complex of two membrane proteins, BmOR1 and BmOrco, in GUVs and demonstrated that this receptor functions in GUVs using the patch clamp technique [Fig. 3(a)].[17] As shown in Fig. 3(b), at a folding potential of −70 mV, inward currents of several picoamperes were recorded while the pheromone was added to the GUVs. Since the current was within the range of the amplitude for the single-channel conductance of insect pheromone receptors found in living cells,[18] it was indicated that BmOR1 and BmOrco fold properly and form heteromeric complexes on the vesicular membrane. Further research on GV-based sensors with high substrate specificity and reaction specificity is expected to continue to rapidly increase.

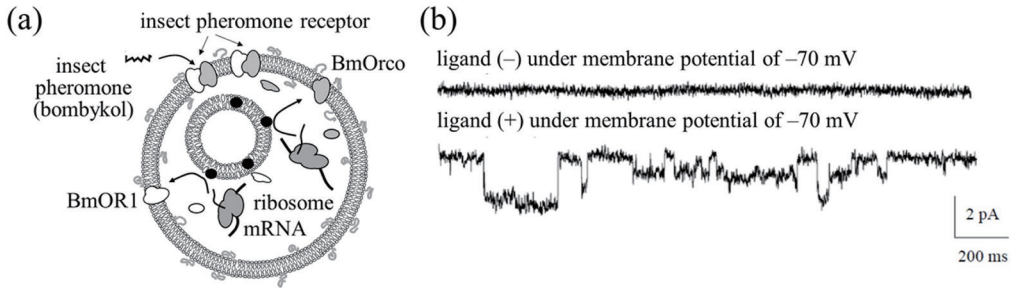

Fig. 3. (a) Schematic illustration of a GUV synthesizing an insect pheromone receptor. (b) Ion current traces of the GUV before ligand (bombykol) stimulation and during ligand stimulation recorded with a voltage clamp at −70 mV.

4. GV-based Cell/Tissue Markers

In recent years, with a declining birthrate and an aging population in many countries, minimally invasive treatments such as endoscopic surgery are in high demand for improving patients' quality of life. Palpation of objects is no longer available with these techniques, and surgeons need to locate target lesions inside organs only with the aid of video images of the objects. This situation complicates surgical procedures. For example, laparoscopic surgeries in patients with early gastric cancer occasionally require additional assistance from a gastroendoscope to localize the lesion during surgery.[19,20] Toyota and coworkers have proposed

the concept of a cell/tissue marker based on GV constructs, in which a large amount of multiple contrast agents can be encapsulated using the W/O emulsion template method (Fig. 4).[21,22] First, GV aggregates containing a near-infrared fluorescent (NIRF) dye are preoperatively administered in a spot manner for identifying the margin of the lesion of interest using a gastroendoscope from inside of the stomach. Second, the location and extent of the lesion are shown as a dotted line on the NIRF laparoscopic images observed from outside of the stomach during surgery. In fact, injection points of GV aggregates have been shown to be clearly detected without severe blurring up to 18 h after administration on animal models. Hayashi *et al.* demonstrated that preoperative administration of the same GV aggregates with an X-ray contrast medium as well as an NIRF dye also enables the identification of lesions undetectable with X-ray CT examination.[23] Early-stage malignant diseases as targets for such minimally invasive surgeries cannot be visualized by conventional laparoscopy or X-ray CT. However, this novel tissue marker encapsulating both an NIRF dye and X-ray media in a single material could contribute to precise preoperative planning and intraoperative navigation for various surgeries. The same group also started to develop a novel active tissue marker based on GV aggregates that are activatable with ultrasound irradiation and release a liposomal tracer for detecting sentinel lymph nodes or the first draining site from a main tumor.[24]

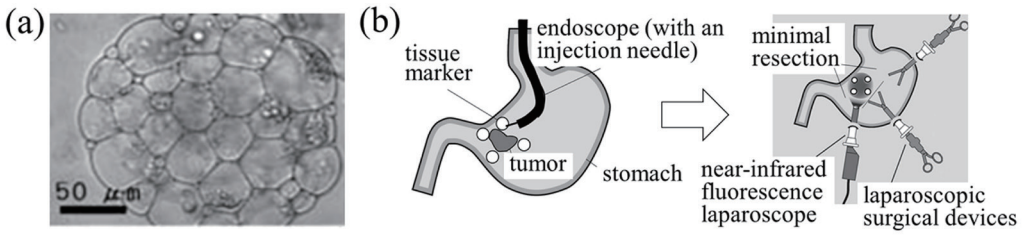

Fig. 4. (a) Phase contrast microscopy image of a GV aggregate containing vesicles with a NIRF dye. (b) Schematic illustration of the conceptual process of laparoscopic gastrectomy using a novel tissue marker of a GV aggregate.

5. GV-based Controlled Releasers

Further research on GVs with regard to medical applicability was conducted by Chen *et al.*, which included the construction of artificial pancreatic β cells that released insulin in response to glucose.[25] The GV used in their study encapsulated polymer-coated large vesicles containing insulin (Fig. 5). Since the inner leaflet of the GV and the outer leaflet of the large vesicles were modified with membrane-fusion peptides, the polymer coating the large vesicles prevented the membrane fusion unless the polymer was decomposed upon exposure to a low pH. In response to glucose stimulation, insulin was released from the inside of the GV during the sequential process of pH decrease by glucose oxidase and catalase and the subsequent membrane fusion of the large vesicles driven by decomposition of the coating polymer. The pH in the GV could be repeatedly recovered by gramicidin A embedded in the GV membrane. When this GV was

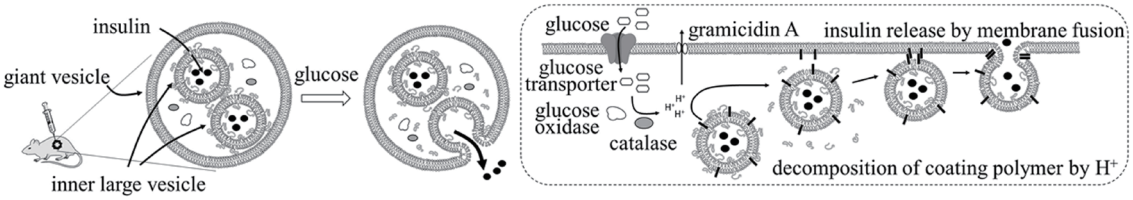

Fig. 5. Schematic illustration of insulin release from the GUV–large vesicle system in response to glucose.[25]

transplanted to mice with high blood glucose levels, the blood glucose levels decreased and returned to normal levels. Recently, Luo *et al.* demonstrated another medical applicability of GVs comprising a plasma membrane and modified by functional DNA for targeting tumor cells.[26] The GVs contained anticancer drugs and near-infrared phototherapy reagents. After administrating the GVs into a tumor in mice and irradiating the tumor with near-infrared light, the weight of the tumor efficiently decreased. Such multimodality of GVs will attract more interest with regard to their medical applications.

6. Summary

Functionalized GVs have an increasing number of applications similar to those of cells and organelles as the GV technology continues to develop. GV-based sensors and controlled releasers constructed from known molecules may be inexpensive, easier to control, and have greater utility in future medical applications.

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