An intelligent liposome which may deliver drug molecules in a well–controlled fashion

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The passage of molecules, especially of large ones, through cellular membrane is a very important problem for some biotechnological applications, such as drug delivery. The pore appearance in lipid bilayer following some controlled mechanisms may be an adequate and interesting way.

Some pores, named stochastic pores, can appear due to structural and dynamic properties of lipid bilayer, but others may be favored by mechanical tension induced by different ways. Recently, a sequence of 30–40 pores was observed in the same vesicle, a pore at a time, which can appear in vesicles stretched by optical induced mechanical tension[1,2].

There are two very interesting biotechnological applications which request the increase of membrane permeability: gene therapy and targeted drug delivery. In the first one, the transport of DNA fragments through cellular and nuclear membranes is requested[3]. The second application uses drug molecules encapsulated in vesicles, which have to be transported to a target place[4].

Having reached that point, one supposes that the liposome discharges its content by its breakdown.

In this paper, we will write about how a lipid vesicle has to release the drug molecules, in a well-controlled fashion.

Such liposome is named pulsatory liposome and it makes a cyclic activity. We will demonstrate that this liposome may be programmed to work a certain number of cycles, settled in advance. Also, we will calculate the amount of drug delivered during each cycle.

In fact, a pulsatory liposome may be conceived as a drug dose micro device, which works according to a medical prescription established a priori.

In the following we will describe the phenomenological base of a pulsatory liposome.
A pulsatory liposome is realized by insertion into a hypotonic aqueous medium of an unilamellar lipid vesicle filled with aqueous solution of a solute to which the vesicle membrane is impermeable.
Osmotic pressure created by the gradient of solute concentration determines an influx of water molecules through the liposome membrane. The supplementary water entered inside the liposome has two direct consequences: the dilution of internal solution and the swelling of the liposome. The vesicle increases up to a critical size, when suddenly a transbilayer pore appears. The appearance of this transbilayer pore is a very important event for the vesicle life and function. Its evolution may be described as follows. In the first stage this event is followed by two simultaneous processes: the pore radius increases and the internal material leaks out the vesicle through the pore, due to excess Laplace pressure. Both these phenomena, the pore increase and the internal liquid leakage, determine the membrane relaxation, due to the reducing of the membrane mechanical tension. The pore dynamics is leaded by the difference between the membrane tension and line tension. The internal liquid continues to leak out the liposome, even after the line tension equals the membrane tension.

Here Fig.1

From the moment when the line tension equals the membrane tension the second stage of pore dynamics starts, therefore the pore radius decreases up to its disappearance, and the vesicle reaches the initial diameter and becomes healthy. At the beginning of each cycle, the vesicle is in a complete relaxed state ($\sigma = 0$), has a radius equal to R_0 and the surface bilayer is smooth. The vesicle critical state is the vesicle state just before the pore appearance (or in some violent evolution just before its rupture), when its radius is R_c and membrane tension is σ_c.

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The cyclic dynamics of lipid vesicle is accompanied by continuous decrease of internal solute concentration. We are focusing our attention only on the inside solute concentration change during a cycle.

Let us suppose that at the beginning of its activity, the vesicle contains solute and water with molar concentrations c_{s0} and c_{w0}, respectively. Due to the water influx, the vesicle swells itself and its radius increases from R_0 to R_c. The water quantity which enters into the vesicle in each cycle is:

$$N^+ = \frac{4\pi\left(R_c^3 - R_0^3\right)}{3V_{\mu w}} = \frac{4\pi R_c^3}{3V_{\mu w}}\left(1 - \frac{R_0^3}{R_c^3}\right) = N(1-f) \qquad (1)$$

In this formula we have introduced the following notations: $N = V_c / V_{\mu w} = 4\pi R_c^3 / \left(3V_{\mu w}\right)$ which is the water quantity, measured in moles, that would fill the stretched vesicle just before the pore appearance only if water is present. The molar volume of water is noted with $V_{\mu w}$. We define the swelling ratio as the ratio between the vesicle volumes in the stretched state just before pore formation and in the complete relaxed state. It results that f is the reversal of the swelling ratio:

$$\frac{1}{f} = \frac{V_c}{V_0} = \frac{R_c^3}{R_0^3} \qquad (2)$$

The internal liquid composition changes after each cycle. Let us analyze the first cycle in both stages.

a) *Swelling stage*. At the beginning of the first cycle, the vesicle contains $N_{s1} = c_{s0}V_0$ moles of solute and $N_{w1} = c_{w0}V_0$ moles of water. At the end of the swelling stage, just before the pore opening, the same amount of solute is present in the vesicle, but it contains a larger amount of water:

$$N_{w1} = N_{w1} + N^+ = c_{w0}V_0 + N(1-f) \qquad (3)$$

The new molar fractions at the end of the first cycle are:

$$c_{s1} = \frac{N_{s1}}{V_c} = \frac{c_{s0}V_0}{V_c} = fc_{s0} \qquad (4)$$

$$c_{w1} = \frac{N_{w1}}{V_c} = \frac{\left(c_{w0}V_0 + N(1-f)\right)}{V_c} = fc_{w0} + \frac{1-f}{V_{\mu w}} \qquad (5)$$

b) *Relaxation stage*. After the pore opening, the pore radius increases up to a maximum value, then it decreases and finally the pore closes. During the pore evolution an amount of internal liquid leaks out. At the beginning of the second cycle, which is the same with the end of the first cycle and, the vesicle is in a complete state of relaxation and it contains:

$$N_{s2} = V_0 c_{s1} = f V_0 c_{s0} \text{ moles of solute and} \tag{6}$$

$$N_{w2} = V_0 c_{w1} = f V_0 c_{w0} + \frac{V_0(1-f)}{V_{\mu w}} \text{ moles of water.} \tag{7}$$

Making the same reasoning as for the first cycle, one finds the following recurrent formula in characterizing the inside composition of vesicle at the end of the nth cycle:

$$N_{sn} = f^n V_0 c_{s0}; \qquad N_{wn} = f^n V_0 c_{w0} + \frac{V_0(1-f^n)}{V_{\mu w}} \tag{8}$$

$$c_{sn} = f^n c_{s0}; \qquad c_{wn} = f^n c_{w0} + \frac{1-f^n}{V_{\mu w}} \tag{9}$$

The n cycles liposome programming. The motrice force of a pulsatory liposome is generated by the transmembrane osmotic gradient. The internal solute concentration decreases along the cycle and with the cycle rank in sequence, and as a consequence the osmotic pressure decreases too. The liposome will swell up to its critical radius only if the osmotic pressure at the end of cycle is greater than the excess Laplace pressure. Taking this into account we can program a pulsatory liposome to have *n* cycles in its activity life by the condition:

$$\sigma_c \left(\frac{1}{R-h} + \frac{1}{R+h} \right) \le \Re T \left(c_{sn}^{in} - c_{sn}^{out} \right) \tag{10}$$

where c_{sn}^{in} and c_{sn}^{out} are the solute concentrations at the end of swelling stage of the n-th cycle, inside and outside the liposome. Considering that at the beginning the solute external concentration is equal to zero and that the external medium composition is so little influenced by the vesicle running, we can take c_{sn}^{out}=0. Taking into account that c_{sn}^{in} is equal to c_{sn}, the condition (10) becomes:

$$\frac{2\sigma_c R}{R^2 - h^2} \le \Re T f^n c_{son} \tag{11}$$

where R is the radius of the sphere between the two monolayers of the liposome bilayer, σ_c is the monolayer surface tension, $2h$ is the hydrophobic core thickness, $\Re$ is the universal gas constant, and T is the absolute temperature. Taking into account that the vesicle considered here is sufficiently large and to obtain a simpler formula we will cut h from now on. Also, R will be equal to R_c. Therefore, from (11), it results that the initial solute concentration inside the liposome c_{son}, such as this liposome to produce n cycles, is equal to:

$$c_{s0n} = \frac{2\sigma_c}{\Re T R_c f^n} \tag{12}$$

The indices have the following significances: s – solute; 0 – initial; n – the number of programmed cycles.

Pulse solute content. We have named the quantity of internal material leaked out in a cycle as material pulse, or simply pulse. Making the difference between the solute contained inside the vesicle after two successive cycles we obtain the quantity of solute contained in the pulse of the internal solution delivered during the last cycle. Assuming that a liposome is programmed for n cycles, the solute content of the p-th pulse is:

$$\Delta N_{sp} = N_{s(p-1)} - N_{sp} = f^{p-1}(1-f)V_0 c_{s0n} = \frac{2\sigma_c V_0 (1-f)}{\Re T R_c f^{n-p+1}} \tag{13}$$

We will apply our above theoretical results to the giant vesicles obtained experimentally[5]. Such giant vesicle's radius in relaxed state is R_0 = 19.7 μm and the value of the critical radius is R_c = 20.6 μm. Let us consider a vesicle in a closed chamber which contains water.

Also, we consider the maximum value of bilayer tension just before pore appearance as being[5] $\sigma_c = 10^{-5}$ N/m. The constant group $\sigma_c / \Re T$ is equal to $4 * 10^{-9}\ mol.m^{-2}$ where T=300 K.

The initial solute concentration. If one introduces the above values in the formula (12) the initial solute concentration inside the vesicle, measured in μM/l, of running *n* cycles is:

$$c_{s0n} = \frac{0.389}{f^n} \tag{14}$$

The calculated value for the reversal swelling coefficient f is equal to 0.8746 for the considered vesicle. The dependence of the initial solute concentration on the programmed activity life, measured in cycle numbers is represented in Fig. 2.

Here fig.2

Solute content. If we introduce the known values in formula (13) it results in the amount of solute as a function of the number of programmed cycles and the rank of the pore in the sequence:

$$\Delta N_{sp} = \frac{12.46(1-f)}{f^{n-p+1}} \cdot 10^{-18} \text{ moles} \qquad (15)$$

Here fig.3

We have considered a liposome programmed to have 40 cycles. The number of cycles is equal to the number of successive pores. The solute content delivered in the p-th cycle is represented in Fig. 3.

We are thinking that the pulsatory liposome is an interesting and useful periodic device. The cycle duration can be calculated as the sum of the pore life and the swelling time. The pore life is short[2]. It is correct that it increases with the viscosity of the internal liquid up to 10 s. For the liposome analyzed in this paper, we make an approximate calculus and have found that the swelling time is nearly constant for all cycles and is equal to 23.44 minutes.

It is known that the water barely passes through the lipid bilayer. This situation is found at the beginning of the each cycle, but surely the water molecule will pass through lipid bilayer for two reasons:

a) The bilayer is composed from a mixture of lipid molecules. As such, it is heterogeneously, containing microclusters determined by selective dynamic association of lipid molecules[6,7]. The bilayer is not smooth or static and does not have uniform thickness;

b) The lipid vesicle must be larger, in order to contain more solute molecules.

As a consequence, the vesicle is more easily deformable in the relaxed state at the beginning of each cycle. So, it is very possible to appear to have very small structural

defects which to be used by water molecules to come into vesicle[8]. On the other hand, the pores are very large[2], up to10 μm. Larger molecules or greater amounts of internal liquid can leak out the vesicle. It is a good thing for a device to deliver drugs, or special substances in a fixed place and to follow a program. A very interesting application of pulsatory liposomes filled with drugs is in the case of hepatic cells. The endothelial pores (also known as fenestrate) control the exchange of fluids, solutes and particles between the sinusoidal blood and the space of Disse. The pulsatory liposomes, free or included inside to other vesicle, may reach hepatocyte due to hydrodynamic effects of the blood circulation[9]. Also, the transient pores in liposomes could be used for the compensation of neurotransmitter deficiency in the synaptic cleft[10]. In conclusion, there is a biotechnological problem: getting the liposome there.

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Legend of figures

Fig.1. The dynamics of a pulsatory liposome during a cycle. Firstly, the vesicle grows from R_0 to R_c, when a pore appearances, then decreases to its initial state. The pore radius increases to a maximum value, r_m, then decreases up to the pore closing. The pore radius is the same when the vesicle has R_{11} radius, or has R_{13} radius.

Fig.2. The dependence of initial solute concentration, c_{s0n}, on working time measured in the number of cycles, n, of a programmed liposome.

Fig.3. The solute content delivered in the p-th cycle by pulsatory liposome programmed to have 40 cycles.