ELSEVIER

Biochimica et Biophysica Acta 1564 (2002) 343–348

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Effect of liposome charge and PEG polymer layer thickness on cell–liposome electrostatic interactions

Nily Dan*

Department of Chemical Engineering, Drexel University, 3141 Chestnut St., Philadelphia, PA 19104, USA

Received 11 March 2002; received in revised form 28 May 2002; accepted 30 May 2002

Abstract

Targeted drug delivery requires binding to (and subsequent uptake by) the carrier and target cell. In this paper, we calculate the work required to bring into contact liposomal carriers and cells as a function of the liposome and cell electrostatic characteristics. We find that cell–liposome adhesion is sensitive to the cell type and optimized at a cell to liposome charge ratio which depends on the degree of cell charge regulation. As a result, uptake (which is dependent on the occurrence of binding) is also optimized. Incorporation of a (poly)ethylene glycol (PEG) layer enhances liposome adhesion in cases where the cell–liposome interactions are repulsive, and suppresses adhesion in systems where the interactions are attractive. Our results, which are in agreement with experimental observations, show that electrostatic interactions may be designed to enable targeted drug delivery by liposomes to a specific cell population.

Keywords: Drug delivery; Liposome; Stealth; Electrostatics; Theory

1. Introduction

Recent achievements in drug development, coupled to advances in biomaterial design, have led to the development of new therapy approaches. One such strategy is targeted drug delivery, whereby drugs are delivered only to a specific cell population or tissue. Targeted delivery is expected to increase the efficiency and effectiveness of the drug, as well as enable the use of new (and more potent) drugs. For example, targeted delivery may be utilized in cancer therapy to deliver anticancer agents specifically into tumor sites, thereby increasing efficacy and reducing the toxic effects of chemotherapy on healthy cells. To achieve this goal, however, the drug carrier must (1) be able to efficiently bind to, and be internalized by, the specific target cell population and (2) have little interactions and no uptake by nontarget cells.

The unique properties of liposomes, which include a large aqueous interior and a biocompatible lipid exterior, make them into ideal candidates for drug delivery (see, for example, Refs. [1–8]). This has led to intensive investigations of the interactions between liposomes and cells (see, for example Refs. [1–3,8–14]). Liposome uptake by cells has been directly linked to the binding stage [1–3]. Internalization may occur through either endocytosis [1–3] or fusion, depending on the liposome and cell characteristics [8–10]. The efficacy of cell uptake (by either method) has been shown to be quite sensitive to cell type [1–3,8–10]. Another parameter that has been shown to affect cell uptake is the liposome charge: As a rule, cationic liposomes bind to cells more easily that anionic cells, due to their opposite charge.[1]

The use of liposomes in vivo is limited due to their short circulation time, which has been linked to their recognition by the immune system [1–8]. Incorporation of polymeric (poly)ethylene glycol (PEG) chains has been shown to increase the circulation time of these sterically stabilized liposomes appreciably when compared to conventional (PEG-less) liposomes.

How does the PEG affect liposome properties? Models analysing PEG carrying liposomes divide the interaction potential into two contributions: One arising from the direct, van der Waals interactions between the protein (or a surface) and the lipid bilayer surface, and another due to steric repulsion provided by the PEG polymer layer [16–19].

* Tel.: +1-215-895-6624; fax: +1-215-895-5837.
E-mail address: Dan@coe.drexel.edu (N. Dan).

[1] It should be noted that anionic liposomes have been found to bind and be internalized in appreciable numbers by various cell types [1–3,8–15], thereby possibly indicating the presence of nonelectrostatic mechanisms.

0005-2736/02/$ - see front matter
PII: S0005-2736(02)00468-6

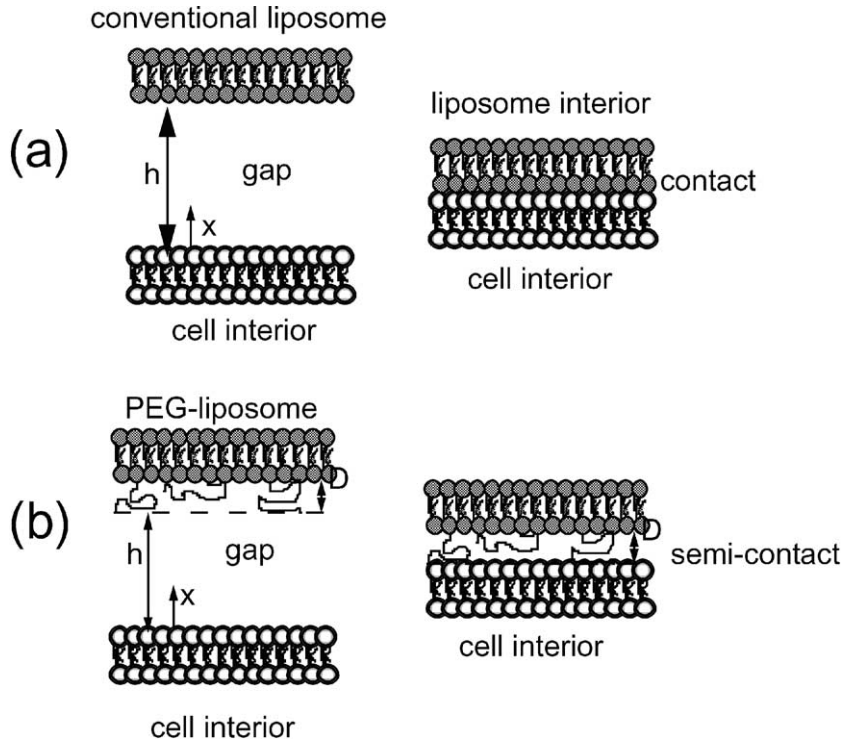

Fig. 1. A sketch of our system: We model the liposome as a hydrophobic, ion-impenetrable layer carrying a fixed charge, and, possibly, attached PEG polymer chains. The polymer layer is denoted *D*. The cell is modeled as an anionic, ion-impenetrable layer. Contact is defined when the edge of the polymer layer comes into contact with the cell (but is not compressed). The polymer layer is taken to be ion-penetrable, allowing free exchange of salt and counter-ions.

Thus, these models predict that the polymer layer should significantly suppress protein adsorption onto the liposome surface, in agreement with experiments [20,21].

While successfully predicting the interactions between proteins and PEG-carrying membranes, current models [16–19] cannot explain the sensitivity of either conventional or sterically stabilized liposome uptake to cell line or the effect of liposome charge on cellular uptake. In this paper, we examine the long-range, electrostatic interactions between cells and liposomes, as a function of the liposome surface charge and the PEG polymer layer thickness. Cell properties are accounted for via the cell's surface charge density and its degree of charge regulation, namely sensitivity to the electrochemical potential.

Contact between a conventional (i.e., PEG-less) liposome and a cell is easily defined as the limit where the separation between the liposome membrane and the cell membrane is of order zero (see Fig. 1a). The work required to bring the cell and liposome into contact is defined as the work of adhesion; for repulsive interaction potentials, the work of adhesion is positive, and for attractive interactions negative. As may be expected, the time spent in contact increases with decreasing work of adhesion, thereby increasing the probability of internalization (whether by endocytosis or fusion).

Defining contact in the case of PEGilated liposomes is somewhat more complex, since the PEG chains prevent direct contact between the cell and the liposome membrane. However, it is reasonable to assume that, as in the case of conventional liposomes, the probability of internalization is proportional to the time the liposome spends in the close vicinity of the cell. We therefore define, somewhat arbitrarily, that contact in the case of PEG-carrying liposomes correspond to the case where the outer edge of the *uncompressed* PEG layer is in contact with the cell membrane (see Fig. 1b). Thus, the work of adhesion in this case is calculated as the work to bring the liposome membrane to within a distance *D*, the PEG layer thickness, from the cell. We neglect the role of PEG compression (which has been discusses elsewhere [16–19]), choosing to focus on the effect of the electrostatic interactions.[2]

2. Model

We model the cell membrane as a hydrophobic, ion-impenetrable core carrying a net surface anionic charge. The curvature of the cell surface is neglected since the cell diameter is orders of magnitude larger than any other

[2] As has been shown [16–19], the PEG layer gives rise to repulsive interactions between the liposomes and substrates. Accounting for this resistance is essential when discussing liposome internalization through fusion, where direct contact between the liposome membrane and the cell membrane is required. However, in the case of endocytosis, internalization requires only contact between the PEG layer and the cell membrane [1–8].

length-scale in the system. The liposome is similarly modeled as an ion-impenetrable hydrophobic core that may carry a charge and/or an ion penetrable layer composed of the attached polymeric chains, as sketched in Fig. 1. We make several simplifying assumptions, including taking the effective cell charge density and liposome charge density to be relatively low, the solution salt concentration to be high, and discuss only solutions of monovalent ions. These assumptions allow us to apply the Debye–Huckel limit of the Poisson–Boltzmann model [22,23], where the electrostatic potential is taken to be smaller than the entropic energy. It should be emphasized that we focus here only on the long-range electrostatic interactions, neglecting all other possible contributions (e.g. van der Waals, molecular/biochemical, or the PEG chain compression). Therefore, the model is valid only for cell–liposome core–core separations larger than, or equal to, the grafted polymer layer thickness, and at relatively short time scales before cellular adjustment and molecular bonding can take place.

The electrostatic potential is defined by the Poisson relationship. For a one-dimensional system [22]

$$\frac{d^2\psi}{dx^2} = \frac{-e\rho(x)}{\varepsilon} \tag{1}$$

where ψ is the dimensionless electrostatic potential, e an electron charge, ρ is the density of charges, x the distance from the surface, and ε the dielectric constant of the medium (i.e. water). The charge distribution ρ is given by the Boltzmann distribution, which reduces in the limit of low potential to [22,23]

$$\rho(x) = n(-e^{e\beta\psi} + e^{-e\beta\psi}) \approx -2ne\beta\psi \tag{2}$$

where n is the overall salt concentration and $\beta=1/kT$ where k is the Boltzmann coefficient and T the temperature. It is common to describe the range of electrostatic interactions in such solutions using the Debye screening length $1/\kappa$, where $\kappa=\sqrt{(2n\beta e/\varepsilon)}$. Boundary conditions define the potential at the cell surface and at the liposome surface, a function of their charge densities [22,23].

The force (per unit area) between two surfaces is given, in this low potential, Debye–Huckel limit, by [22–24]

$$p\beta \approx n\psi_0^2 \tag{3}$$

where ψ_0 is the potential at the point where $d\psi/dx=0$. The work of adhesion, namely the work required to bring the two surfaces into contact or semicontact (defined as the point where the separation between the liposome surface and the cell surface is equal to the thickness of the unperturbed polymer layer) is given, therefore, by

$$W \approx \int_0^\infty p\,dx \tag{4}$$

The net charge on the cell surface is not fixed, however, but is charge regulated. That means that the surface ionic groups are subject to an equilibrium between dissociated and nondissociated states determined by the electrochemical potential. The fraction of dissociated charges, α, depends therefore on the electrostatic potential, given in the Debye–Huckel limit by [24]

$$\alpha \approx \alpha_0 + \alpha_1\, e\beta\psi_s \tag{5}$$

where α_0, α_1 are constants, a function of the ionization equilibrium coefficient, and ψ_s is evaluated at the appropriate surface. Thus, the effective charge of the cell surface is given by $\Sigma_c=\Sigma_c^*(1+\alpha_1 e\beta\psi_s/\alpha_0)$, where Σ_c^* is the surface charge on an isolated cell (namely, when $\psi_s=0$).

3. Results

We first calculate the work of adhesion required to bring a conventional liposome (namely, one that is not carrying a PEG polymer layer) into contact with a cell, as a function of the nominal liposome to cell surface charge density ratio Σ_L/Σ_c^*.

$$W = \frac{\alpha_0\Sigma_c^*\left(\left[\frac{\Sigma_L}{\Sigma_c^*}+1\right]^2 + 2A\frac{\Sigma_L}{\Sigma_c^*} - A^2\left[\frac{\Sigma_L}{\Sigma_c^*}\right]^2\right)}{2\alpha_1\beta^2e^2n^2(1+A)} \tag{6}$$

where $A=\kappa\alpha_1\Sigma_c^*/2\alpha_0 n$. We see that the work to bring into contact such surfaces is infinite in the case of nonregulating cell surface where $\alpha_1=0$ and $A=0$, unless the surface charges exactly match so that $\Sigma_L/\Sigma_c^*=-1$. This is in agreement with the calculations of Parsegian and Gingel [25] who examined the interactions between two solid, nonregulating charged surfaces. For a given surface charge ratio, the work of adhesion decreases rapidly with the degree of the cell charge regulation, namely A and α_1. It should be noted that the work of adhesion may be repulsive (namely, positive) even if the cell and the liposome are oppositely charged so that $\Sigma_L/\Sigma_c^*<0$.

Examining the effect of the polymer layer thickness, D, on the work to bring into contact a cell with an uncharged liposome we find

$$W = \left(\frac{\Sigma_c^*}{en\beta}\right)^2 \frac{\kappa}{2n(1+A)((1+A)e^{2\kappa D}+A-1)} \tag{7}$$

Thus, in the case of uncharged liposomes, increasing the layer thickness decreases the work required to bring the liposome and cell to contact.

The work to bring a liposome carrying a relatively thin PEG polymer layer into contact (as defined in Fig. 1) with a cell is plotted in Fig. 2 as a function of the liposome to cell charge ratio. We see that the minimum in the work is not obtained when the liposome charge is exactly opposite to the cell charge (namely, when $\Sigma_L/\Sigma_c^*=-1$). Calculating the

charge density ratio at which the minimum work occurs, we find

$$\left[\frac{\Sigma_L}{\Sigma_c^*}\right]_{min} = \frac{-e^{\kappa D}}{(1-A)} \tag{8}$$

and the corresponding work is given by

$$W_{min} = -\left(\frac{\Sigma_c^*}{en\beta}\right)^2 \frac{\kappa}{2n(1-A^2)} \tag{9}$$

Thus, accounting for either the PEG layer thickness or the cell's charge regulation shifts the location of the minimum in the adhesion energy from the symmetrical case ($\Sigma_L=-\Sigma_c^*$) to an asymmetrical one where the charge density on the liposome is higher than the (nominal) charge density of the cell. However, the work associated with the optimal liposome surface charge, W_{min}, is independent of the PEG polymer layer thickness.

From Eq. (9), we see that the work of adhesion at the optimal liposome to cell surface charge ratio is independent of the PEG polymer layer thickness. However, in most cases, the liposome charge is unlikely to be the optimal one. What role does the PEG layer play? In our model (which applies only for uncompressed layers), the polymer layer acts as a barrier. Thus, the smallest distance between the liposome membrane and the cell membrane is defined by D, and the work of adhesion calculated as the integral over the potential up to that point. In Fig. 3a, we plot the pressure, or interaction potential, as a function of membrane separation. We see that for similarly charged liposome–cell pairs, the repulsion increases exponentially with decreasing separation. As a result, the work of adhesion for this system (see Fig. 3b) increases with decreasing PEG layer thickness. In the case of oppositely charged liposomes of identical charge density, the attraction increases with decreasing separation, so that the work 'gained' by liposome adhesion to the cell decreases with increasing D.

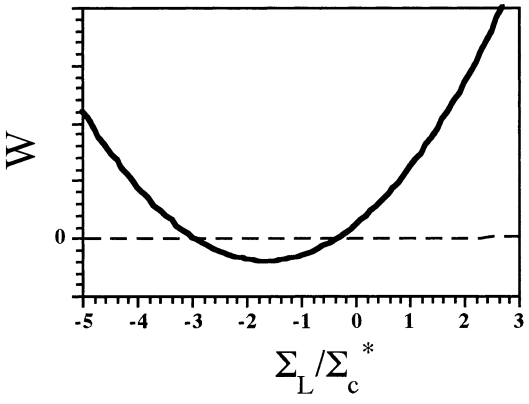

Fig. 2. The work of adhesion for liposomes carrying a thin polymer layer as a function of the liposome to cell charge ratio. The work is given in arbitrary units, and the cell degree of charge regulation α_1=0.1. κD=1/2 so that in a solution of 0.1 M, D=5 nm.

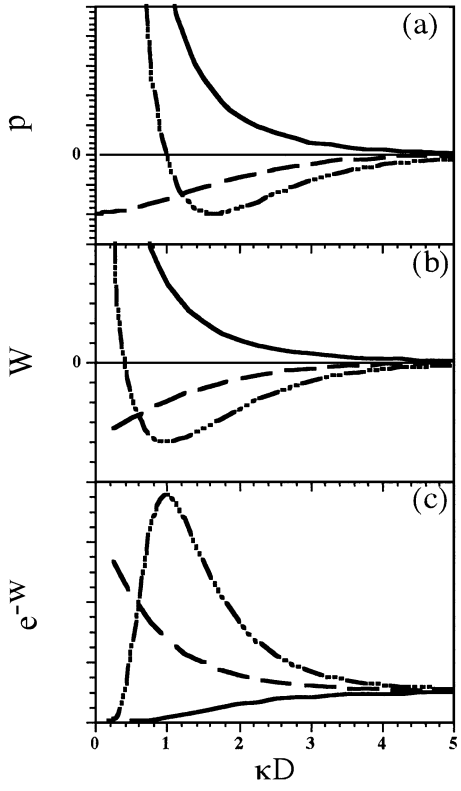

Fig. 3. The effect of the polymer layer thickness on the (a) pressure, (b) work and (c) the probability of adhesion. The solid line denotes $\Sigma_L=\Sigma_c^*$, the dashed line $\Sigma_L=-\Sigma_c^*$ and the dot–dash line $\Sigma_L=3\Sigma_c^*$. The work is given in arbitrary units, and the probability is normalized to unity. α_1=0.1. Note that in 0.1 M solutions, κ=1 nm^{-1}.

The most interesting case, perhaps, is that of oppositely charged liposomes where the density of liposome charges is very different from that of the cell. As can be seen in Fig. 3a, the interaction between the liposome and the cell is attractive for large separations, but switches to a strong repulsion at shorter distances. Therefore, the work of adhesion favors contact for liposomes with large polymer layer thicknesses, and inhibits contact in systems where D is small.

In Fig. 3c, we translate the work of adhesion into a Boltzmann probability that may be taken to describe either the probability of contact, or, alternately, the 'residence' time distribution of liposomes at contact with the cell. As expected, the probability of contact (or residence time) increases with D for oppositely charged cell–liposome pairs and increases with D for similarly charged pairs. In the case of oppositely charged but mismatched pairs, the probability peaks at a finite PEG layer thickness.

4. Discussion

In this paper, we examine the effect of liposome charge density and the thickness of the PEG polymer layer on the long-range electrostatic interactions with cells. Specifically,

we focus on the work of adhesion, namely the work required to bring the liposome into contact with the cell. Our model predicts that liposome internalization should be sensitive to the cell surface charge and degree of charge regulation, parameters that have been shown to vary not as a function of cell and/or bacteria type, but also for different strains of the same type (see, for example, Ref. [26]). Thus, accounting for electrostatics can explain the marked differences in the uptake of a given liposome by different cells [1–11].

Examining the effect of (conventional, PEG-less) liposome charge on the interactions with a specific cell line, we find that the work is most attractive at a finite charge ratio, and can be repulsive even for oppositely charged cell–liposome pairs [24]. Plotting the effect of the charge ratio on the probability of adhesion, we see (Fig. 4) that the probability peaks at a finite charge ratio. Outside the peak area, there is a large range of liposome to cell charge ratios (both positive and negative) where uptake is rather insensitive to the liposome charge. This is in qualitative agreement with the experiments of Miller et al. [11], who found that liposome uptake by either human ovarian carcinoma cells (HeLa) or by mononuclear macrophage cells (J774) is similar for both anionic and cationic liposomes. An exception was observed in the case of HeLa cells, where a peak in uptake was obtained for a specific cationic lipid content, which may correspond to the optimal cell–liposome charge ratio[3] [11].

Pires et al. [27], find that uptake of cationic liposomes by monocytic THP-1 cells is only slightly higher for 100% cationic lipid content relative to 50%, but is significantly lower when the cationic lipid content was further reduced to 33%. Is it possible that reducing the liposome cationic charge by a factor of 2 would not affect uptake significantly, but a factor of 3 would? Examining Fig. 4 we see that, indeed, such a behavior is possible; for example, if the fully cationic liposomes correspond to a liposome–cell charge ratio of (−1.4), their uptake would be only slightly higher than that of liposomes with only half that charge (−0.7), while the uptake of liposomes with a third that value (−0.45) would be very low[4].

Incorporating a PEG polymer layer mitigates the electrostatic interactions, and thus, moderates the work of adhesion (Fig. 3); in the case of either similarly charged cell–liposome pairs, or uncharged liposomes (Eq. (7)), increasing the PEG layer thickness increases the volume available to the confined counter-ions, thereby reducing their osmotic pressure and the work required to bring them into contact.

In the case of cationic liposomes with surface charge density similar to that of the cell (namely, in the vicinity of the optimal value where attraction is maximal), the strength of attractive interactions decreases with increasing PEG layer thickness. However, if the cationic liposomes are overcharged compared to the cell, the probability of adhesion is maximized at a finite polymer layer thickness. Ross and Hui [12] examined the effect of PEG incorporation on the adhesion of liposomes to Chinese hamster ovary cells, finding that adhesion increased with increasing PEG content for both cationic and anionic liposomes, in agreement with our predictions (assuming that the cationic liposomes were overcharged). Our predictions are also in agreement with Miller et al. [11] who find that HeLa cell uptake of moderately cationic liposomes increased slightly upon the incorporation of PEG chains. It should be noted that our model cannot account for the PEG-induced suppression of anionic liposome uptake (see, for example, Refs. [11,13]) since the attractive interaction, leading to adhesion, is not electrostatic in these systems.

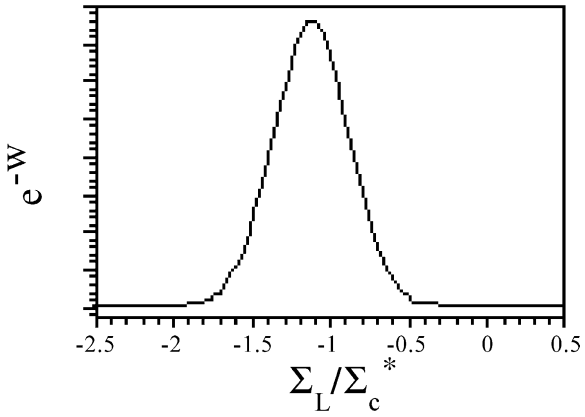

Fig. 4. The probability of adhesion as a function of the liposome to cell charge ratio, for conventional liposomes where no polymer layer is present. α_1=0.1.

In a recent paper, Carignano and Szleifer [28] developed a molecular model for the effects of electrostatics on the adsorption of charged proteins onto uncharged surfaces carrying grafted polymer chains. This system is similar to our analysis of the interactions between uncharged, PEG carrying liposomes interacting with a (charged) cell. They find [25] that the adsorption of proteins on top of the polymer layer (i.e., adhesion by our definition) is enhanced by the polymer layer, in agreement with our Eq. (7).

In conclusion, we present here a simple model for the electrostatic interactions between liposomes and cells, focusing on the work of adhesion as a function of the liposome–cell charge ratio, the cell's degree of charge regulation, and the thickness (if present) of the PEG polymer layer. We show that the probability of adhesion is sensitive to the cell type, as expressed through its charge density and degree of charge regulation. We also find that cationic liposomes may not effectively bind to anionic cells if their charge ratio is high. The optimal liposome–cell charge ratio varies with the cell's degree of charge regu-

[3] A minimum uptake was observed in J774 cells and neutral liposomes, which cannot be explained by our model [11]. However, this minimum seems an anomaly when compared to either very weakly cationic or weakly anionic liposomes.

[4] Pires et al. [27] find that lipid chemistry significantly affects uptake, a phenomena that cannot be described by our purely electrostatic model.

lation and the PEG layer thickness. Incorporation of a PEG layer reduces the barrier for adhesion for mismatched cell–liposome pairs, thereby increasing uptake of anionic liposomes or highly cationic ones. However, the PEG layer is predicted to reduce the attraction, and therefore uptake, in systems where the liposome charge is opposite to, and of the same magnitude, as that of the cell.

How do these results relate to the design of liposomes for targeted delivery? As mentioned in the Introduction, targeted delivery requires efficient internalization of drug-carrying liposomes by the target cell population only. Thus, it requires suppression of any nonselective adhesion, and maximization of selective uptake (e.g., through specific ligand incorporation into the liposomes). Generally, it was assumed that electrostatics work against these requirements: Cationic liposomes will bind nonselectively to all cell types, while anionic ones would suffer from low efficiency due to the long-range electrostatic repulsion. Our calculation shows that designing conventional cationic liposomes so that their surface charge matches the optimal value for delivery for a specific cell type (Fig. 4), or manipulating the PEG polymer layer thickness of overcharged, sterically stabilized cationic liposomes so that it matches the maximal value (Fig. 3) can lead to optimization of delivery to a specific cell population, where delivery to other types of cells would be largely suppressed.

Acknowledgements

This work was supported by the NSF Career Grant BES 0096004.

References

[1] T.M. Allen, G.A. Austin, A. Chonn, L. Lin, K.C. Lee, Biochim. Biophys. Acta 1061 (1991) 56–64.
[2] K.D. Lee, K. Hong, D. Papahadjopoulos, Biochim. Biophys. Acta 1103 (1992) 185–197.
[3] K.D. Ee, S. Nir, D. Papahadjopoulos, Biochemistry 32 (1993) 889–899.
[4] O.G. Mouritsen, K. Jorgensen, Pharm. Res. 15 (1998) 1507–1518.
[5] M.B. Bally, H. Lim, P.R. Cullis, L.D. Mayer, J. Liposome Res. 8 (1998) 299–335.
[6] T. Lian, R.J.Y. Ho, J. Pharm. Sci. 90 (2001) 667–680.
[7] R. Banerjee, J. Biomater. Appl. 16 (2001) 3–21.
[8] N. Duzgunes, S. Nir, Adv. Drug Deliv. Rev. 40 (1999) 3–18.
[9] N. Higashi, J. Sunamoto, Biochim. Biophys. Acta 1243 (1995) 386–392.
[10] E. Papadimitriou, S.G. Antimisiaris, J. Drug Target. 8 (2000) 335–340.
[11] C.R. Miller, B. Bondurant, S.D. McLean, K.A. McGovern, D.F. O'Brien, Biochemistry 37 (1998) 12875–12883.
[12] P.C. Ross, S.W. Hui, Biochim. Biophys. Acta 1421 (1999) 273–283.
[13] H. Du, P. Chandroy, S.W. Hui, Biochim. Biophys. Acta 1326 (1997) 236–248.
[14] D. Baczynska, K. Widerak, M. Ugorski, M. Langer, Zeit Naturforsch. C 56 (9–10) (2001) 872–877.
[15] D. Needham, D.H. Kim, Colloids Surf., B Biointerfaces 18 (2000) 183–195.
[16] I. Szleifer, M.A. Carignano, Macromol. Rapid Commun. 21 (2000) 423–448.
[17] M.A. Carignano, I. Szleifer, Colloids Surf., B Biointerfaces 18 (2000) 169–182.
[18] F. Fang, I. Szleifer, Biophys. J. 80 (2001) 2568–2589.
[19] A. Halperin, Langmuir 15 (1999) 2525–2533.
[20] D. Needham, T.J. MacIntosh, D.D. Lasic, Biochim. Biophys. Acta 1108 (1992) 40–48.
[21] N.V. Efremova, B. Bondurant, D.F. O'Brien, D.E. Leckband, Biochemistry 39 (2000) 3441–3451.
[22] J.N. Israelachvili, Intermolecular and Surface Forces: With Applications to Colloidal and Biological Systems, Academic Press, London, 1985.
[23] S. Safran, Statistical Thermodynamics of Surfaces, Interfaces and Membranes, Addison-Wiley, New York, 1994.
[24] B.W. Ninham, V.A. Parsegian, J. Theor. Biol. 31 (1971) 405–428.
[25] V.A. Parsegian, D. Gingel, Biophys. J. 12 (1972) 1192–1204.
[26] H. Morisaki, Y. Kashara, T. Hattori, J. Gen. Appl. Microbiol. 39 (1993) 65–74.
[27] P. Pires, S. Simoes, R. Gaspar, N. Duzgones, M.C. Pedroso de Lima, Biochim. Biophys. Acta 1418 (1999) 71–84.
[28] M.A. Carignano, I. Szleifer, Mol. Phys. (2002) in press.