The liposomal delivery of cancer therapeutics, including gene therapy vectors, is an area of intense study. Poor penetration of liposomes into interstitial tumor spaces remains a problem, however. In this work, the penetration of different liposomal formulations was examined. Spheroid penetration was assessed by confocal microscopy of fluorescently labeled liposomes. The impact of liposomal surface charge, mean diameter, lipid bilayer fluidity and fusogenicity on spheroid penetration was examined. A variety of different liposome systems relevant to clinical or preclinical protocols were studied, including classical zwitterionic (DMPC:chol) and sterically stabilized liposomes (DMPC:chol:DOPE-PYPEG2000), both used clinically or in preclinical studies. GM1-directed liposomes (DMPC:DOPE:DC-chol) led to improvements in the observed intratumoral penetration of liposomes into interstitial tumor spaces. The inclusion of the fusogenic lipid DOPE and use of a cationic lipid of lower surface charge density (DOTAP instead of DC-chol) led to improvements in the observed intratumoral penetration. Sterically stabilized liposomes did not interact with the tumor spheroids, whereas small unilamellar classical liposomes exhibited extensive distribution deeper into the tumor volume. Engineering liposomal delivery systems with a relatively low charge molar ratio and enhanced fusogenicity, or electrostatically neutral liposomes with fluid bilayers, offered enhanced intratumoral penetration. This study shows that a delicate balance exists between the strong affinity of delivery systems for the tumor cells and the efficient penetration and distribution within the tumor mass, similar to previous work studying targeted delivery by ligand-receptor interactions of monoclonal antibodies. Structure-function relationships from the interaction of different liposome systems with 3-dimensional tumor spheroids can lead to construction of delivery systems able to target efficiently and penetrate deeper into the tumor interstitium and act as a screening tool for a variety of therapeutics against cancer.

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roids are continuously being utilized as models for the study of early tumor development, models of avascular micrometastatic tumors, and, particularly in scope of the present study, as models of the tumor interstitial space (despite the absence of vasculature and endogenous humoral agents). In this work, the multicellular tumor spheroid model has been utilized to study passive (nonspecific) targeting through electrostatic surface binding and interstitial penetration and diffusion of a variety of liposome systems. Passive targeting of tumor vasculature by electrostatic binding has recently been studied in vivo using cationic liposomes alone or in combination with targeting ligands. In the present study, different types of fluorescently labeled liposomes were constructed and allowed to interact with tumor spheroids under controlled conditions; 200 μm diameter spheroids were chosen as these are at the edge of exhibiting hypoxic and necrotic cores, the presence of which would be expected to complicate characterization of liposome penetration due to the accumulation of fluid and cellular debris. Using a quantitative evaluation based on confocal laser scanning microscopy and image analysis, the affinity for binding (nonspecific, passive targeting) and penetration (intratumoral diffusion) exhibited by the different liposome systems was studied.

**MATERIAL AND METHODS**

### Lipids and liposomes

Different types of liposomes, representing liposomes commonly used for biomedical applications (delivery of anthracyclines, amphotericin, plasmid DNA) were allowed to interact with tumor spheroids. All liposome compositions were prepared as multilamellar vesicles (MLVs) and small unilamellar vesicles (SUVs), differing in the mean particle size of the respective liposome populations. Light and electron microscopy indicated that the mean vesicle diameter for all MLV systems ranged between 800 and 1,000 nm, and for all SUV systems between 50 and 150 nm (not shown). The total lipid composition in the liposome systems was kept constant at 1 mg/ml throughout the study. All liposome compositions were prepared as multilamellar vesicles prepared by solubilization of all lipids into laboratory-grade chloroform (USP) and subsequent evaporation under high pressure to form a lipid film. Hydration of the lipids by addition of either PBS (in experiments not involving cells) or RPMI medium (for cellular experiments) produced multilamellar vesicles (MLVs). Extrusion cycles (10) through polycarbonate filters (Millipore, Bedford, MA) using a LiposetFast extruder (Avestin, Ottawa, Canada) was used to form small unilamellar liposomes according to a previously described protocol.

### Liposome surface characterization

The surface properties of the liposomes were characterized using a DELSA 440 Zetasizer instrument by Beckman-Coulter (Fullerton, CA). Ten different measurements for each liposome system were carried out and all 4 different angles of detection were used to obtain the ζ potential at the liposome surface by employing the Smoluchowski approximation on the electrophoretic mobility data obtained when a 5 V electric field was applied to the liposome suspension: $U = \frac{\xi}{\eta}$, where $U$ is electrophoretic mobility, $\xi$ is permiittivity of the medium ($\epsilon = \epsilon r D$, where $\epsilon r$ is permittivity of free space and $D$ is dielectric constant), $\eta$ is viscosity and $\xi$ is zeta potential.

### Cells and spheroids

Multicellular spheroids consisting of the LNCap-LN3 prostate tumor cell line were prepared according to the liquid overlay technique of Yuhas et al. as described in detail previously. Approximately 10^6 LNCap-LN3 cells, obtained by trypsination from growing monolayer cultures, were seeded into 100 mm dishes coated with a thin layer of 1% agar (Bacto Agar; Difco, Detroit, MI) with 15 ml of RPMI-1640, supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. After 3–5 days in the agar culture, spheroids of 200 ± 50 μm in diameter were selected under an inverted phase-contrast microscope with an ocular scale using an Eppendorf pipette. The selected spheroids were transferred to 35 mm bacteriologic Petri dishes in 2 ml of medium.

### Interaction between liposomes with spheroids

Multicellular spheroids were coinubated with liposomes in 35 mm diameter Petri dishes for 2, 5 and 24 hr at 37°C. All incubations were undertaken in an orbital shaker incubator. At the specified time points, spheroids were washed 3 times with PBS and placed in fresh incubation medium before fluorescence imaging was carried out; selected spheroids were not washed prior to imaging. Five spheroids were studied in each condition.

### Confocal laser scanning fluorescence microscopy

CLSM imaging (Zeiss LSM 510; Carl Zeiss, Oberkochen, Germany) was carried out by acquiring 3 μm thick optical sections of the liposomes under study from the top toward the center of the spheroids until approximately scanning 120 μm deep into the spheroid. Dil fluorescence was observed red using standard rhodamine optics (excitation filter at 546 nm, dichroic mirror at 580 nm and barrier filter at 590 nm), as previously described.

### Image analysis

The fluorescence profile of each spheroid as a function of depth was determined from the average intensity along 50 equally spaced spheroid diameters using the image analysis software Intelligent View (version 1.2 by Bokwon Yoon). For each spheroid image, the obtained radial profiles were corrected for background fluorescence due to scattering and absorption within the spheroid. The linear attenuation coefficient was determined in separate experiments from spheroids of autofluorescent cells transfected with a fluorescent protein. Attenuation was modeled by the expression $I = I_0 \exp(-\alpha r)$, where $r$ is a variable representing the perpendicular distance between the focal plane and the proximal surface of the spheroid, $\alpha$ is the attenuation coefficient and $I_0$ is the fluorescent intensity at the rim of the spheroid (in which attenuation is assumed negligible). Using this expression, the attenuation coefficient, $\alpha$, was found to be 0.003165/μm. Quantitative fluorescence intensity data are from spheroids from at least 2 separate experiments. Five spheroids were included per condition per experiment. The fluorescence data collected were then analyzed to yield the following parameters: binding, taken as the percentage of total fluorescence intensity found in a 20 μm shell surrounding the spheroid rim (~2–3 cell layers); affinity, taken as the total fluorescence intensity signal from each image. The percentage of fluorescence intensity (relative to peak intensity) found at 50 and 100 μm radial distance from the outer spheroid periphery is used to obtain a 2-component description of diffusion or penetrative capacity (i.e., penetration at equilibrium state) for each liposome system examined.

It is important to note that the CM-based methodology used to evaluate vesicle penetration into spheroids is an alternative to the...
RESULTS

Various types of liposome systems were constructed and allowed to interact with the tumor spheroids. The liposome systems prepared and their surface charge (z potential) data from the laser electrophoresis experiments are depicted in Table I. The variation in liposome surface and lipid bilayer characteristics and the ensuing ability to interact with cellular membranes should be noted. In Figure 1, the confocal laser scanning microscopy (CLSM) and differential interference contrast images of the equatorial slice images of LNCaP-LN3 spheroids following interaction with fluoro-ducally labeled SUVs formed with DMPC:chol (Fig. 1a), DMPC:DC-chol (Fig. 1b), DPPC:chol (Fig. 1c) and DMPC:chol: DOPE-PEG$_{2000}$ (Fig. 1d) are shown. Apart from the notable association and intraspheroid diffusion exhibited by the DMPC:chol SUVs (Fig. 1a), what is also striking is the limited interaction of the cationic DMPC:DC-chol SUVs (Fig. 1b) at the very edge of the outer spheroid rim. DPPC:chol liposomes (Fig. 1c) indicated that hardly any interaction was taking place between them and the spheroid. Similarly, in the case of sterically stabilized DMPC:chol:DOPE-PEG$_{2000}$ (Fig. 1d), even though present in the vicinity of the spheroid, the polymer layer at its surface seems to exclude any binding or penetration within the spheroid. The fluorescence signals in the environment surrounding the spheroid in Figure 1(d) are apparent as no washing of this spheroid preceded the microscopic study.

A quantitative comparative analysis of the fluorescence intensity data obtained from the spheroid images depicts the dramatically different pattern of interaction occurring with the DMPC:chol and DMPC:DC-chol SUVs (Fig. 2). The latter, a strongly cationic liposome system, primarily binds to the outer cells at the spheroid surface, exhibiting a sharp decline in fluorescence intensity to almost zero values at approximately 30 μm within the spheroid. Contrary to that, the DMPC:chol liposomes not only associate with the tumor spheroids, but also penetrate extensively almost throughout the interstitial space. Figure 3 shows that only a moderate improvement in the penetrative capacity of DMPC:chol SUVs was obtained, when allowing interaction with the spheroids for 2 and 5 hr. This is more clearly illustrated in the analysis of fluorescence intensity profiles (Fig. 3c); a 40–50% increase in the fluorescence intensity toward the spheroid center is observed between 2 and 5 hr.

To elaborate on the construction and properties of the liposome systems, we engineered several different positively charged (cationic) vesicles containing the fusogenic lipid DOPE. In Figure 4, equatorial slice images of spheroids following interaction with multilamellar vesicles for 2 and 5 hr are shown. The cationic MLVs of DMPC:DC-chol adhere to the surface of the spheroid (Fig. 4a) and after 5 hr only a moderate increase in the accumulation of vesicles at the spheroid surface was observed (Fig. 4b). Addition of the fusogenic lipid DOPE in the vesicle content led to an improvement in the vesicle interaction with the outer tumor cells; however, no dramatic augmentation of penetration of the spheroids was obtained (Fig. 4c). After 5 hr, more DMPC:DC-chol:DOPE MLVs were binding strongly onto the spheroid, but without any appreciable intratumoral diffusion occurring (Fig. 4d).

Altering the cationic lipid used to DOTAP, MLVs of DMPC:DOPE:DOTAP exhibited a dramatic increase in both the amount of vesicles binding and fusing with the spheroids (Fig. 4e). Moreover, allowing the vesicles to interact with the spheroid for 5 hr seemed to improve the extent of fusion taking place with the tumor cells (Fig. 4f). In Figure 5, the quantitative depiction of fluorescence intensity for a series of images using the liposome systems shown in Figure 4 is represented. Overall, DOPE inclusion in the lipid bilayers offered a homogeneous interaction with the spheroids, improving both electrostatic targeting and diffusion within the spheroid by 20% compared to lipid bilayer liposomes that did not contain any DOPE (Fig. 5). A further, almost 2-fold improvement in both affinity for the spheroid surface and diffusion within the model tumor tissue was obtained by using the cationic lipid DOTAP instead of DC-cholesterol.

Small unilamellar vesicles of the 3 cationic systems were allowed to interact with the tumor spheroids for 2 hr. Representative CLSM equatorial images of the spheroids are shown in Figure 6. The DMPC:DC-chol and DMPC:DOPE:DC-chol SUVs (Fig. 6a and b) displayed binding limited to the surface of the spheroid. The addition of the fusogenic DOPE led to an evident increase in the amount of fluorescence intensity at the spheroid rim, particularly evident in the quantitative results of Figure 7. DOPE inclusion, however, only led to a 2-fold increase of fluorescence intensity signal obtained at the outer cell layers of the spheroid, without any evident improvement in the intratumoral diffusion of liposomes. Interestingly, DOPE in the MLV systems had a pronounced effect in both total affinity and diffusion. The DMPC:DOPE:DOTAP SUV system exhibited the most homogeneous distribution throughout the tumor spheroid and also an evident fusogenic capability. In Figure 6(c), in a representative CLSM image of a spheroid interacting with the DMPC:DOPE:DOTAP system, individual cells deep within the spheroidal cluster are fluorescently tagged by the liposomes. From the data obtained quantitatively and depicted in Figure 7, the penetrative capabilities of the DMPC:DOPE:DOTAP SUV system can be observed with fluorescence intensities up to 100 μm toward the core of the spheroid, almost at the core of the tumor mass.

In Table II, the fluorescence signal intensity profiles of liposome-spheroid interactions have been analyzed to provide indications of the overall affinity for the tumor spheroid, as well as other intratumoral distribution characteristics (i.e., binding and diffusion). It can be observed that all cationic liposomes exhibited strong overall affinity for the tumor spheroid. The extent of diffu-

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**Table I: Liposome Systems Studied and Their Surface Charge Characteristics from Zeta Potential Measurements Carried Out Using Laser Scattering Microelectrophoresis and the Lipid Bilayer Characteristics (as Determined by the Recorded 22 Phase Transition Temperatures) of the Selected Lipid Molecules at 37°C.**

<table>
<thead>
<tr>
<th>Liposome systems</th>
<th>Surface charge (mV)</th>
<th>Liposome bilayer characteristics (at 37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC:chol (2:1)</td>
<td>−9.3 ± 2.2</td>
<td>Liquid crystalline</td>
</tr>
<tr>
<td>DMPC:DC-chol (2:1)</td>
<td>51.7 ± 3.9</td>
<td>Liquid crystalline</td>
</tr>
<tr>
<td>DPPC:chol (2:1)</td>
<td>−55 ± 3.2</td>
<td>Gel</td>
</tr>
<tr>
<td>DMPC:chol:DOPE-PEG$_{2000}$ (10:5:1)</td>
<td>4.8 ± 0.4</td>
<td>Liquid crystalline</td>
</tr>
<tr>
<td>DMPC:DOPE:DC-chol (2:1:0.5)</td>
<td>55 ± 6.7</td>
<td>Fusogenic$^1$</td>
</tr>
<tr>
<td>DMPC:DOPE:DOTAP (2:1:0.5)</td>
<td>49 ± 5.0</td>
<td>Fusogenic$^1$</td>
</tr>
</tbody>
</table>

$^1$The difference between those liposome systems is the charge:molecular weight ratio, which is 1:504 and 1:732 for the DC-chol and DOTAP, respectively.
DISCUSSION

We have investigated the binding, permeability and diffusive distribution of liposome systems of various characteristics into multicellular spheroids used as in vitro models of avascular tumor bodies. Previously reported work, relevant to this study, involved the evaluation of the antiproliferative effects that free or liposome-encapsulated retinoic acid had on squamous carcinoma monolayers and spheroids, and free or liposome-TNF-α on glioma (A172) multicellular spheroids. Both of these studies reported effective delivery of the therapeutic molecules using liposomes compared to free drug, but did not investigate or attempt to correlate liposome characteristics with the interaction patterns and delivery into the tumor spheroid body. More recently, a study compared the improvement in doxorubicin diffusion within tumor spheroids between the free drug and the encapsulated drug in micelles and liposomes. Strategies like that to improve the homogeneous distribution of drugs in tumor volumes by appropriately designed delivery systems can help overcome the barriers posed by the tumor physiology (such as interstitial pressure and pH and pO2 levels).

From the different types of liposomes tested here, only the ones with neutral and positive surface charge were able passively to target and diffuse within the spheroids in an efficient way. Sterically stabilized liposomes were not able to interact with the tumor cell cluster due to the PEG polymer coat on their surface (Fig. 1d) acting as a repulsive barrier against any attractive force with the cell surface. This result suggests that liposome vesicles surface-coated with large groups (polymeric, sugar, or other) essential to attain long-circulating properties will exhibit a pronounced limitation to travel intratumorally after extravasation from the tumor microcapillaries. This incapability of intratumoral transport has indeed been suggested previously in studies looking at the localization of stealth liposomes within the tumors in vivo. Such observations, in combination with our findings, suggest that improvement of the therapeutic index of drugs (e.g., liposome-encapsulated anthracyclines) can be achieved by delivery systems that once at the tumor interstitium lose their steric or stealth components, e.g., by detachment or stimulated cleavage, become able actively to transport or diffuse more efficiently within the tumor.

FIGURE 1 – The effect of different liposome types. CLSM images of spheroids (fluorescent and differential interference contrast images of the same field in each case) following interaction with small unilamellar vesicles composed of (a) DMPC:chol (washed); (b) DMPC:DC-chol (washed); (c) DPPC:chol (washed); (d) DMPC:chol:DOPE-PEG (unwashed). Spheroids and liposomes in all shown systems were coincubated for 2 hr at 37°C.

FIGURE 2 – The effect of liposome surface charge. Comparative fluorescence intensity (arbitrary units) profiles relative to distance from the spheroid rim to the center (0 μm is the edge of the spheroid rim) for the DMPC:chol and DMPC:DC-chol SUVs.
Contrary to the well-documented time-dependent interaction between monoclonal antibodies and tumor spheroids toward developing actively targeted modalities against tumors, liposomes seemed to reach an end state of interaction following rapid kinetics within the first 2 hr. Moreover, after 5 hr of interaction, minimal changes in both diffusion and binding were found throughout this study. However, as in the case of antibodies, the time necessary for maximum uptake of liposomes, or other delivery systems, within a tumor mass in vivo will normally be longer than within multicellular spheroids due to the different composition of the extracellular matrix and the interstitial fluid pressure, both reported to act as barriers to the delivery of therapeutic agents. Such differences in the kinetic constants between actively or passively targeted carriers and spheroids or in vivo tumor xenografts highlight the caution in extrapolating clinically relevant information from such studies.

Another apparent finding from this study is the pronounced ability of all cationic liposomes to interact with the spheroids (Figs. 4–7). MLV cationic liposomes exhibited enhanced binding for the multicellular spheroids, strongly binding and aggregating on the cellular surfaces. Cationic MLV systems despite their heavy binding, as expected from their large size dimension (0.8–1 μm in mean diameter, measured by quasielastic light scattering; not shown), lacked the ability to diffuse through the interstitial space of the spheroid, limiting their intratumoral penetration to the outer cell layers. Substantial (at least by a factor of 2) improvement in the diffusive capabilities of cationic liposomes was obtained when...

**Figure 3** – The effect of duration of interaction. CLSM images of DMPC:cholester (a) 2 and (b) 5 hr. (c) Their comparative fluorescence intensity profiles from the spheroid rim to the center.
lipid bilayers were engineered to include the fusogenic DOPE lipid. Moreover, the retention and diffusion of cationic liposomes within the spheroids also depended on the molecular characteristics of the cationic lipid used to engineer the delivery vehicles. Optimization of the interaction was obtained when the cationic lipid DOTAP with a charge:molecular weight (1:732) ratio was used, instead of the cationic lipid DC-chol (1:504) with a higher molecular charge density. Inclusion of DOTAP improved the overall affinity for the spheroids by a factor of 2 (i.e., 100%) compared to the DC-chol containing lipid bilayers, and the diffusion by almost a factor of 10 (Fig. 7), delivering fluorescence up to the spheroid core. The cationic liposomes containing DOTAP and the neutrally charged fusogenic DOPE lipid proved the most effective of all cationic vesicles formed and studied, both in terms of the targeting (binding) and diffusion (penetration) within the tumor spheroids.

Cationic liposomes have systematically been studied only in the last 15 years, principally due to their universal affinity for cell surfaces, an outcome of the electrostatic attractive force between their surface and the plasma membrane. Their biologic and medical significance is getting established, as more types of cationic liposomes are engineered to deliver genetic material (DNA, RNA, oligonucleotides, artificial chromosomes) intracellularly. In fact, the most popular commercial transfection agents and the majority of the nonviral gene therapy vectors in clinical trials today include DC-chol, DOPE, DOTAP, or their mixtures. Even though highly surface-charged cationic liposomes exhibit much shorter blood circulation half-lives than other more pharmacokinetically robust liposome systems when administered intravenously, there are clinical protocols being developed for their use in tumor treatments. Particularly in terms of targeted cancer therapeutics, cationic liposomes have been shown to be internalized specifically by tumor endothelial cells and efficiently target the tumor neovasculature compared to anionic and neutral liposomes, rendering them attractive delivery systems for antiangiogenic agents. In view of such applications, the present study suggests that use of cationic lipids with high molecular charge density to form liposomes can lead to strong binding with the cellular membranes. However, when delivery within more complex 3-dimensional structures (such as tumors or other tissues) is required, cationic liposomes should be engineered using molecules of lower charge density and enhanced fusogenic characteristics.

An important parameter of the interaction between tumor cell clusters and delivery vehicles is the extent of the binding-site barrier effect, detrimental to effective distribution within the tumor volume. The binding-site barrier effect has been reported to become an increasingly significant factor in active (specific) targeting of tumors using monoclonal antibodies (MoAbs), particularly as the affinity of the targeting moieties for the cellular receptors increases as the number of receptors increases. The importance of such an effect in the case of cationic liposomes interacting with the tumor spheroids has become evident in this study, as the strong electrostatic force between the vesicle and the cell surface saturates and congests the outer cell layers of the tumor spheroids (Figs. 4 and 5). In the systems that this effect was evident (namely, all DC-chol containing cationic liposomes), limited intratumoral distribution characteristics were observed. Passive targeting strategies, therefore, may be particularly susceptible to any physical (e.g., electrostatic) binding-site barrier effects, as they utilize universal nonspecific interactions that may easily lead to unselective uneven distribution of the therapeutic agent within the target tissue.

The analysis presented in Table II illustrates the following points. One, positive charge at the liposome surface offered markedly effective binding and overall affinity for cell surfaces, an outcome of the electrostatic attractive force between their surface and the plasma membrane. Their biologic and medical significance is getting established, as more types of cationic liposomes are engineered to deliver genetic material (DNA, RNA, oligonucleotides, artificial chromosomes) intracellularly. In fact, the most popular commercial transfection agents and the majority of the nonviral gene therapy vectors in clinical trials...
geneous distribution throughout the avascular cell mass, is a much more intricate process requiring small particle size and a balance between liposome surface charge and bilayer fluidity. It is interesting to note that only 2 SUV types of different surface and bilayer properties (neutral–DMPC: chol- and cationic/fusogenic-DMPC:DOPE:DOTAP) yielded notable fluorescence toward the spheroid core. This is particularly relevant to the efficacy of liposome-encapsulated drugs and suggests one possible mechanism for the low therapeutic indices that have been achieved. In the case of sterically stabilized liposomes that passively accumulate in solid vascularized tumors \textit{in vivo}, their reported heterogeneous distribution close to perivascular regions and limited movement across the tumor interstitium and mass\textsuperscript{19,20,60} limit their therapeutic potential. Recent reports have indicated that improvements in the intratumoral distribution can be achieved in the case of conjugating monoclonal antibody fragments targeting against the herceptin (anti-HER2) receptor, leading to improved therapeutic effects.\textsuperscript{61} The interaction of such immunoliposomes with a variety of avascular tumor spheroids will be extremely interesting toward evaluation of their capability to treat metastatic lesions and systematically draw liposome structure (spheroid binding, affinity and diffusion functions) similarly to the present study. Moreover, such work is also of value in the eventual use of liposomes as delivery vehicles for therapeutic radionuclides.\textsuperscript{62,63}

This is the first systematic study on the ability of liposomal delivery systems to passively target and diffuse within multicellular tumor spheroids. Taking into account the inherent limitations and restrictions of the tumor spheroid model compared to an \textit{in vivo} or clinical situation, as well as the limitations of the present investigations posed by use of spheroids from a single (LNCaP-LN3) cell line, valuable insight can be obtained on the interaction of delivery systems with 3-dimensional avascular tumor tissue.
TABLE II – QUANTITATIVE EXPRESSION OF BINDING ONTO THE SPHEROIDS, OVERALL AFFINITY FOR THE SPHEROIDS AND THE EXTENT OF LIPOSOME DIFFUSION WITHIN THE TUMOR SPHEROIDS FOLLOWING IMAGE ANALYSIS

<table>
<thead>
<tr>
<th>Liposome system</th>
<th>Binding (%)</th>
<th>Affinity (a.u.)</th>
<th>Diffusion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLV (DMPC:DC-chol)</td>
<td>73.3</td>
<td>7.27</td>
<td>12.3</td>
</tr>
<tr>
<td>MLV (DMPC:DOPE-DC-chol)</td>
<td>60.0</td>
<td>18.3</td>
<td>23.3</td>
</tr>
<tr>
<td>MLV (DMPC:DOPE:DOTAP)</td>
<td>50.2</td>
<td>48.0</td>
<td>29.4</td>
</tr>
<tr>
<td>SUV (DMPC:chol)</td>
<td>38.5</td>
<td>7.3</td>
<td>71.0</td>
</tr>
<tr>
<td>SUV (DMPC:DC-chol)</td>
<td>84.7</td>
<td>3.1</td>
<td>3.4</td>
</tr>
<tr>
<td>SUV (DMPC:DOPE:DC-chol)</td>
<td>76.5</td>
<td>10.3</td>
<td>4.4</td>
</tr>
<tr>
<td>SUV (DMPC:DOPE:DOTAP)</td>
<td>35.6</td>
<td>31.9</td>
<td>62.0</td>
</tr>
</tbody>
</table>

1Fluorescence intensity up to 20 µm deep within the spheroid as a percentage of the total fluorescence intensity signal. 2Total fluorescence intensity signal in arbitrary units. – Fluorescence intensity at a particular depth as a percentage of the peak fluorescence intensity.

More work is required employing spheroids from various tumor cell lines to determine the generality of the liposome-spheroid interactions observed here. This is essential towards rational engineering of delivery systems and optimization in the design of in vivo experimentation and clinical assessment of any cancer therapeutics, as has been shown towards angiogenesis and neutrophil chemotaxis observed here. This is essential towards rational engineering of delivery systems and optimization in the design of in vivo experimentation and clinical assessment of any cancer therapeutics.

REFERENCES


