International Journal of Pharmaceutics xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Doxorubicin-loaded lipid-quantum dot hybrids: Surface topography and release properties

Bowen Tian [a], Wafa' T. Al-Jamal [a], Khuloud T. Al-Jamal [a,b], Kostas Kostarelos [a,*]

[a] Nanomedicine Lab, Centre for Drug Delivery Research, The School of Pharmacy, University of London, London WC1N 1AX, United Kingdom
[b] Drug Delivery Group, Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, London SE1 9NH, United Kingdom

ARTICLE INFO

Article history:
Received 1 December 2010
Received in revised form 22 January 2011
Accepted 25 January 2011
Available online xxx

Keywords:
Nanomedicine
Biological imaging
Theranostic
Multimodality
Nanotechnology

ABSTRACT

A few studies have attempted to combine the physicochemical versatility offered by the liposome structure with the superior optical characteristics of quantum dots (QD) for the construction of multifunctional nanoparticles. We are reporting the construction of drug-loaded liposome-QD hybrid vesicles (L-QD) by incorporating TOPO-capped, CdSe/ZnS QD into the two types of lipid bilayers: the 'rigid' disteroylphosphatidylcholine (DSPC:Chol:DSPE-PEG_{2000}) and a fluid-phase bilayer of egg PC (EPC:Chol:DSPE-PEG_{2000}). Structural characterization of L-QD hybrid vesicles using atomic force microscopy (AFM) revealed that the incorporation of QD took place by hydrophobic self-association within the membranes. The encapsulation of hydrophilic small molecules in the internal aqueous phase of the L-QD hybrids showed different degrees of carboxyfluorescein (CF) release in buffer and serum, depending on the type of lipid used. The presence of QD in the lipid bilayer increased the CF release from EPC fluid bilayer. On the other hand, (DSPC) L-QD hybrids showed a higher stability under the same conditions with minimal CF leakage. Furthermore, (DSPC) L-QD hybrids showed a stable mean diameter up to three weeks stored at 4 °C, 25 °C, and 40 °C, determined by photo correlation spectroscopy (PCS) analysis. Finally, doxorubicin (Dox) was loaded into L-QD hybrids using the osmotic gradient technique and with at least 97% loading efficiency. The fluorescence spectrum of Dox was simultaneously detected with that of green-emitting QD that indicated the coexistence of QD and Dox in a single vesicle system. In conclusion, the drug-loaded L-QD-Dox hybrid vesicles presented here constitute a promising multifunctional delivery vector capable of transporting combinations of therapeutic and diagnostic modalities.

1. Introduction

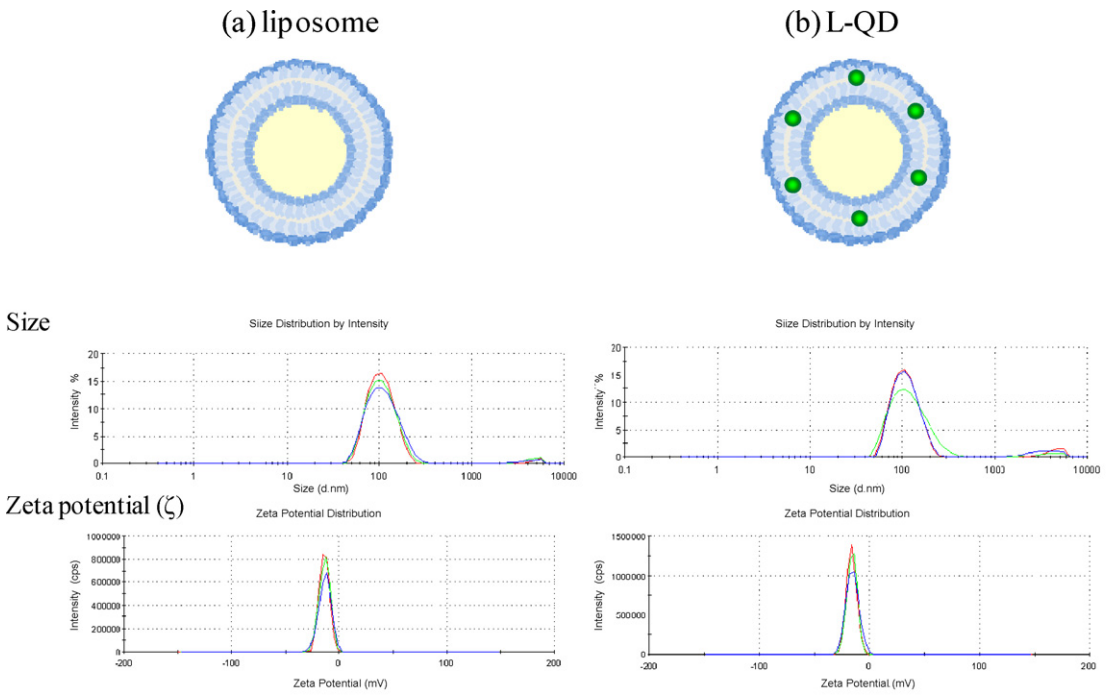
QD have been extensively investigated as optical probes for a variety of biomedical applications *in vitro* and *in vivo* (Alivisatos et al., 2005; Alivisatos, 2004; Gao et al., 2004; Kim et al., 2004; Rhyner et al., 2006; Smith et al., 2008; Wu et al., 2003). However, the low hydrophilicity of QD is considered a major obstacle that impedes their widespread use in biology. Many strategies have been proposed to improve the QD hydrophilicity and biocompatibility, including exchanging the surface ligands with hydrophilic moieties (Bruchez et al., 1998; Chan and Nie, 1998; Gerion et al., 2001), encapsulation within amphiphilic copolymers (Gao et al., 2004; Larson et al., 2003; Wu et al., 2003) and phospholipid micelles (Dubertret et al., 2002). Our group previously presented an alternative strategy based on the self-assembly of lipids and hydrophobic (non-functionalised) quantum dots (L-QD) into hybrid bilayers, leading to the formation of nanometer scaled hybrid vesicles (Al-Jamal et al., 2008). The embedding of QD into the bilayers rendered hydrophobic QD biocompatible and efficiently labeled tumor cells *in vitro* and *in vivo*. These findings have been also confirmed by others (Gopalakrishnan et al., 2006). In the present work, we offer further systematic characterization of the L-QD hybrid vesicles, such as surface topography and colloidal stability of the L-QD hybrids, and developed these delivery systems a step further by loading their internal aqueous phase with doxorubicin molecules. In this way, explore the potential use of L-QD hybrids as theranostic devices for the simultaneous delivery of therapeutic and diagnostic agents.

2. Results and discussion

L-QD hybrids were prepared following the thin lipid film hydration protocol as previously described (Al-Jamal et al., 2008). Briefly, 1.68×10^{15} p/ml CdSe/ZnS QD (green emitting (520 nm) Evidot®, Evident Technologies, USA) were mixed with 8 μmol of DSPC:Chol:DSPE-PEG_{2000} (1.8:1:0.2) phospholipid molecules in chloroform, and the organic solvent was evaporated using the rotovaporator (BUCHI, Switzerland). (DSPC) L-QD hybrid vesicles

* Corresponding author. Tel.: +44 207 753 5861; fax: +44 207 753 5942.
E-mail address: kostas.kostarelos@pharmacy.ac.uk (K. Kostarelos).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.01.057



Nanoparticle Type	Mean diameter (nm)[a]	Polydispersity index[a]	Surface charge (mV)[a]
DSPC liposome	103±1.87	0.214±0.012	-13.5±0.42
L-QD [DSPC]	123±8.50	0.259±0.016	-15.8±1.76
EPC liposome	119±1.12	0.208±0.011	-8.9±0.85
L-QD [EPC]	132±6.21	0.277±0.03	-10.6±2.01

[a]Mean ± standard deviation; n = 3. Mean diameter is the hydrodynamic diameter; polydispersity index (PI) indicates size distribution of the sample population.; Zeta potential (ζ) indicates average surface charge.

Fig. 1. Size and surface charge characteristics of (DSPC) L-QD hybrid vesicles. The mean average diameter (nm), polydispersity index and surface charge of (DSPC) L-QD hybrids, DSPC:Chol:DSPE-PEG (1.8:1:0.2 molar ratio) as obtained using the Nanosizer ZS.

were formed by hydrating the lipid film in 1 ml of dH_2O and bath sonication for 10 min at 60 °C (ultrasonic cleaner, VWR, UK). The L-QD hybrid vesicles exhibited 100 nm average diameter and negative surface charge (−15 mV) (Fig. 1b), similar to liposome alone (Fig. 1a).

The structure of L-QD hybrids has been previously elucidated by our laboratory and others using cryo-EM (Al-Jamal et al., 2008; Gopalakrishnan et al., 2006). In the present study we hypothesized that if the QD were incorporated within the lipid bilayer of liposomes, they could be detected on the surface of dehydrated L-QD vesicles using atomic force microscopy (AFM), as has been done for the elucidation of the surface topography of other nanoparticles (Spyratou et al., 2009). A droplet of L-QD sample was deposited on freshly cleaved mica surface (Agar Scientific, Essex, UK) for 30 s and then washed with dH_2O, followed by drying with a nitrogen stream. (DSPC) L-QD hybrids were structurally elucidated in air, using tapping-mode AFM in order to avoid sample damage (see Supplementary information). Fig. 2 depicts the structural elucidation of L-QD hybrid vesicles by AFM. Interestingly, the amplitude image showed that the incorporation of QD into DSPC:Chol:DSPE-PEG_{2000} lipid bilayers resulted in a rough surface. This was in sharp contrast to the liposome control which showed a smooth surface. 3D image analysis indicated that QD associated with the lipid bilayers and distributed throughout the vesicle surface. Furthermore, the cross-section analysis suggested that QD incorporation increased the height of the liposome from 8 nm to almost 20 nm. All this was considered evidence that confirmed the previously reported cryo-EM data and indicated that QD were mainly incorporated throughout the lipid bilayers of the vesicle population by hydrophobic self-association within the lipid molecules. However, we cannot exclude the possibility of QD aggregation in the bilayer as different heights were observed in the case of L-QD hybrids.

To further investigate the effect of QD on the membrane integrity of the hybrid vesicles, QD were incorporated into two types of lipid bilayers, disteroyl phosphatidylcholine (DSPC:Chol:DSPE-PEG_{2000}) and egg PC (EPC:Chol:DSPE-PEG_{2000}). The hydrophilic, membrane-impermeable carboxyfluorescein (CF) dye was encapsulated in these two types of L-QD vesicles, and CF release was monitored from L-QD in either HBS buffer, pH 7.4 or 50% serum at room temperature for 6 h. Fig. 3 depicts the cumulative release (%) of CF from the L-QD hybrid vesicles. The

Please cite this article in press as: Tian, B., et al., Doxorubicin-loaded lipid-quantum dot hybrids: Surface topography and release properties. Int. J. Pharm. (2011), doi:10.1016/j.ijpharm.2011.01.057

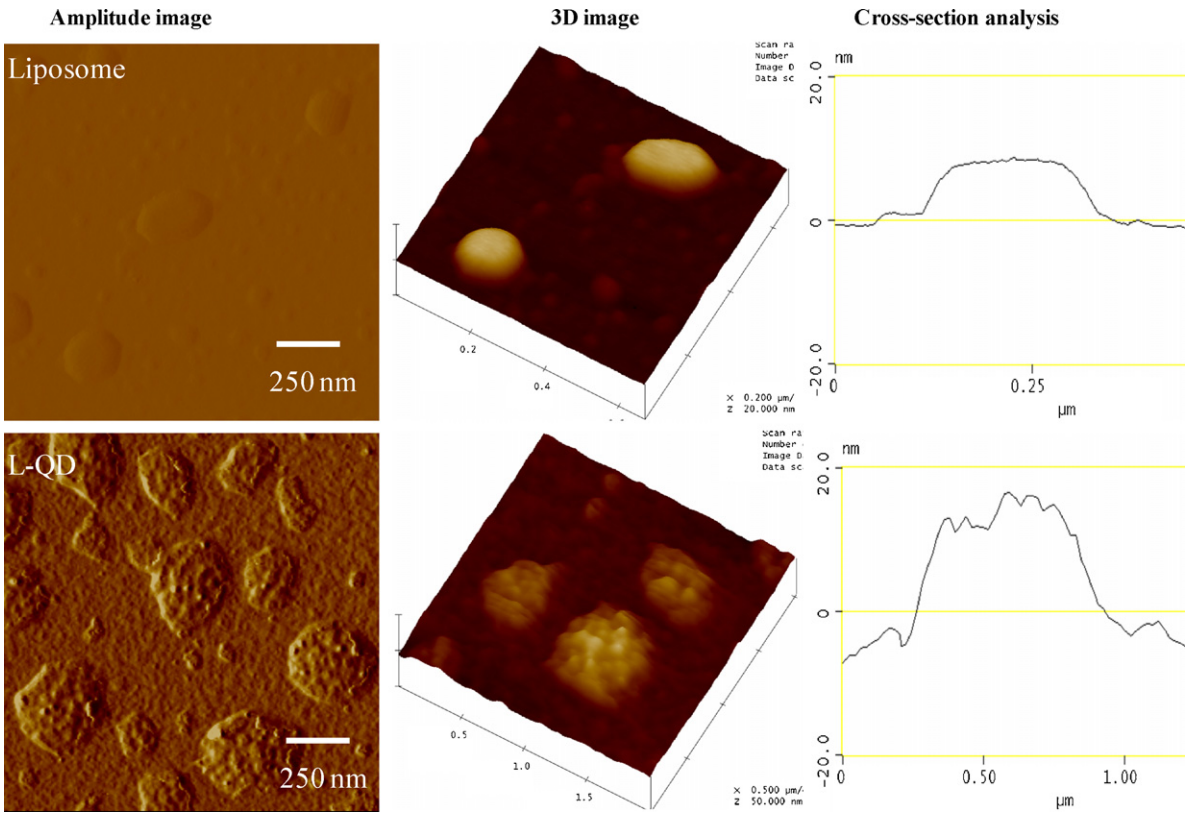


Fig. 2. Surface characterization of (DSPC) L-QD hybrid vesicles by atomic force microscopy. Amplitude image, 3D image, and cross-section analysis (left to right) of (a) liposome alone and (b) L-QD hybrids. Scale bars are 250 nm.

results show that QD incorporation destabilized EPC lipid membrane, as evidenced by the dramatic CF release from (EPC) L-QD (52%) compared to EPC liposome (7%) (Fig. 3a, squares and diamonds, respectively) in HBS buffer over 6 h. In agreement with CF release in HBS buffer, CF release in 50% serum was found to be constantly faster from (EPC) L-QD than EPC liposomes (Fig. 3b, squares and diamonds, respectively). The presence of serum, which has been reported to destabilize fluid lipid bilayers (Ravily et al., 1996) was found to further accelerate CF release from both (EPC) L-QD hybrids and EPC liposomes in 50% serum compared to those in HBS buffer, especially at the initial period of time (Fig. 3, squares and diamonds, respectively). As CF is membrane impermeable at neutral pH, these results indicated that although QD can be incorporated into EPC liposomes by self-assembly with the lipid molecules, this embedding can lead to disruption in the packing of the lipid bilayer in the case of fluid phase membranes. In comparison, QD incorporated into DSPC bilayers demonstrated no CF release, similar to the liposome control (Fig. 3, triangle and axis, respectively) in both HBS buffer and 50% serum. L-QD hybrids constructed from (DSPC) bilayers, in contrast to (EPC), can maintain lipid membrane tight packing, high stability and encapsulation of hydrophilic small molecule cargos in their inner aqueous core. Further biophysical studies are warranted to explore in more detail the molecular interactions between hydrophobic nanoparticles, such as QD, and lipids self-assembled into hybrid lipid bilayers.

The stable CF encapsulation observed from the (DSPC) L-QD encouraged us to further investigate the colloidal stability of the hybrid over a long period of time at different temperatures (4°C, 25°C and 40°C) in HBS buffer, pH 7.4. In Table 1, (DSPC) L-QD hybrids demonstrated pronounced colloidal stability and no mean vesicle diameter increase at both 4°C and 25°C over three weeks. A minor increase in mean diameter was observed after three weeks at 40°C (from 125 nm to 135 nm in diameter). Overall, this study indicated that (DSPC) L-QD hybrid vesicles were stable and no aggregation was observed, presumably due to effective steric stabilization offered by the polyethylene glycol chains on the liposome surface (Torchilin, 2005).

Following the observation that the L-QD hybrid vesicles were stable in serum, (DSPC) L-QD hybrids were loaded with the anticancer drug doxorubicin (Dox) using the osmotic gradient technique as previously described (Haran et al., 1993; Ishida et al., 2001; Lasic et al., 1995; Li et al., 1998; Mayer et al., 1990). Free Dox was removed after loading using a desalting column, and Dox encapsualtion efficiency was quantified by measuring UV absorbance at 480 nm following lysis of liposomes with 1% Triton, pH 7.4. Dox was successfully encapsulated into L-QD hybrid vesicles with a loading efficiency up to 97%, similar to liposomes alone (data not shown). The fluorescence characteristics of Dox-loaded L-QD hybrid (L-QD-Dox) were investigated using fluorescence spectrophotometry (Perkin Elmer Luminescence Spectrometer LS 50B). Fig. 4a depicts the emission spectra of L-QD hybrids at 350 nm excitation wavelength. The spectral features of green-emitting L-QD were not dramatically affected compared to that obtained for QD in toluene, except the few nanometer shift in the emission peak as previously reported (Al-Jamal et al., 2008), whereas green QD

Table 1
Mean average diameter and polydispersity index of (DSPC) L-QD hybrids suspensions stored at 40 °C, 25 °C and 4 °C in HBS buffer, pH 7.4.

	4 °C		25 °C		40 °C	
	Size (nm)	PI	Size (nm)	PI	Size (nm)	PI
Week 1	122	0.151	124	0.167	125	0.161
Week 2	120	0.130	125	0.132	128	0.177
Week 3	119	0.123	129	0.206	135	0.217

Please cite this article in press as: Tian, B., et al., Doxorubicin-loaded lipid-quantum dot hybrids: Surface topography and release properties. Int. J. Pharm. (2011), doi:10.1016/j.ijpharm.2011.01.057

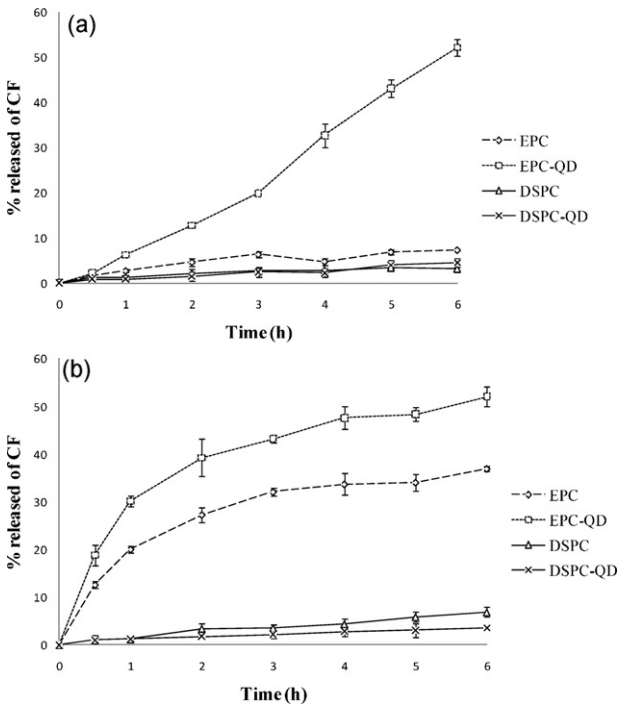


Fig. 3. CF release from L-QD hybrid vesicles. CF release from (DSPC) and (EPC) L-QD hybrids and corresponding empty liposomes was monitored in (a) HEPEs buffer and (b) 50% serum by dialysis tubing assay. L-QD hybrids were prepared at 8 mM lipid with 1.68×10^{15} particles/ml of QD.

in dH_2O emitted almost no fluorescence. The fluorescence spectra of doxorubicin-loaded L-QD hybrids (L-QD-Dox) showed that both Dox and QD could be simultaneously detected (Fig. 4b, green line), indicating the coexistence of both QD and Dox in the hybrid vesicle population. This was the first evidence we obtained that the L-QD hybrid vesicles could be further explored as theranostic systems with a capability to carry both imaging (QD) and therapeutic (Dox) agents in their lipid bilayer and internal aqueous phase simultaneously.

Previously, Bagalkot et al. (2007) reported quantum dot–aptamer–Dox conjugates as a first attempt to construct a targeted imaging and therapy system against cancer. However, in that system Dox fluorescence was quenched due to the conjugation onto PSMA aptamers. Only upon release of Dox from the QD surface was its fluorescence restored. In another attempt closer to the design currently described in this study, Weng et al. have covalently conjugated hydrophilic, polymer-functionalised QD at the outer tips of the polyethylene glycol chains coating the surface of liposomes and then loaded Dox into the aqueous core of these vesicles (Weng et al., 2008). Although such construct exhibited improved prolonged blood circulation half-life and therapeutic efficacy compared to QD-Dox alone, conjugation of such polymer-coated QD to the liposome surface also adversely increased liposome size (from 112 nm to 212 nm) and greatly lowered Dox loading efficiency (30%). In comparison to such previous attempts to design drug-loaded liposome-QD conjugates, we have demonstrated in this study that incorporation of hydrophobic QD into the lipid bilayer of liposomes maintains the mean vesicle diameter consistently between the desirable 100–120 nm range (Fig. 1), allows loading efficiencies of Dox using the established osmotic gradient technique, above 95%, and has minimal effects on the Dox fluorescence characteristics (Fig. 4). The improved Dox loading efficiency achieved for this L-QD-Dox hybrid vesicle system may allow opportunities for greater therapeutic efficacy. Further studies are needed to assess the cytotoxic activity and the pharmacokinetic profile of such Dox-loaded L-QD hybrids. Also, such vesicle systems can be further modified with different targeting ligands on the liposome surface to offer binding specificity and target cancer cell receptors.

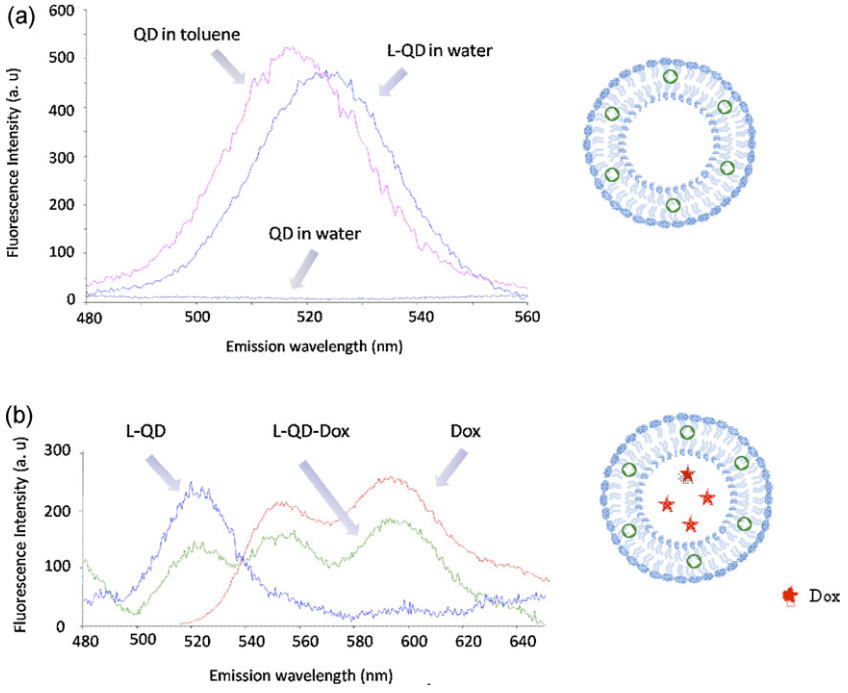


Fig. 4. Fluorescence characterization of (DSPC) L-QD hybrid vesicles. L-QD were prepared at 8 mM lipid DSPC:Chol:DSPE-PEG (1.8:1:0.2 molar ratio) with 1.68×10^{15} particles/ml of QD in 1 ml dH_2O. The fluorescence spectra of (DSPC) L-QD hybrids (a) before and (b) after Dox loading were analyzed by spectrofluorometry. QD and L-QD hybrids were excited at the wavelengths of 350 nm, Dox at 480 nm and L-QD-Dox at 450 nm.

Please cite this article in press as: Tian, B., et al., Doxorubicin-loaded lipid-quantum dot hybrids: Surface topography and release properties. Int. J. Pharm. (2011), doi:10.1016/j.ijpharm.2011.01.057

3. Conclusion

Hybrid Dox-loaded, PEGylated L-QD vesicles were successfully engineered in an attempt to combine the physicochemical properties and flexibility offered by liposomes in order to carry QD (optical imaging) and Dox (therapeutic) for combinatory applications. This is the first attempt to design drug-loaded hybrid L-QD vesicles in the 100 nm diameter range with diagnostic and therapeutic capabilities.

Acknowledgements

This work was partially supported by The School of Pharmacy, University of London. The authors acknowledge Lipoid Co. (Germany) for the lipid sample gifts. B.T. is the recipient of the Overseas Research Student Award Scheme (ORSAS) from the University of London.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.01.057.

References

Al-Jamal, W.T., Al-Jamal, K.T., Tian, B., Lacerda, L., Bornans, P.H., Frederik, P.M., Kostarelos, K., 2008. Lipid-quanturn dot bilayer vesicles enhance tumor cell uptake and retention in vitro and in vivo. Acs Nano 2, 408–418.
Alivisatos, A.P., Gu, W., Larabell, C., 2005. Quantum dots as cellular probes. Annu. Rev. Biomed. Eng. 7, 55–76.
Alivisatos, P., 2004. The use of nanocrystals in biological detection. Nat. Biotechnol. 22, 47–52.
Bagalkot, V., Zhang, L., Levy-Nissenbaum, E., Jon, S., Kantoff, P.W., Langer, R., Farokhzad, O.C., 2007. Quantum dot – aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on Bi-fluorescence resonance energy transfer. Nano Lett. 7, 3065–3070.
Bruchez Jr., M., Moronne, M., Gin, P., Weiss, S., Alivisatos, A.P., 1998. Semiconductor nanocrystals as fluorescent biological labels. Science 281, 2013–2016.
Chan, W.C.W., Nie, S.M., 1998. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281, 2016–2018.
Dubertret, B., Skourides, P., Norris, D.J., Noireaux, V., Brivanlou, A.H., Libchaber, A., 2002. In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science 298, 1759–1762.
Gao, X., Cui, Y., Levenson, R.M., Chung, L.W., Nie, S., 2004. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat. Biotechnol. 22, 969–976.
Gerion, D., Pinaud, F., Williams, S.C., Parak, W.J., Zanchet, D., Weiss, S., Alivisatos, A.P., 2001. Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. J. Phys. Chem. B 105, 8861–8871.
Gopalakrishnan, G., Danelon, C., Izewska, P., Prummer, M., Bolinger, P.Y., Geissbuhler, I., Demurtas, D., Dubochet, J., Vogel, H., 2006. Multifunctional lipid/quantum dot hybrid nanocontainers for controlled targeting of live cells. Angew. Chem. 45, 5478–5483.
Haran, G., Cohen, R., Bar, L.K., Barenholz, Y., 1993. Transmembrane ammonium-sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim. Biophys. Acta 1151, 201–215.
Ishida, T., Kirchmeier, M.J., Moase, E.H., Zalipsky, S., Allen, T.M., 2001. Targeted delivery and triggered release of liposomal doxorubicin enhances cytotoxicity against human B lymphoma cells. Biochim. Biophys. Acta 1515, 144–158.
Kim, S., Lim, Y.T., Soltesz, E.G., De Grand, A.M., Lee, J., Nakayama, A., Parker, J.A., Mihaljevic, T., Laurence, R.G., Dor, D.M., Cohn, L.H., Bawendi, M.G., Frangioni, J.V., 2004. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat. Biotechnol. 22, 93–97.
Larson, D.R., Zipfel, W.R., Williams, R.M., Clark, S.W., Bruchez, M.P., Wise, F.W., Webb, W.W., 2003. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science 300, 1434–1436.
Lasic, D.D., Ceh, B., Stuart, M.C., Guo, L., Frederik, P.M., Barenholz, Y., 1995. Transmembrane gradient driven phase transitions within vesicles: lessons for drug delivery. Biochim. Biophys. Acta. 1239, 145–156.
Li, X., Hirsh, D.J., Cabral-Lilly, D., Zirkel, A., Gruner, S.M., Janoff, A.S., Perkins, W.R., 1998. Doxorubicin physical state in solution and inside liposomes loaded via a pH gradient. Biochim. Biophys. Acta 1415, 23–40.
Mayer, L.D., Tai, L.C., Bally, M.B., Mitilenes, G.N., Ginsberg, R.S., Cullis, P.R., 1990. Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients. Biochim. Biophys. Acta. 1025, 143–151.
Ravily, V., Santaella, C., Vierling, P., 1996. Membrane permeability and stability in buffer and in human serum of fluorinated di-O-alkylglycerophosphocholine-based liposomes. Biochim. Biophys. Acta 1285, 79–90.
Rhyner, M.N., Smith, A.M., Gao, X., Mao, H., Yang, L., Nie, S., 2006. Quantum dots and multifunctional nanoparticles: new contrast agents for tumor imaging. Nanomedicine (Lond.) 1, 209–217.
Smith, A.M., Duan, H., Mohs, A.M., Nie, S., 2008. Bioconjugated quantum dots for in vivo molecular and cellular imaging. Adv. Drug Deliv. Rev. 60, 1226–1240.
Spyratou, E., Mourelatou, E.A., Makropoulou, M., Demetzos, C., 2009. Atomic force microscopy: a tool to study the structure, dynamics and stability of liposomal drug delivery systems. Expert Opin. Drug Deliv. 6, 305–317.
Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov. 4, 145–160.
Weng, K.C., Noble, C.O., Papahadjopoulos-Sternberg, B., Chen, F.F., Drummond, D.C., Kirpotin, D.B., Wang, D., Hom, Y.K., Hann, B., Park, J.W., 2008. Targeted tumor cell internalization and imaging of multifunctional quantum dot-conjugated immunoliposomes in vitro and in vivo. Nano. Lett. 8, 2851–2857.
Wu, X., Liu, H., Liu, J., Haley, K.N., Treadway, J.A., Larson, J.P., Ge, N., Peale, F., Bruchez, M.P., 2003. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat. Biotechnol. 21, 41–46.

Please cite this article in press as: Tian, B., et al., Doxorubicin-loaded lipid-quantum dot hybrids: Surface topography and release properties. Int. J. Pharm. (2011), doi:10.1016/j.ijpharm.2011.01.057