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Research Paper

Investigations on cellular interaction of polyelectrolyte based nano-walled reservoir using MCF-7 cell lines: a novel chemotherapeutic approach

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Abstract

Objectives A polyelectrolyte (PE) based nano-walled reservoir (NwR) was developed using alternate deposition of natural polyions on a decomposable core ($CaCO_3$). The system was charged with paclitaxel (PTX) using the trigger property of an organic solvent (NwR-PTX). In addition, the surface of the nano-walled reservoir was modified with PE-PEG2000 (NwR-PTX-PEG)) in order to investigate any changes in the interaction of surface-modified polyelectrolyte shells with breast cancer cells, since surface chemistry greatly influences the performance of microcapsules in the biological environment.

Methods The surface modification was confirmed by differential scanning calorimetry studies, which showed a shifting of the endothermic peak after pegylation. Layer-by-layer (LBL) growth of the system was confirmed by the sequential change in the ζ-potential. The release of paclitaxel from the formulations followed first order kinetics ($r^2 = 0.9$), indicating matrix diffusion. The interaction of NwR-PTX with MCF-7 cell lines was investigated by coating the system with FITC-dextran (NwR-PTX-FITC) and quantitated using flow cytometry.

Key findings Cellular uptake of positively charged NwR reached 56% after 4 h and 76% after 24 h. This was reduced significantly after pegylation. The negatively charged NwR reached only 49% after 24 h.

Conclusions This study opens the possibility of specific targeting of tumour cells that can control the release of chemotherapeutic agent either by means of a physiological or chemical trigger. This suggests potential application of this system as a novel approach for the delivery of chemotherapeutic agents.

Keywords cell–shell interaction; chemotherapy; layer-by-layer; nano-walled reservoir; surface modification

Introduction

Treatment of cancer mainly involves chemotherapy accompanied by surgical procedures used to reduce the tumour burden. In cancer chemotherapy, it is usually advised that, for maximal efficacy, normal tissue should be minimally affected while administering chemotherapeutic agents. Use of cytotoxic drugs, with their non-specific tissue distribution and toxicity on normal proliferating cells, results in life-threatening side effects and patient discomfort, restricting high dose strategies.[1] Paclitaxel (PTX) is the drug most commonly used to treat breast and ovarian cancers.[2] Development of a safe and efficacious formulation of paclitaxel has been hindered because of its low aqueous solubility[3] and non-specificity towards tumour cells. Furthermore, the marketed formulation, containing a cremophor EL/ethanol mixture, has been associated with serious adverse side effects such as bronchospasm and hypersensitivity and it has also been reported that ethanol and cremophor leach diethylhexylpthalate from the PVC infusion bags and administration sets.[4] Once diluted, it also tends to precipitate out of solution if stored and it therefore requires in-line filtration during administration to patients.[5]

Controlled and targeted delivery of PTX to tumour cells could reduce required dosages and also the possibility of life-threatening side effects. Attempts have been made to prepare safer formulations by incorporating the drug into lipid emulsions,[6] mixed micelles,[7,8]

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liposomes[9] and taxol–albumin conjugates,[10] and poly (γ-glutamic acid)-poly (lactide) nanoparticles,[11] but there is still tremendous scope to develop formulations that are safer and more selective than those on the market.

One novel approach in this regard is the fabrication of ultrathin nano- and micro-compartments using layer-by-layer (LBL) assembly to sequentially adsorb oppositely charged polyelectrolytes (PEs) onto removable inorganic core particles.[12,13] Considerable efforts have been dedicated to the study of surface modifications of microcapsules and the creation of multilayer coated colloids that will not be recognized by hosts as foreign materials or that can be targeted to specific sites or cells.

Our study was focused on the interactions of surface-modified polyelectrolyte shells and breast cancer cells, since surface properties, particularly surface charge and composition, greatly influence the performance of microcapsules in the biological environment. We assembled PTX-loaded nano-walled reservoirs (NwR) and assessed its effect on human MCF-7 breast cancer cells by means of an in-vitro cell uptake study. In addition, the reservoir surfaces were modified with PE-PEG2000 and the effects on cell interactions, biocompatibility and stability in the physiological environment were studied.

Materials and Methods

Materials

Paclitaxel (PTX, purity >99%) was obtained as a gift sample from Dabur Research Foundation (Ghaziabad, India). Sodium alginate (SA), protamine sulfate (PRM), pluronic F-68 and FITC-dextran sulfate (FITC-DEX) were purchased from Sigma (St Louis, MO, USA). PE-PEG2000 was generously provided as a gift sample from Lipoid, AG (Ludwigshafen, Germany). For cell cultures, modified Eagle medium (MEM) with glutamine, fetal bovine serum, antibiotic solution (penicillin/streptomycin, 0.1% v/v), ethylenediamine tetraacetic acid (EDTA) and trypsin were purchased from Sigma (St Louis, MO, USA). Well plates, for in-vitro cell uptake studies, were obtained from Greiner Bio-One (Frickenhausen, Germany). All materials were used without further purification. The water used in all experiments was prepared in a three-stage Millipore Milli-Q plus 185 purification system (Bradford, MA, USA) and had a resistivity greater than 18.2 mΩ/cm.

Preparation of the paclitaxel-loaded nano-walled reservoir

Alternate assembly of natural PEs over preformed porous calcium carbonate ($CaCO_3$ MP, 3–5 μm) was carried out according to a previously described method employing PF-68 with sodium carbonate at 2% w/v.[14,15] Preformed porous $CaCO_3$ MP was alternately coated with five bilayers of PRM and SA. Intact NwR was obtained by dissolving the core in 0.1 M HCl (pH 1.2) followed by centrifugation (18 000 rev/min, 5 min) and washing three times with triple distilled water (TDW).[16] PTX loading was carried out by incubating an ethanolic solution of PTX (1 mg/ml) in pelleted NwR for 2 h with constant stirring.[17] The NwR-PTX thus obtained was subjected to three repeated cycles of centrifugation at 18 000 rev/min for 5 min followed by washing and re-dispersion using TDW. The PTX encapsulation was back-calculated in the supernatant using the RP-HPLC method as reported by Jain *et al.*[18] with slight modification and correlated with their respective standard curves. To study cellular uptake, the developed NwR-PTX was alternately inter-layered with FITC-dextran (NwR-PTX-FITC) and quantitated using flow cytometry (Becton Dickinson, Oxford, UK).

Paclitaxel estimation

PTX concentration in the samples was measured by RP-HPLC as reported elsewhere by our group.[18] The HPLC system was equipped with 10 ATVP binary gradient pumps (Shimadzu, Japan), a Rheodyne (Cotati, CA, USA) model 7125 injector with a 20 μl loop and SPD-M10 AVP UV detector (Shimadzu). HPLC separation was achieved on a Lichrospher Lichrocart C_{18} column (250 mm, 4 mm, 5 μm) (Merck, Darmstadt, Germany). The mobile phase consisted of a mixture of acetonitrile and triple distilled water (55:45, v/v) and the flow rate was 1 ml/min. The injection volume was 20 μl and the detection was made at 227 nm. Data was acquired and processed using Class-VP (Shimadzu, Japan) software.

Surface modification of NwR-PTX with PE-PEG

Surface modification (NwR-PTX-PEG) was carried out by incubating the appropriate quantity of NwR-PTX with PE-PEG2000 (2 mg/ml) in chloroform for 2 h with constant stirring. The excess PE-PEG2000 was removed by centrifugation (18 000 rev/min, 5 min) and washed three times with TDW. The surface modification was confirmed by differential scanning calorimetry (DSC; Diamond DSC, Perkin Elmer, Degussa, Germany) and ζ-potential measurements using photon correlation spectroscopy (Malvern Nano ZS, Worcestershire, UK).

Characterization

The surface morphology of the prepared $CaCO_3$ MP with alternately layered NwR was examined by scanning electron microscopy (SEM). Samples were prepared by applying a drop of NwR suspension to a glass slide and then drying overnight. Then samples were sputtered with gold and measurements were taken using a Gemini Leo VP 435 instrument at an operation voltage of 3 KeV. Layer-by-layer growth was determined by measuring the ζ-potential of each adsorbing PE layer on $CaCO_3$ MP dispersed in milli Q water using a Zetasizer Nano ZS (UK). The ζ-potential was taken as the average value of three successive measurements. Surface modification of multilayered NwR was evaluated using differential scanning calorimetry to assess any changes in endothermic events of PE-PEG2000 and NwR-PTX-PEG. The endothermic events were monitored by heating the sample from 25 to 150°C at a rate of 10°C/min. The endothermic event was recorded during the first heating. An empty pan was used as a reference standard. Analysis was performed under a nitrogen purge; triple runs were carried out on each sample.

In-vitro drug release profile

The in-vitro drug release profile of entrapped PTX from the different formulations (NwR-PTX, NwR-PTX-PEG) was studied using an artificial dialysis bag (cut off mol. wt. 12 000 Dalton, Sigma). One millilitre of the reservoir suspension of each formulation was taken into a treated dialysis bag, which was tied securely at both the ends and placed in a beaker containing 199 ml of phosphate buffered saline (PBS) (pH 7.4). The beaker was placed over a magnetic stirrer at 100 rev/min and the temperature of the assembly was maintained at 37°C throughout the study. Samples (2 ml) were withdrawn at 0.5, 1, 2, 3, 6, 12, 24 and 48 h and replaced with the same volume of PBS (pH 7.4). The percentage of PTX released in the dissolution volume was determined considering the payload efficiency of PTX in the formulation (72 ± 3.13%). The amount of formulation was so adjusted that the PTX concentration in the buffer remained below the limit of saturation solubility even after complete release of PTX. The percentage of drug release at each sampling point was determined by RP-HPLC and statistically analysed using a Kruskal–Wallis test.

Cell uptake study and flow cytometry

Human breast cancer MCF-7 cells (passage no. 53–57) were maintained in 90% MEM-glutamine supplemented with 5% heat-inactivated fetal bovine serum and 0.1% antibiotic solution (penicillin/streptomycin), and the cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. To differentiate uptake of NwR by cell adhesion or phagocytosis, similar experiments were carried out at 4°C (data not shown). For experimentation, MCF-7 cells were seeded into 12-well plates at a density of 4×10^5 cells/well under these conditions for 1 day, before addition of FITC inter-layered nano-walled reservoirs, with and without surface modification. The cellular uptake of NwR-PTX was measured by flow cytometry, using a slight modification of the method reported by Ai *et al.*[19]. The FITC-tagged formulation (NwR-PTX-FITC and NwR-PTX-FITC-PEG) was suspended at a density of 2×10^7 NwR/ml (calculated using a haemocytometer) in 1 ml of culture medium and added to each well containing MCF-7 cells and incubated for 4 and 24 h. After the indicated time, the medium was removed and the cells were detached using EDTA and trypsin. Cell pellets were collected by centrifugation (500g, 5 min) and resuspended in PBS (pH 7.4). To remove NwR attached to the cell surface, the cells were washed following a previous protocol (5 mM EDTA, pH 5.0 for 15 min). The cell suspensions were analysed using a flow cytometer (FACS Caliber, Becton Dickinson, USA). The data are presented as the mean fluorescence obtained from a population of 10 000 cells.

Statistical analysis

All results are given as means ± SD ($n = 3$). The percentage drug release at each sampling point was analysed using a Kruskal-Wallis test. The effect of time on the cell uptake for each formulation has been analysed using the Mann–Whitney U test. Differences on the ζ-potential of each formulation and comparison of the percentage cell uptake between formulations, at 4 and 24 h was statistically analysed using the Kruskal–Wallis test. Individual differences between means were determined using Dunn's post-hoc test. $P < 0.05$ denotes significance in all cases.

Results

Preparation of paclitaxel-loaded nano-walled reservoirs

The colloidal core particles used are required to maintain their sphericity and monodispersity throughout the process. We therefore employed a method of co-precipitation to prepare spherical $CaCO_3$ core particles, as reported previously by our group.[15] Briefly, sodium carbonate with 2% w/v PF-68 was added to calcium chloride dihydrate solution, with continuous stirring, to achieve more than 80% spherical (vaterite polymorph) and monodisperse core particles. An intact polyelectrolyte assembly was prepared by electrostatic attraction over colloidal core particles followed by core removal to get intact NwR. This core removal step generates a system with wall thickness in the range of ~450 nm, as determined by differences in size distribution data of the bared core particles and the coated particles. The average size distributions of bare $CaCO_3$ and the coated particles was found to be 3558 nm and 4110 nm, respectively). After core removal the assembly attained a matrix-based architecture comprising PEs. Furthermore, the PTX was loaded in to this architecture using a solvent-induced chemical trigger. The payload efficiency of PTX in NwR was found to be 72 ± 3.13%.

Characterization

The developed system was characterized for surface morphology, LBL growth and surface modification. The SEM output shown in Figure 1 illustrates the surface texture of the porous $CaCO_3$ MP and the fabricated nano-walled reservoir. The developed core particles were perfectly spherical, non-aggregated and monodisperse in the size range of 3–5 μm. However in some formulations we observed clusters of PEs, which signifies interaction beyond charge neutralization.

As oppositely charged species are adsorbed onto the surface of the particles, it is expected that there will be a reversal in the measured ζ-potentials of the particles. LBL growth was therefore confirmed by successful recharging of the particle surface after each deposition cycle. Stepwise PE assembly onto these negatively charged core particles was monitored via ζ-potential reversal. The ζ-potential of NwRs alternated between –26.75 mV for SA and +10.06 mV for PRM, as shown in Figure 2 (layer 0; –22.4 mV).

Differential scanning calorimetry was performed to understand related thermal events related to LBL growth, i.e. melting, recrystallization, decomposition, out-gassing, or a change in heat capacity due to nature of the polymer material (PE-PEG 2000) that was used to modify the surface of LBL-based NwR. NwR-PTX-PEG showed a single endothermic event at 60°C (Figure 3, Curve B), whereas for PE-PEG2000 a sharp endothermic peak was obtained at 51°C, corresponding to its melting temperature (Figure 3, Curve A).

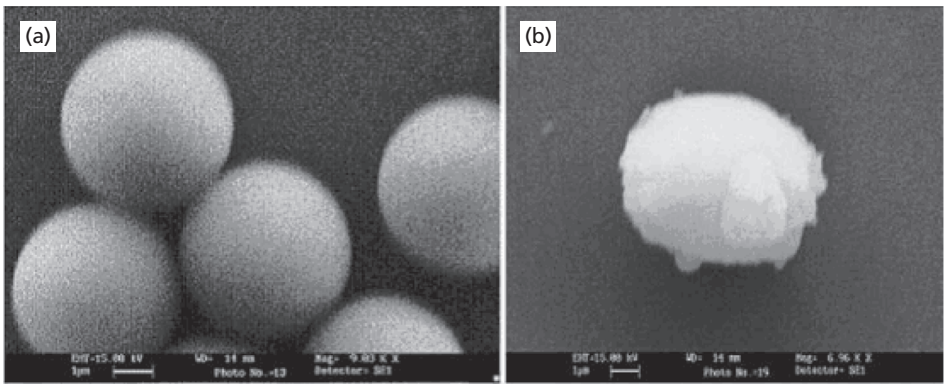

Figure 1 Scanning electron microscopy of nano-walled reservoirs. (a), porous $CaCO_3$ MP; (b), after five bilayers of polyelectrolytes adsorbed, showing rough surface. Scale bar:1 μm.

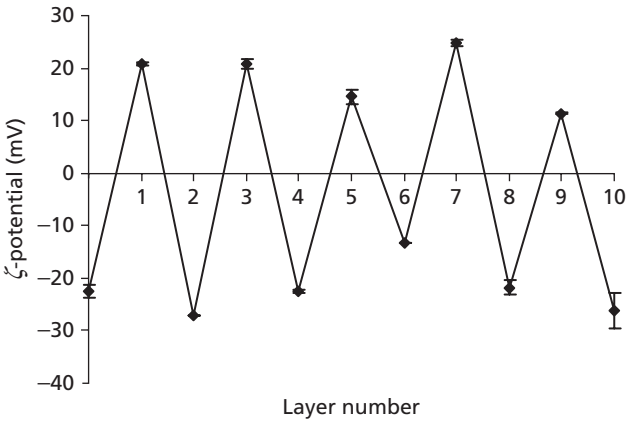

Figure 2 Layer-by-layer growth after each adsorbing of a polyelectrolyte layer shown as a function of ζ-potential. The error bar indicates ± SD of three sets of experiments.

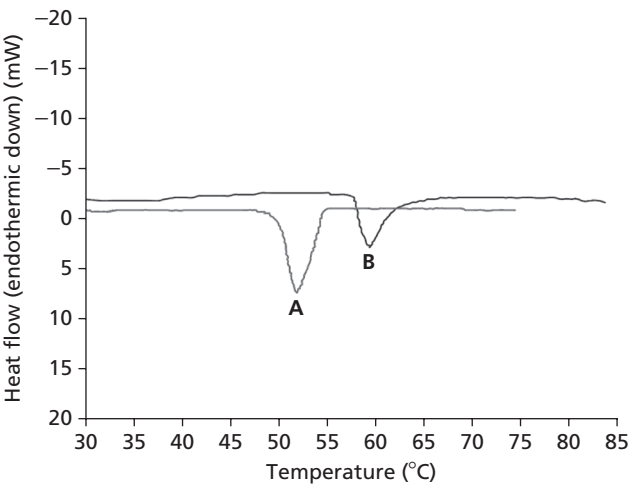

Figure 3 Transition temperatures shown by differential scanning calorimetry. A, PE-PEG2000; B, surface-modified NwR-PTX.

In-vitro drug release profile

In-vitro drug release of NwR-PTX formulations was studied using dialysis bags. After 48 h NwR-PTX and NwR-PTX-PEG released 76.0 ± 5.1% and 62.7 ± 3.2% of PTX, respectively, as shown in Figure 4. The release profile (pH 7.4) from both the formulations was best fitted by first order kinetics (r^2 = 0.9), indicating matrix diffusion kinetics.

Cell uptake study of the formulations in MCF-7 cell line and flow cytometry

To gain an insight into the behaviour of the NwR-PTX with reference to its interaction with MCF-7 cells, the nano-walled reservoirs were charged with a fluorescent probe and quantitated using flow cytometry. Figure 5 illustrates representative flow cytometry overlay for the uptake of FITC-dextran inter-layered nano-walled reservoirs (NwR-PTX-FITC and NwR-PTX-FITC-PEG) by the MCF-7 cell line after 24 h. The cellular uptake of positively charged NwR-PTX (ζ-potential +13.2 ± 4.3 mV) was found to peak (56.48 ± 3.12% and 76.35 ± 4.1%) after 4 and 24 h, respectively, as shown in Figure 6. On the other hand, the

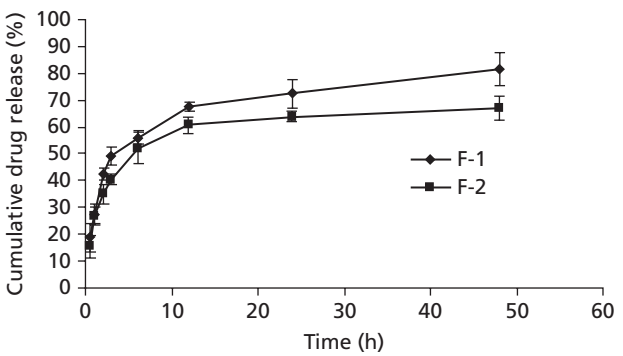

Figure 4 In-vitro release profiles of paclitaxel from NwR-PTX and NwR-PTX-PEG. F-1, NwR-PTX; F-2, NwR-PTX-PEG. Data shown for phosphate buffered saline (pH = 7.4). The error bars indicate ± SD of three sets of experiments (n = 3).

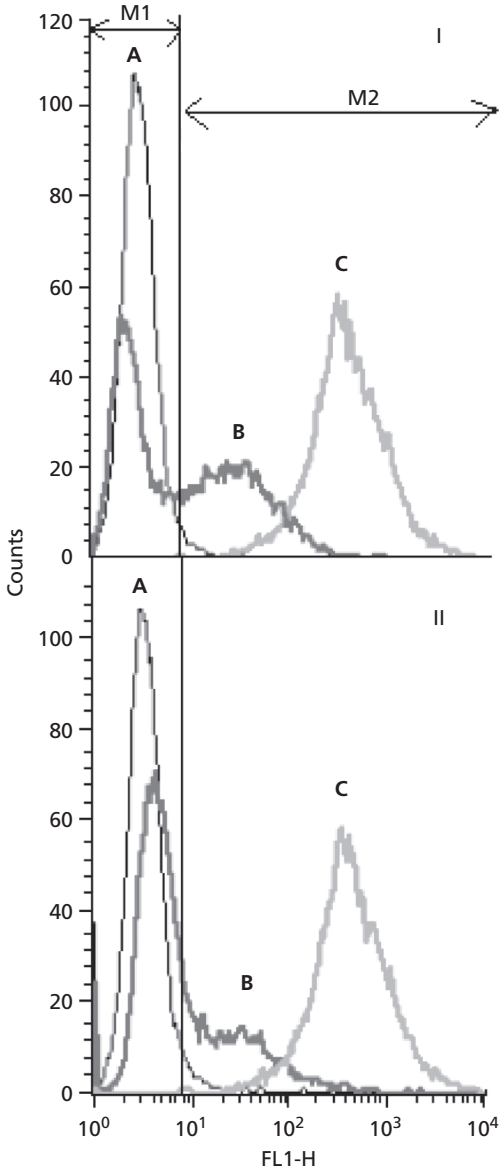

Figure 5 Flow cytometry for the uptake of FITC-dextran inter-layered nano-walled reservoirs. I and II: positively charged NwR-PTX-FITC and NwR-PTX-FITC-PEG, respectively. Data shown for MCF-7 cells after 24 h. M2 and M1 zones represent cells that have and have not taken up the NwR formulation, respectively. A, Control; B, interacted reservoir with MCF-7 cells; C, plain NwR-PTX-FITC.

uptake of negatively charged NwR-PTX (ζ-potential –25.8 ± 2.95 mV) was found to be 49.21 ± 2.4% after 24 h. After pegylation (NwR-PTX-FITC-PEG) the uptake was significantly reduced ($P < 0.05$) to about 22.94 ± 1.9% (2.5-fold) and 54.64 ± 4.5% (1.4-fold) after 4 and 24 h.

Discussion

Nano-walled reservoirs (NwR) fabricated by layer-by-layer assembly on porous colloidal templates followed by core removal has attracted significant attention as having potential application in drug delivery, especially in chemotherapeutic

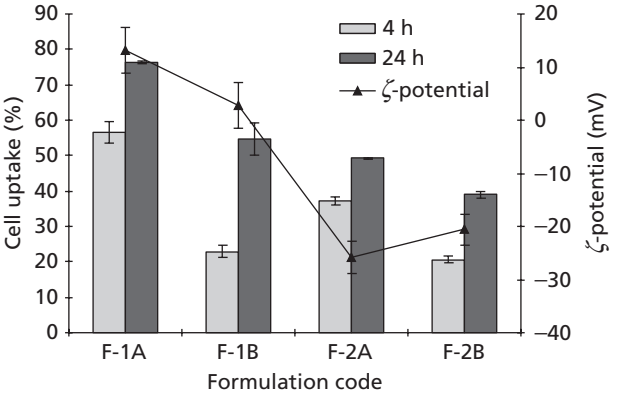

Figure 6 Cell uptake of different nano-walled reservoir formulations by the MCF-7 cell line. Data shown after 4 and 24 h. F-1A, NwR with positive charge; F-2A, NwR with negative charge; F-1B, NwR-PEG with positive charge; F-2B, NwR-PEG with negative charge. The error bars indicate ± SD of three sets of experiments ($n = 3$).

approaches.[19] The system is well known for being able to encapsulate and deliver different drug molecules and enzymes.[20,21] In this study we have investigated PTX-loaded nano-walled reservoirs (NwR-PTX) with different surface chemistries and their physiological consequences on interaction with MCF-7 cell lines.

Previously[15] we have demonstrated that $CaCO_3$ MP has a porous structure that is anticipated to provide deep penetration of PE to give a matrix-based architecture. The striated surface of the fabricated nano-walled reservoirs clearly indicates that a coating of PEs has formed over the smoother porous MP, as shown in Figure 1B.

Layer-by-layer deposition results in continuous growth of the PE film without inducing further aggregation. These observations demonstrate that the LBL technique can be successfully used to fabricate charged colloidal particles coated with thin PE layers. In our previous study (unpublished data) it was observed that there was a huge deviation in ζ-potential values when LBL assembly was carried out using PEs on a heterogeneous population of $CaCO_3$ core particles. This means that the system sometimes provides a dipolar surface due to formation of clusters. It has been reported that when a polyion interacts with a macro ion the complexation reaches beyond charge neutralization and co-ordination reaches its maximum in the region of charge inversion where large clusters are formed.[22] Using spherical and monodisperse $CaCO_3$ core particles the ζ-potential values are consistent and uniform according to the type of PE used.

DSC thermograms show a shifting of the endothermic peak after pegylation, indicating surface modification. The modification of the NwR surface with PE-PEG2000 would have possibly resulted from adsorption and/or hydrophobic interaction between phospholipids alkyl chains and the charged surface of the NwR, providing additional stability to the system and making it more biocompatible. The higher transition temperatures obtained for the surface modified NwR indicate increased inter- and intra-molecular interaction between PE-PEG-2000 and the surface of the NwR.

The dialysis membrane acts like a semi-permeable diffusion barrier that limits the passage of drug from the delivery system to surrounding media. The in-vitro drug release pattern obtained from both formulations displayed an initial burst release during the first 6 h, followed by sustained release up to 48 h, regardless of formulation type. The initial burst release shown by both the formulations may be due to leaching of the surface adsorbed drug, since we have used a post-loading method for PTX encapsulation. The release profile from both the formulations was fitted best by first order kinetics showing an r^2 value close to 0.9, indicating matrix diffusion kinetics (graphical data not shown). After 48 h NwR-PTX and NwR-PTX-PEG released 76.0 ± 5.1% ($P < 0.005$) and 62.7 ± 3.2% ($P < 0.005$) of PTX, respectively, as shown in Figure 4. The reduction in PTX release from the NwR-PTX-PEG formulation could be due to there being a steric barrier posed by PE-PEG 2000. The striking advantage of such a diffusion barrier is the control of the drug release rate and fluxes by simply tuning the number and type of PE layers.

The interaction of the NwR-PTX with MCF-7 cell lines was investigated by coating the system with FITC-dextran (NwR-FITC) and quantitated using flow cytometry. It is evident from this data that the uptake of particles is greatly influenced by particle surface chemistry. The uptake was found to be a time-dependent phenomenon, as uptake progressed linearly with time when incubated for 4 and 24 h. It has been observed that the interaction of these nanoreservoirs with biological cells basically depends on three factors: (a) presence or absence of any steric hindrance, (b) type of surface charge and (c) size. When tested against plain J774 macrophages, the cell viability of plain NwR was found to be more than 90% at the given concentration (10–100 μg/ml, equivalent to the concentration of the core particles) indicating biocompatibility.

Figure 5 shows a representative overlay of the flow cytometry for the uptake of FITC-dextran inter-layered nano-walled reservoirs (I and II: positively charged-NwR-PTX and NwR-PTX-PEG, respectively) in MCF-7 cells after 24 h. Each shows two zones, M2 and M1, which correspond to the number of cells with and without uptake of the NwR formulations, respectively. The broad M2 zone indicates the heterogenous population of MCF-7 cells, with different numbers of internalized NwR formulations. The ratio M2/(M1 + M2) represents the percentage of cells with internalized NwR formulations. NwR with an outermost positive charge results in higher cellular uptake than that with an outermost negative charge. The cellular uptake of positively charged NwR (ζ-potential +13.2 ± 4.3mV) was found to reach 56.48 ± 3.12% and 76.35 ± 0.41% after 4 and 24 h respectively, whereas the uptake of negatively charged NwR (ζ-potential –25.8 ± 2.95 mV) was found to be only 49.21 ± 2.4% after 24 h. This clearly indicates the role played by charge of the nanoreservoir. The uptake of this NwR can be viewed as a two-step process: first binding on the cell membrane and second the internalization step.[23] The attachment of the NwR to the cell membrane as the first step seems to be most affected by the surface charge.[24] Nanoassemblies show a high affinity for cellular membranes, mainly due to electrostatic interaction.[23] After adsorption on the cellular membrane the uptake occurs, with several mechanisms possible: pinocytosis, nonspecific or receptor mediated endocytosis or phagocytosis.[24] To rule out the possibility of active uptake of the NwR-PTX as a result of physicochemical interaction, the same experiment was performed at 4°C (data not shown). At this temperature the physiological processes are inhibited and no interaction was observed.

The cell membrane possesses large negatively charged domains, which should repel negatively charged nanoassemblies. It is therefore very interesting to note that the uptake of *negatively* charged NwR is quite good, at 49.21 ± 2.4%, although this is lower than *positively* charged NwR. The uptake of negatively charged NwR may be supported by the fact that the cells possess few cationic sites for adsorption of the negatively charged particles.[23] It has also been suggested that the negatively charged particles bind at the cationic site in the form of clusters because of their repulsive interactions with the large, negatively charged domains of the cell surface. In addition, the NwRs already bound on the cell surface present a reduced charge density that may favour adsorption of other free nanoassemblies, therefore uptake of negatively charged NwR is related first to the non-specific processing of adsorption on the cell membrane and second to the formation of clusters.[23] After surface modification of positively charged NwR-PTX with PE-PEG2000 (NwR-PEG; ζ-potential +2.8 ± 1.3 mV), the uptake was reduced significantly ($P < 0.05$) to about 22.94 ± 1.9% after 4 h and 54.64 ± 4.5% after 24 h (2.5-fold and 1.4-fold reductions compared to positively charged NwR, respectively). These results are in agreement with observations of Deshpande *et al*., who reported that PEG chains reduce cellular binding of cationic polymer to COS-7 cells to one third of non-pegylated complexes.[25] It has been observed that the assembly of a final monolayer of PE-PEG onto the PRM or SA-terminated multilayers can impart protein resistance to the surface, strongly demonstrating the stealth effect of PEG chains as reported in other studies.[19] One of the other important requirements for biomedical applications is the escape of clearance by the mononuclear phagocytic system. It was found that the PE-PEG coating had a significant effect on cellular recognition, possibly due to PEG density being sufficient to effectively overcome phagocytosis. The quality of the PE-PEG layer, which greatly influences the protein resistance of the surface on the LBL microcapsules, depends on both the density of PEG and type of charge present on the surface. The ζ-potential of NwR without surface modification was found to be +13.2 ± 4.3 mV, while after surface modification with PE-PEG the ζ-potential was reduced to +2.8 ± 1.3 mV. This reduction in surface charge is anticipated to decrease NwR interaction with MCF-7 cells. These data indicate that the reduction in uptake of NwR-PTX-PEG compared to NwR-PTX by MCF-7 cells is being governed by two processes: the stealth effect of PEG chains and a reduction in surface charge. It will be interesting to use a different graft ratio of PE-PEG 2000 to determine the nature of these phenomena, an investigation which is ongoing in our laboratory.

Conclusions

The present paper describes the development and characterization of nano-walled reservoirs followed by variation of

their surface chemistry and subsequent interaction with MCF-7 cells *in vitro*. Frequently used LBL self-assembled materials, including SA and PRM, were introduced as an outermost layer on the reservoir surface along with pegylation. It is obvious from the data that precise engineering of the LBL surface might well be beneficial in a variety of biological applications. Sufficient reduction in serum protein adsorption (opsonization) on pegylation provides evidence that these reservoirs can be transformed into stealth systems, without using the more tedious formulation approaches. Furthermore, the developed system may find potential application in the field of chemotherapy.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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