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Teaching an old dog new tricks: A global approach to enhancing the cytotoxicity of drug-loaded, non-responsive micelles using oligoelectrolytes

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Abstract

Polymeric micelles have been extensively studied as vectors for the delivery of hydrophobic drugs for the treatment of cancer and other diseases. Despite intensive research, few formulations provide significant benefits and even fewer have been clinically approved. Whilst many traditional non-responsive micelles have excellent safety profiles, they lack the ability to respond to the intracellular environment and release their cargo in a spatiotemporally defined manner to effectively deliver large doses of cytotoxic drugs into the cytosol of cells that overwhelm efflux pumps. As a novel and adaptable strategy, we hypothesized that well-established non-responsive polymeric micelles could be augmented with a *pH*-trigger *via* the co-encapsulation of cytocompatible oligoelectrolytes, which would allow rapid cargo release in the endosome leading to increased cytotoxicity. Herein, we demonstrate how this strategy can be applied to render non-responsive micelles *pH*-responsive, resulting in abrupt cargo release at specific and tuneable *pH* values compatible with endosomal delivery, which significantly increased their cytotoxicity up to 3-fold in an ovarian adenocarcinoma (SKOV-3) cell line compared to non-responsive micelles. In comparison, the oligoelectrolyte-loaded micelles were significantly less toxic to healthy 3T3 fibroblasts, indicating selective cargo release in cancer cell lines. Oligoelectrolytes can be co-encapsulated in the micelles along with drugs at high encapsulation efficiency percentages, which are both ejected from the micelle core upon oligoelectrolyte ionisation. Mechanistically, the increase in cytotoxicity appears to also result from accelerated endosomal escape of the cargo caused by disruption of the endosomal membrane by the simultaneous release of the oligoelectrolytes from the micelles. Furthermore, we show how this approach is broadly applicable to non-responsive micelles regardless of their composition, and various classes of hydrophobic chemotherapeutics. The preliminary studies presented here reveal the versatility and wide scope of oligoelectrolyte-mediated, *pH*-triggered drug release as a compelling and powerful strategy to enhance the cytotoxicity of non-responsive polymeric micelles.

Key words: Polymeric micelle; oligoelectrolyte; co-encapsulation; *pH*-triggered; drug release; cytotoxicity.

Introduction

Over the last fifty years, advances in nanomedicine have emerged as promising platforms for the diagnosis and treatment of disease.[1, 2] Specifically, nanomedicine offers unique opportunities to deliver therapeutics that are poorly soluble, sensitive to degradation, rapidly eliminated or cause systemic toxicity. Various polymeric vectors, including dendrimers, conjugates, micelles and polymersomes have been extensively studied for the delivery of therapeutic payloads. Polymeric micelles prepared from amphiphilic copolymers offer numerous advantages for the delivery of hydrophobic therapeutics, including improved drug stability and aqueous solubility, relatively high drug-loadings, enhanced circulation times, and when combined with targeting ligands, significantly reduced off-target toxicity.[3, 4] Despite these favourable characteristics, polymeric micelles have seen limited translation to the clinic.[5, 6]

One of the major limitations associated with traditional polymeric micelles (and other self-assembled delivery systems such as liposomes and polymersomes) is their inefficient release of physically entrapped payloads at the desired target site as they are unresponsive to changes in their environment.[7] For example, numerous studies have reported that the release rates of hydrophobic payloads are insensitive to endosomal *pH* values, and only a small fraction (< 5%) is released within suitable timeframes (i.e., hours), with substantial release typically requiring many days to weeks.[8-13] Thus, upon cellular internalisation *via* endocytosis, micelles may be trafficked to the perinuclear lysosomal compartments for degradation prior to releasing their cargo. Migration to these lysosomal compartments has been reported to occur within a few hours,[14] and therefore, endosomal escape and cytosolic delivery of drugs requires rapid release from micelles during the trafficking process to avoid entrapment and degradation.[15, 16] Furthermore, any small doses of drugs that are released from the micelles during endosomal migration and sorting, and escape the endosome, may be insufficient to inhibit their targets or overcome the action of efflux pumps.[17] The limited ability of non-responsive micelles for cytosolic delivery of their payloads is likely a contributing factor towards their relatively modest cytotoxicity when compared to free drugs *in vitro*.[10-12]

A common approach used to promote the release of drugs from polymeric micelles during endosomal trafficking is to prepare them from the self-assembly of stimuli-responsive polymers, which can be designed to respond to specific environmental cues such as changes in *pH*, temperature and redox potential.[18] Generally, *pH*-sensitive micelles are engineered to respond to the tumour interstitial microenvironment (*pH* 6.5–6.9) and/or the endosomal compartments (*pH* 4.5–6.5).[19] This often involves the preparation of micelles from bespoke copolymers that have a *pH*-sensitive core-forming block that can switch from hydrophobic to hydrophilic as the *pH* is lowered. For example, micelles prepared from amphiphilic block copolymers with acid-labile acetal groups were found to be

responsive below *pH* 5 due to hydrolysis of the acetal groups.[20, 21] While significant hydrolysis (~ 75%) of the acetal groups was observed after 5 h at *pH* 4, drug release was < 50% (*cf.* ~20–25% for micelles at *pH* 7.4) and the micelles only displayed moderate cytotoxicity compared to the free drug.[20] Similarly, Tang *et al.* prepared micelles from copolymers containing ortho ester groups that displayed similar *pH*-mediated drug release.[22] Micelles prepared from copolymers containing dimethyl maleamidic groups displayed exceptional drug release (~80–90%) within 5 h at *pH* 5.4, although surprisingly, they only displayed a slight increase in their cytotoxicity relative to non-responsive micelles of similar composition.[23]

An alternative strategy to acid-labile groups is to use copolymers where one block is ionisable through protonation at endosomal *pH*. Copolymers with tertiary alkylamine functionalised blocks have been extensively investigated in combination with PEG blocks, although it is also common to include a non-*pH*-responsive hydrophobic block to promote self-assembly and form mixed micelles.[24-27] The release of hydrophobic payloads from these types of micelles is typically reported to be slow (< 30% at *pH* 5 over 5 h),[24-26] presumably due to encapsulation in the non-responsive block, negating the potential advantage of the *pH*-responsive block. In addition, mixed micelles are significantly larger and have higher critical micellization concentrations (CMCs) leading to reduced stability under dilution as compared to their non-responsive, which can negatively impact their biodistribution and bioaccumulation in target tissues.[2]

Alternatively, Brewer *et al.* prepared micelles from poly[(ethylene glycol)-*b*-(2-vinyl pyridine)] (PEG-PVP) copolymers, avoiding the need for a non-responsive hydrophobic block.[28] While these micelles were shown to efficiently encapsulate a range of different drugs, release was highly dependent on the type of drug, drug-polymer interactions and *pH* of the surrounding environment.

While the application of *pH*-responsive copolymers has been shown to be effective for improving drug release compared to their non-responsive counterparts, there are several hurdles to their clinical development. In particular, these highly specialised copolymers can be complex to synthesize and may not be suitable for scale-up,[29] they lack detailed safety testing and profiles in humans,[30, 31] and display variable drug release kinetics, limiting their broad applicability. Furthermore, the rate of drug release at endosomal *pH* values is still relatively slow compared to endosomal trafficking, increasing the likelihood that their payloads become entrapped and degraded in lysosomal compartments. Compared to their *pH*-responsive counterparts, there are several non-responsive copolymer micelle systems[32-36] that have been exhaustively tested *in vivo* (including humans) and exhibit excellent safety profiles,[37-39] but unfortunately lack the *pH*-responsiveness for triggered drug release.

Considering these limitations, we contemplated approaches to augment well-established non-responsive micelles with a novel *pH*-trigger mechanism through the physical co-encapsulation of oligoelectrolytes that could be ejected from the micelle core along with the payload at a desired *pH*. Thus, we hypothesised that it would be possible to render traditionally non-responsive micelles *pH*-responsive through the co-encapsulation of oligoelectrolytes that could respond to changes in environmental *pH* to trigger payload release leading to significant increases in the cytotoxicity. To demonstrate this concept and its broad applicability, we initially investigated oligo(2-vinyl pyridine) (OVP) as the oligoelectrolyte in combination with various traditional non-responsive micelles and drug classes. In all cases, OVP mediated significant *pH*-triggered release of payloads within hours of being exposed to acidic conditions, leading to large increases in cytotoxicity as compared to the corresponding non-responsive micelles. Furthermore, we demonstrate how the *pH* responsiveness of the micelles can be tuned through the selection of appropriate oligoelectrolytes.

Results and discussion

Encapsulation and pH-triggered release of oligoelectrolytes from copolymer micelles

To test our hypothesis, we initially investigated the *pH*-mediated encapsulation and release of an OVP (number-average molecular weight ($M_{n,\mathrm{NMR}}$) = 1.3 kDa, dispersity ($Đ$) = 1.2, average repeat units (RU_{ave}) = 10) from poly[(ethylene glycol)-*b*-(ε-caprolactone)] (PEG-PCL) diblock copolymer micelles (PEG-PCL; $M_{n,\mathrm{NMR}}$ = 20 kDa, $Đ$ = 1.2, weight fraction of PCL (f_{PCL}) = 0.5). Narrow $Đ$ OVP (Supplementary Information (SI), **Figure S1**) and PEG-PCL copolymer (SI, **Figure S2** and **Table S1**) were prepared *via* conventional radical polymerization using a chain transfer agent and ring-opening polymerization, respectively. OVP-loaded PEG-PCL micelles (PEG-PCL-OVP$_{0.3}$) with an OVP loading of 0.3 mg/mg of copolymer were prepared *via* the solvent evaporation approach in either phosphate buffered saline in ultrapure water (PBS; 10 mM, *pH* 7.4) or deuterium oxide (PBS-d; 10 mM, *pH* 7.3). Blank micelles were prepared in an identical fashion in the absence of OVP. All micelle solutions were optically transparent with no macroscopic evidence of precipitation. In contrast, attempts to micellize OVP in PBS in the absence of the copolymer resulted in precipitation, indicating that OVP is encapsulated in the core of the copolymer micelles. The CMC of the PEG-PCL copolymer in PBS was determined *via* ratiometric fluorescence emission of a pyrene probe at λ_{em} = 372 and 384 nm (I_{384}/I_{372}) to be ~ 0.5 mg/L (SI, **Figure S3** and **Table S2**),[40] which is consistent with previously reported CMC values.[41] The addition of OVP had negligible influence on the CMC of the copolymer. In contrast, pH-responsive mixed micelle systems have significantly higher CMCs as compared to their non-responsive counterparts, which can lead to poorer stability.[26, 42] Low CMCs are particularly important for preventing premature micelle disassembly upon dilution (e.g., in the bloodstream) and the release of encapsulated cargo.

The encapsulation of OVP into the micelles was further supported by dynamic light scattering (DLS) and synchrotron small-angle X-ray scattering (SAXS) at ambient temperature, which revealed an increase in the size of the PEG-PCL-OVP$_{0.3}$ micelles (*cf.* blank PEG-PCL micelles) at *pH* 7.3–7.4. For DLS experiments, micelles were prepared in PBS-d to allow the same samples to also be analysed *via* ultraviolet-visible (UV-vis) spectrophotometry and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (SI, **Table S3**). DLS revealed monomodal particle size distributions (PSDs) with narrow polydispersity index (PDI) values (**Figure 1a** and SI, **Figure S4** and **Table S4**). While the blank PEG-PCL micelles presented with a hydrodynamic diameter (D_h) of 42 ± 1 nm (PDI = 0.11), there was a moderate increase to 53 ± 1 nm (PDI = 0.15) for the PEG-PCL-OVP$_{0.3}$ micelles consistent with OVP encapsulation (**Figure 1b**). In comparison, a doubling or greater is size is often reported for the preparation of mixed micelles as compared to their respective non-responsive copolymer micelles.[43-45] To provide an indication of the stability of the micelles in solution, the D_h was measured again after 42 d, which

revealed an increase of 20–30% for the PEG-PCL (D_h = 52 ± 2 nm, PDI = 0.18) and PEG-PCL-OVP$_{0.3}$ micelles (D_h = 71 ± 2 nm, PDI = 0.23) and implied that the encapsulation of OVP has negligible influence on the micelle stability (SI, **Figure S5**). Modelling of the SAXS profiles revealed that the blank and OVP-loaded micelles adopted oblate ellipsoidal morphologies in PBS at *pH* 7.4 (SI, **Tables S5**–**6**). Incorporation of OVP into the PEG-PCL micelles led to a significant increase in the equatorial radius from 31 ± 1 to 48 ± 2 nm (**Figure 1c**) and suppressed secondary aggregation of the PEG-PCL micelles, characterised by an upturn in the low scattering vector (Q) region for the blank micelles (SI, **Tables S5**–**6**).

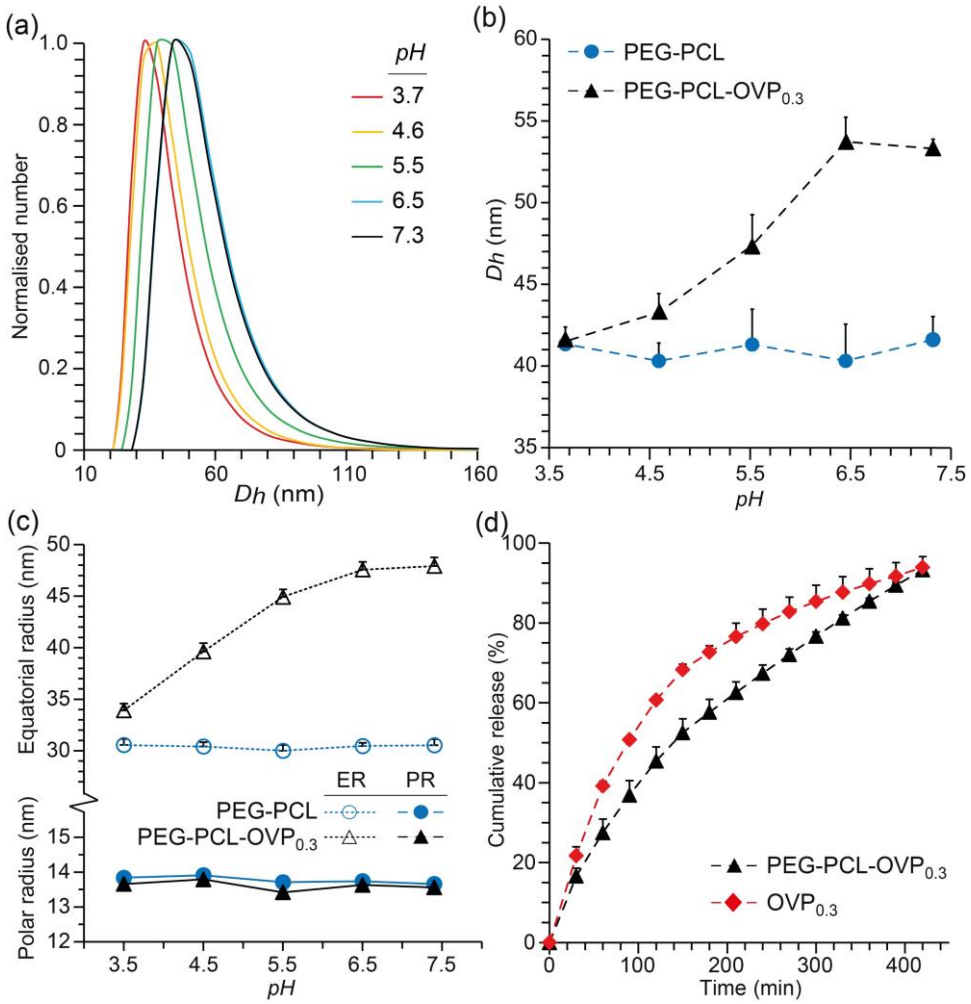


Figure 1: **(a)** Number particle size distributions (PSDs) for PEG-PCL-OVP$_{0.3}$ micelles at various *pH* values in D_2O as determined *via* DLS; all *pH** values recorded in D_2O using a H_2O-calibrated probe were converted to their equivalent *pH* values using the formula report by Krężel and Bal.[46] **(b)** Hydrodynamic diameter (D_h) as determined from DLS number PSDs (n = 3) and **(c)** equatorial radius and polar radius as determined from small-angle X-ray scattering (SAXS) experiments *versus pH* for PEG-PCL and PEG-PCL-OVP$_{0.3}$ micelles. **(d)** *In vitro* OVP release from control solutions (OVP$_{0.3}$) and PEG-PCL-OVP$_{0.3}$ micelles in acetate buffer (*pH* 4.5) at 22 ± 1 °C using a dialysis membrane assay and quantified *via* UV-vis spectrophotometry; data are shown as the average cumulative release (%) ± SD (n = 3).

The *pH*-dependent release of OVP from the PEG-PCL-$OVP_{0.3}$ micelles was followed by measuring the change in micelle size as a function of solution *pH*. DLS of the blank micelle solutions revealed that the D_h was insensitive to the *pH* value, remaining relatively constant at 40 nm. In comparison, a sharp decrease in D_h of the PEG-PCL-$OVP_{0.3}$ micelles from 54 to 41 nm was observed when the solution *pH* was changed from 6.5 to 4.5 (**Figure 1b** and SI, **Figure S6**), indicating that the OVP is ejected from the core of the micelle leading to core collapse. SAXS experiments revealed a similar trend for the PEG-PCL-$OVP_{0.3}$ micelles, albeit with a more gradual change in equatorial radius from 48 to 39 nm as the solution *pH* was decreased from 6.5 to 4.5 (**Figure 1c** and SI, **Tables S5**–**6**). Interestingly, the polar radius of the OVP-loaded micelles remained constant at 14 nm regardless of the *pH* (SI, **Table S5-6**), resulting in a change in the aspect ratio of the PEG-PCL-$OVP_{0.3}$ micelles from 0.29 at *pH* 7.5 to 0.36 at *pH* 3.5. Thus, the OVP would appear to be ejected from the equatorial plane resulting in less flattened (more spherical) oblate ellipsoids. Both the DLS and SAXS results indicate that the OVP is excluded from the PEG-PCL micelles when the *pH* < 6.5, resulting in shrinkage of the micelle core.

To unequivocally prove that the OVP is released from the core of the PEG-PCL-$OVP_{0.3}$ micelles, dialysis experiments were conducted, whereby the micelles in PBS (10 mM, *pH* 7.4) were placed in a membrane (molecular weight cut-off (MWCO) = 100 kDa) that was suspended in an acetate buffered *pH* 4.5 solution. Regular monitoring of the receiving solution *via* UV-vis spectrophotometry revealed the rapid release of OVP into the receiving solution (**Figure 1d** and SI, **Figures S7**–**8**). Although the release curve would suggest a more gradual release of the OVP over a period of 7 h, the release through the membrane is diffusion limited as control experiments with OVP at *pH* 4.5 in the absence of micelles revealed a similar release profile (**Figure 1d** and SI, **Figure S8**). To confirm that release of OVP from the micelles is triggered by *pH*, control experiments were conducted using PBS as the receiving solution, which resulted in no release of the OVP.

Mechanistically, release of OVP from the PEG-PCL-$OVP_{0.3}$ micelles bears some resemblance to *pH*-mediated disassembly of *pH*-responsive block copolymer micelles. For instance, Brewer *et al.* reported that the D_h of PEG-PVP diblock copolymer micelles decreased as the *pH* was lowered below 6, and when the weight fraction of PVP (f_{PVP}) was ≥ 0.5 unimers were obtained at *pH* 4.5.[28] Similar trends for PEG-PVP diblock copolymer micelles in response to *pH* changes have also been reported by others,[47, 48] and in all cases attributed to protonation of the PVP block resulting in electrostatic repulsion.

The light scattering and dialysis results of the PEG-PCL-$OVP_{0.3}$ micelles are consistent with OVP release being driven by protonation and electrostatic repulsion of the core encapsulated OVP as the *pH* approaches the pK_a (~ 5) of the pyridyl moieties,[49] leading to core collapse and micelle shrinkage (*vide infra*), which was further confirmed by UV-vis spectrophotometry and NMR spectroscopy (**Figure 2**). OVP was found to display two absorption maxima at λ_{max} = 206 and 264 nm, similar to PVP homopolymers, with the latter π-π* transition being *pH*-sensitive as a result of protonation.[50] While OVP is insoluble at *pH* 7.4, it does display slight solubility at *pH* 6.5, which increases as the solution *pH* becomes more acidic. UV spectra revealed that the absorbance of OVP at 264 nm followed a near linear, inverse relationship with *pH* from 3.7 to 6.5, deviating marginally at *pH* 6.5 because of limited solubility (**Figure 2a** and SI, **Figure S9**). Subsequently, the *pH*-dependent absorbance of the PEG-PCL-$OVP_{0.3}$ micelles was measured, which revealed a similar trend and confirmed protonation of the OVP. To confirm that the PEG-PCL copolymer did not contribute towards this trend, the blank micelles were also analysed, which revealed a weak absorbance at 264 nm that was *pH*-insensitive (**Figure 2a** and SI, **Figure S9**). These results indicate that despite the hydrophobic core of the micelles, protons readily diffuse into the core resulting in OVP protonation.

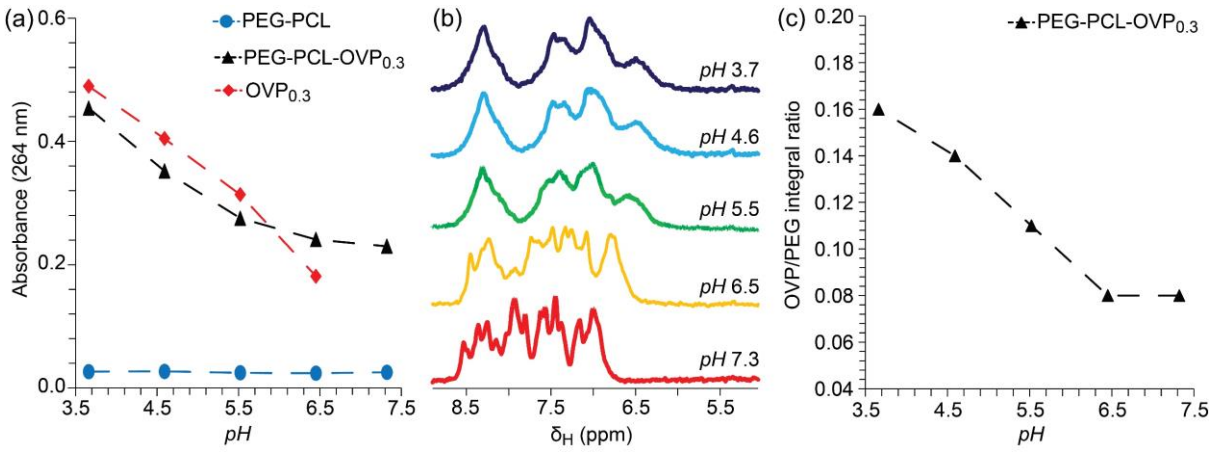


Figure 2: **(a)** UV absorbance (λ_{264}) versus *pH* for OVP, and PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles. (**b**) ^{1}H NMR spectra of PEG-PCL-$OVP_{0.3}$ micelles at various *pH* values in D_2O; spectral range (δ_H 5.0–9.0 ppm) limited to show OVP pyridyl proton resonances. **(c)** *pH*-dependent change in the intensity of the OVP pyridyl proton resonances with reference to the PEG repeat unit methylene proton resonance (OVP/PEG integral ratio) observed in ^{1}H NMR spectra.

^{1}H NMR spectra of the blank and OVP-loaded micelles in PBS-d revealed characteristic resonances corresponding to the PEG and PCL blocks, with the latter also displaying resonances corresponding to the OVP pyridyl moieties (δ_H 6.0–8.5 ppm) (SI, **Figure S10**). As reported previously, the appearance of PCL resonances in the NMR spectra indicates that part of the hydrophobic core is

semi-hydrated, allowing sufficient rotational mobility of protons and long enough relaxation times for detection.[51] With a decrease in the *pH*, the intensity of the OVP resonances in the PEG-PCL-$OVP_{0.3}$ micelles increased (relative to the PEG repeat unit resonance) and there was a characteristic change in their chemical shift resulting from protonation at *pH* values < 6.5 (**Figure 2b**), which is consistent with that observed for pure OVP (SI, **Figure S11**). The increase in intensity, accompanied by an improvement in the resolution, was consistent with the release of the OVP from the micelle core and increased proton mobility as a result of solvation (**Figure 2c**).

Oligoelectrolyte-mediated, pH-triggered release of doxorubicin from copolymer micelles

Having demonstrated the *pH*-mediated expulsion of OVP from the micelle core upon protonation, the final test of our hypothesis was to confirm if this process could also be used to trigger the rapid release of an encapsulated cargo (i.e., therapeutic), resulting in greater cytotoxicity. Blank PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles were prepared in PBS (10 mM, *pH* 7.4) containing doxorubicin (DOX; 0.1 mg/mg of copolymer) as a model drug, with encapsulation efficiency percentages (EE%) of 98.2 ± 0.2 (PEG-PCL-$DOX_{0.1}$) and 89.5 ± 0.4% (PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$), respectively (SI, **Figures S12**–**13**, and **Table S7**). The small reduction in DOX EE% for the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles was attributed to the co-encapsulation of OVP, which had an EE% of 82.1 ± 0.3% (*cf.* OVP EE% of 89.3% for PEG-PCL-$OVP_{0.3}$). Loading of DOX resulted in a slight increase in the D_h as determined from DLS number PSDs from 40 ± 1 to 43 ± 1 nm for the PEG-PCL-$DOX_{0.1}$ micelles and 53 ± 1 to 59 ± 2 nm for the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles, consistent with the encapsulation of the drug (**Figure S14**).

To assess the *pH*-mediated release of DOX, the micelles were placed in a dialysis membrane (MWCO 100 kDa) at 22 ± 1 °C and initially dialysed against PBS for 120 min before switching to an acetate buffer at *pH* 4.5 (SI, **Figure S15** and **Table S8**). The concentration of DOX in the receiving solution was measured every 30 min *via* UV-vis spectrophotometry, with the receiving solution being completely replaced to maintain sink conditions. While some release of DOX (~ 20%) from the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles was noted at *pH* 7.4, a change in *pH* to 4.5 resulted in the triggered release of the DOX, with the gradient of the release curve approximating that of control experiments with the free drug (**Figure 3a**). For the free drug, the release through the dialysis membrane is diffusion limited,[52] and therefore, the release curve marks the fastest possible rate at which the drug can transverse the membrane at a particular concentration gradient. Thus, the similarity in the gradients of the DOX release curves for the free drug and PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles indicates that the core encapsulated DOX is instantaneously ejected from the micelles along with OVP when the *pH* is switched to 4.5 (SI, **Figure S16**). The rapid increase in the release rate of DOX can also be observed from the first derivative plots (SI, **Figure S17**). In comparison, the PEG-PCL-$DOX_{0.1}$ micelles did not

display any *pH*-triggered release of the DOX, but rather a slow and sustained release ('leakage') regardless of the *pH* (**Figure 3a**). To assess potential temperature effects on the observed DOX release profiles that may manifest under physiological temperatures above the glass transition (T_g) of the OVP (SI, **Figure S1**), the release experiments were also conducted at 37 ± 1 °C, which revealed little change in the release profiles (**Figure 3a**) and confirmed that release studies conducted at ambient temperature (22 ± 1 °C) are a close approximation to what would be expected at physiological temperatures.

To confirm that the core encapsulated DOX is immediately ejected from the micelles when the *pH* is switched to 4.5, the fluorescence lifetime decay of the DOX was recorded *via* time-correlated single photon counting (TCSPC) and fitted with a bi-exponential model to determine the amount of free and encapsulated DOX. In water, the fluorescence lifetime (τ_1) of free DOX is ~ 1 ns, while encapsulation in hydrophobic environments results in an increase in the τ.[53] For the PEG-PCL-$DOX_{0.3}$ and PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles in PBS (10 mM, *pH* 7.4), the encapsulated DOX was found to have a τ_2 of ~ 3.4 ns, which is similar to other reported polymeric micelles and nanoparticles.[54] While a decrease in the *pH* to 4.5 had negligible effect on the τ_2 amplitude of the PEG-PCL-$DOX_{0.3}$ micelles, a sharp decrease in the τ_2 amplitude was observed for the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles corresponding to the immediate release of 62% of the encapsulated DOX and confirming *pH*-triggered, OVP-mediated release from the micelle core. These results are in stark contrast to other drug-loaded non-responsive micelles systems whereby drug release occurs over days,[8-13] potentially resulting in entrapment and degradation of their payloads in the lysosomal compartments and limiting their ability to overwhelm the efflux pumps of cells with high doses of cytotoxic drugs.[55]

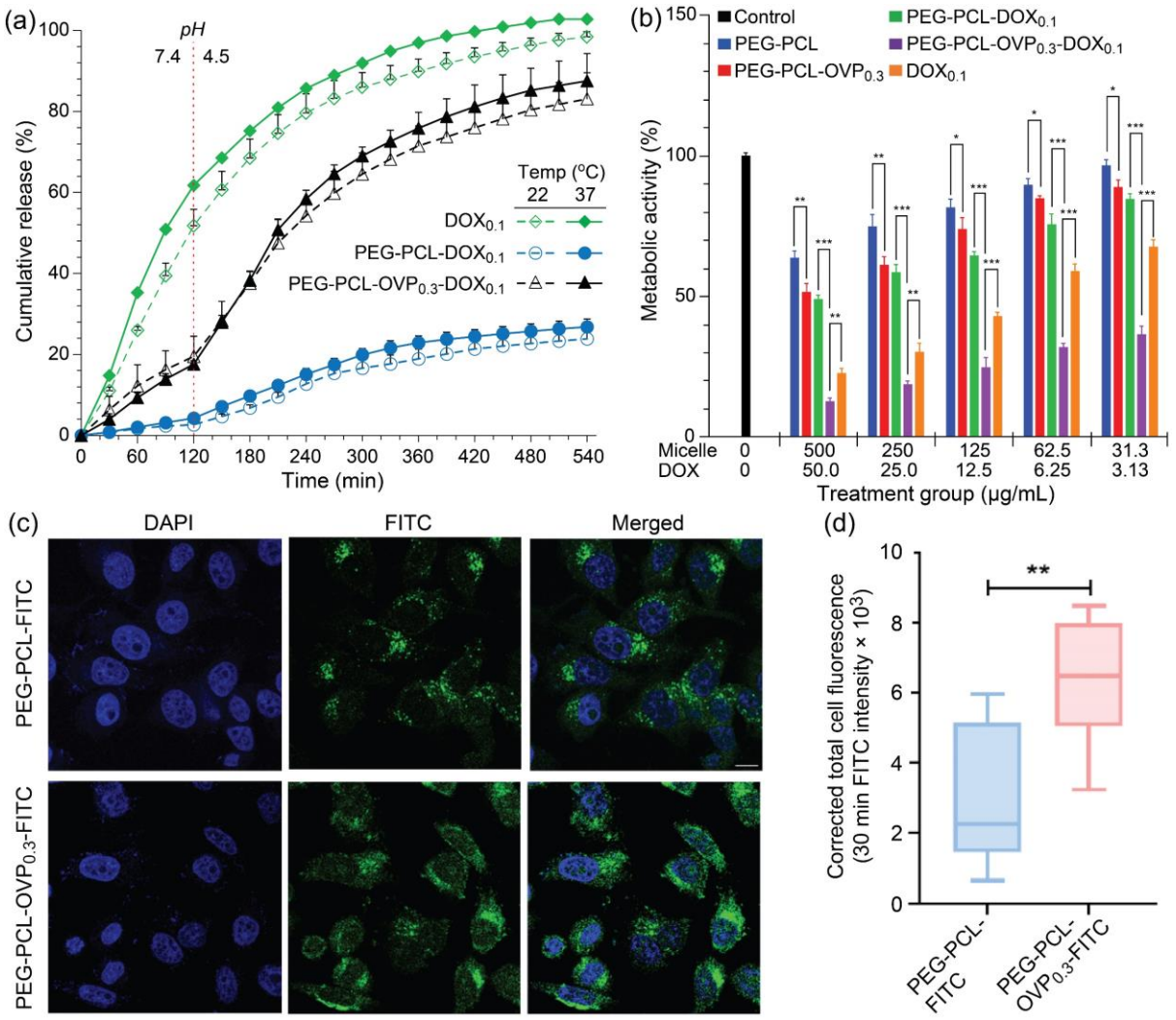


Figure 3: **(a)** *In vitro* DOX release from PEG-PCL-$DOX_{0.1}$ and PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles, and free DOX using a dialysis assay against PBS (10 mM, *pH* 7.4) for the first 2 h followed by acetate buffer (10 mM, *pH* 4.5) for 7 h; experiments were conducted at 22 ± 1 and 37 ± 1 °C, and the DOX release was measured *via* UV-vis spectroscopy (DOX λ_{max} = 479 nm). Data are shown as the average cumulative release (%) + SD (n = 3); some error bars are smaller than markers. **(b)** *In vitro* metabolic activity assay with SKOV-3 cells when untreated (negative control) and treated with free DOX (3.13−50.0 μg/mL) and the micelles (PEG-PCL, PEG-PCL-$DOX_{0.1}$, PEG-PCL-$OVP_{0.3}$ and PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$) for 48 h; treatment group concentrations refer to the micelle and DOX concentration if present. Data are shown as the mean metabolic activity (%) + SD (n = 3); *, ** and *** stand for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. **(c)** Fluorescence imaging of lysosome inhibited HeLa cells following 30 min incubation with FITC-labelled PEG-PCL (top) and PEG-PCL-$OVP_{0.3}$ micelles (bottom). Scale bars are 20 μm. **(d)** Total cell fluorescence intensity (λ_{ex} = 488) of HeLa cells following 30 min incubation with FITC-labelled PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles ($p < 0.01$).

With these promising results in hand for the *pH*-triggered, OVP-mediated release of DOX from the micelles, we assessed their dose dependent cytotoxicity against a human Caucasian ovarian adenocarcinoma (SKOV-3) cell line using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) metabolic assay (**Figure 3b**). SKOV-3 cells were treated with PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ and PEG-PCL-$DOX_{0.1}$ micelles at copolymer concentrations ranging from 0.03 to 0.5 mg/mL, which equated to a DOX dose of 3.13 to 50 μg/mL, respectively (uncorrected for EE%). In addition, PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelle solutions (31.3 to 500 μg/mL) were also tested to assess any inherent cytotoxicity of the micelle platform. Negative and positive controls consisted of untreated cells and free DOX treated cells at concentrations ranging from 3.13 to 50 μg/mL.

All formulations displayed dose dependent cytotoxicity over the concentration range studied relative to the negative control. At the highest copolymer concentration (500 μg/mL), the blank PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles resulted in cell metabolic activities of ~ 64 and 52%, implying that the copolymer and OVP (0.15 mg/mL) have some inherent cytotoxicity. At copolymer concentrations ≤ 125 μg/mL, the cell metabolic activity increased to > 80% for both the PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles. The apparent increase in cytotoxicity of PEG-PCL-$OVP_{0.3}$ micelles at higher concentrations (≥ 125 μg/mL) may result from OVP release from the micelles in lower *pH* environments following endocytosis, resulting in the disruption of endosomal and lysosomal membranes and a temporary reduction in metabolic function (*vide infra*). In contrast, the non-responsive PEG-PCL-$DOX_{0.1}$ micelles only displayed a small increase in cytotoxicity when compared to their blank PEG-PCL counterparts, indicating limited or inefficient drug release, as the free drug (positive control) resulted in a significant reduction in the cell metabolic activity (25–60%). Similar results have been reported by others, whereby drug-loaded, non-responsive micelles have shown limited improvements in cytotoxicity as compared to their blank micelle counterparts.[10-12]

In comparison, the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles significantly outperformed the PEG-PCL-$DOX_{0.1}$ micelles and free drug, with a large reduction in the cell metabolic activity ranging from 15 to 35%. These results imply that the encapsulated OVP is responsible for the triggered release of DOX from the micelles once internalized by the cells, resulting in increased cytotoxicity. While the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles outperform the free DOX at comparable DOX concentrations, significantly greater than previous reports by Lei *et al.* for DOX-loaded poly(lactide-co-glycolide) nanoparticles with SKOV-3 cells,[56] a direct comparison does not account for possible differences in cellular uptake mechanisms that may play a role.[57] Specifically, nanoparticulate formulations are predominantly taken up through endocytosis while free drugs pass through membranes, by-passing endocytic pathways.[58] While these two processes may differ significantly in their kinetics of cellular uptake, nanoencapsulation has the potential to overcome limitations associated with renal and hepatic

clearance of free drugs during circulation[59] leading to greater accumulation of cytotoxic doses at the target site.[60]

The performance of the micelles was also assessed on a healthy cell line, NIH 3T3 fibroblasts, with surprising results (SI, **Figure S18**). While the free drug and PEG-PCL-$DOX_{0.1}$ micelles displayed a similar reduction in metabolic activity with the fibroblasts as compared to the SKOV-3 cells (**Figure 3b**), the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles were significantly less cytotoxic to the fibroblasts. Across the concentration range studied, metabolic activities varied from 30 to 80% for the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles (*cf.* 15 to 35% for the SKOV-3 cells). These differences may arise from disparate pH gradients across the endosomal-lysosomal systems and intracellular trafficking mechanisms.[57] Although further studies are required to understand the differences in cytotoxicity between the different cell lines, these preliminary results indicate a level of selectivity towards cancer cell lines offered by oligoelectrolyte-loaded micelles.

Cellular uptake of micelles is often influenced by their surface charge.[61] To rule out differences in cellular uptake due to the surface charge as a possible cause for the differences in cytotoxicity of the PEG-PCL-$DOX_{0.1}$ and PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles, their zeta potential (ζ) was determined at an ionic strength of 1 mM (i.e., lower than that of 10 mM PBS solution) at *pH* 7 and provided similar values of –2.6 and –0.2 mV, respectively. The small, negative ζ values for both micelles at this ionic strength are consistent with previously reported values and the slight difference would not be expected to influence cellular uptake at physiological conditions (ionic strength of 154 mM) as the Debye length is smaller than 1 nm and the absolute value of ζ would be very small.[62]

The significantly higher cytotoxicity observed for the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles as compared to the PEG-PCL-$DOX_{0.1}$ micelles further supports the rapid, *pH*-triggered, OVP-mediated release of the drug into the acidic organelles (endosome and lysosome) of the cells. While diffusion of the released drug through the endosomal or lysosomal membrane would be expected[15, 63] the protonated OVP likely contributes to pore formation or membrane disruption, accelerating the release of the drug into the cytosol. Similar behaviour has been observed for micelles that co-encapsulate cationic amphiphilic drugs with small nucleic acids, whereby the former have been shown to contribute to endosomal escape of the latter into the cytosol.[64]

To assess the influence of the OVP on the endosomal escape of micelles, lysosome inhibited HeLa cells were treated with fluorescein isothiocyanate (FITC)-labelled PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles (SI, **Figures S19** and **S20**). The HeLa cells were initially treated with concanamycin A, which inhibits lysosomal acidification,[65, 66] and then incubated with the micelles for 30 min before the cells were fixed and imaged (**Figure 3c**). Inhibition of lysosomal acidification would be expected to result in accumulation of the micelles in the lysosomal compartments if they had not previously escaped from

the endosome. For cells treated with the FITC-tagged PEG-PCL micelles, the fluorescence response was predominately restricted to spherical structures clustered around the nucleus, consistent with endosomal/lysosomal accumulation.[67] In contrast, the fluorescent signal from the FITC-labelled PEG-PCL-$OVP_{0.3}$ micelles was significantly more dispersed throughout the cytosol, indicating that the micelles escape from the endosome, potentially due to OVP-mediated pore formation or membrane disruption.

Endosomal escape was further confirmed by comparing the total cell fluorescence intensity, which was significantly higher for the PEG-PCL-$OVP_{0.3}$ micelles (**Figure 3d**). The fluorescence intensity of the FITC-labelled copolymer micelles increased exponentially with increasing *pH* (SI, **Figure S21**), and therefore, micelles in an acidic environment would be expected to have a lower fluorescence intensity. Thus, the lower fluorescence intensity for the PEG-PCL micelles implies that they reside in the acidic *pH* environment of the endosome, whereas the PEG-PCL-$OVP_{0.3}$ micelles have escaped into the more neutral *pH* environment of the cytosol. These findings suggest that the increased cytotoxicity observed for the PEG-PCL-$OVP_{0.3}$-$DOX_{0.1}$ micelles likely originates from the *pH*-triggered release of the drug and disruption of the endosomal membrane, allowing the drug to escape into the cytosol prior to entrapment and degradation in the lysosome. Thus, the OVP serves a dual role in mediating the release of the drug from both the micelle and the endosome.

These release and cell studies confirm our hypothesis that the co-encapsulation of a suitable oligoelectrolyte and drug in a non-responsive micelle renders the micelle *pH*-responsive, allowing the *pH*-triggered release of the encapsulated cargo, which significantly increases the cytotoxicity of the micelles. To demonstrate the versatility and broad scope of this approach, we further investigated the application of OVP-loaded micelles to a range of commonly used non-responsive copolymers and different drug classes.

Application of oligoelectrolyte-mediated, pH-triggered release to compositionally diverse non-responsive micelles

OVP- and DOX-loaded (0.3 and 0.1 mg/mg of copolymer, respectively) micelles were prepared *via* the solvent evaporation approach in PBS using a range of non-responsive copolymers with constant hydrophilic PEG blocks and compositionally diverse hydrophobic blocks, including poly[(ethylene glycol)-*b*-(ε-decalactone)] (PEG-PDL), poly[(ethylene glycol)-*b*-(styrene)] (PEG-PS) and poly[(ethylene glycol)-*b*-(DL-lactic acid)] (PEG-PDLLA). The block copolymer molecular weight and weight fraction of the hydrophobic block were kept approximately constant at M_n ≈ 20 kDa and 0.5, respectively (SI, **Figures S22**–**24**). Control micelles (PEG-PDL-$DOX_{0.1}$, PEG-PS-$DOX_{0.1}$ and PEG-PDLLA-$DOX_{0.1}$) were prepared in the absence of OVP and provided DOX EE% values of 96.9 ± 0.5, 98.1 ± 0.1 and 95.8 ±

0.8%, respectively. For the OVP-loaded PEG-PDL-$OVP_{0.3}$-$DOX_{0.1}$, PEG-PS-$OVP_{0.3}$-$DOX_{0.1}$ and PEG-PDLLA-$OVP_{0.3}$-$DOX_{0.1}$ micelles, the DOX EE% values were reduced slightly to 82.6 ± 0.8%, 89.3 ± 2.1% and 80.7 ± 1.6%, while the OVP EE% values were 79.9 ± 1.5%, 83.1 ± 1.4% and 78.9 ± 2.1%, respectively. As observed previously for the PEG-PCL micelles, co-encapsulation of OVP and DOX resulted in a reduction in the DOX loading regardless of the hydrophobic block composition.

The triggered release of DOX from the micelles was assessed using the same dialysis assay as described previously (**Figure 4**). For all of the OVP loaded micelles, an initial release of 15–20% DOX was observed at *pH* 7.4, followed by an accelerated release at *pH* 4.5, with the release curve approximating that of control experiments with the free drug and indicating instantaneous release of DOX regardless of the hydrophobic core composition, which was further evidenced from the first derivatives plots (SI, **Figure S25**). DOX-loaded micelles in the absence of OVP did not display any *pH*-triggered release of the DOX. These results demonstrate that the oligoelectrolyte-mediated, *pH*-triggered release of DOX from micelles is broadly applicable to various non-response micelles, regardless of the core composition.

To assess the performance of OVP- and DOX-loaded micelles, the same metabolic assay as described previously was conducted using SKOV-3 cells with the relevant micelle and free drug controls (**Figure 4**). In general, there was no significant difference between the metabolic activity of cells exposed to OVP-loaded micelles and their blank counterparts, except for at very high doses, indicating that OVP has negligible cytotoxicity. The DOX-loaded micelles displayed a small increase in cytotoxicity compared to the blank micelles, particularly at higher concentrations, whereas the OVP- and DOX-loaded micelles resulted in a significant increase in the cytotoxicity at all concentrations, consistent with the *pH*-triggered release of DOX. These results demonstrate that the OVP-mediated release of drugs is broadly applicable to compositionally diverse non-responsive micelles.

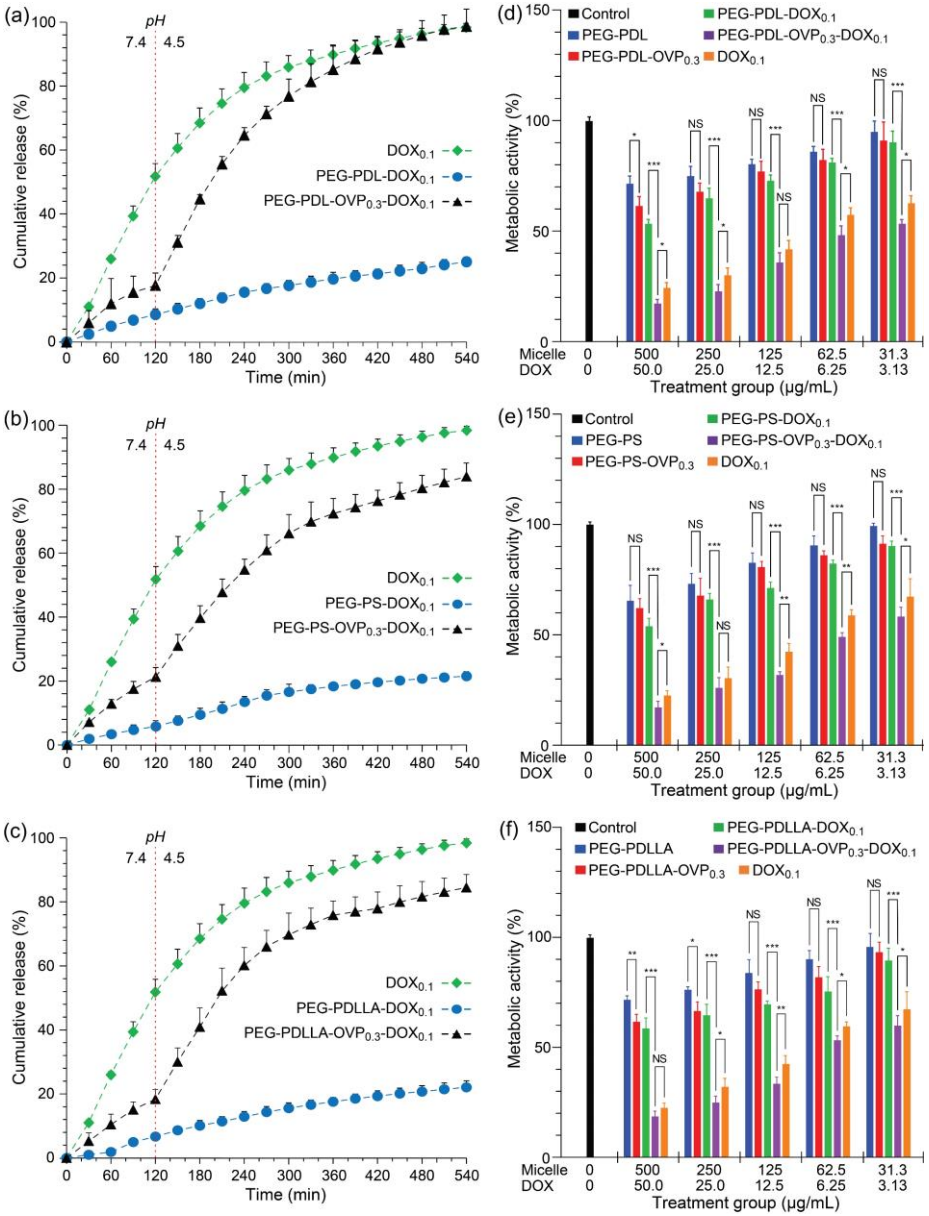


Figure 4: *In vitro* DOX release from **(a)** PEG-PDL-$DOX_{0.1}$ and PEG-PDL-$OVP_{0.3}$-$DOX_{0.1}$ micelles, **(b)** PEG-PS-$DOX_{0.1}$ and PEG-PS-$OVP_{0.3}$-$DOX_{0.1}$ micelles, and **(c)** PEG-PDLLA-$DOX_{0.1}$ and PEG-PDLLA-$OVP_{0.3}$-$DOX_{0.1}$ micelles and free DOX using a dialysis assay against PBS (10 mM, *pH* 7.4) for the first 2 h followed by acetate buffer (10 mM, *pH* 4.5) for 7 h; experiments were conducted at 22 ± 1 °C and the DOX release was measured *via* UV-vis spectrophotometry (DOX λ_{max} = 479 nm). Data are shown as the average cumulative release (%) ± SD (n = 3). *In vitro* metabolic activity assay with SKOV-3 cells when untreated (negative control) and treated with free DOX (3.13 −50 μg/mL) and **(d)** PEG-PDL, PEG-PDL-$OVP_{0.3}$, PEG-PDL-$DOX_{0.1}$ and PEG-PDL-$OVP_{0.3}$-$DOX_{0.1}$ micelles, **(e)** PEG-PS, PEG-PS-$OVP_{0.3}$, PEG-PS-$DOX_{0.1}$ and PEG-PS-$OVP_{0.3}$-$DOX_{0.1}$ micelles, and **(f)** PEG-PDLLA, PEG-PDLLA-$OVP_{0.3}$, PEG-PDLLA-$DOX_{0.1}$ and PEG-PDLLA-$OVP_{0.3}$-$DOX_{0.1}$ micelles for 48 h; treatment group concentrations refer to the micelle and DOX concentrations if present. Data are shown as the mean metabolic activity (%) ± SD (n = 3); NS (not significant), *, ** and *** stands for $p > 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Application of oligoelectrolyte-mediated, pH-triggered release to various drug classes

The scope of the oligoelectrolyte-mediated, *pH*-triggered release strategy to different drug classes was further demonstrated with a selection of chemotherapy drugs, including highly cytotoxic drugs that are typically limited in their application due to their extremely poor water solubility.[68, 69] Using the PEG-PCL-$OVP_{0.3}$ micelle platform, gossypol (GP), paclitaxel (PX) and 7-ethyl-10-hydroxycamptothecin (SN38) drug loaded micelles were prepared, and the *pH*-triggered release of drugs was monitored using the previously described dialysis assay (**Figure 5**). In the absence of OVP, the drug EE% for the PEG-PCL-$GP_{0.1}$, PEG-PCL-$PX_{0.1}$ and PEG-PCL-$SN38_{0.1}$ micelles was ≥ 94 % (SI, **Table S7** and **S9**, and **Figures S26**–**31**). In comparison, the co-encapsulation of OVP resulted in a drug-dependent decrease in the drug EE% values to 90, 86, and 72% for the PEG-PCL-$OVP_{0.3}$-$GP_{0.1}$, PEG-PCL-$OVP_{0.3}$-$PX_{0.1}$ and PEG-PCL-$OVP_{0.3}$-$SN38_{0.1}$ micelles, respectively, which was accompanied by OVP EE% values of 84, 83 and 77%, respectively.

In general, GP, PX and SN38 were observed to release slowly from the non-responsive PEG-PCL micelles independent of *pH*, with ~ 5-10% release at *pH* 7.4 over 2 h and a continued gradual leakage over 7 h at *pH* 4.5. In comparison, the co-encapsulation of OVP with the drugs resulted in a slightly higher release of the drugs (10–20%) at *pH* 7.4 consistent with a slightly lower EE%. However, a switch in the *pH* to 4.5 resulted in a significantly faster release of the drugs (**Figure 5** and SI, **Figure S32**). For the PEG-PCL-$OVP_{0.3}$-$GP_{0.1}$ micelles, a rapid release of GP was observed at *pH* 4.5, similar to the DOX release profiles (**Figure 4a**). In comparison, the apparent release of PX and SN38 from PEG-PCL-$OVP_{0.3}$-$PX_{0.1}$ and PEG-PCL-$OVP_{0.3}$-$SN38_{0.1}$ micelles appeared more gradual, although still significantly accelerated as compared to micelles held at *pH* 7.4 (**Figure 5**). While more detailed studies are required to understand the intricate differences in the release profiles of different drugs from the OVP-loaded micelles, it is evident that as a general concept, OVP-mediated, *pH* triggered release can be broadly applied to different drug classes.

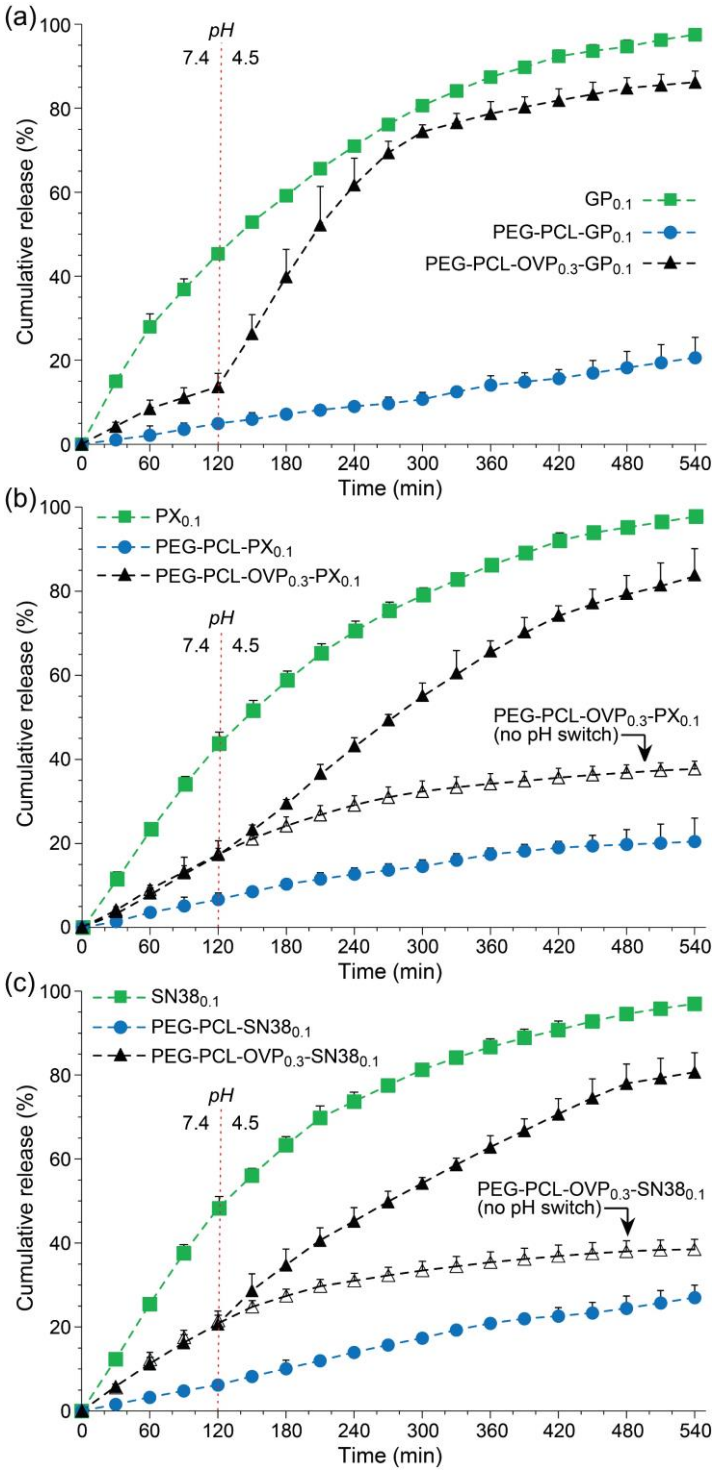


Figure 5: *In vitro* release of **(a)** GP, **(b)** PX and **(c)** SN38 from PEG-PCL and PEG-PCL-$OVP_{0.3}$ micelles and as the free drugs using a dialysis assay against PBS (10 mM, *pH* 7.4) for the first 2 h followed by acetate buffer (10 mM, *pH* 4.5) for 7 h; experiments were conducted at 22 ± 1 °C and the drug release was measured *via* UV-vis spectrophotometry (GP and SN38) or high-performance liquid chromatography (PX). **(C)** Release from PEG-PCL-$OVP_{0.3}$-$PX_{0.1}$ and PEG-PCL-$OVP_{0.3}$-$SN38_{0.1}$ at *pH* 7.4 also shown without *pH* switch after 2 h. Data are shown as the average cumulative release (%) + SD (n = 3); some error bars are smaller than the symbols.

Tuning the pH of oligoelectrolyte-mediated, pH-triggered release

The release of drugs from *pH*-sensitive micelles is typically dependent on the environmental *pH*. While the *pH* of the endosome gradually becomes more acidic as it matures prior to fusion with the lysosome,[70, 71] there is also a significant difference in the endosomal *pH* ranges across different types of cells and tissues.[72, 73] To ensure endosomal release of payloads from *pH*-sensitive micelles prior to fusion with the lysosome the micelle should ideally be responsive as soon as the *pH* becomes acidic in the early endosome. Therefore, micelle platforms with tunable *pH*-responsivity are highly desirable, particularly if they can respond to mildly acidic conditions (e.g., *pH* 5.5–6.5) without significant loss of their payload during circulation in the blood at *pH* 7.4. Although the OVP-loaded micelles clearly demonstrate the oligoelectrolyte-mediated, *pH*-triggered release concept, the release kinetics and responsivity are closely correlated to the OVP pK_a (~5). While rapid release of the encapsulated payload along with the release of OVP is observed at *pH* 4.5, no triggered release is noted at *pH* 5.5 and 5 (SI, **Figure S33**). Nevertheless, it was hypothesized that the concept could be extended to other oligoelectrolytes and that the *pH*-triggered release could be tuned through selection of oligoelectrolytes with appropriate pK_a values.

To test this hypothesis we synthesized oligo(2-vinyl benzimidazole) (OVB; $M_{n,\mathrm{NMR}}$ = 1.8 kDa, $Đ$ = 1.5, RU_{ave} = 11) (SI, **Figures S34**–**39**), which would be expected to have a pK_a value of ~6 when considering structurally related benzimidazoles.[74] The OVB was then encapsulated in PEG-PCL micelles along with DOX to afford PEG-PCL-$OVB_{0.3}$-$DOX_{0.1}$ micelles, with an EE% of 81.2 ± 0.4 and 80.4 ± 0.8% for DOX and OVB, respectively. In dialysis assays, the PEG-PCL-$OVB_{0.3}$-$DOX_{0.1}$ micelles provided triggered release of the DOX when the *pH* was switched from 7.4 to 5.5 (**Figure 6a** and SI, **Figure S40**), as compared to the slow leakage of DOX observed for control PEG-PCL-$DOX_{0.1}$ micelles. In cytotoxicity assays with SKOV-3 cells, the PEG-PCL-$OVB_{0.3}$-$DOX_{0.1}$ micelles outperformed the PEG-PCL-$DOX_{0.1}$ micelles with significantly lower metabolic activities (increased cytotoxicity) across the concentration range studied (**Figure 6b**). These results clearly demonstrate the *pH* tunability of the oligoelectrolyte mediated drug release concept.

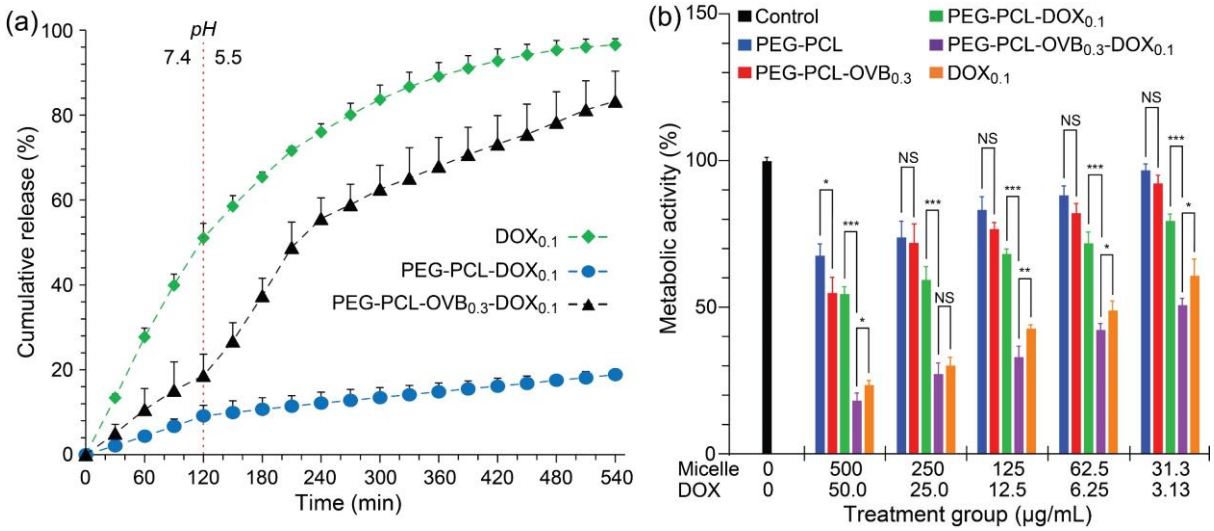


Figure 6: **(a)** *In vitro* DOX release from PEG-PCL-$DOX_{0.1}$ and PEG-PCL-$OVB_{0.3}$-$DOX_{0.1}$ micelles, and free DOX using a dialysis assay against PBS (10 mM, *pH* 7.4) for the first 2 h followed by citrate buffer (10 mM, *pH* 5.5) for 7 h; experiments were conducted at 22 ± 1 °C and the DOX release was measured *via* UV-vis spectroscopy. Data are shown as the average cumulative release (%) ± SD (n = 3). **(b)** *In vitro* metabolic activity assay with SKOV-3 cells when untreated (negative control) and treated with free DOX (3.13 −50.0 μg/mL) and the micelles (PEG-PCL, PEG-PCL-$DOX_{0.1}$, PEG-PCL-$OVB_{0.3}$ and PEG-PCL-$OVB_{0.3}$-$DOX_{0.1}$) for 48 h; treatment group concentrations refer to the micelle concentration and DOX concentration if present. Data are shown as the mean metabolic activity (%) ± SD (n = 3); NS, *, ** and *** stands for $p > 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Conclusions

In this study, we demonstrate the novel concept of oligoelectrolyte-mediated, *pH*-triggered drug release from traditionally non-responsive polymeric micelles and how this strategy can be employed to significantly enhance the cytotoxicity of the micelles. Oligoelectrolytes, such as oligo(2-vinyl pyridine) were readily encapsulated in the hydrophobic core of polymeric micelles at physiological *pH* and retained the ability to respond to changes in the environmental *pH*. The encapsulation of oligoelectrolytes resulted in a slight increase in the micelle size and polydispersity index, but also retarded the formation of secondary aggregates. A decrease in the *pH* below the pk_a of the oligoelectrolyte triggered its abrupt release from the micelle core due to ionisation and increasing hydrophilicity. When co-encapsulated with hydrophobic drugs, the release of the oligoelectrolyte mediated the simultaneous burst release of the drug. This behaviour was observed for a range of traditional polymeric micelles prepared from different non-responsive block copolymers, and for different drug classes, demonstrating the versatility and broad applicability of this approach. Furthermore, the *pH* of drug release could be tailored through the selection of oligoelectrolyte. *In vitro* cell studies revealed that co-encapsulation of oligoelectrolytes and drugs resulted in significant increases in the cytotoxicity of micelles to cancer cell lines as compared to the standard drug-loaded micelles or the free drugs. This increase in cytotoxicity not only results from more efficient intracellular drug release, but the ability of the released oligoelectrolyte to mediate endosomal escape of the drugs, although further detailed studies are required to establish the exact mechanism of this process. In comparison, the oligoelectrolyte- and drug-loaded micelles displayed significantly higher metabolic activities with healthy cell lines, indicating selective cytotoxicity to cancer cell lines, while the drug-loaded micelles in the absence of oligoelectrolytes were equally cytotoxic to both healthy and cancerous cell lines. In summary, these findings provide a proof-of-principle for a facile, adaptable, and versatile approach to enhance drug release from traditional micelles and significantly increase their cytotoxicity, without the need for the synthesis of specialized *pH*-responsive copolymers. Nevertheless, further studies are required to evaluate if the exceptional performance observed *in vitro* translates *in vivo*, and the potential therapeutic benefits this novel approach may provide.

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Supporting Information available

Experimental details, including materials, instrumentation, methods and procedures. Characterisation of OVP (**Figure S1**) and the $PEG_{10}PCL_{10}$ block copolymer (**Figure S2** and **Table S1**), including ^{1}H NMR spectroscopy, GPC and DSC data. Characterisation of the PEG_4PCL_4 (**Figures S19-20**), PEG-PDL (**Figure S22**), PEG-PS (**Figure S23**) and PEG-PDLLA (**Figure S24**) block copolymers, including ^{1}H NMR spectroscopy and GPC data. NMR spectra (**Figures S34-38**) for the synthesis of OVB and its precursors, and the GPC DRI chromatogram of OVB (**Figure S39**). Fluorimetry data for determination of micelle CMC (**Figure S3**). DLS PSDs (**Figures S4-5** and **S14**), PDI values (**Table S4**) and D_h (**Figure S6**) of the micelles. SAXS scattering profiles of micelles and model fitting (**Tables S5-6**). UV-vis spectra (**Figure S9**) and NMR spectra (**Figures S10-11**) of micelles as a function of pH. Fluorimetry of labelled micelles as a function of pH (**Figure S21**). Concentrations and volumes of stock solutions to prepare micelles for CMC (**Table S2**), EE% (**Table S7**) and drug release (**Table S8**) studies. Concentrations and volumes of stock solutions for pH adjustment of micelle solutions (**Table S3**). EE% values for drug-loaded micelles (**Table S9**). UV-vis spectra and calibration curves for OVP (**Figures S7** and **S13**) and drugs (**Figures S12**, **S15**, **S26-31**). OVP release curves from micelles (**Figure S8**) and UV-vis spectra of receiving solutions (**Figure S16**). Drug release curves (**Figure S33**) and first derivative drug release curves from micelles (**Figures S17**, **S25**, **S32** and **S40**). *In vitro* metabolic activity assay with fibroblasts (**Figure S18**).

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