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Alginate hydrogels functionalized with β-cyclodextrin as a local paclitaxel delivery system

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Abstract

Modification of drug delivery materials with beta-cyclodextrins (β-CyD) is known to increase solubility of poorly water-soluble drugs, protect drugs from degradation and sustain release. In this study, we developed a hydrogel drug delivery system for local paclitaxel delivery using the natural polysaccharide alginate functionalized with β-CyD-moieties. Paclitaxel was chosen due to its ability to form inclusion complexes with cyclodextrins. The rheological and mechanical properties of the prepared hydrogels were characterized, as well as in vitro release of the paclitaxel and in vitro activity on PC-3 prostate cancer cells. Introduction of β-CyD-moieties into the hydrogel reduces the mechanical properties of the gels compared to nonmodified gels. However, gelation kinetics were not markedly different. Furthermore, the β-CyD-modified alginate helped to reduce undesired crystallization of the paclitaxel in the gel and facilitated paclitaxel diffusion out of the gel network. Remarkably, the β-CyD grafted alginate showed increased capacity to complex paclitaxel compared to free HPβ-CyD. Release of both paclitaxel and degradation products were measured from the gels and were shown to have cytotoxic effects on the PC-3 cells. The results indicate that functionalized alginate with β-CyDs has potential as a material for drug delivery systems.

KEYWORDS
alginate, cytotoxicity, drug delivery system, hydrogel, paclitaxel, β-cyclodextrin

1 | INTRODUCTION

Local drug delivery gives an advantage compared to systemic therapy due to increased drug concentration at delivery site, less detrimental systemic side effects, and ease of administration.[1] Local drug delivery strategies include micro- and nanocarriers, implants, and injectable depots. Injectable systems are advantageous due to their minimally invasive delivery, capacity to fill the cavity they are placed in, and ability to form a drug-loaded depot that can protect payload from enzymatic degradation and sustain drug release over a long period of time.[2] Many injectable in situ forming hydrogels have been developed, and their superior efficacy in animal models over conventional systemic therapy has been demonstrated.[3] As an example, an injectable hydrogel has been used for local delivery of paclitaxel for the treatment of glioblastoma in mice.[4] To design an efficacious hydrogel-based injectable system many parameters should be controlled, including biocompatibility, mechanical, and viscoelastic properties.[3]

Alginate, a polysaccharide found in brown algae, is an excellent candidate for an injectable hydrogel system. The polymer has the ability to form a hydrogel by crosslinking with divalent ions at

physiological conditions, and the sol/gel transition is gentle enough that cells and biomolecules will be functional after entrapment in the hydrogel.[5] Furthermore, alginate can be tailored to present different functional groups[6-10] and the mechanical properties can be adjusted.[11-13] In addition, the polymer is biocompatible and non-toxic.[14] Different approaches for using alginate as an injectable delivery system are currently being investigated, often in combination with other polymers or components in the quest to achieve optimal delivery capability.[15-20]

The hydrophilic nature of the hydrogel presents certain challenges for drug delivery.[21,22] Small water-soluble molecules will often quickly diffuse out of the hydrogel matrix, giving a high burst release. On the other hand, hydrophobic molecules can be difficult to load into the hydrogel and their release in a controlled manner also possesses a challenge. To gain better control over the loading and release of therapeutic agents from a hydrogel, cyclodextrins (CyDs) can be incorporated into the gel.[23,24]

CyDs are well known cyclic structures built from glucopyranose units produced by enzymatic treatment of starch.[25] Due to the cyclic structure of the formed CyD molecules, the cavity of the ring structure is hydrophobic while the exterior is hydrophilic. The cavity can then give a local environment in the hydrogel that is better suited for entrapment of hydrophobic molecules and moieties.[24,26,27]

Paclitaxel is a highly hydrophobic anticancer drug that can form inclusion complexes with CyDs, including β-CyD.[28,29] It should be noted however that paclitaxel has a limited penetration into the hydrophobic cavity of β-CyD and the obtained inclusion complex is characterized by a low binding constant.[29,30] Given intravenously, paclitaxel is not specifically targeted to the tumor, and thus only low therapeutic levels of the drug can be achieved at the tumor site.[31,32] Moreover, paclitaxel has been shown to have poor penetration across the blood–brain-barrier in preclinical models.[31,33] Hence, paclitaxel use in treatment of brain tumors and brain metastases is limited, although it was shown to be active against various tumor types.[33] Interestingly, it has been shown recently that in addition to the antiproliferative activity, paclitaxel has a capacity to promote antitumor immunity.[34] This makes paclitaxel a very promising candidate for drug delivery systems. To enhance the therapeutic potential of paclitaxel several delivery strategies and formulations of paclitaxel have been developed,[35,36] including only a few injectable hydrogel systems based on alginate.[37,38]

CyDs have previously been grafted to alginate by attachment to the carboxyl- or OH-group,[39-46] often for drug delivery applications. Examples include photoresponsive CyD-grafted alginate hydrogels where UV-light can be used to control the inclusion complex between CyDs and azobenzene moieties, giving control over gel morphology and subsequent release from the gel,[39] release of the antiemetic drug ondansetron by mechanical stimulation of a cyclodextrin-crosslinked alginate gel,[41] and release of the poorly soluble anti-inflammatory agent hydrocortisone acetate from β-CyD grafted alginate hydrogels.[43]

We have recently shown that functionalization of alginate hydrogels with β-CyD moieties allows to slow down the release rate of a small model compound methyl orange.[9] Based on this finding, we hypothesized that with the β-CyD-grafted alginate, it might be possible to modulate the release of paclitaxel. While numerous studies have investigated the entrapment of paclitaxel in hydrogel systems, to the best of our knowledge, this study for the first time describes a paclitaxel delivery system based on alginate hydrogels functionalized with β-CyDs. In this study, we focused on, first, the development of an injectable hydrogel system and characterized it with respect to rheological and mechanical properties. Second, in vitro release of the paclitaxel and in vitro cytotoxicity toward PC-3 prostate cancer cells was studied.

2 | MATERIAL AND METHODS

2.1 | Materials

Alginate (Alg) from *Laminaria hyperborea* stipe ($F_G = 0.7$, $N_{G>1} = 11$, $\bar{M}_w = 126$ kDa) was obtained from FMC Health and Nutrition, Sandvika, Norway. For cell studies, ultrapure stipe alginate from Novamatrix™, Norway (UPLVG, $F_G = 0.67$, $N_{G>1} = 13$, $\bar{M}_w = 221$ kDa) was used. 6-monoazido-6-monodeoxy-β-cyclodextrin was kindly provided from Aalborg University.[47] The oxidized and grafted alginate materials were made in-house from stipe alginate and ultrapure stipe alginate at NTNU as previously described.[9] The following materials were synthesized for this work: partially oxidized alginate (POA, $P_O = 8\%$, $\bar{M}_w = 102$ kDa), POA grafted with β-CyD (POAβCyD, DS = 3.7% mol CyD/mol monomer, $\bar{M}_w = 55$ kDa). For the cell studies, POA ($\bar{M}_w = 127$ kDa) and POAβCyD (DS = 3.4% (mol/mol), $\bar{M}_w = 61$ kDa) were produced from UPLVG. Hydroxypropyl-β-cyclodextrin (HPβ-CyD, CAVASOL® W7 HP) and β-CyD (CAVAMAX® W7) were obtained from Wacker Chemie AG (Burghausen, Germany). Physiological buffer was made according to literature,[48] without bovine serum albumin. High glucose Dulbecco's Modified Eagle Medium without sodium pyruvate (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, phosphate buffered saline pH 7.4 (PBS), 0.25% Trypsin–EDTA solution were from Sigma-Aldrich. AlamarBlue™ cell viability reagent was from Invitrogen. All the other chemicals used were obtained from commercial sources and were analytical or high-performance liquid chromatography (HPLC) grade and were used without further purification.

2.2 | Methods

2.2.1 | Preparation of hydrogels

The calcium alginate hydrogels were prepared (with different ratios of unmodified alginate mixed with modified alginates) by internal gelation by mixing alginate solution, $CaCO_3$ ($d = 4$ μm) and the slowly hydrolyzing glucono-δ-lactone (GDL, freshly prepared), as previously reported.[49] After addition of the $CaCO_3$ alginate solutions were degassed to prevent air bubbles in the hydrogel. The final

concentration of components in the gelling solution was 1% (w/v) alginate, 15 mM $CaCO_3$, and 30 mM GDL. The hydrogels for the cell studies were made differently as described in the methods section detailing the cell experiments.

2.2.2 | Gelation kinetics

In addition to testing a stipe alginate sample (Alg), a 1:1 (w/w) mixture of the stipe alginate and POA (Alg/POA [1:1]) were tested. Within 3 min after dissolution of GDL (at time point zero), an aliquot of the sample was applied onto the lower plate and gelation was followed by Kinexus Rheometer (Malvern instruments, Uppsala, Sweden) using a 2 cm flat probe and a flat plate geometry with 0.1 mm gap, 0.005 strain, and 0.5 Hz frequency. The gelation kinetics was determined by repeated measurement of storage modulus G', loss modulus G'', and phase angle δ at 1-min intervals within the first 20 min and at 5 min intervals for approximately 5 hr. At least two repetitions were done for each sample.

2.2.3 | Syneresis and compression measurements

Young's modulus, stress at rupture, deformation at rupture and syneresis of the Alg, Alg/POA (1:1), a 1:3 (w/w) mixture of the stipe alginate and POA (Alg/POA [1:3]), and a 1:1 (w/w) mixture of the stipe alginate and POAβCyD (Alg/POAβCyD (1:1)) samples were assessed. Additionally, since the molecular weight of monomers with attached CyDs is higher than for ungrafted monomers, we evaluated a 1:0.8 (w/w) mixture of the stipe alginate and POA (Alg/POA [1:0.8]) sample which contained the same molar amount of alginate units as a Alg/POAβCyD (1:1) sample. Hereafter, a freshly made GDL solution was added to the alginate mixture to initiate gelation, the mixture was immediately aliquoted into silicone forms (1.5 mL/well) and left to mature for 1 day. The next day gel cylinders were carefully wiped down to remove separated water and mass measured. Syneresis was determined as $100 \times (W_0 - W)/W_0$, where W_0 and W are initial and final weights of the cylinders, respectively. Diameter and height of the cylinders were measured with digital calipers prior to compression measurements. The response of the gels to uniaxial compression was evaluated using a Stable Micro Systems TA-XT2 texture analyzer equipped with a P/35 probe, 5 kg pressure capacity load cell, and at a compression rate of 0.1 mm/s and 1 g trigger force. The stress deformation curves were recorded at room temperature.[50] Young's modulus (Pa) was calculated as $G \times (h/A)$, where G is the initial slope (N/m) of the curve, h is the height of the cylinder (m), and A is the area (m^2) of the cylinder. At least four cylinders of each gel type were measured.

2.2.4 | Release studies of paclitaxel from Ca-alginate gels into physiological buffer

The alginate hydrogels were loaded with 25 μM paclitaxel and release of paclitaxel was studied in the physiological buffer. Five different 1% (w/v) alginate solutions were prepared for gelation: Alg, Alg/POAβCyD (1:1) containing 0.8 mM β-CyD residues, Alg/POAβCyD (1:3) containing 1.1 mM β-CyD residues, Alg + free β-CyD (0.9 mM β-CyD), and Alg + free HPβ-CyD (7.2 mM HPβ-CyD). The solutions were divided into 1.5 mL polypropylene tubes. Paclitaxel dissolved in ethanol was mixed into the solutions to a final concentration of 25 μM paclitaxel, 2.5% (v/v) ethanol, and an end volume of 200 μL of gel (paclitaxel was added after mixing the solution with $CaCO_3$ and degassing but before adding GDL). Three parallels of each sample were made. Samples were left to gel at room temperature for 23 hr (day 0). Physiological buffer of 300 μL was then added, and gels were incubated at 37°C.

After 1, 3, 6, 9, 15, and 21 days, 200 μL supernatant was removed and pictures of the gels were taken with light microscopy (40× magnification). Thereafter, 200 μL fresh physiological buffer was again added to the gel-vials. The 200 μL supernatant was diluted in acetonitrile (1:1). Internal standard docetaxel in the samples was 5 μM. Gels were weighed and dissolved in 400 μL EDTA (100 mM) overnight after sample collection at day 21. The dissolved gels were then diluted with acetonitrile (1:1 the weight of the gels was taken into account), and internal standard docetaxel was added to a final concentration of 5 μM. All samples were stored at −20°C. Samples were thawed and centrifuged for 15 min at 23,000×g before analyzed with ultra-high performance liquid chromatography coupled to tandem quadrupole mass spectrometry using an ACQUITY I-class UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA) (Appendix S1).

2.2.5 | Stability of paclitaxel

For the stability studies, 195 μL physiological buffer was mixed with 5 μL paclitaxel dissolved in ethanol (375 μM). This gave 9.4 μM paclitaxel aqueous solutions with 2.5% (v/v) ethanol as a starting point. This was done to mimic the making of the hydrogels were 195 μL was water containing alginate and gelling components, while 5 μL of paclitaxel dissolved in ethanol was mixed in to give a final volume of 200 μL. physiological buffer or water was used as the aqueous medium and left at 37°C for 0, 1, 3, 6, 9, 15, or 21 days. Samples were then diluted 1:1 with acetonitrile and stored in freezer. Samples with physiological buffer were then centrifuged (15 min 23,000×g), and the supernatant analyzed with liquid chromatography coupled to tandem quadrupole mass spectrometry (Appendix S1). Samples with water were directly analyzed without further sample preparation.

2.2.6 | Cell line and subculture

PC-3 human prostate carcinoma cell line (ATCC® CRL-1435™) was chosen as a model cells for in vitro studies. Cells were cultured in the DMEM supplemented with 10% FBS and 1% (v/v) penicillin–streptomycin at 37°C and 5% CO_2, 95% air, and complete humidity. Cells were maintained in exponential growth phase until they were

detached by trypsin–EDTA solution, counted, and seeded into experimental plates/flasks.

2.2.7 | Cytotoxicity of β-CyD-grafted alginate

The test solutions of 40 mg/mL of POAβCyD and the solution of free β-CyD (13.2 mM) in 40 mg/mL POA were prepared in PBS supplemented with 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and sterilized through 0.2 μm syringe PES filters prior use. Nine 1:1 serial dilutions of the solutions were then made in 40 mg/mL POA to ensure equal concentration of alginate in all tested solutions after serial dilutions.

PC-3 cells were seeded in a concentration of 5×10^5 cells into each well of 96-well clear bottom TC-treated black plates (100 μL/well) and incubated at 37°C overnight. After 24 hr, old medium was replaced with 50 μL/well of nine 1:1 serial dilutions of the test solutions. PC-3 cells were incubated with the test solutions for 2 hr, after which the plates were centrifuged at 150×*g* for 5 min. Then, the tested solutions were carefully aspirated and 100 μL of fresh culture medium was added. The plates were incubated for additional 48 hr and cell metabolic activity was assessed using resazurin assay. Maximal tested concentrations were 13.2 mM of free β-CyD and 40 mg/mL of POAβCyD (5.65 mM of β-CyD-moieties). The 40 mg/mL POA a solution was used as a control. All the samples and controls were run in four replicates.

2.2.8 | Hydrogels for cell studies

Hydrogels based on ultrapure Alg and on Alg/POAβCyD (1:1) were prepared for cell studies under sterile conditions. Hydrogels contained 2% (w/v) alginate, 30 mM $CaCO_3$, 60 mM GDL, and paclitaxel. Ultrapure alginate solutions and GDL solutions were sterilized through 0.2 μm syringe PES filters. $CaCO_3$ particles (0.7 μm) were autoclaved at 121°C for 30 min and then suspended in a sterile deionized water. Paclitaxel was pre-dissolved in ethanol. For burst release studies, hydrogels ($V = 200$ μL) were loaded with 25 μM paclitaxel. For the cytotoxicity studies, hydrogels ($V = 50$ μL) were loaded with 0, 0.9, 7.2, and 25 μM paclitaxel. After fabrication, the gels were left to gel at 4°C for at least 1 day.

2.2.9 | Burst release in culture medium

PC-3 cells were seeded in a concentration of 3200 cells/well into 96-well plates (50 μL/well) and incubated at 37°C overnight. Next day, the 200 μL gels containing 25 μM paclitaxel were incubated with 300 μL of DMEM supplemented with 10% FBS at 37°C for 1 hr. After 1 hr, the medium was aspirated, diluted (six 1:1 serial dilutions), and added to the PC-3 cells (50 μL/well). After 72 hr, PC-3 cells metabolic activity was assessed using resazurin assay. The obtained values of cell metabolic activity were used to determine concentration of the released drug. To generate a standard curve, 0–200 nM paclitaxel solutions in media were used (Figure A1, Supporting Information S1). All the samples and controls were run in triplicates.

2.2.10 | Cytotoxicity of paclitaxel-loaded hydrogels

PC-3 cells were seeded in a concentration of 1×10^5 cells into each well of 6-well plates (3 mL/well) and incubated at 37°C. After 24 hr, 1.45 mL of fresh medium and 50 μL hydrogels (or free paclitaxel solution) were added into each well of PC-3 seeded plates, and plates were incubated at 37°C for 72 hr. After 72 hr, cell metabolic activity was assessed using resazurin assay. Hydrogels contained 0, 0.9, and 7.2 μM of paclitaxel. Free paclitaxel (final concentrations in the plate were 1, 5, 10, 40, and 80 nM) was used as a positive control. Drug-free medium and the hydrogel without the drug were used as negative controls. All the samples and controls were run in triplicate. Gels were examined by optical microscopy before and after experiment.

For the gels loaded with 25 μM paclitaxel, 300 cm^2 tissue culture treated flasks were used and the total (volume of medium was increased up to 125 mL). PC-3 cells were seeded in a concentration of 3×10^6 cells into 300 cm^2 tissue culture treated flasks (50 mL/flask) and incubated at 37°C overnight. Next day, 75 mL of fresh medium and 50 μL of gels loaded with 25 μM paclitaxel were added into the flasks. Cells were incubated with gels at 37°C for 72 hr and cell metabolic activity was assessed using resazurin assay. Drug-free medium was used as a negative control. All the samples and controls were run in triplicates. Gels were examined by optical microscopy before and after experiment.

2.2.11 | Resazurin cell metabolic activity assay

Cells were incubated with 50% diluted AlamarBlue™ reagent at 37°C for 1–8 hr, as it is recommended by a supplier. Then, 100 μL aliquots were transferred into black 96-well plates with clear bottom and fluorescence was read with Spectra Max i3x (Molecular Devices) at 560 nm excitation and 600 nm emission wavelengths. Cell metabolic activity was expressed as percentage of fluorescent intensities relative to a control (cells not exposed to paclitaxel were defined as 100%) after subtraction of non-cell-derived background.

2.2.12 | Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 365 or SigmaPlot Version 14.0 (Systat Software Inc., CA) and differences between groups were compared applying a two-tailed *t*-test. The significance level was set at 0.05. Differences between more than two groups were tested using one-way (or two-way when necessary) analysis of variance (ANOVA). After ANOVA, the Tukey's post hoc test or a two-tailed *t*-test was used for comparison. All results are expressed as means ± *SD*.

3 | RESULTS AND DISCUSSION

We have previously shown that CyDs grafted to alginate were able to interact with the model compound methyl orange, a small guest molecule that can form inclusion complexes with β-CyD.[9] This new study relies on the hypothesis that introduction of β-CyD moieties into alginate mediates the uptake of highly hydrophobic drugs like paclitaxel and their release from the alginate hydrogel. For this study the alginate was functionalized with β-CyD moieties in a three-step synthesis as previously described.[9] The partial oxidation of alginate performed in the first step is known to lead to noticeable structural changes, such as partial depolymerization and ring opening (cleavage of the C2–C3 bonds).[11,51-53] Such changes in the alginate structure have been shown to affect the gelation capability of the polymer and mechanical properties of the hydrogels fabricated from this polymer.[11,12,40,54] Therefore, we conducted a series of experiments to characterize gelation kinetics and mechanical properties of the hydrogel system based on the modified alginate.

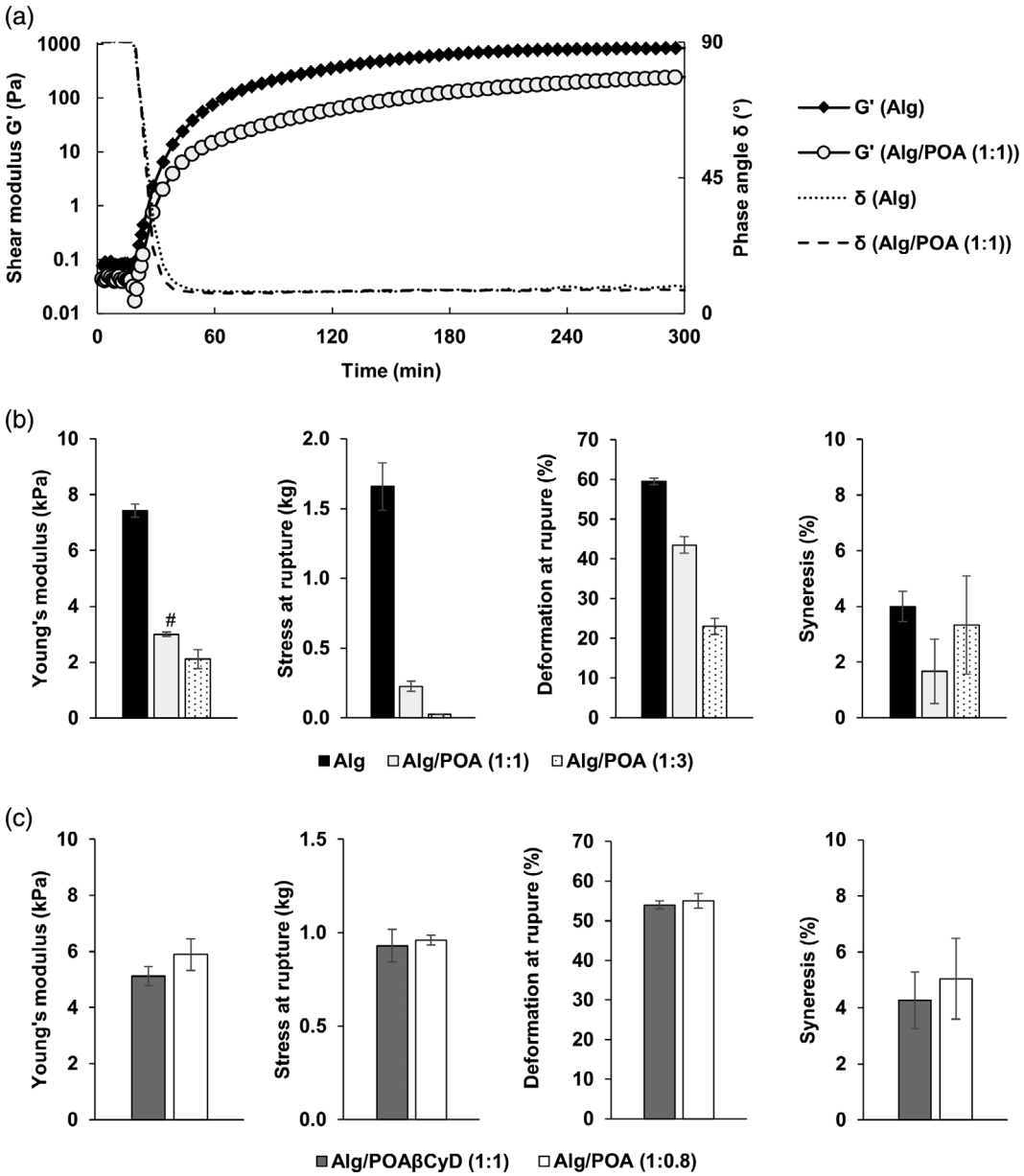

FIGURE 1 Gelation kinetics and mechanical properties of the 1% (w/v) calcium-unsaturated alginate hydrogels. (a) Gelation kinetics of Alg and Alg/POA (mean, $n = 3$). Young's modulus, rupture strength, deformation at rupture and syneresis of the Alg and Alg/POA hydrogels: (b) Effect of 1:1 and 1:3 (w/w) mix of Alg/POA on properties of the hydrogels (mean ± *SD*, $n = 4–8$); (c) Comparison of the mixed hydrogels containing equal number of unmodified alginate monomers (mean ± *SD*, $n = 4–8$). #—it was not possible to measure dimensions of the gels due to extreme softness of the Alg:POA (1:3) mixed gels, therefore theoretical area of the cylinders was used to calculate Young's modulus

3.1 | Gelation kinetics and mechanical properties of Ca-alginate hydrogels

The well-established system of alginate gelation, using slowly releasing calcium ions from $CaCO_3$ particles induced by the slow release of H^+ from GDL, was used to allow for slow gelation of alginate.[55] Using this technique, it is possible to make soft alginate gels with homogeneous and reproducible structure at physiological conditions, while the end products of the reaction, namely CO_2 and D-glucuronic acid, are nontoxic.[49] Importantly, this method allows to have control over gelation kinetics of alginate by varying the release rate of calcium ions from the calcium carbonate particles. The size of $CaCO_3$ particles have previously been shown to largely influence the gelation kinetics.[49] For an injectable hydrogel, the gelation time will influence how easy it is to inject the gel, as well as its propensity to gel at the injection site instead of leakage before the gel network can form.[56,57]

The rate of gelation was followed by oscillation rheometry showing similar values for the sol–gel transition time for the gels with stipe alginate only (Alg) and in 1:1 w/w mix with oxidized alginate (Alg/POA (1:1)) (25.1 ± 2.8 min and 24.3 ± 2.4 min, respectively) (Figure 1a). Hence the gelation kinetics seems not to be influenced when mixing in the modified alginate. The sol–gel transition time in the studied conditions was about 25 min, but it can be significantly extended/reduced by minor modification of the gelling solution composition to fulfill time-requirements for systemic administration. As expected, the storage modulus (G'_{max}) of the Alg/POA (1:1) mixed gel was significantly lower than for the Alg hydrogels: 266 ± 17 Pa for (1:1) mixture and 875 ± 108 Pa for the unmodified alginate. The pronounced difference in storage modulus reflects the reduced capacity of the oxidized material to form ionic crosslinks which has also been shown previously, where the storage modulus was measured for 12 hr.[12]

To further investigate the mechanical properties of the formed gels, the gels were cast in cylinders and mechanical properties investigated by compression measurements (Figure 1b). The Ca-unsaturated hydrogels casted from Alg maintained their shape upon handling, whereas introduction of POA resulted in softer and weaker gels. Gel elasticity (Young's elastic modulus), deformation and stress at rupture, as well as volume reduction upon gel formation (syneresis) of the formed hydrogels were measured in a series of compression tests. Increase in POA content showed a clear trend toward weaker hydrogels with lower Young's modulus, stress at rupture, deformation at rupture, and syneresis compared to the gels made from unmodified alginate alone. This trend was previously also observed for the same type of mixed Alg/POA gels for Ca-saturated hydrogels.[12] To see if introduction of β-CyD would influence the mechanical properties relative to the oxidized sample, two samples containing equal molar ratio of uronic acid residues between stipe alginate and modified alginates (POA or POAβCyD) were prepared (Figure 1c). Mechanical properties of Alg/POAβCyD (1:1) gels (E = 5.1 kPa, stress at rupture = 0.93 kg) were not significantly different from the Alg/POA (1:0.8) gels (E = 5.9 kPa, stress at rupture = 0.96 kg), indicating that introduction of linker and β-CyD moieties onto POA did not significantly change gel-forming properties of the material for the Ca-unsaturated hydrogels. When comparing the Alg/POAβCyD (1:1) gels with the Alg/POA (1:1) gels (E = 3 kPa, stress at rupture = 0.23 kg), the stress needed to rupture the Alg/POA gels were lower compared to the grafted gels. The opposite is observed for Ca-saturated gels[12] where Young's modulus decreased for Alg/POAβCyD gels (1:1, ~15 kPa) compared to Alg/POA gels (1:1, 24 kPa), while the stress at rupture was approximately the same (~1 kg). This was also observed for mixed hydrogels grafted with other substituents than β-CyD. This indicates that for Ca-unsaturated gels Young's modulus for mixed Alg/POAβCyD gels is not largely affected by grafting, but for Ca-saturated gels the covalent coupling of β-CyD affects the network formation/rearrangement due to steric effects, and may hinder optimal interaction with Ca-ions for the crosslinking units of the alginate.

3.2 | Loading of paclitaxel into alginate gels

To load paclitaxel into alginate hydrogels, the drug was solubilized in ethanol and mixed with alginate solution before gelation was initiated. All the loaded drugs were maintained within the gel, hence, the final concentration of paclitaxel in the gels was 25 μM. The strategy of internal gelation was chosen as this has been shown to give homogeneous distribution of polymer in the gels.[49] Similarly, it was assumed that homogenous distribution of paclitaxel within the gels would also be achieved in addition to facilitate formation of inclusion complexes between the drug and POAβCyD. Here, precipitation of paclitaxel was utilized as an indirect indicator of inclusion complex formation.

Crystallization of paclitaxel is known to occur in both aqueous solution and hydrogels[58] and was clearly observed in the alginate gels (Figure 2). The Alg/POAβCyD (1:1) and Alg/POAβCyD (1:3) hydrogels loaded with 25 μM of paclitaxel showed variable crystallization of the drug (Figures 2 and A2, Supporting Information S1): Paclitaxel crystals were present in the 1:1 hydrogels, whereas very little crystal formation was observed for the 1:3 gels. The resulted paclitaxel:β-CyD molar ratios were 0.03 and 0.02 for 1:1 and 1:3 gels, respectively. This indicates that a paclitaxel:β-CyD molar ratio of 0.02 or lower should be used to prevent paclitaxel crystallization in the alginate hydrogels. Crystals of paclitaxel were also present in the gels mixed with free β-CyD (0.9 mM) and also HPβ-CyD (7.2 mM) (Figure 2) where the drug:cyclodextrin molar ratios were 0.03 and 0.004, respectively. Interestingly, although drug:cyclodextrin molar ratio in HPβ-CyD sample was 10 times lower than in the Alg/POAβCyD (1:3) hydrogels, more crystallization was observed in the gels containing HPβ-CyD. This could indicate that the nongrafted CyDs are showing limited usefulness in helping dissolve the paclitaxel in the studied conditions or may have inferior inclusion complex ability with paclitaxel compared to POAβCyD. This is surprising, in particular for HPβ-CyD which was chosen due to its higher water solubility compared to β-CyD, as well as its more frequent use in pharmaceutics due to it being suitable for parenteral injection.[59] The phenomenon of the increase in complexation ability of β-CyD upon grafting has been also observed for β-CyD-grafted hyaluronic acid.[60] Authors reported paclitaxel:cyclodextrin

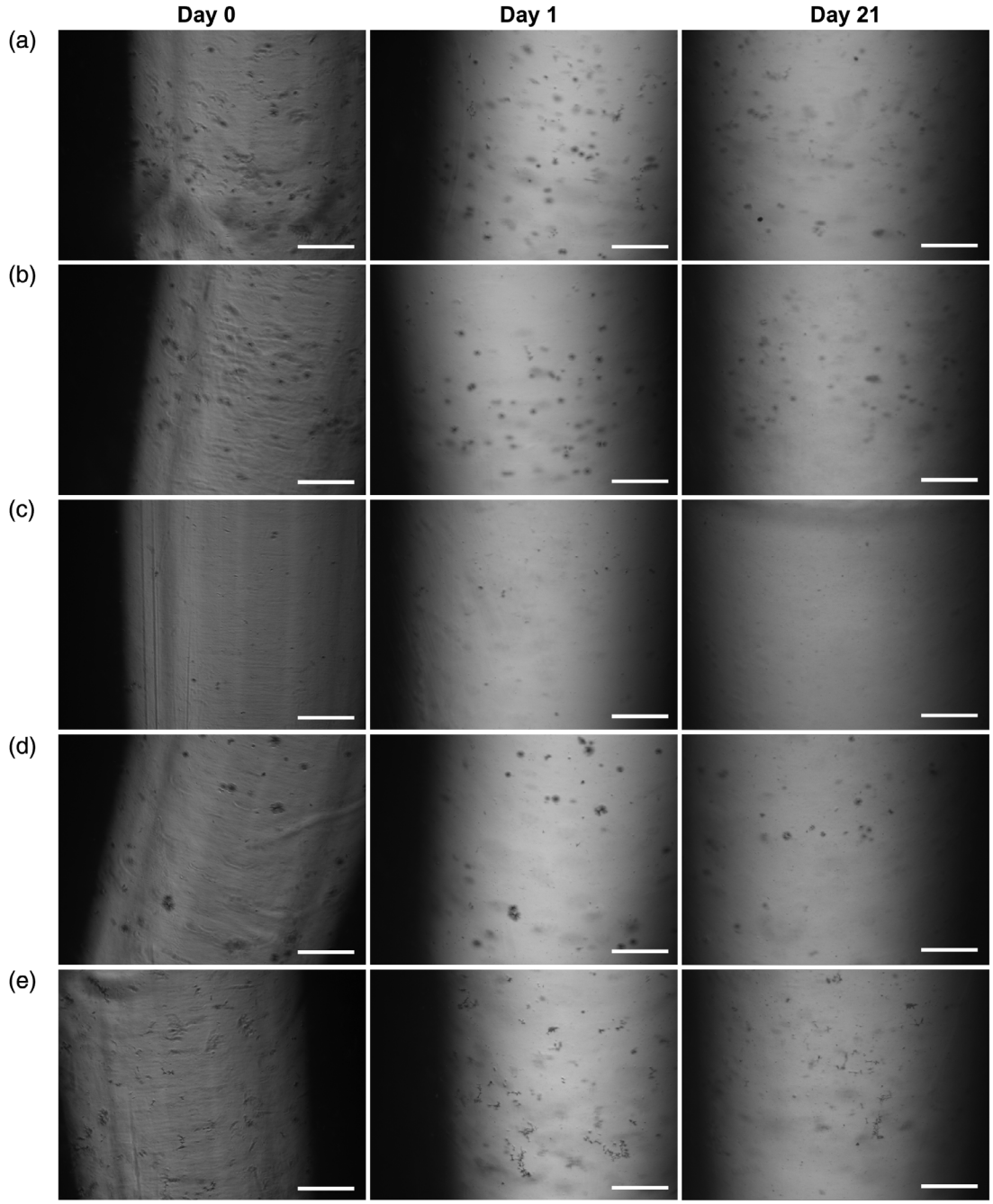

FIGURE 2 Paclitaxel crystals in alginate hydrogels loaded with 25 μM paclitaxel. Hydrogels shown at days 0 (1 day after gelation), 1 and 21. (a) Alg. (b) Alg/POAβCyD (1:1 (w/w), 0.8 mM β-CyD moieties). (c) Alg/POAβCyD (1:3 (w/w), 1.1 mM β-CyD moieties). (d) Alg + β-CyD (0.9 mM β-CyD). (e) Alg + HPβ-CyD (7.2 mM HPβ-CyD). Scale bar 500 μm

molar ratios of 0.011, 0.00026, and 0.007 for the hyaluronic acid grafted β-CyD, β-CyD, and dimethyl-β-CyD, respectively.

The paclitaxel crystals have been reported to be stable in aqueous environment up to 2 months and are seen as a limitation for achieving a therapeutic effect from the drug.[58] Therefore, the presence of crystals is not desirable, especially for controlled drug release systems. Here, paclitaxel crystals could be observed in the gels after 21 days after exposure to physiological buffer with same observations of the different gels as mentioned above (Figure 2).

3.3 | Release of paclitaxel from alginate gels

The ability of the developed hydrogel system to release paclitaxel was studied in vitro in the physiological buffer (Figure 3). Paclitaxel concentration was measured based on a standard curve of paclitaxel dissolved in extraction solvent, however the presence of paclitaxel degradation products (7-epi-taxol, 10-deacetyltaxol, and baccatin III) in the release samples was obvious throughout the time of the experiment (21 days) and was measured as areas due to lack of standards. In

the experiment, 200 μL of 300 μL medium was removed at each time point and analyzed, and 200 μL new medium added. This means that measured paclitaxel and degradation products from day 3 to 21 also includes remains from previous time points, hence a mix of newly and previously released drug.

After 1 day, the concentration of paclitaxel (Figure 3a) found in physiological buffer was much lower for the samples containing β-CyD-grafted polymer (328–333 nM) than the concentration observed for Alg and Alg + HPβ-CyD samples (756 and 665 nM, respectively) and was comparable with the sample Alg + β-CyD, which contained free β-CyD (315 nM). When looking at the degradation products (Figure 3b–d), a slight increase could be seen for the Alg/POAβCyD (1:3) sample relative to the Alg gels. For baccatin III (Figure 3d) both grafted samples generally showed higher areas of this degradation product compared to Alg samples. The Alg/POAβCyD (1:3) gels showed significantly higher areas of baccatin III compared to Alg sample at days 6, 9, and 15 (p value less than 0.01 for all samples, $n = 3$). This could indicate that the grafted alginate influences the release and/or degradation of the paclitaxel. For the degradation products, no accurate quantification can be made due to the lack of standards, however, it can be seen that 7-epi-taxol largely follows the release of paclitaxel, whereas 10-deacetyltaxol and baccatin III show higher amounts at the early timepoints (day 1–6) relative to later (day 9–21). Interestingly, it has been shown previously that the epimer 7-epi-taxol still shows anti-cancer properties.[61]

At day 1, Alg and Alg + HPβ-CyD showed higher concentrations of paclitaxel in physiological buffer than other gels. For Alg samples, the observed increase cannot be explained by higher initial release from this sample, because the areas of the degradation products did not increase accordingly, and vice versa, they were not significantly different from those for Alg/POAβCyD (1:1) and Alg/POAβCyD (1:3) samples. The similar increase in paclitaxel concentration at day 1 was observed in one of the control samples that contained POA instead of grafted material (Alg/POA (1:3)) (Figure A3, Supporting Information S1).

For the Alg + HPβ-CyD sample more degradation products were found at day 1–6 compared to the other samples, indicating faster release of paclitaxel in presence of free HPβ-CyD compared to the

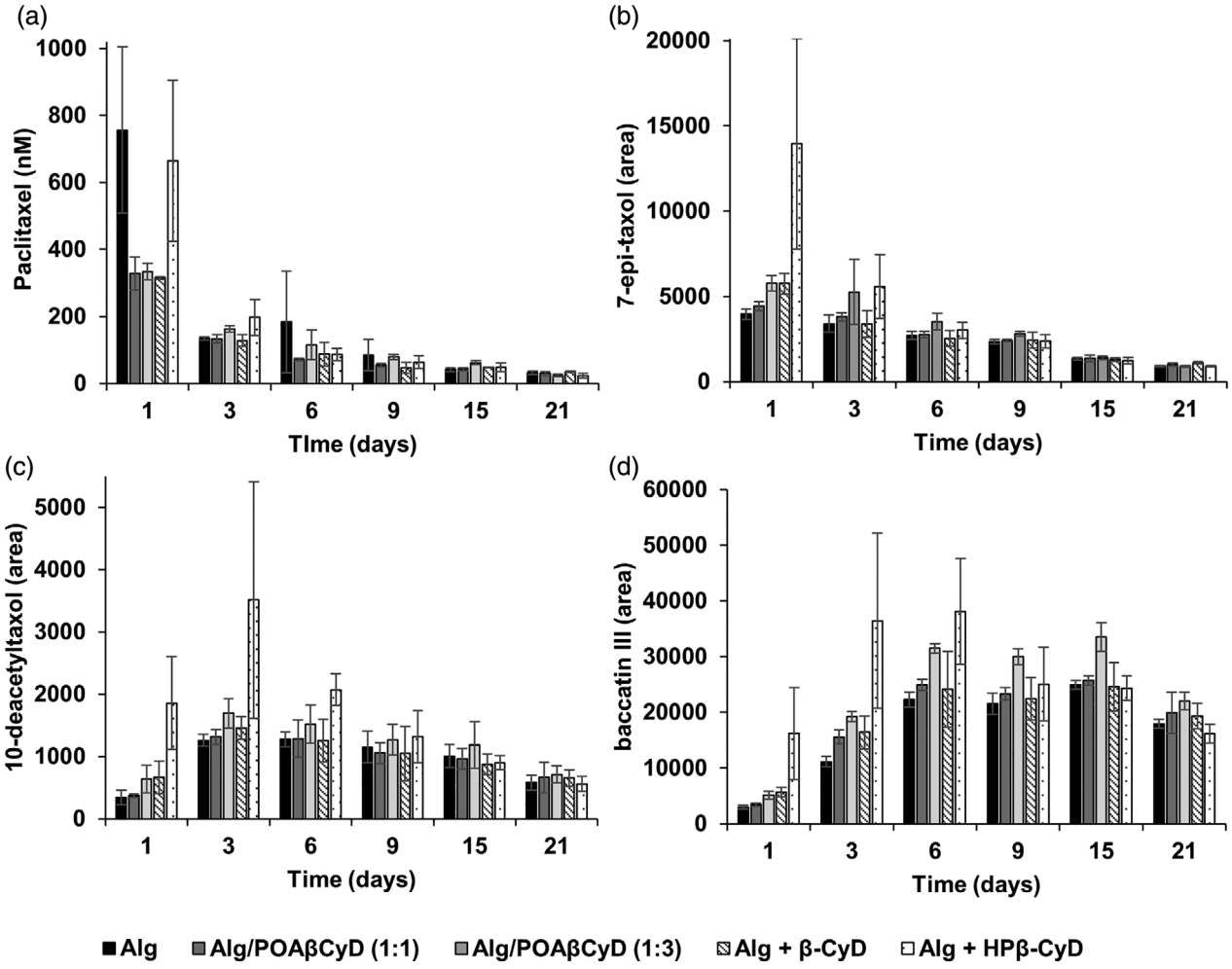

FIGURE 3 Drug release from hydrogels into physiological buffer at 37°C for 21 days. (a) Concentration of paclitaxel in release medium. (b,c) Integrals of the paclitaxel degradation products found in release medium: (b) 7-epi-taxol, (c) 10-deacetylytaxol, and (d) baccatin III. Mean ± *SD*, $n = 3$

other samples. Furthermore, the release of paclitaxel from Alg + HPβ-CyD was much higher compared to the gels containing free β-CyD. There are several important factors to consider here: higher concentration of HPβ-CyD than that of β-CyD (7.2 vs. 0.9 mM, respectively) and higher water solubility of HPβ-CyD (>860 mM compared to 16 mM for β-CyD).[62] It was therefore expected to be easier for HPβ-CyD to transport paclitaxel out of the gel *via* diffusion compared to the β-CyD.

At the end of the release study, the remaining paclitaxel in the gels were measured (Figure 4). All samples contained unreleased paclitaxel, with more in the Alg gels compared to grafted gels. Looking at the average values, about 5% of the loaded drug was found in the Alg/POAβCyD (1:3) hydrogels, about 18% in the Alg/POAβCyD (1:1) hydrogel and about 24% in the Alg hydrogel. For the degradation products found in these gels, only baccatin III and 10-deacetyltaxol were found in the Alg gels whereas also 7-epi-taxol was found in the Alg/POAβCyD gels (Figure A4, Supporting Information S1).

For the Alg gels mixed with free β-CyD or HPβ-CyD, the variation in the amount of remaining paclitaxel in the gels was very large, compared to the other gels. This may reflect differences in the release of free CyD from the gels and hence drug released or potential differences in initial crystallization (Figure A2, Supporting Information S1). On average, the paclitaxel remaining in the Alg gels with free β-CyD was higher (20%) than in the Alg gels with free HPβ-CyD (9%), which again indicates faster release from the gels in presence of free HPβ-CyD and agrees with the observations made in the release study. In these gels, baccatin III, 10-deacetyltaxol, and 7-epi-taxol were also found at day 21 (Figure A4, Supporting Information S1).

Taken together, our results indicate that more paclitaxel is solubilized in the gels with grafted alginate (Figure 2), and more is released over time, relative to nongrafted alginate and alginate gels with free β-CyD (Figures 3 and 4). In total, 82–95% of the paclitaxel was released into physiological buffer and/or underwent degradation at day 21 for the grafted material (Figure 4). Inclusion complexes between β-CyDs and paclitaxel are characterized by low association constants as paclitaxel molecules have a limited penetration into the hydrophobic cavity of cyclodextrin.[29,30] On the other hand, one can expect faster release rate of paclitaxel in presence of β-CyDs. Indeed, paclitaxel in vitro release from the different drug delivery systems functionalized with β-CyD moieties has been reported to be 30–100% during the first 24 hr.[60,63,64]

Other factors that may influence the release of paclitaxel from the gels could be different mechanical properties of the gels with different concentration of the grafted material (see the Section 3.1). The gels in which POAβCyD is substituted with POA can be considered as a good control for mechanical properties of the gels. For the Alg/POA (1:3), which also showed high concentrations of both paclitaxel and degradation products in physiological buffer, concentration of the remaining drug was found to be ~11%, whereas more drug (~ 26%) was found in the stiffer Alg/POA (1:1) gel (Figure A5, Supporting Information S1). Stiffer gels are recognized by a higher crosslinking density, that could influence both degradation of the gels[12] as well as gel permeability. Although no direct degradation of the gels was measured in this study, differences in stability were observed as the grafted alginate samples were more difficult to handle than the non-modified samples. Also, slight decrease in gels weight was found at day 21 (Figure A6, Supporting Information S1). The permeability of the gels in this study is not known, although one may speculate that lower crosslinking density will lead to more permeable gels. However, for Ca-saturated alginate gels, the opposite is found, as alginates with a low content of guluronic acid were shown to be less permeable to, for example, albumin, than alginate with a higher content of guluronic acid and thus higher crosslinking density.[65] The alginate gel network as such is not expected to influence the release of paclitaxel, as Ca-saturated alginate hydrogels are open for diffusion for larger molecules such as antibodies (IgG, 150 kDa).[13] Hence, paclitaxel, as a non-polar and small molecule (854 Da), is expected to readily diffuse through the alginate hydrogels. However, differences in release could also be caused by differences in the drug loading procedure (see the discussion above). The crystallization of paclitaxel must also be considered. For a system containing only paclitaxel dissolved in aqueous medium (no gel), only very little of the free drug (less than 10%) has

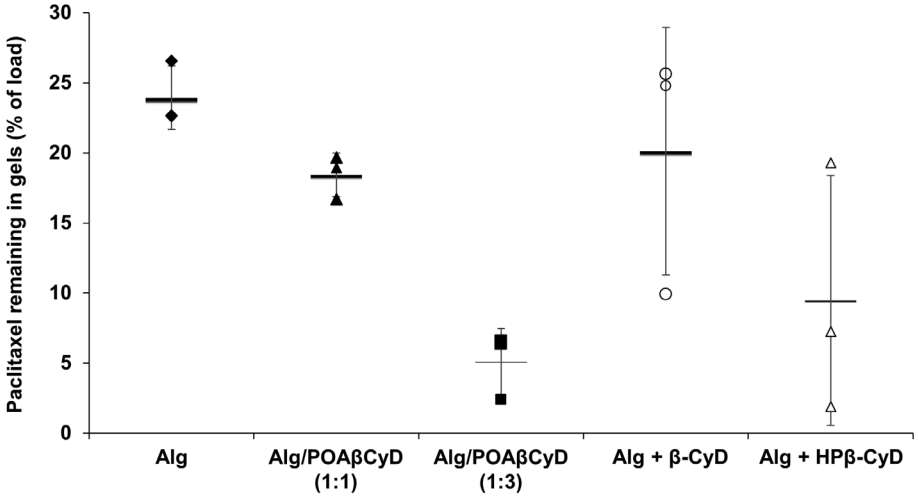

FIGURE 4 Paclitaxel remaining in the alginate hydrogels after being exposed to physiological buffer for 21 days. Values show amount of paclitaxel found in dissolved gels relative to the amount of the paclitaxel loaded into the hydrogels. Mean ± *SD*, $n = 3$

been shown to be released within 10 days in PBS pH 7.4, which is likely due to the low solubilization/crystallization of paclitaxel in water.[66]

It should also be noted that the release study was not conducted under sink conditions. The release medium used for drug release studies should ideally obtain sink conditions, that is to say the volume of the medium should be three times higher than the volume required to solubilize the drug to ensure free diffusion out of the drug delivery system.[67,68] The solubility of paclitaxel in physiological buffer has not been tested in this study. But one could expect that paclitaxel solubility in physiological buffer is comparable to the solubility in PBS buffer, which is reported[67] to be within the range of 0.3–10 μg/mL (0.35–12 μM) at 37°C. If all the paclitaxel releases at once from the tested hydrogel, the concentration in physiological buffer will reach 10 μM, which corresponds to the reported solubility limit. However, the release media used in our study also contained traces of ethanol (below 1% (v/v) from the gels), which increases the solubility of paclitaxel.

As degradation of paclitaxel seemed to be a major issue in determining release from the gels, stability studies of paclitaxel were performed in the physiological buffer and water for comparison (Figure 5). At day 1, about 90% of the paclitaxel was found in both water and physiological buffer. Thereafter, the degradation was especially prevalent in the physiological buffer where only 33%, 11%, and 2% of the initial concentration could be found after 3, 9, and 15 days, respectively. In water, about 80% of the paclitaxel remained in the solution after 3 days and to the end of the study. Hence, limited degradation was seen in water. The degradation products 10-deacetyltaxol and baccatin III[6169] were found in both solutes (Figure A7, Supporting Information S1), whereas the epimer 7-epitaxol was found only in physiological buffer. This underlines the difficulties in determining release of paclitaxel in relevant fluids. Here, we identified three degradation products, but more degradation products of paclitaxel are reported,[69] hence illustrating the complexity in determining released paclitaxel. Since the degradation of paclitaxel was also observed in water, it was therefore decided to cast paclitaxel-loaded hydrogels at 4°C for further experiments.

3.4 | Cytotoxicity of paclitaxel-loaded hydrogels

The effect of the released drug was further studied in in vitro cell culture on the prostate cancer cell line PC-3. For the cell studies, the total concentration of alginate in the gels was increased from 1% (w/v) up to 2% (w/v) while keeping the ratio of Alg/POAβCyD 1:1 (w/w) and paclitaxel loading remained unchanged. This allowed for a drug:cyclodextrin molar ratio in the gels below 0.02 and thus to avoid crystallization of paclitaxel within the gels. Another benefit of increasing the alginate concentration was higher stiffness of the gels and their reduced vulnerability to syneresis in culture medium (Figure A8, Supporting Information S1).

The potential toxic effect of the grafted polymer by itself was first studied, followed by examining the burst release effects. Thereafter, the cytotoxicity of the paclitaxel-loaded hydrogels on metabolic activity of PC-3 cells was studied. The reasoning behind studying cytotoxicity of the functionalized polymer itself was as follows: Firstly, although cyclodextrins are widely used as excipients in pharmaceutic applications, it has been shown previously that β-CyDs can cause dose-dependent hemolysis and cytotoxicity.[70] Secondly, formation of the Alg/POAβCyD gel is not instant, meaning that upon injection in vivo the surrounding tissue and cells can interact with nongelled polymers. And lastly, possible leakage of POAβCyD from the gels over time[12] can lead to unwanted cytotoxic effects. To address this question, PC-3 cells were exposed to nongelled POAβCyD polymer. Free β-CyD dissolved in POA (β-CyD + POA sample) was used as a control. The cells exposed to nongelled POAβCyD showed viability ≥90% for concentrations up to 1 mM of β-CyD, which equals 5 mg/mL of the polymer (Figure 6a). A decrease in the cells metabolic activity was seen above this concentration in a concentration-dependent manner. The cytotoxic effect of POAβCyD was nearly two times higher than that one of free β-CyD dissolved in POA solution (β-CyD + POA sample). The half maximal inhibitory concentrations (IC50) were not obtained in this experiment, because high viscosity of the polymers (POAβCyD is highly viscous at concentrations >40 mg/mL) and low water solubility of free β-CyD (water solubility is 16.3 mM[62]) limited

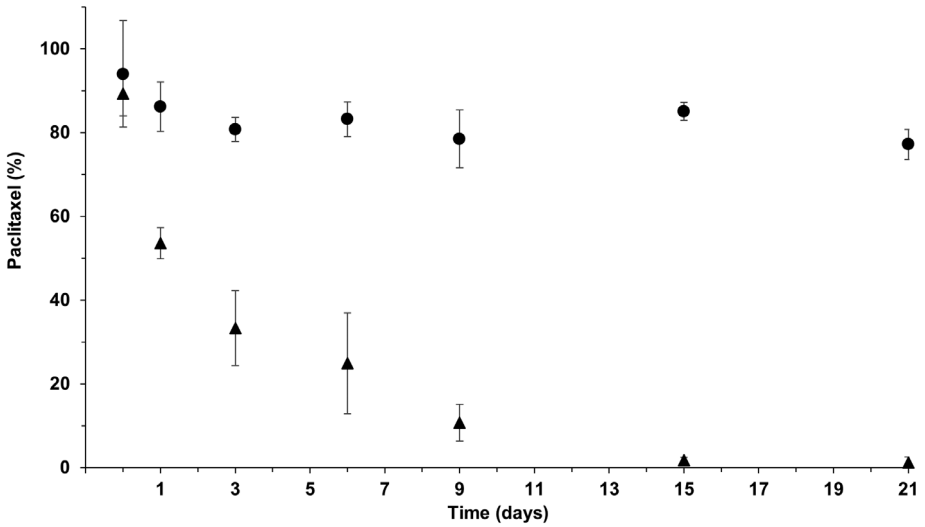

FIGURE 5 Stability of paclitaxel over time in water (circle) and physiological buffer (triangle) at 37°C

further increase of samples concentration. It has been previously reported that substituents can attenuate cytotoxicity of the native CyDs.[71,72] However, our results with POAβCyD show the opposite effect. We hypothesize that the observed effect might be connected to the difference in interaction between free βCyD and grafted βCyD moieties with the cellular cholesterol, since the cytotoxicity of various CyD derivatives is known to depend on their capacity to extract cholesterol from the cell membrane.[70]

Next, we formed alginate gels loaded with paclitaxel and studied burst release of the drug in culture media and hydrogels cytotoxicity in vitro. To study the burst release of paclitaxel, Alg and Alg/POAβCyD (1:1) hydrogels were loaded with 25 μM of paclitaxel and incubated in the culture media for 1 hr. The concentration of the released drug was then determined by exposing PC-3 cells to serial dilutions of the collected media and subsequent conversion of the obtained cytotoxicity values into the drug concentration using a standard curve. The standard curve was prepared using free paclitaxel in media (Figure A1, Supporting Information S1). The effects of the collected medium on metabolic activity of the PC-3 cells are shown on Figure 6b. The release-medium from both grafted and nongrafted alginates decreased cell metabolic activity in a concentration dependent manner. The paclitaxel burst release was found to be 6.2 ± 2.7% for Alg gel and 11.2 ± 2.5% for Alg/POAβCyD (1:1) gel. Under light microscope, the Alg/POAβCyD (1:1) mixed gels did not contain visible crystals of paclitaxel, whereas the Alg gels had clearly visible crystals. Thus, it is very likely that the Alg gels released less drug during the first hour due to crystal formation of the drug within these gels.

Next, to demonstrate efficacy of the developed delivery system in vitro, free paclitaxel, empty gels and gels loaded with paclitaxel were added to PC-3 cells and cell metabolic activity was determined after 72 hr of co-incubation (Figure 6c–d). Two gel types, namely Alg gels and Alg/POAβCyD (1:1) gels, and three different loadings (0.9, 7.2, and 25 μM) as well as placebo (without drug) were compared. The gels of both grafted and nongrafted alginate without paclitaxel showed no effect

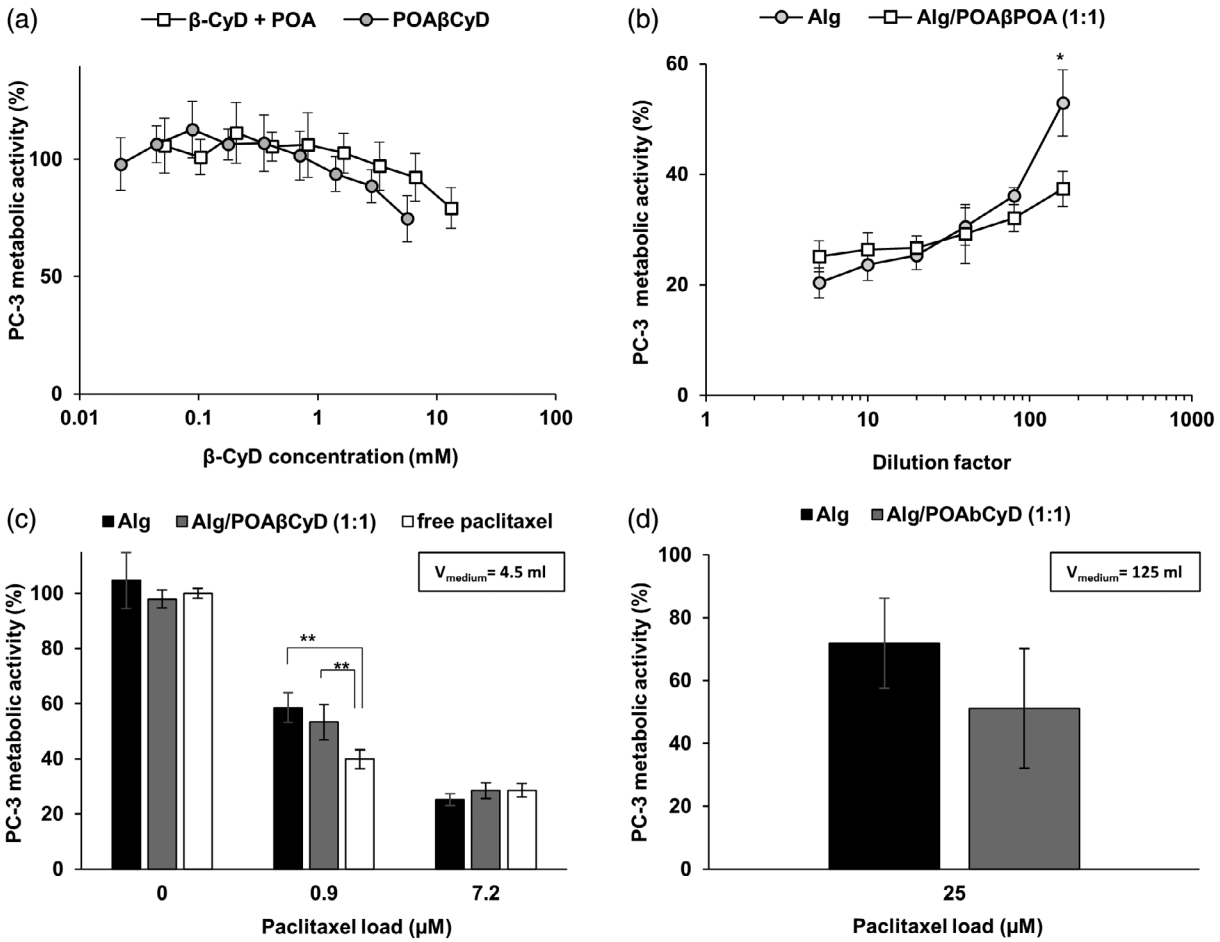

FIGURE 6 Metabolic activity of PC-3 cells. (a) Cells were incubated with alginate functionalized with β-CyD (POAβCyD) solution and free β-CyD mixed with POA solution; (b) cells were exposed to serial dilutions of 1-hr release media for paclitaxel loaded gels (25 μM) for 72 hr; (c) Cells were exposed to the 50 μL gels loaded with 0, 0.9 and 7.2 μM paclitaxel for 72 hr (total volume of medium 4.5 mL); and (d) cells were exposed to the 50 μL gels loaded with 25 μM paclitaxel for 72 hr (total volume of medium 125 mL). Cell metabolic activity is expressed as fluorescent intensities relative to a control (cells not exposed to paclitaxel were defined as 100%) after subtraction of non-cell-derived background. Data are expressed as mean ± *SD*, $n = 2–8$. * and ** denote statistically significant differences ($p < 0.05$ and $p < 0.01$, respectively) between two groups

on the metabolic activity of the cells (Figure 6c). The drug-loaded gels as expected decreased metabolic activity of PC-3 cells in dose-dependent manner relative to the loading concentration of paclitaxel. The Alg gel and Alg/POAβCyD (1:1) mixed gels loaded with 0.9 μM paclitaxel decreased cell viability down to 53.5 ± 4.8% and 60.4 ± 4%, respectively, whereas the gels loaded with 7.2 μM paclitaxel decreased cell viability down to 28.2 ± 1.9% and 24.3 ± 1.9%, respectively. Thus, the difference between two gel types (with and without POAβCyD) was not noticeable when 0.9 and 7.2 μM loadings were tested. These samples did not contain visible crystals of paclitaxel neither inside Alg nor Alg/POAβCyD (1:1) gels. In contrast, upon 25 μM paclitaxel loading, crystallization of the drug was visible inside the Alg gels, but not within the gels comprising grafted β-CyD. The mixed gels of Alg/POAβCyD (1:1) decreased cell metabolic activity to 51.1 ± 19%, whereas the Alg gels reduced it to 71.8 ± 14.3%; (Figure 6d). The Alg gels still contained undissolved paclitaxel crystals after 72 hr of incubation with cells, whereas the grafted gels remained transparent. Although the difference between Alg and Alg/POAβCyD (1:1) gels was not statistically significant ($p = 0.11$ ($n = 2$) and $p = 0.2$ ($n = 2$) for two independent experiments), we assume that the observed slightly higher cytotoxic activity of Alg/POAβCyD (1:1) gels could be related to a slightly faster paclitaxel release from these gels as well as higher burst release found in culture media.

In this work, we evaluated the in vitro biological activity of the functionalized alginate hydrogels loaded with paclitaxel on prostate cancer cells. This cell model was chosen because paclitaxel is known to be efficacious against various prostate cancer cells, including PC-3 cells.[73] At present, however, paclitaxel is only approved for the treatment of breast cancer, cancer of the ovaries, nonsmall cell lung cancer, and Kaposi's sarcoma, and is being currently used off-label for the treatment of castration-resistant prostate cancer and some other malignant tumors.[74] In situ forming hydrogel systems can serve as a local depot slowly releasing paclitaxel and may be beneficial for the treatment of patients with prostate tumors. A recent preclinical study demonstrates therapeutic efficacy of local delivery system based on an injectable polymer paste with paclitaxel in LNCaP human prostate cancer xenografts.[75] In addition, the efficacy of the developed paclitaxel delivery system would be interesting to study in models of brain tumor and brain metastases. As mentioned earlier, paclitaxel has strong anticancer activity against glioma cells and many other cancers in vitro.[33] However, paclitaxel is a substrate for the P-gp/ABCB1, and therefore has limited access to the central nervous system.[33] Since local delivery systems have already shown antitumor efficacy against glioblastoma,[4] delivery of drugs to brain tumors is a relevant follow-up of the studied gel system. In addition, other poorly water-soluble chemotherapeutic agents that can form inclusion complexes with β-CyD, for instance cisplatin, doxorubicin, curcumin, camptothecin, and so on, may be considered as potential payloads for the developed depot hydrogel.

4 | CONCLUSIONS

In this study, we developed a hydrogel-based delivery system employing β-CyD functionalized alginate, where alginate acted as the gelling material and the β-CyD moieties were responsible for formation of inclusion complexes with a poorly soluble drug (paclitaxel). The introduction of β-CyD-moieties into the delivery system generally decreased the mechanical properties of the gels (compared to nonmodified alginate hydrogels) and modulated paclitaxel behavior but did not influence gelation kinetics. The significance of the present work is that it shows increased capacity of β-CyD grafted alginate to complex paclitaxel as compared to free HPβ-CyD. Although the paclitaxel is not an ideal payload for β-CyD-based systems because of its relatively poor retention within β-CyD hydrophobic cavity, and because it is difficult to quantify due to rapid degradation, our findings support that the β-CyD-grafted alginate can modulate release of the paclitaxel from the gels. The β-CyD-grafted alginate prevented crystallization of the paclitaxel by retaining a complexed dispersion of the drug and facilitated paclitaxel diffusion out of the gel network. This effect was observed at paclitaxel:β-CyD molar ratios not exceeding 0.02. Furthermore, the paclitaxel-loaded hydrogels comprising modified alginate were shown to have cytotoxic activity. The results indicate that β-CyD functionalized alginates have potential to be used as a material for drug delivery systems. The hydrogel system could also be applied in future work for delivery of other hydrophobic drugs and molecules that can form inclusion complexes with β-CyD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The relevant data generated or analyzed during the current study are included within the published article (and its supplementary information) or are available from the corresponding author upon reasonable request.

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REFERENCES

1. Darge HF, Andrgie AT, Tsai HC, Lai JY. Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical

applications. *Int J Biol Macromol*. 2019;*133*:545-563. https://doi.org/10.1016/j.ijbiomac.2019.04.131.
2. Dimatteo R, Darling NJ, Segura T. In situ forming injectable hydrogels for drug delivery and wound repair. *Adv Drug Deliv Rev*. 2018;*127*:167-184. https://doi.org/10.1016/j.addr.2018.03.007.
3. Cirillo G, Spizzirri UG, Curcio M, Nicoletta FP, Iemma F. Injectable hydrogels for cancer therapy over the last decade. *Pharmaceutics*. 2019;*11*(9):486. https://doi.org/10.3390/pharmaceutics11090486.
4. Bastiancich C, Bianco J, Vanvarenberg K, et al. Injectable nanomedicine hydrogel for local chemotherapy of glioblastoma after surgical resection. *J Control Release*. 2017;*264*:45-54. https://doi.org/10.1016/j.jconrel.2017.08.019.
5. Draget KI, Taylor C. Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocoll*. 2011;*25*(2):251-256. https://doi.org/10.1016/j.foodhyd.2009.10.007.
6. Sandvig I, Karstensen K, Rokstad AM, et al. RGD-peptide modified alginate by a chemoenzymatic strategy for tissue engineering applications. *J Biomed Mater Res A*. 2015;*103*(3):896-906. https://doi.org/10.1002/jbm.a.35230.
7. Arlov Ø, Aachmann FL, Sundan A, Espevik T, Skjåk-Bræk G. Heparin-like properties of sulfated alginates with defined sequences and sulfation degrees. *Biomacromolecules*. 2014;*15*(7):2744-2750. https://doi.org/10.1021/bm500602w.
8. Donati I, Draget KI, Borgogna M, Paoletti S, Skjåk-Braek G. Tailor-made alginate bearing galactose moieties on mannuronic residues: selective modification achieved by a chemoenzymatic strategy. *Biomacromolecules*. 2005;*6*(1):88-98. https://doi.org/10.1021/bm040053z.
9. Omtvedt LA, Dalheim MØ, Nielsen TT, Larsen KL, Strand BL, Aachmann FL. Efficient grafting of cyclodextrin to alginate and performance of the hydrogel for release of model drug. *Sci Rep*. 2019;*9*(1):9325. https://doi.org/10.1038/s41598-019-45761-4.
10. Dalheim MØ, Vanacker J, Najmi MA, Aachmann FL, Strand BL, Christensen BE. Efficient functionalization of alginate biomaterials. *Biomaterials*. 2016;*80*:146-156. https://doi.org/10.1016/j.biomaterials.2015.11.043.
11. Kristiansen KA, Schirmer BC, Aachmann FL, Skjåk-Braek G, Draget KI, Christensen BE. Novel alginates prepared by independent control of chain stiffness and distribution of G-residues: structure and gelling properties. *Carbohydr Polym*. 2009;*77*(4):725-735. https://doi.org/10.1016/j.carbpol.2009.02.018.
12. Dalheim MØ, Omtvedt LA, Bjørge IM, et al. Mechanical properties of ca-saturated hydrogels with functionalized alginate. *Gels*. 2019;*5*(2):23. https://doi.org/10.3390/gels5020023.
13. Mørch YA, Donati I, Strand BL, Skjåk-Braek G. Effect of Ca2+, Ba2+, and Sr2+ on alginate microbeads. *Biomacromolecules*. 2006;*7*(5):1471-1480. https://doi.org/10.1021/bm060010d.
14. Andersen T, Strand BL, Formo K, Alsberg E, Christensen BE. Alginates as biomaterials in tissue engineering. In: Rauter AP, Lindhorst T, eds. *Carbohydrate chemistry: chemical and biological approaches*. Cambridge: The Royal Society of Chemistry; 2012:227-258. https://doi.org/10.1039/9781849732765-00227.
15. Shin EY, Park JH, Shin ME, et al. Injectable taurine-loaded alginate hydrogels for retinal pigment epithelium (RPE) regeneration. *Korean J Couns Psychother*. 2019;*103*:109787. https://doi.org/10.1016/j.msec.2019.109787.
16. Criado-Gonzalez M, Fernandez-Gutierrez M, San Roman J, Mijangos C, Hernández R. Local and controlled release of tamoxifen from multi (layer-by-layer) alginate/chitosan complex systems. *Carbohydr Polym*. 2019;*206*:428-434. https://doi.org/10.1016/j.carbpol.2018.11.007.
17. Etter JN, Oldinski RA. Synthesis of a tri-network alginate hydrogel for use as an injectable cell carrier. *Biomed Phys Eng Express*. 2018;*5*(1):015017. https://doi.org/10.1088/2057-1976/aaeb6f.
18. Ferreira NN, Caetano BL, Boni FI, et al. Alginate-based delivery systems for bevacizumab local therapy: *in vitro* structural features and release properties. *J Pharm Sci*. 2019;*108*(4):1559-1568. https://doi.org/10.1016/j.xphs.2018.11.038.
19. Brudno Y, Pezone MJ, Snyder TK, et al. Replenishable drug depot to combat post-resection cancer recurrence. *Biomaterials*. 2018;*178*:373-382. https://doi.org/10.1016/j.biomaterials.2018.05.005.
20. Zhang Y, Li X, Zhong N, Huang Y, He K, Ye X. Injectable in situ dual-crosslinking hyaluronic acid and sodium alginate based hydrogels for drug release. *J Biomater Sci Polym Ed*. 2019;*30*(12):995-1007. https://doi.org/10.1080/09205063.2019.1618546.
21. Hoare TR, Kohane DS. Hydrogels in drug delivery: progress and challenges. *Polymer*. 2008;*49*(8):1993-2007. https://doi.org/10.1016/j.polymer.2008.01.027.
22. McKenzie M, Betts D, Suh A, Bui K, Kim LD, Cho H. Hydrogel-based drug delivery systems for poorly water-soluble drugs. *Molecules*. 2015;*20*(11):20397-20408. https://doi.org/10.3390/molecules201119705.
23. Nielsen AL, Madsen F, Larsen KL. Cyclodextrin modified hydrogels of PVP/PEG for sustained drug release. *Drug Deliv*. 2009;*16*(2):92-101. https://doi.org/10.1080/10717540802605129.
24. Woldum HS, Larsen KL, Madsen F. Cyclodextrin controlled release of poorly water-soluble drugs from hydrogels. *Drug Deliv*. 2008;*15*(1):69-80. https://doi.org/10.1080/10717540701829267.
25. Szejtli J. Introduction and general overview of cyclodextrin chemistry. *Chem Rev*. 1998;*98*(5):1743-1754. https://doi.org/10.1021/cr970022c.
26. Peng K, Tomatsu I, Korobko AV, Kros A. Cyclodextrin-dextran based in situ hydrogel formation: a carrier for hydrophobic drugs. *Soft Matter*. 2010;*6*(1):85-87. https://doi.org/10.1039/B914166A.
27. Grimaudo MA, Nicoli S, Santi P, Concheiro A, Alvarez-Lorenzo C. Cyclosporine-loaded cross-linked inserts of sodium hyaluronan and hydroxypropyl-β-cyclodextrin for ocular administration. *Carbohydr Polym*. 2018;*201*:308-316. https://doi.org/10.1016/j.carbpol.2018.08.073.
28. Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. *Int J Pharm*. 2002;*235*(1–2):179-192. https://doi.org/10.1016/s0378-5173(01)00986-3.
29. Alcaro S, Ventura CA, Paolino D, et al. Preparation, characterization, molecular modeling and *in vitro* activity of paclitaxel-cyclodextrin complexes. *Bioorg Med Chem Lett*. 2002;*12*(12):1637-1641. https://doi.org/10.1016/s0960-894x(02)00217-2.
30. Sharma US, Balasubramanian SV, Straubinger RM. Pharmaceutical and physical properties of paclitaxel (Taxol) complexes with cyclodextrins. *J Pharm Sci*. 1995;*84*(10):1223-1230. https://doi.org/10.1002/jps.2600841015.
31. Sparreboom A, van Tellingen O, Nooijen WJ, Beijnen JH. Tissue distribution, metabolism and excretion of paclitaxel in mice. *Anticancer Drugs*. 1996;*7*(1):78-86. https://doi.org/10.1097/00001813-199601000-00009.
32. Wolinsky JB, Colson YL, Grinstaff MW. Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers. *J Control Release*. 2012;*159*(1):14-26. https://doi.org/10.1016/j.jconrel.2011.11.031.
33. Fellner S, Bauer B, Miller DS, et al. Transport of paclitaxel (Taxol) across the blood-brain barrier *in vitro* and *in vivo*. *J Clin Invest*. 2002;*110*(9):1309-1318. https://doi.org/10.1172/JCI15451.
34. Wanderley CW, Colón DF, Luiz JPM, et al. Paclitaxel reduces tumor growth by reprogramming tumor-associated macrophages to an M1 profile in a TLR4-dependent manner. *Cancer Res*. 2018;*78*(20):5891-5900. https://doi.org/10.1158/0008-5472.CAN-17-3480.
35. Wang F, Porter M, Konstantopoulos A, Zhang P, Cui H. Preclinical development of drug delivery systems for paclitaxel-based cancer chemotherapy. *J Control Release*. 2017;*267*:100-118. https://doi.org/10.1016/j.jconrel.2017.09.026.

36. De Clercq K, Xie F, De Wever O, et al. Preclinical evaluation of local prolonged release of paclitaxel from gelatin microspheres for the prevention of recurrence of peritoneal carcinomatosis in advanced ovarian cancer. *Sci Rep*. 2019;*9*(1):14881. https://doi.org/10.1038/s41598-019-51419-y.
37. Ranganath SH, Kee I, Krantz WB, Chow PK, Wang CH. Hydrogel matrix entrapping PLGA-paclitaxel microspheres: drug delivery with near zero-order release and implantability advantages for malignant brain tumour chemotherapy. *Pharm Res*. 2009;*26*(9):2101-2114. https://doi.org/10.1007/s11095-009-9922-2.
38. Abe T, Sakane M, Ikoma T, Kobayashi M, Nakamura S, Ochiai N. Intraosseous delivery of paclitaxel-loaded hydroxyapatitealginate composite beads delaying paralysis caused by metastatic spine cancer in rats. *J Neurosurg Spine*. 2008;*9*(5):502-510. https://doi.org/10.3171/SPI.2008.9.11.502.
39. Chiang C-Y, Chu C-C. Synthesis of photoresponsive hybrid alginate hydrogel with photo-controlled release behavior. *Carbohydr Polym*. 2015;*119*:18-25. https://doi.org/10.1016/J.CARBPOL.2014.11.043.
40. Gomez CG, Chambat G, Heyraud A, Villar M, Auzely-Velty R. Synthesis and characterization of a beta-CD-alginate conjugate. *Polymer*. 2006;*47*(26):8509-8516. https://doi.org/10.1016/j.polymer.2006.10.011.
41. Izawa H, Kawakami K, Sumita M, Tateyama Y, Hill JP, Ariga K. Beta-cyclodextrin-crosslinked alginate gel for patient-controlled drug delivery systems: regulation of host-guest interactions with mechanical stimuli. *J Mater Chem B*. 2013;*1*:2155-2161. https://doi.org/10.1039/C3TB00503H.
42. Miao T, Fenn SL, Charron PN, Oldinski RA. Self-healing and Thermoresponsive dual-cross-linked alginate hydrogels based on supramolecular inclusion complexes. *Biomacromolecules*. 2015;*16*:3740-3750. https://doi.org/10.1021/acs.biomac.5b00940.
43. Tan L, Li J, Liu Y, Zhou H, Zhang Z, Deng L. Synthesis and characterization of β-cyclodextrin-conjugated alginate hydrogel for controlled release of hydrocortisone acetate in response to mechanical stimulation. *J Bioactive Compatible Polym*. 2015;*30*:584-599. https://doi.org/10.1177/0883911515590494.
44. Zhang S, Qiao X, Hu B, Gong Y. Formation and controlled release of the inclusion complex of water soluble model drug neutral red with β-cyclodextrin grafted sodium alginate. *J Control Release*. 2011;*152*:e116-e118. https://doi.org/10.1016/J.JCONREL.2011.08.161.
45. Moncada-Basualto M, Matsuhiro B, Mansilla A, Lapier M, Maya JD, Olea-Azar C. Supramolecular hydrogels of β-cyclodextrin linked to calcium homopoly-l-guluronate for release of coumarins with trypanocidal activity. *Carbohydr Polym*. 2019;*204*:170-181. https://doi.org/10.1016/J.CARBPOL.2018.10.010.
46. Pluemsab W, Sakairi N, Furuike T. Synthesis and inclusion property of α-cyclodextrin-linked alginate. *Polymer*. 2005;*46*:9778-9783. https://doi.org/10.1016/J.POLYMER.2005.08.005.
47. Nielsen TT, Wintgens V, Amiel C, Wimmer R, Larsen KL. Facile synthesis of beta-cyclodextrin-dextran polymers by "click" chemistry. *Biomacromolecules*. 2010;*11*(7):1710-1715. https://doi.org/10.1021/bm9013233.
48. Bastiancich C, Vanvarenberg K, Ucakar B, et al. Lauroyl-gemcitabine-loaded lipid nanocapsule hydrogel for the treatment of glioblastoma. *J Control Release*. 2016;*225*:283-293. https://doi.org/10.1016/j.jconrel.2016.01.054.
49. Draget KI, Østgaard K, Smidsrød O. Homogeneous alginate gels: a technical approach. *Carbohydr Polym*. 1990;*14*:159-178. https://doi.org/10.1016/0144-8617(90)90028-Q.
50. Mørch YA, Holtan S, Donati I, Strand BL, Skjåk-Braek G. Mechanical properties of C-5 epimerized alginates. *Biomacromolecules*. 2008;*9*(9):2360-2368. https://doi.org/10.1021/bm8003572.
51. Smidsrød O, Painter T. Effect of periodate oxidation upon the stiffness of the alginate molecule in solution. *Carbohydr Res*. 1973;*26*(1):125-132. https://doi.org/10.1016/S0008-6215(00)85029-6.
52. Lee KY, Bouhadir KH, Mooney DJ. Evaluation of chain stiffness of partially oxidized polyguluronate. *Biomacromolecules*. 2002;*3*(6):1129-1134. https://doi.org/10.1021/bm025567h.
53. Vold IM, Kristiansen KA, Christensen BE. A study of the chain stiffness and extension of alginates, *in vitro* epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors. *Biomacromolecules*. 2006;*7*(7):2136-2146. https://doi.org/10.1021/bm060099n.
54. Gomez CG, Rinaudo M, Villar MA. Oxidation of sodium alginate and characterization of the oxidized derivatives. *Carbohydr Polym*. 2007;*67*:296-304. https://doi.org/10.1016/j.carbpol.2006.05.025.
55. Draget KI, Østgaard K, Smidsrød O. Alginate-based solid media for plant tissue culture. *Appl Microbiol Biotechnol*. 1989;*31*:79-83. https://doi.org/10.1007/BF00252532.
56. Espona-Noguera A, Ciriza J, Cañibano-Hernández A, et al. Tunable injectable alginate-based hydrogel for cell therapy in type 1 *Diabetes mellitus*. *Int J Biol Macromol*. 2018;*107*:1261-1269. https://doi.org/10.1016/j.ijbiomac.2017.09.103.
57. Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. *Nat Rev Mater*. 2016;*1*(12):16071. https://doi.org/10.1038/natrevmats.2016.71.
58. Castro JS, Tapia LV, Silveyra RA, Martinez CA, Deymier PA. Negative impact of paclitaxel crystallization on hydrogels and novel approaches for anticancer drug delivery systems. In: Ozdemir O, ed. *Current cancer treatment—Novel beyond conventional approaches*. London: InTech; 2011:767-782.
59. Del Valle EMM. Cyclodextrins and their uses: a review. *Process Biochem*. 2004;*39*(9):1033-1046. https://doi.org/10.1016/S0032-9592(03)00258-9.
60. Jing J, Szarpak-Jankowska A, Guillot R, Pignot-Paintrand I, Picart C, Auzély-Velty R. Cyclodextrin/paclitaxel complex in biodegradable capsules for breast cancer treatment. *Chem Mater*. 2013;*25*(19):3867-3873. https://doi.org/10.1021/cm4019925.
61. Amini-Fazl MS, Mobedi H, Barzin J. Investigation of aqueous stability of taxol in different release media. *Drug Dev Ind Pharm*. 2014;*40*(4):519-526. https://doi.org/10.3109/03639045.2013.771646.
62. Saokham P, Muankaew C, Jansook P, Loftsson T. Solubility of cyclodextrins and drug/cyclodextrin complexes. *Molecules*. 2018;*23*(5):1161. https://doi.org/10.3390/molecules23051161.
63. Mognetti B, Barberis A, Marino S, et al. *In vitro* enhancement of anticancer activity of paclitaxel by a cremophor free cyclodextrin-based nanosponge formulation. *J Incl Phenom Macrocycl Chem*. 2012;*74*:201-210. https://doi.org/10.1007/s10847-011-0101-9.
64. Zhang X, Zhang X, Wu Z, et al. A hydrotropic β-cyclodextrin grafted hyperbranched polyglycerol co-polymer for hydrophobic drug delivery. *Acta Biomater*. 2011;*7*(2):585-592. https://doi.org/10.1016/j.actbio.2010.08.029.
65. Martinsen A, Storrø I, Skjårk-Braek G. Alginate as immobilization material: III diffusional Properties. *Biotechnol Bioeng*. 1992;*39*(2):186-194. https://doi.org/10.1002/bit.260390210.
66. Choi SG, Lee SE, Kang BS, Ng CL, Davaa E, Park JS. Thermosensitive and mucoadhesive sol-gel composites of paclitaxel/dimethyl-β-cyclodextrin for buccal delivery. *PLoS One*. 2014;*9*(9):e109090. https://doi.org/10.1371/journal.pone.0109090.
67. Abouelmagd SA, Sun B, Chang AC, Ku YJ, Yeo Y. Release kinetics study of poorly water-soluble drugs from nanoparticles: are we doing it right? *Mol Pharm*. 2015;*12*(3):997-1003. https://doi.org/10.1021/mp500817h.
68. Phillips DJ, Pygall SR, Cooper VB, Mann JC. Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. *J Pharm Pharmacol*. 2012;*64*(11):1549-1559. https://doi.org/10.1111/j.2042-7158.2012.01523.x.
69. Tian J, Stella VJ. Degradation of paclitaxel and related compounds in aqueous solutions I: epimerization. *J Pharm Sci*. 2008;*97*(3):1224-1235. https://doi.org/10.1002/jps.21112.

70. Kiss T, Fenyvesi F, Bácskay I, et al. Evaluation of the cytotoxicity of beta-cyclodextrin derivatives: evidence for the role of cholesterol extraction. *Eur J Pharm Sci*. 2010;*40*(4):376-380. https://doi.org/10.1016/j.ejps.2010.04.014.
71. Szente L, Singhal A, Domokos A, Song B. Cyclodextrins: assessing the impact of cavity size, occupancy, and substitutions on cytotoxicity and cholesterol homeostasis. *Molecules*. 2018;*23*(5):1228. https://doi.org/10.3390/molecules23051228.
72. Wang L, Ruffner DE. Cyclodextrin grafted biocompatible amphilphilic polymer and methods of preparation and use thereof. 2001.
73. Castilla C, Flores ML, Medina R, et al. Prostate cancer cell response to paclitaxel is affected by abnormally expressed securin PTTG1. *Mol Cancer Ther*. 2014;*13*(10):2372-2383. https://doi.org/10.1158/1535-7163.MCT-13-0405.
74. Weaver BA. How taxol/paclitaxel kills cancer cells. *Mol Biol Cell*. 2014;*25*(18):2677-2681. https://doi.org/10.1091/mbc.E14-04-0916.
75. Jackson JK, Gleave ME, Yago V, Beraldi E, Hunter WL, Burt HM. The suppression of human prostate tumor growth in mice by the intratumoral injection of a slow-release polymeric paste formulation of paclitaxel. *Cancer Res*. 2000;*60*(15):4146-4151.

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