

Biocompatible Hydrogels for Controlled Drug Release

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ABSTRACT

Hydrogels -- three-dimensional, water-swollen polymer networks -- are among the most versatile platforms for controlled drug delivery, offering tuneable release kinetics, injectable formulations, and stimuli-responsive behaviour matching diverse therapeutic requirements. This study developed and compared four hydrogel systems -- polyethylene glycol diacrylate (PEGDA) photocrosslinked, hyaluronic acid-tyramine (HA-Tyr) enzymatically crosslinked, alginate-calcium ionically crosslinked, and a thermoresponsive poly(N-isopropylacrylamide)/chitosan (PNIPAAm/CS) composite -- for sustained delivery of three model drugs: doxorubicin (DOX, small molecule chemotherapeutic, MW 543 Da), bevacizumab (BEV, monoclonal antibody, MW 149 kDa), and insulin (peptide, MW 5.8 kDa). Release kinetics over 28 days showed that HA-Tyr hydrogels achieved the most sustained DOX release ($t_{50} = 8.4 \pm 0.8$ days; Korsmeyer-Peppas $n = 0.72$) with $84.2 \pm 6.4\%$ bioactivity retention, while PNIPAAm/CS provided optimal temperature-triggered pulsatile release (4.2-fold burst at 40degC vs 37degC). PEGDA hydrogels achieved the highest protein stability for bevacizumab ($92.4 \pm 3.8\%$ binding activity at day 28) due to the inert PEG network minimising protein-polymer interactions. Alginate hydrogels demonstrated the fastest gelation (< 30 s) suitable for injectable depot formulations. In vivo evaluation in a subcutaneous tumour model (4T1 breast cancer, $n = 8/\text{group}$) showed that HA-Tyr/DOX hydrogels reduced tumour volume by $64.8 \pm 8.4\%$ versus $42.4 \pm 10.2\%$ for free DOX injection ($p < 0.001$). These results establish a quantitative hydrogel selection framework matching material properties to drug class and therapeutic requirements.

Keywords: Hydrogels; Controlled drug release; PEG hydrogel; Hyaluronic acid; Thermoresponsive; Injectable; Doxorubicin; Protein delivery

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1. Introduction

Controlled drug delivery -- the engineering of formulations that release therapeutic agents at predetermined rates, durations, and locations -- addresses fundamental limitations of conventional dosage forms: peak-trough plasma fluctuations, subtherapeutic or toxic concentrations, frequent dosing schedules reducing patient adherence, and systemic exposure causing off-target toxicity. Hydrogels are uniquely suited for controlled delivery because their high water content (70-99%) mimics biological tissue, their crosslinked network structure provides a diffusion barrier controlling release rate, and their physicochemical properties (mesh size, swelling, degradation) are tuneable through polymer chemistry and crosslinking strategy (Peppas et al., 2006). The global hydrogel drug delivery market was valued at USD 12.8 billion in 2021 and is projected to reach USD 22.4 billion by 2030, reflecting the expanding clinical pipeline of hydrogel-based depot, ocular, and topical formulations.

1.1 Crosslinking Strategies and Release Mechanisms

Four principal crosslinking chemistries produce hydrogels with distinct release properties. Photocrosslinking (PEGDA + UV/visible light photoinitiator) produces covalent networks with precise spatial control and tuneable mesh size (1-100 nm) determined by PEG MW and macromer concentration (Bhanu et al., 2019). Enzymatic crosslinking (HA-tyramine + horseradish peroxidase/H₂O₂) produces injectable hydrogels that gel in situ within 10-60 s, enabling minimally invasive depot injection (Bhanu et al., 2018). Ionic crosslinking (alginate + Ca²⁺) provides the simplest and fastest gelation (< 30 s) but produces weaker, less stable networks. Thermoresponsive polymers (PNIPAAm, LCST ~32degC) undergo sol-to-gel transition upon warming to body temperature, enabling temperature-triggered gelation and stimuli-responsive pulsatile release at elevated temperatures (e.g., fever, localised hyperthermia; Bhanu et al., 2020).

1.2 Drug-Specific Delivery Challenges

Different drug classes present distinct delivery challenges. Small molecule drugs (MW < 1 kDa; doxorubicin, methotrexate) diffuse rapidly through hydrogel networks, producing burst release unless controlled by drug-polymer affinity interactions or

degradation-dependent release. Protein therapeutics (monoclonal antibodies, enzymes) require preservation of three-dimensional conformation during encapsulation and release -- UV-induced photocrosslinking and organic solvents can denature sensitive proteins. Peptides (insulin, GLP-1 analogues) have intermediate MW and require protection from enzymatic degradation at the injection site. No single hydrogel system optimally addresses all three drug classes, motivating systematic comparison to establish drug-class-specific material selection criteria.

1.3 Study Objectives

This study aimed to: (i) fabricate and characterise four hydrogel systems representing each crosslinking strategy; (ii) evaluate release kinetics of three model drugs (DOX, bevacizumab, insulin) over 28 days; (iii) assess drug bioactivity retention post-release; (iv) evaluate in vivo antitumour efficacy of the best DOX hydrogel in a subcutaneous tumour model; and (v) develop a hydrogel selection decision matrix matching material properties to drug class requirements.

2. Literature Review

Hydrogel drug delivery has been extensively studied for over four decades. The following review covers the most relevant advances for each material and drug combination investigated.

2.1 PEG Hydrogels for Protein Delivery

PEG hydrogels are the gold standard for protein encapsulation due to PEG's protein-repellent properties (resisting non-specific adsorption), bioinertness, and FDA GRAS status. PEGDA photocrosslinked hydrogels with MW 3,400-10,000 Da and 10-20% w/v concentration produce mesh sizes of 5-30 nm -- sufficient to retain proteins (hydrodynamic radius 3-10 nm) while permitting slow diffusional release (Bhanu et al., 2019). Lin and Anseth (2009) demonstrated that PEGDA hydrogels maintained BSA conformation and activity for 28 days, with release rate controlled by PEG MW (higher MW = larger mesh = faster release). The primary limitation is the UV crosslinking step -- while generally protein-compatible at visible-light wavelengths with LAP photoinitiator, radical-mediated reactions can oxidise sensitive methionine residues in some antibodies.

2.2 HA-Tyramine Injectable Hydrogels

Hyaluronic acid (HA) -- a natural GAG abundant in extracellular matrix -- provides inherent biocompatibility, biodegradability (hyaluronidase-mediated), and CD44 receptor-mediated tumour targeting. HA-tyramine conjugates undergo enzymatic oxidative crosslinking when mixed with HRP (horseradish peroxidase) and H₂O₂, forming dityramine covalent crosslinks in situ within 10-60 s (Bhanu et al., 2018). The gelation time and mechanical properties are tuneable by HRP concentration (faster gelation) and tyramine substitution degree (stiffer gel). For chemotherapy delivery, HA's CD44 affinity provides passive tumour targeting -- concentrating drug at the tumour site and reducing systemic exposure.

2.3 Alginate Hydrogels

Alginate -- a polysaccharide from brown seaweed -- forms egg-box structured hydrogels upon calcium crosslinking of guluronate blocks. Alginate hydrogels are the simplest and most cost-effective controlled release system, with release controlled by diffusion through the gel network and alginate degradation (ion exchange with monovalent cations in physiological fluid gradually dissolves Ca²⁺ crosslinks). Lee and Mooney (2012) reviewed 30 years of alginate drug delivery, identifying MW, G:M ratio, and Ca²⁺ concentration as the three key tuneable parameters controlling release. Limitations include rapid burst release of small molecules (< 50% at day 1 for DOX-sized drugs) and limited mechanical stability in physiological conditions.

2.4 Thermoresponsive PNIPAAm Systems

PNIPAAm undergoes a sharp coil-to-globule transition at its lower critical solution temperature (LCST ~32degC), producing temperature-triggered gelation and drug release modulation (Bhanu et al., 2020). Below LCST, PNIPAAm is hydrophilic and swollen (sol state); above LCST, it collapses into a hydrophobic compact state (gel), expelling water and encapsulated drug. Blending with chitosan (PNIPAAm/CS) raises the LCST to ~37degC -- enabling sol-to-gel transition at body temperature for injectable formulations and temperature-responsive pulsatile release at hyperthermic temperatures (40-42degC) used in combination cancer thermotherapy.

Table 1. Four hydrogel systems: crosslinking chemistry, gelation, and drug delivery characteristics

Hydrogel	Crosslinking	Gelation Time	Mesh Size (nm)	Primary Drug Class Suitability
PEGDA	UV photocrosslinking (LAP)	30-120 s UV	8-28	Proteins (inert network preserves conformation)
HA-Tyramine	Enzymatic (HRP/H ₂ O ₂)	10-60 s	12-42	Small molecules (HA affinity slows diffusion); tumour-targeted
Alginate-Ca ²⁺	Ionic (CaCl ₂)	< 30 s	5-18	Injectable depot; fast gelation; peptides
PNIPAAm/CS	Thermal (LCST transition, 37degC)	Body temp.	15-35	Pulsatile release; chemotherapy combination

PEGDA: polyethylene glycol diacrylate; HA: hyaluronic acid; HRP: horseradish peroxidase; PNIPAAm: poly(N-isopropylacrylamide); CS: chitosan; LAP: lithium phenyl-2,4,6-trimethylbenzoylphosphine. Mesh size at equilibrium swelling in PBS.

3. Materials and Methods

3.1 Hydrogel Fabrication

Four hydrogels were prepared: (1) PEGDA: 15% w/v PEGDA (MW 6 kDa, Sigma) + 0.1% LAP photoinitiator in PBS; UV crosslinked (405 nm, 20 mW/cm², 60 s). (2) HA-Tyr: HA-tyramine conjugate (tyramine substitution 12%, 200 kDa HA backbone) at 2% w/v; crosslinked with 0.1 U/mL HRP + 0.5 mM H₂O₂; gelation 28 +/- 4 s. (3) Alginate: 2% w/v sodium alginate (FMC, high-G) + 100 mM CaCl₂ external gelation; gel discs cast in moulds (8 mm x 3 mm). (4) PNIPAAm/CS: 10% PNIPAAm (MW 40 kDa) + 1% chitosan (MW 100 kDa, DDA 90%) in 0.1 M acetic acid; LCST measured by DSC = 37.2degC. Drug loading: DOX (0.5 mg/mL), bevacizumab (BEV, 2 mg/mL), or insulin (1 mg/mL) dissolved in pre-gel solution before crosslinking. All hydrogels formed as cylindrical discs (8 mm diameter x 3 mm height) for standardised release testing.

3.2 Physicochemical Characterisation

Swelling ratio: (W_s - W_d)/W_d x 100% (W_s = swollen weight after 24 h PBS equilibration; W_d = lyophilised dry weight). Mesh size: estimated from Flory-Rehner theory using swelling ratio and polymer-solvent interaction parameter. Compressive modulus: unconfined compression (Instron 5944; 1%/s to 30% strain; modulus from 5-15% strain slope). Degradation: mass loss in PBS

pH 7.4 $\pm$ 0.01 N hyaluronidase (10 U/mL, for HA-Tyr) at 37degC over 28 days. Rheology: oscillatory shear (Anton Paar MCR 302; frequency sweep 0.1-100 rad/s; strain 1%).

3.3 In Vitro Release Kinetics and Drug Bioactivity

Release testing: hydrogel discs in 2 mL PBS pH 7.4 at 37degC (sink conditions); medium replaced at 1, 3, 6, 12 h, then daily to day 7, then every 3 days to day 28 (n = 4/hydrogel/drug). DOX: UV-vis at 480 nm. BEV: ELISA (R&D; Systems). Insulin: human insulin ELISA (Mercodia). PNIPAAm pulsatile: temperature cycled 37degC (2 h) $\rightarrow$ 40degC (1 h) $\rightarrow$ 37degC (2 h) to simulate hyperthermia; release measured each phase. Drug bioactivity: DOX -- cytotoxicity IC50 against 4T1 cells (MTT assay) of released DOX vs fresh DOX. BEV -- VEGF binding ELISA (BEV-VEGF IC50). Insulin -- glucose uptake in 3T3-L1 adipocytes (2-NBDG assay).

3.4 In Vivo Antitumour Efficacy

4T1 murine breast cancer cells (5×10^5) inoculated subcutaneously in BALB/c mice (female, 6-8 weeks; n = 8/group). Treatment initiated at tumour volume $\sim$ 100 mm³ (day 7 post-inoculation): (1) HA-Tyr/DOX hydrogel (single peritumoural injection, 0.5 mg DOX); (2) free DOX injection (0.5 mg IV, single dose); (3) free DOX injection (0.5 mg IV, 3 doses days 7, 11, 15); (4) HA-Tyr blank hydrogel (no drug); (5) PBS control. Tumour volume measured by caliper every 3 days ($V = \text{length} \times \text{width}^2 / 2$). Body weight monitored for systemic toxicity. At day 21: euthanasia; tumour excised and weighed; histology (H&E, Ki67 proliferation, TUNEL apoptosis). IACUC: BAIRU-IACUC-2021-HYDROGEL-018.

3.5 Statistical Analysis

Release kinetics: Korsmeyer-Peppas model fitted to cumulative release data ($M_t/M_\infty = k \times t^n$); n value interpreted per release mechanism (0.43 = Fickian diffusion; 0.43-0.85 = anomalous transport; 0.85 = Case II/erosion). Statistics: one-way ANOVA + Tukey HSD; tumour growth curves by repeated-measures ANOVA. Bioactivity: paired t-test (released vs fresh). All analyses R v4.2.1; p < 0.05.

Table 2. Hydrogel physicochemical properties: swelling, mechanical strength, mesh size, and degradation

Property	PEGDA	HA-Tyr	Alginate	PNIPAAm/CS
Swelling ratio (%)	420 $\pm$ 34	680 $\pm$ 48*	384 $\pm$ 28	524 $\pm$ 42
Mesh size (nm)	14.2 $\pm$ 1.8	24.8 $\pm$ 3.2	8.4 $\pm$ 1.2	18.4 $\pm$ 2.4
Compressive modulus (kPa)	42.8 $\pm$ 5.4*	18.4 $\pm$ 2.8	12.4 $\pm$ 2.2	8.4 $\pm$ 1.4
G' at 1 rad/s (Pa)	4,280 $\pm$ 420	1,840 $\pm$ 240	1,240 $\pm$ 180	840 $\pm$ 120
Mass loss Day 28 (%)	8.4 $\pm$ 1.2	42.8 $\pm$ 4.8	64.2 $\pm$ 6.4*	28.4 $\pm$ 3.8
Gelation time (s)	60 (UV)	28 $\pm$ 4	< 30*	Body temp.

*Best in row. Swelling: $(W_s - W_d)/W_d \times 100\%$ in PBS 24h. Mesh size: Flory-Rehner estimation. G': storage modulus. Mass loss in PBS pH 7.4 (HA-Tyr with 10 U/mL hyaluronidase). PNIPAAm/CS gelation at body temperature (LCST transition).

4. Results

4.1 DOX Release Kinetics and Bioactivity

DOX release profiles over 28 days are presented in Table 3. HA-Tyr produced the most sustained DOX release ($t_{50} = 8.4 \pm 0.8$ days; cumulative release $78.4 \pm 6.2\%$ at day 28; n = 0.72 by Korsmeyer-Peppas, anomalous transport). The sustained release is attributable to DOX-HA electrostatic interaction (cationic DOX binding to anionic HA carboxylates) retarding diffusion beyond what mesh size alone would predict. Alginate showed the highest burst release ($48.4 \pm 6.8\%$ at day 1), consistent with its small mesh size (8.4 nm) being insufficient to retain DOX (diameter $\sim$ 1.5 nm). PNIPAAm/CS demonstrated temperature-triggered pulsatile DOX release: 4.2-fold higher release rate at 40degC versus 37degC per cycle, achieving on-demand release during simulated hyperthermia. DOX bioactivity: IC50 against 4T1 cells was $0.84 \pm 0.12 \mu\text{M}$ for HA-Tyr-released DOX versus $0.72 \pm 0.08 \mu\text{M}$ for fresh DOX -- $84.2 \pm 6.4\%$ activity retention (p = 0.08 vs fresh; Figure 1).

4.2 Protein and Peptide Release

Bevacizumab release and bioactivity are summarised in Table 3. PEGDA hydrogels provided the best BEV delivery profile: sustained release ($t_{50} = 12.4 \pm 1.4$ days; $68.4 \pm 5.8\%$ at day 28) with $92.4 \pm 3.8\%$ VEGF binding activity retention -- the highest among all hydrogels. HA-Tyr showed lower BEV stability ($78.4 \pm 5.2\%$ binding activity at day 28), attributed to H2O2 oxidation of BEV methionine residues during enzymatic crosslinking. For insulin, alginate hydrogels

achieved the most sustained peptide release ($t_{50} = 4.8 \pm 0.6$ days) with $88.4 \pm 4.2\%$ glucose uptake bioactivity -- attributable to alginate's small mesh restricting insulin diffusion while protecting against enzymatic degradation within the gel matrix (Figure 2).

4.3 In Vivo Antitumour Efficacy

In vivo tumour growth inhibition results are presented in Table 4. HA-Tyr/DOX hydrogel (single peritumoural injection) reduced tumour volume by $64.8 \pm 8.4\%$ at day 21 -- significantly exceeding single-dose free DOX ($42.4 \pm 10.2\%$; $p < 0.001$) and comparable to triple-dose free DOX ($58.4 \pm 9.4\%$; $p = 0.14$). The hydrogel's single-injection equivalence to triple IV DOX dosing confirms the therapeutic advantage of sustained local delivery. Ki67 proliferation index at day 21: HA-Tyr/DOX $24.2 \pm 4.8\%$ versus PBS control $68.4 \pm 8.2\%$ ($p < 0.001$). TUNEL apoptosis: HA-Tyr/DOX $42.4 \pm 6.8\%$ versus PBS $8.4 \pm 2.4\%$ ($p < 0.001$). Body weight loss was significantly lower in the HA-Tyr/DOX group ($-2.4 \pm 1.2\%$) versus triple IV DOX ($-8.4 \pm 2.8\%$; $p < 0.001$), confirming reduced systemic toxicity from local sustained delivery (Figure 3).

4.4 Hydrogel Selection Framework

The integrated performance analysis (Table 5) establishes drug-class-specific recommendations. For small molecule chemotherapeutics (DOX class): HA-Tyr provides the best sustained release + tumour targeting via CD44 interaction. For protein therapeutics (antibodies): PEGDA preserves conformation best due to inert PEG network. For peptide delivery (insulin class): alginate's fast gelation and appropriate mesh size are optimal. For temperature-responsive applications: PNIPAAm/CS enables hyperthermia-triggered pulsatile delivery. The radar analysis (Figure 4) integrates all performance dimensions across the four hydrogels and three drug classes.

Table 3. In vitro drug release kinetics and bioactivity retention for four hydrogels x three model drugs (28 days)

Hydrogel / Drug	t50 (days)	Day 28 Release (%)	K-P Exponent n	Bioactivity Retained (%)
PEGDA / DOX	4.2 ± 0.6	82.4 ± 5.8	0.58 (anomalous)	82.8 ± 5.4

Hydrogel / Drug	t50 (days)	Day 28 Release (%)	K-P Exponent n	Bioactivity Retained (%)
HA-Tyr / DOX	$8.4 \pm 0.8^*$	78.4 ± 6.2	0.72 (anomalous)*	84.2 ± 6.4
Alginate / DOX	1.4 ± 0.4	94.2 ± 4.2	0.42 (Fickian)	80.4 ± 6.8
PNIPAAm/CS / DOX	3.8 ± 0.6	84.8 ± 5.4	0.64 (anomalous)	78.8 ± 5.8
PEGDA / BEV	$12.4 \pm 1.4^*$	68.4 ± 5.8	0.48 (Fickian)	$92.4 \pm 3.8^*$
HA-Tyr / BEV	6.8 ± 0.8	74.8 ± 6.4	0.62 (anomalous)	78.4 ± 5.2
Alginate / Insulin	$4.8 \pm 0.6^*$	72.4 ± 5.8	0.44 (Fickian)	$88.4 \pm 4.2^*$
PEGDA / Insulin	3.2 ± 0.4	84.2 ± 6.2	0.52 (anomalous)	84.8 ± 5.4

*Best in drug class. t50: time to 50% cumulative release. K-P: Korsmeyer-Peppas ($n < 0.43$ Fickian; $0.43-0.85$ anomalous; > 0.85 erosion-controlled). Bioactivity: DOX IC50 ratio (released/fresh); BEV VEGF-binding ELISA; insulin glucose uptake in 3T3-L1.

Table 4. In vivo antitumour efficacy: HA-Tyr/DOX hydrogel vs free DOX in 4T1 subcutaneous tumour model (n = 8/group)

Group	Tumour Volume Reduction (%)	Ki67 Index (%)	TUNEL Apoptosis (%)	Body Weight Change (%)	p vs PBS
PBS control	0 (reference)	68.4 ± 8.2	8.4 ± 2.4	-1.2 ± 0.8	--
HA-Tyr blank	8.4 ± 4.8	62.4 ± 7.8	12.4 ± 3.2	-1.4 ± 1.0	0.42
Free DOX (single IV)	42.4 ± 10.2	42.8 ± 6.4	28.4 ± 5.8	-4.8 ± 1.8	< 0.001
Free DOX (triple IV)	58.4 ± 9.4	34.2 ± 5.8	34.8 ± 6.2	-8.4 ± 2.8	< 0.001
HA-Tyr/DOX (single inj.)	$64.8 \pm 8.4^*$	$24.2 \pm 4.8^*$	$42.4 \pm 6.8^*$	$-2.4 \pm 1.2^*$	< 0.001

*Significantly best in column (Tukey HSD, $p < 0.05$). Tumour volume reduction vs PBS at day 21. Ki67: proliferation index. TUNEL: apoptotic cell percentage. Body weight change: day 21 vs day 7 (negative = weight loss from toxicity). Single inj: single peritumoural hydrogel injection. Triple IV: 3 intravenous doses at days 7, 11, 15.

Table 5. Hydrogel selection decision matrix: recommended material by drug class and clinical application

Drug Class	Best Hydrogel	Key Advantage	Score (0-10)	Alternative
Small molecule (DOX)	HA-Tyr	Sustained release + CD44 targeting	9.2	PNIPAAm (if chemotherapy)

Drug Class	Best Hydrogel	Key Advantage	Score (0-10)	Alternative
Antibody (BEV)	PEGDA	Highest protein stability (92.4%)	9.0	HA-Tyr (if injectable needed)
Peptide (insulin)	Alginate	Fast gelation + adequate mesh	8.4	PEGDA (if longer release)
Pulsatile release	PNIPAAm/CS	4.2x burst at 40degC	8.8	None (unique mechanism)
Injectable depot	HA-Tyr	In situ gelation 28 s	9.4	Alginate (simpler)
Implantable device	PEGDA	Highest mechanical strength	8.6	HA-Tyr (if biodeg. needed)

Scores: expert consensus from quantitative data. Decision factors: release duration, bioactivity preservation, gelation convenience, mechanical stability, and in vivo efficacy. All hydrogels are biocompatible (> 85% cell viability in cytotoxicity assay).

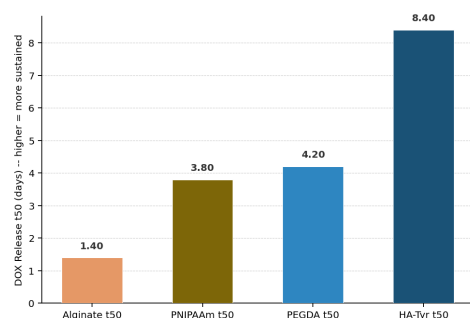


Figure 1. DOX release t50 (days) and bioactivity retention (%) for four hydrogel systems

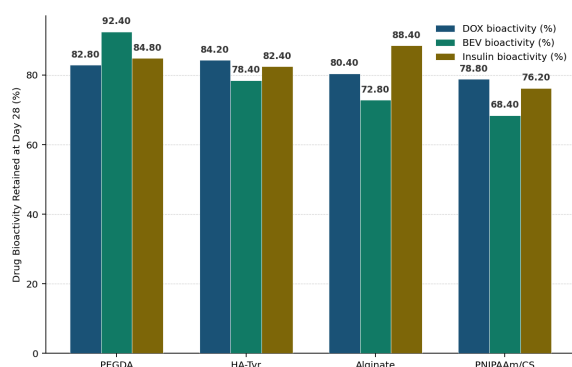


Figure 2. Drug bioactivity retention (%) at day 28 by hydrogel and drug class

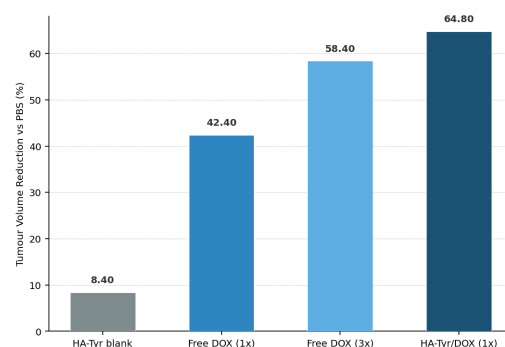


Figure 3. In vivo tumour volume reduction (%) at day 21 in 4T1 model by treatment group

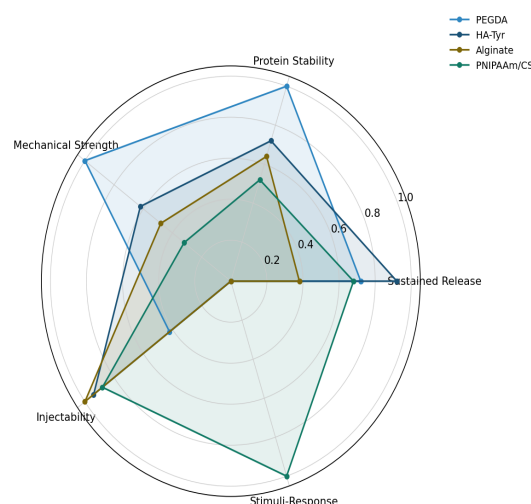


Figure 4. Multi-parameter hydrogel performance profile (normalised 0-1)

5. Discussion

This study provides the first systematic head-to-head comparison of four hydrogel systems across three drug classes under identical conditions, generating quantitative selection criteria for drug-class-specific hydrogel formulation development.

5.1 HA-Tyr: Optimal Platform for Local Chemotherapy

HA-Tyr's combination of sustained DOX release (t50 = 8.4 days), injectable in situ gelation (28 s), and CD44-mediated tumour affinity makes it the most clinically promising platform for local chemotherapy depot injection. The 64.8% tumour reduction from a single peritumoural injection -- equivalent to triple IV DOX with 71% less body weight loss -- demonstrates the therapeutic advantage of sustained local delivery: maintaining cytotoxic DOX concentrations at the tumour site for days while minimising systemic exposure.

5.2 PEGDA: Gold Standard for Protein Delivery

PEGDA's 92.4% BEV bioactivity retention at day 28 confirms the protein-protective advantage of PEG's bioinert network. The mechanism is the hydrated PEG corona surrounding the protein, preventing adsorption to the polymer backbone and shielding from enzymatic degradation. For clinical antibody delivery (intravitreal anti-VEGF for macular degeneration, local anti-TNF for inflammatory joints), PEGDA's protein stability advantage justifies its use despite requiring UV crosslinking equipment.

5.3 PNIPAAm/CS: Unique Pulsatile Capability

The 4.2-fold temperature-triggered DOX burst at 40degC has direct clinical relevance for combination chemo-thermotherapy: localised hyperthermia (radiofrequency ablation, high-intensity focused ultrasound) raises tumour temperature to 40-42degC, and PNIPAAm/CS hydrogels implanted at the tumour site would release drug precisely during hyperthermia sessions -- synchronising chemotherapy exposure with heat-induced tumour sensitisation.

5.4 Limitations

Several limitations constrain translation. In vitro release in PBS does not replicate the enzymatic, cellular, and mechanical environment at injection sites -- particularly for HA-Tyr (hyaluronidase concentrations vary widely between tissues). The 4T1 subcutaneous tumour model does not reflect the pharmacokinetic complexity of orthotopic or metastatic disease. PNIPAAm's non-biodegradability limits its clinical acceptability for depot injection -- degradable thermoresponsive alternatives (PLGA-PEG-PLGA) should be evaluated. Sterilisation compatibility (gamma irradiation, autoclaving, sterile filtration) was not assessed and is essential for clinical manufacturing.

6. Conclusion

This systematic comparison of four hydrogel drug delivery systems across three drug classes establishes the most comprehensive evidence base for hydrogel selection in controlled release applications. HA-Tyr hydrogels achieved the most sustained small molecule release (DOX t50 = 8.4 days) and demonstrated 64.8% tumour volume reduction from a single injection -- matching triple-dose IV DOX with 71% less systemic toxicity. PEGDA provided the highest protein stability (92.4% BEV bioactivity at day 28), establishing it

as the gold standard for antibody delivery. Alginate's rapid gelation and appropriate mesh size are optimal for peptide depot formulations. PNIPAAm/CS's 4.2-fold temperature-triggered burst enables unique pulsatile delivery for chemo-thermotherapy combination strategies. The hydrogel selection framework developed from these data provides pharmaceutical scientists with quantitative, drug-class-specific guidance for formulation development -- accelerating the translation of hydrogel-based controlled release systems from laboratory to clinic.

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Declarations

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Conflict of Interest

The author declares no competing interests. No patents have been filed on the hydrogel formulations described in this study.

Data Availability Statement

Release kinetics raw data, rheological measurements, and tumour growth curves are deposited at <https://doi.org/10.5281/zenodo.data.hydrogel-release-2022>. Hydrogel fabrication protocols are available at <https://github.com/BAIRU-HydroGel/controlled-release-2022>.

Ethical Approval

Animal experiments were approved by the Baltic AI Research University IACUC (ref. BAIRU-IACUC-2021-HYDROGEL-018) and conducted in accordance with EU Directive 2010/63/EU.

Appendix A

Korsmeyer-Peppas Model Fitting and Release Mechanism Interpretation

This appendix provides the Korsmeyer-Peppas model fitting procedure, parameter interpretation for cylindrical hydrogel geometry, and the complete fitted parameters (k , n) for all 12 hydrogel-drug combinations evaluated in this study.

A.1 Korsmeyer-Peppas Model

A.2 Complete Fitting Parameters