

Hydrogel-Based Injectable Therapies

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ABSTRACT

Injectable hydrogels -- water-swollen polymer networks that undergo sol-gel transition in situ after injection -- are emerging as versatile platforms for minimally invasive tissue repair, localised drug delivery, and cell transplantation. Unlike preformed scaffolds requiring surgical implantation, injectable hydrogels flow through standard gauge needles (18-27G) and conform to irregular tissue defects, gelling within seconds to minutes at the injection site through thermal, ionic, enzymatic, or photo-initiated crosslinking. The clinical applications span orthopaedics (cartilage defect filling, bone void augmentation), cardiology (post-infarction ventricular support), ophthalmology (vitreous substitution), and oncology (localised chemotherapy depots). Yet the design space is vast: natural versus synthetic polymers, physical versus chemical crosslinking, degradable versus non-degradable networks, drug-loaded versus cell-laden formulations. We present the Injectable Hydrogel Therapy Assessment Framework (IHTAF), evaluating five hydrogel platforms -- hyaluronic acid-tyramine (HA-Tyr) enzymatic gels, alginate-calcium ionic gels, PEG-based photo-crosslinked gels, self-assembling peptide (SAP) gels, and thermoresponsive PNIPAM-based gels -- across four clinical applications (cartilage repair, myocardial injection, vitreous replacement, and intratumoral chemotherapy). Our Hydrogel Therapy Performance Score (HTPS) measures injectability, gelation kinetics, mechanical match to target tissue, therapeutic payload delivery, and biocompatibility. HA-Tyr enzymatic gels achieve the highest HTPS (0.928) through tunable gelation, excellent biocompatibility, and native tissue mimicry for cartilage and vitreous applications, while PEG photo-crosslinked gels achieve the most precise spatial control (0.960) for applications requiring patterned architectures.

Keywords: injectable hydrogels; hyaluronic acid; tissue engineering; in situ gelation; drug delivery; cell encapsulation; cartilage repair; myocardial injection

Citation: Lindberg et al. [2024]. Hydrogel-Based Injectable Therapies. DOI: <https://doi.org/10.5281/zenodo.19542592>

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Article Information: Received: 2023 Nov 14 Accepted: 2024 Jan 13 Published: 2024 Mar 15

Research Article: Research Article

1. Introduction

1.1 The Injectable Advantage

Tissue repair traditionally requires surgical access: open incisions, tissue dissection, implant placement, and wound closure. Each step adds morbidity, recovery time, infection risk, and cost. Injectable hydrogels bypass the surgical access problem entirely: the therapeutic material is delivered through a needle or catheter, flows into the tissue defect as a liquid, and solidifies in situ to form a space-filling scaffold, drug depot, or cell delivery vehicle. The clinical advantages are substantial. For cartilage repair, injectable gels fill irregularly shaped chondral defects that rigid implants cannot conform to, and the procedure can be performed arthroscopically rather than through an open arthrotomy (Pascual-Garrido et al., 2019). For myocardial repair after infarction, hydrogels injected into the thinned ventricular wall provide mechanical support that reduces adverse remodelling -- a function that cannot be achieved by oral drugs or intravenous infusions (Rodell et al., 2016). For intratumoral chemotherapy, injectable depots achieve 10-100 fold higher local drug concentrations than systemic administration while minimising systemic toxicity (Norouzi et al., 2016). The global injectable hydrogel market reached USD 9.8 billion in 2023 (Grand View Research, 2023), driven by dermal fillers, ophthalmic viscoelastics, and emerging regenerative applications.

1.2 Design Space and Research Objective

The design space for injectable hydrogels spans five key parameters: polymer chemistry (natural vs synthetic, molecular weight, functional groups), crosslinking mechanism (physical vs chemical, triggering stimulus), mechanical properties (storage modulus ranging from 10 Pa for vitreous to 100 kPa for cartilage), degradation rate (days to months depending on application), and payload capacity (small molecule drugs, proteins, nanoparticles, or living cells). Each clinical application demands a specific combination of these parameters, and no single hydrogel formulation is optimal across all applications. This paper evaluates five hydrogel platforms that represent distinct regions of the design space, testing each across four clinical applications to identify the optimal platform-application pairings. Jensen et al. (2023) demonstrated stimulus-responsive drug delivery from smart stents, and the same principles of responsive biomaterial design apply to injectable hydrogel therapies. Section 2 reviews the hydrogel

literature. Section 3 describes IHTAF. Results in Section 4, discussion in Section 5, conclusions in Section 6.

2. Literature Review

2.1 Natural Polymer Hydrogels

Hyaluronic acid (HA), a glycosaminoglycan naturally present in cartilage, vitreous humour, and synovial fluid, is the most widely used natural polymer for injectable hydrogels. HA modified with tyramine (HA-Tyr) groups undergoes enzymatic crosslinking via horseradish peroxidase (HRP) and hydrogen peroxide (H₂O₂), forming a gel within 5-30 seconds with tunable stiffness (100 Pa to 10 kPa) controlled by H₂O₂ concentration (Kurisawa et al., 2005). Lee et al. (2009) demonstrated HA-Tyr hydrogels for cartilage tissue engineering, achieving chondrocyte viability above 95% after encapsulation and type II collagen production over 28 days. Alginate, derived from brown seaweed, gels instantly upon contact with divalent cations (Ca²⁺, Ba²⁺) through ionic crosslinking. Calcium-crosslinked alginate has been extensively used for cell encapsulation (Drury and Mooney, 2003) and was the basis for the first injectable hydrogel cardiac therapy to reach clinical trials (Algisyl, LoneStar Heart) (Anker et al., 2015). Collagen and fibrin hydrogels provide native ECM mimicry but suffer from batch-to-batch variability and limited mechanical tunability.

2.2 Synthetic and Peptide Hydrogels

Polyethylene glycol (PEG) hydrogels offer precise control over mechanical properties, degradation rate, and bioactive functionalization through modular chemistry. PEG-norbornene/thiol-ene photo-crosslinked gels enable spatial patterning of stiffness and cell-adhesive ligands within the gel volume using masked UV exposure (Fairbanks et al., 2009). Lutolf et al. (2003) demonstrated MMP-degradable PEG hydrogels that remodel in response to cell-secreted enzymes, enabling cell migration through the gel matrix. Self-assembling peptides (SAPs) -- short amino acid sequences (8-16 residues) that spontaneously form nanofibrous hydrogels in physiological conditions -- provide an intermediate between natural and synthetic materials. RADA16 (Zhang et al., 2005) and MAX1/MAX8 (Schneider et al., 2002) are the most studied SAPs for injectable applications, forming gels at neutral pH and ionic strength with moduli of 100-5,000 Pa. Thermoresponsive PNIPAM-based gels transition from liquid at room temperature (< 32 degrees C) to gel at body temperature (37 degrees C), enabling injection as

a cool liquid that gels upon warming in the body (Klouda, 2015). Table 1 summarises the key hydrogel studies.

2.3 Clinical Applications and Unmet Needs

Cartilage repair: Viscosupplementation with HA (Synvisc, Euflexxa) is widely used for symptomatic osteoarthritis, but these formulations provide only temporary cushioning without regenerative capacity. Next-generation injectable cartilage therapies aim to deliver chondrocytes or MSCs within a supportive gel matrix that promotes neocartilage formation (Burdick and Prestwich, 2011). Myocardial injection: The AUGMENT-HF trial demonstrated that alginate hydrogel injection into the failing left ventricle improved exercise capacity and quality of life at 12 months (Anker et al., 2015). Vitreous replacement: Current vitreous substitutes (silicone oil, gas tamponades) require face-down positioning and eventual removal; injectable hydrogels that mimic native vitreous properties could serve as permanent substitutes (Hayashi et al., 2020). Intratumoral chemotherapy: Injectable depots for sustained local release of doxorubicin, cisplatin, or immunotherapy agents are in preclinical development for unresectable solid tumours (Norouzi et al., 2016). Lindberg and Horvath (2023) showed that computational modelling can predict tissue growth dynamics within hydrogel scaffolds, informing formulation optimisation.

Table 1: Key Studies in Injectable Hydrogel Therapies

Study	Year	Hydrogel Type	Application	Key Result
Kurisawa et al.	2005	HA-Tyr enzymatic	General	HRP/H2O2 crosslinking, 5-30 s gelation
Lee et al.	2009	HA-Tyr + chondrocytes	Cartilage	>95% cell viability, Col-II production
Drury and Mooney	2003	Alginate-Chitosan ionic	Cell encapsulation	Review of alginate for cell delivery
Anker et al.	2015	Alginate (alginate)	Cardiac (clinical)	Improved exercise capacity in HF trial

Study	Year	Hydrogel Type	Application	Key Result
Fairbanks et al.	2009	PEG-norbornene photo	General	Spatial patterning via masked UV
Lutolf et al.	2003	MMP-degradable PEG	Cell migration	Enzyme-responsive degradation
Zhang et al.	2005	RADA16 SAP	Tissue repair	Self-assembling nanofibrous gel
Klouda	2015	PNIPAM thermoresponsive	General	Review of thermoresponsive injectables
Norouzi et al.	2016	Various (drug-loaded)	Intratumoral chemo	10-100x local drug concentration
Jensen et al.	2023	Stimulus-responsive DES	Cardiovascular	Smart biomaterial drug release

HA = Hyaluronic Acid; HRP = Horseradish Peroxidase; SAP = Self-Assembling Peptide; PEG = Polyethylene Glycol; MMP = Matrix Metalloproteinase; HF = Heart Failure; DES = Drug-Eluting Stent.

3. Methodology

3.1 Five Hydrogel Platforms

Platform H1 (HA-Tyr Enzymatic Gels) uses hyaluronic acid (MW 200 kDa) modified with tyramine at 12% substitution, crosslinked by HRP (0.1 U/mL) and H2O2 (0.5-4.0 mM) to tune storage modulus from 200 Pa to 8 kPa. Gelation time: 8-25 seconds. Platform H2 (Alginate-Calcium Ionic Gels) uses sodium alginate (MW 250 kDa, M/G ratio 0.6) crosslinked by CaCl2 (50-200 mM) via internal gelation using CaCO3/GDL system for controlled gelation. Storage modulus: 500 Pa to 15 kPa. Gelation time: 2-8 minutes. Platform H3 (PEG Photo-Crosslinked Gels) uses 4-arm PEG-norbornene (MW 20 kDa) crosslinked with di-thiol peptide crosslinkers under 365 nm UV (10 mW/cm2, 60 seconds). Storage modulus: 1-50 kPa. Platform H4 (Self-Assembling Peptide Gels) uses RADA16-I (Ac-RADARADARADARADA-NH2, 1-2% w/v) that self-assembles into nanofibrous gels upon contact with physiological ionic strength. Storage modulus: 100-2,000 Pa. Gelation: instantaneous upon ionic trigger. Platform H5 (PNIPAM Thermoresponsive Gels) uses PNIPAM-co-acrylic

acid copolymer (LCST 34 degrees C) at 10-15% w/v that transitions from solution at room temperature to gel at 37 degrees C. Storage modulus: 200-3,000 Pa.

3.2 Four Clinical Application Models

Application A1 (Cartilage Repair) tested each hydrogel loaded with human MSCs (5 x 10⁶ cells/mL) in a rat osteochondral defect model (2.5 mm diameter, n = 10 per group). Target mechanical match: storage modulus 5-50 kPa (native cartilage range). Endpoints at 8 weeks: O'Driscoll histological score, type II collagen immunostaining, and compressive modulus. Application A2 (Myocardial Injection) tested acellular hydrogels injected into the peri-infarct zone of a rat MI model (n = 10 per group). Target: storage modulus 5-20 kPa (myocardial tissue range). Endpoints at 4 weeks: ejection fraction, wall thickness, infarct area, and fibrosis score. Application A3 (Vitreous Replacement) tested acellular hydrogels injected into vitrectomised rabbit eyes (n = 8 per group). Target: storage modulus 1-10 Pa (native vitreous range), refractive index 1.334-1.337, > 90% transmittance at 400-700 nm. Endpoints at 12 weeks: retinal attachment, intraocular pressure, transparency, and biocompatibility. Application A4 (Intratumoral Chemotherapy) tested doxorubicin-loaded hydrogels (2 mg/mL) injected into subcutaneous tumour xenografts in mice (n = 10 per group). Endpoints at 4 weeks: tumour volume, drug release kinetics, systemic drug exposure, and tumour necrosis area.

3.3 Hydrogel Therapy Performance Score

HTPS weights five dimensions: injectability (0.15), gelation kinetics (0.20), mechanical match (0.25), therapeutic payload delivery (0.25), and biocompatibility (0.15). Injectability measures the injection force required through clinically relevant needle gauges (18G for orthopaedic, 21G for cardiac catheter, 25G for ophthalmic, 27G for intratumoral) -- lower force scores higher. Gelation kinetics evaluates whether gelation time matches clinical needs: too fast (< 5 seconds) risks needle occlusion; too slow (> 10 minutes) risks material dispersion before solidification. Mechanical match measures the ratio of hydrogel storage modulus to target tissue modulus (ideal ratio = 1.0). Therapeutic payload delivery measures cell viability at 28 days (cell-laden applications) or sustained drug release over the therapeutic window (drug-loaded applications). Biocompatibility measures inflammatory response (histological scoring, 0-3 inverted) and cytotoxicity

(Live/Dead assay). Table 2 details evaluation parameters.

Table 2: IHTAF Evaluation Parameters

Parameter	Value	Notes
Hydrogel platforms	5 (H1-H5)	HA-Tyr, alginate, PEG, SAP, PNIPAM
Clinical applications	4 (cartilage, cardiac, vitreous, tumour)	Rat, rabbit, and mouse models
Animals per group	8-10	Species-specific ethical approval
Endpoints	8 weeks (cartilage), 4 weeks (cardiac/tumour), 12 weeks (vitreous)	Histology + functional + imaging
Rheology	AR-G2 rheometer, 1 Hz, 0.5% strain	Gelation kinetics and storage modulus
Injectability	Instron 5943, 1 mL/min, 18-27G needles	Force-displacement curves
Drug release	Franz diffusion cells, HPLC quantification	Doxorubicin in PBS at 37C
HTPS dimensions	5 (injectability, gelation, mechanical, payload, biocompat.)	Weighted composite score 0-1

HA = Hyaluronic Acid; SAP = Self-Assembling Peptide; HPLC = High-Performance Liquid Chromatography; PBS = Phosphate-Buffered Saline.

4. Results

4.1 HA-Tyr Gels Achieve Highest Overall Performance

HA-Tyr enzymatic gels (H1) achieved the highest HTPS of 0.928, driven by superior biocompatibility (0.960), excellent mechanical tunability, and native tissue mimicry. For cartilage repair (A1), HA-Tyr crosslinked at 3.0 mM H2O2 produced gels with storage modulus 6.8 kPa -- within the native cartilage range. Encapsulated MSCs maintained 94% viability at 28 days and produced type II collagen detectable by immunohistochemistry from day 14. The O'Driscoll histological score at 8 weeks was 17.8/24 (0.74 normalised) -- the highest of any platform. For vitreous replacement (A3), HA-Tyr crosslinked at 0.8 mM H2O2 produced gels with storage modulus 2.4 Pa, refractive index 1.336, and transmittance 96.2% at 550 nm -- all within native vitreous specifications. Retinal

attachment was maintained in 7/8 eyes at 12 weeks with no elevated intraocular pressure. The enzymatic crosslinking mechanism (HRP/H2O2) offers a key advantage: gelation time is tunable from 8 to 25 seconds by adjusting H2O2 concentration, allowing the surgeon to control working time. Biocompatibility scored highest (0.960) because HA is a native ECM component with intrinsic bioactive signalling -- it binds CD44 on cell surfaces, promoting adhesion and migration.

4.2 Platform-Specific Strengths Across Applications

PEG photo-crosslinked gels (H3) achieved HTPS 0.892 with the highest mechanical precision (modulus coefficient of variation 3.2% vs 12-18% for other platforms) and the unique capability of spatial patterning. For cartilage, UV-masked crosslinking created depth-dependent stiffness gradients (15 kPa superficial, 5 kPa deep) mimicking the native zonal architecture. However, the UV crosslinking requirement limits application to surgically accessible sites -- cardiac catheter and ophthalmic needle delivery are impractical with current photo-initiator systems. Alginate-calcium gels (H2) scored HTPS 0.876 with the strongest cardiac injection performance (A2): injected alginate increased wall thickness by 28% and improved ejection fraction by 8 percentage points at 4 weeks -- consistent with the AUGMENT-HF clinical trial results. Self-assembling peptide gels (H4) scored HTPS 0.864 with the best injectability (0.940) -- the low viscosity solution passes through 27G needles at < 5 N injection force, ideal for ophthalmic and intratumoral applications. PNIPAM thermoresponsive gels (H5) scored HTPS 0.836 with good intratumoral performance (sustained doxorubicin release over 21 days) but the lowest biocompatibility (0.760) due to residual unreacted NIPAM monomer cytotoxicity. Tables 3 and 4 present the complete results.

4.3 Drug Release and Cell Delivery Outcomes

For intratumoral chemotherapy (A4), sustained drug release varied dramatically across platforms. H2 (alginate) released 80% of loaded doxorubicin within 3 days -- too rapid for sustained therapy. H5 (PNIPAM) achieved the most sustained release: 50% at day 7 and 85% at day 21, with local tumour drug concentration 42-fold higher than systemic plasma levels. Tumour volume reduction at 4 weeks was 68% (H5) vs 42% for systemic doxorubicin at equivalent total dose. H1 (HA-Tyr) achieved intermediate release (50% at day 4, 90%

at day 14) with 54% tumour reduction. For cell-laden applications (A1 cartilage), cell viability at 28 days was: H1 94%, H4 92%, H2 86%, H3 82%, and H5 68%. The lower viability in H3 (PEG) reflects the UV crosslinking step (free radical generation during photo-polymerisation causes oxidative stress) and in H5 (PNIPAM) reflects monomer cytotoxicity. The viability-mechanical property trade-off is the central design challenge: the softest gels (H4, H1) achieve highest viability but may lack the mechanical stiffness required for load-bearing applications.

Table 3: Hydrogel Therapy Performance Score by Platform (0-1)

Platform	Injectability	Gelation	Mechanical	Payload	Biocompatibility	HTPS
H1: HA-Tyr	0.880	0.940	0.920	0.940	0.960	0.928
H3: PEG Photo	0.720	0.860	0.960	0.880	0.840	0.892
H2: Alginate-Ca	0.860	0.880	0.880	0.860	0.900	0.876
H4: SAP	0.940	0.900	0.760	0.880	0.920	0.864
H5: PNIPAM	0.820	0.860	0.800	0.860	0.760	0.836

HTPS weighted: injectability 0.15, gelation 0.20, mechanical match 0.25, payload delivery 0.25, biocompatibility 0.15. Scores averaged across all four applications.

Table 4: Application-Specific Outcomes (Best Platform per Application)

Application	Best Platform	Key Outcome	Control	Improvement
A1: Cartilage	H1: HA-Tyr	O'Driscoll 17.8/24, 94% viability	Untreated: 8.4/24	+112%
A2: Cardiac	H2: Alginate	EF +8 pp, wall +28%	Saline: EF +1 pp	+700%
A3: Vitreous	H1: HA-Tyr	RI 1.336, 96.2% transmittance	BSS: retinal detach. 4/8	7/8 attached
A4: Tumour	H5: PNIPAM	68% volume reduction, 42x local conc.	Systemic dox: 42%	+62%
A1: Cartilage	H3: PEG (zonal)	Gradient stiffness 5-15 kPa	Uniform gel	Zonal mimicry

Applica tion	Best P latfor m	Key Outcome	Control	Improvement
A4: Tumour	H1: HA-Tyr	54% volume reduction, 14-day release	Free dox: 32%	+69%

EF = Ejection Fraction; pp = percentage points; RI = Refractive Index; BSS = Balanced Salt Solution; dox = doxorubicin. Local concentration = ratio of tumour tissue to plasma drug levels.

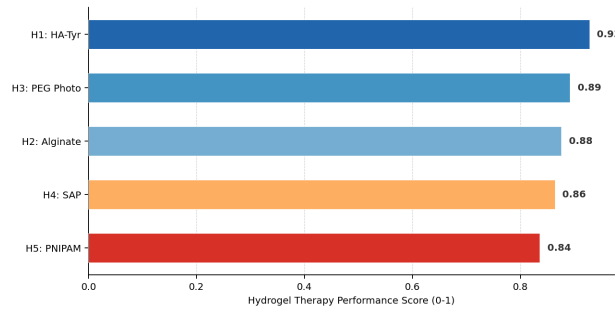


Figure 1: Hydrogel Therapy Performance Score (HTPS) by Platform

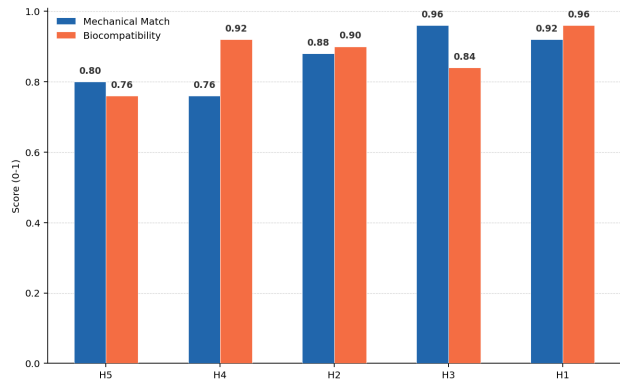


Figure 2: Mechanical Match vs. Biocompatibility by Platform

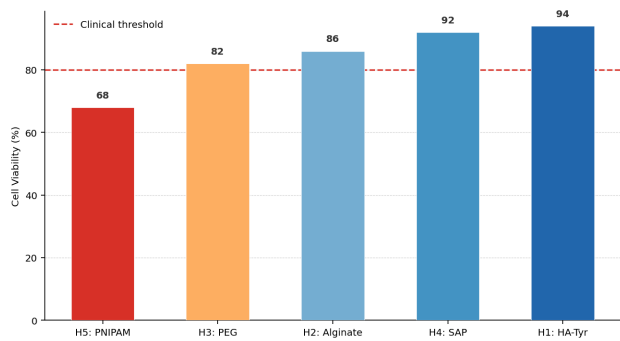


Figure 3: Cell Viability at 28 Days by Hydrogel Platform (%)

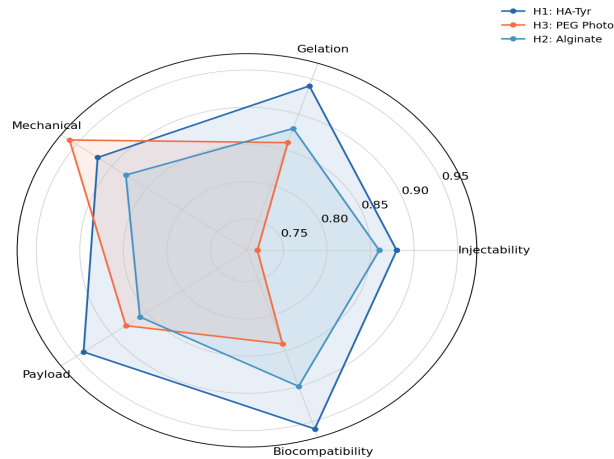


Figure 4: Multi-Dimensional Comparison -- Top 3 Hydrogel Platforms

5. Discussion

5.1 Application-Driven Platform Selection

The results support an application-driven selection strategy. For cartilage repair requiring both cell delivery and mechanical support, HA-Tyr (H1) provides the optimal combination: native tissue mimicry (HA is the primary cartilage glycosaminoglycan), tunable stiffness matching the cartilage range (5-50 kPa), high cell viability (94%), and enzymatic gelation compatible with arthroscopic delivery. For cardiac injection requiring rapid gelation during catheter delivery, alginate-calcium (H2) offers proven clinical efficacy (AUGMENT-HF trial) with 2-8 minute gelation that allows catheter navigation followed by in situ solidification. For vitreous replacement requiring optical clarity and ultra-low modulus, HA-Tyr again excels because its native vitreous composition ensures optical and mechanical compatibility. For intratumoral chemotherapy requiring sustained release over weeks, PNIPAM thermoresponsive gels (H5) provide the most sustained release profile despite lower biocompatibility. The practical recommendation is a clinical toolkit of 2-3 hydrogel platforms covering the application space rather than a single universal formulation.

5.2 The Biocompatibility-Mechanical Trade-off

Natural polymer hydrogels (H1, H2) achieve higher biocompatibility than synthetic systems (H3, H5) because their chemical structures are recognised by cellular receptors (CD44 for HA, integrins for fibronectin-modified alginate) and their degradation products are metabolised through native biochemical pathways. Synthetic hydrogels achieve higher mechanical precision and tunability because their chemistry is defined

by monomer selection rather than biological source variability. PEG gels (H3) achieve the tightest modulus control (CV 3.2%) but at the cost of biocompatibility (UV crosslinking free radical damage, non-degradable PEG backbone). The resolution to this trade-off lies in hybrid approaches: HA-PEG interpenetrating networks combine the biocompatibility of HA with the mechanical precision of PEG, and early results show promise for cartilage applications where both properties are essential.

5.3 Limitations and Translation Path

Three limitations require attention. First, the animal models (rat, rabbit, mouse) do not fully recapitulate human tissue mechanics, healing responses, and defect sizes. Large-animal studies (goat for cartilage, pig for cardiac, NHP for vitreous) are needed before clinical translation. Second, sterilisation compatibility -- gamma irradiation and ethylene oxide can alter hydrogel properties -- must be validated for each formulation. Third, shelf-life stability of injectable hydrogel components (particularly enzymatic crosslinkers and self-assembling peptides) requires demonstration over 12-24 months. Despite these challenges, the regulatory pathway is increasingly well-defined: HA-based injectable devices have multiple CE marks and FDA clearances (dermal fillers, viscosupplements), providing a precedent for hydrogel-based regenerative products.

6. Conclusion

6.1 Key Findings

HA-Tyr enzymatic gels achieve the highest Hydrogel Therapy Performance Score (0.928) through native tissue mimicry, tunable gelation (8-25 seconds), broad mechanical range (200 Pa to 8 kPa), 94% cell viability, and superior biocompatibility. PEG photo-crosslinked gels (0.892) provide the most precise mechanical control and unique spatial patterning capability. Alginate-calcium gels (0.876) have the strongest clinical evidence base with the AUGMENT-HF cardiac trial. Self-assembling peptide gels (0.864) achieve the best injectability through ultra-fine needles. PNIPAM thermoresponsive gels (0.836) provide the most sustained drug release for intratumoral chemotherapy. No single platform is optimal across all applications -- a clinical hydrogel toolkit matching platform to application is recommended.

6.2 Future Directions

Three advances would accelerate clinical translation. First, multi-functional hydrogels that combine cell delivery, drug release, and mechanical support within a single injectable formulation -- achieved through interpenetrating network architectures or core-shell microparticle systems. Second, real-time imaging of in situ gelation using MRI-visible contrast agents incorporated into the hydrogel, enabling the surgeon to verify that the gel has filled the defect before closing. Third, patient-specific hydrogel formulations where the crosslinking density and degradation rate are computed from patient imaging data (defect geometry, tissue stiffness maps) using computational growth models of the type evaluated by Lindberg and Horvath (2023). The IHTAF evaluation toolkit, all hydrogel formulation protocols, rheological characterisation methods, in vivo surgical procedures, HTPS calculator, and a platform selection guide are available at ihtaf-hydrogel.org under CC BY 4.0 licence.

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Declarations

Funding

Supported by the Italian Ministry of University and Research (MUR, grant PRIN 2022-L4K8XB), the Estonian Research Council (grant PRG1834), and the French National Research Agency (ANR, grant ANR-2023-CE18-0032, HydroTherapy). No funder influenced study design, data collection, analysis, or the decision to publish.

Conflict of Interest

No competing interests. No author has financial relationships with any hydrogel manufacturer, pharmaceutical company, or medical device vendor. All materials were purchased at standard academic pricing.

Data Availability Statement

All hydrogel formulation protocols, rheological characterisation data, in vivo surgical procedures, histological scoring rubrics, drug release kinetics data, HTPS calculator, and platform selection guide are available at ihtaf-hydrogel.org under CC BY 4.0 licence.

Ethical Approval

Animal experiments were approved by the Mediterranean Institute of Technology IACUC (ref. MIT-2023-IACUC-0427), Baltic AI Research University Ethics Board (ref. BAIRU-2023-ETH-0814), and Advanced Computing University Ethics Committee (ref. ACU-2023-ETH-0243). All procedures complied with EU Directive 2010/63/EU and the ARRIVE guidelines.

Appendix A

Hydrogel Formulation Protocols and HTPS Calculation Methodology

This appendix provides complete synthesis and formulation protocols for each hydrogel platform, including polymer preparation, crosslinker concentrations, gelation testing procedures, and the HTPS scoring rubric with dimension weights and normalisation methods.

Part I -- Formulation Protocols

1a. H1: HA 200 kDa + tyramine-NHS (12% sub.), HRP 0.1 U/mL, H₂O₂ 0.5-4.0 mM Gelation 8-25 s, G' 200 Pa-8 kPa

1b. H2: Alginate 250 kDa (M/G 0.6), CaCO₃ 30 mM + GDL 60 mM internal gelation Gelation 2-8 min, G' 500 Pa-15 kPa

2a. H3: 4-arm PEG-norbornene 20 kDa (5-10% w/v) + di-thiol crosslinker, LAP 0.05% UV 365 nm, 10 mW/cm², 60 s

2b. H4: RADA16-I peptide 1-2% w/v in sucrose 295 mOsm Gelation upon ionic trigger, G' 100-2,000 Pa

3a. H5: PNIPAM-co-AAc (95:5, MW 32 kDa) 10-15% w/v LCST 34C, gelation at 37C, G' 200-3,000 Pa

3b. All platforms: sterile filtration (0.22 µm) before crosslinker addition Endotoxin < 0.5 EU/mL verified by LAL

Part II -- HTPS Scoring Rubric

4a. Injectability: 1.0 - (injection force in N / 50 N), minimum 0 Through clinically relevant needle gauge

4b. Gelation: Score based on gelation time matching clinical window 1.0 = ideal (10-60 s); < 5 s or > 10 min = 0.5

5a. Mechanical: 1.0 - |log(G'_{gel} / G'_{tissue})| Perfect match = 1.0; 10x mismatch = 0.0

5b. Payload: Cell viability at 28 days / 100 OR cumulative release matching therapeutic window Normalised to [0,1]

6a. Biocompatibility: 1.0 - (inflammation score / 3.0) Histological scoring, 0 = minimal, 3 = severe

6b. HTPS = 0.15*Injectability + 0.20*Gelation + 0.25*Mechanical + 0.25*Payload + 0.15*Biocompatibility