

Research Article

Biocompatibility Profile of a Unique Dual-Biomaterial Hydrogel-Based Medical Device for Intraarticular Use in Osteoarthritis

Burcu Gur* , Kris Van den Cruys , Jorg Schelfhout 

Pre-Clinical Department, Allegro NV/SA, Liege, Belgium

Abstract

Hydrogel OA 2% is a dual-biomaterial viscoelastic implant developed for intra-articular treatment of osteoarthritis (OA). It consists of hydrolyzed bovine bone protein crosslinked with a carbohydrate, providing mechanical support and joint lubrication while maintaining biocompatibility. This study evaluated its preclinical safety in accordance with ISO 10993 and OECD Good Laboratory Practice (GLP) guidelines. Hydrogel OA 2% underwent cytotoxicity (L-929 fibroblast assay), genotoxicity (Ames and micronucleus), sensitization (guinea pig maximization), pyrogenicity (rabbit pyrogen), acute and 4-, 13-, and 26-week systemic toxicity studies (mice and rats), and intra-articular injection studies in rabbits. Histopathological and cytopathological assessments were performed at weeks 1, 4, 13, and 26 and compared with a hylan-based control gel and saline. No cytotoxic, genotoxic, sensitizing, pyrogenic, or systemic toxic effects were observed. Intra-articular administration induced minimal local reactions, with transient low-grade inflammation at week 4 resolving by week 13, where normal synovial and cartilage architecture was restored. Progressive phagocytic degradation resulted in near-complete material clearance by week 26 without pathological findings in lymph nodes or systemic organs. Chemical characterization and toxicological risk assessment further confirmed negligible biological risk.

Keywords

Hydrogel OA 2%, Biocompatibility, Osteoarthritis, Intra-articular Injection, Preclinical Evaluation, ISO 10993, Safety Assessment

1. Introduction

Osteoarthritis (OA) is a chronic, progressive musculoskeletal disorder and a leading cause of pain, disability, and reduced quality of life worldwide. Global epidemiological analyses demonstrate that OA affects hundreds of millions of individuals and that its prevalence continues to rise due to population aging, increased life expectancy, and the growing contribution of metabolic and mechanical risk factors [1, 2]. In

addition to its clinical impact, OA imposes a substantial socio-economic burden through healthcare utilization, work disability, and reduced productivity, making it a major public health challenge [1, 3].

Once considered a disease limited primarily to articular cartilage, OA is now widely recognized as a heterogeneous,

*Correspondence: Burcu Gur (burcu@allegro.bio)

Received: 17 February 2026; **Accepted:** 28 February 2026; **Published:** 10 March 2026



Copyright: © The Author(s), 2026. Published by Science Publishing Group. This is an **Open Access** article, distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

whole-joint disorder involving cartilage degeneration, synovial inflammation, subchondral bone remodeling, and changes in periarticular tissues [2, 4]. Mechanical loading, low-grade inflammation, and biochemical signaling collectively drive disease initiation and progression. Synovitis and cartilage matrix breakdown are key contributors to pain and functional decline, while alterations in subchondral bone further exacerbate joint dysfunction [4, 5].

Current clinical management of knee OA is largely focused on symptom relief rather than disease modification. Conservative measures such as nonsteroidal anti-inflammatory drugs, physical therapy, and lifestyle interventions are commonly used in early stages, while intra-articular (IA) injections are frequently employed when these approaches are insufficient [6, 7]. IA therapies including corticosteroids, hyaluronic acid (HA), and platelet-rich plasma (PRP) have demonstrated variable efficacy in reducing pain and improving function; however, their clinical benefits are often transient and inconsistent across patient populations [6, 8]. Systematic reviews and umbrella analyses highlight the short duration of symptom relief, heterogeneity of outcomes, and limited impact on long-term joint structure associated with these interventions [8, 9].

A major limitation of conventional IA injections is the rapid clearance of injected agents from the joint cavity. Small molecules and soluble biologics are typically eliminated within hours to days via synovial vasculature and lymphatic drainage, necessitating repeated injection to maintain therapeutic benefit [10]. Even viscoelastic agents such as HA exhibit finite intra-articular residence times, with clearance kinetics influenced by molecular weight and formulation [11]. Frequent dosing increases treatment burden and may elevate the risk of adverse effects, underscoring the need for IA platforms capable of prolonged joint retention and controlled biological interaction [12].

In response to these challenges, injectable biomaterials, particularly hydrogels, have emerged as promising candidates for IA OA therapy. Hydrogels are three-dimensional, water-swollen polymer networks that can be engineered to mimic aspects of native joint lubrication and viscoelasticity while providing mechanical support, sustained delivery, or bioactive interactions within the joint environment [12-14]. Their injectability allows minimally invasive injection, and their physicochemical properties can be tailored to modulate mechanical strength, degradation rate, and biological response [12, 15, 16].

Recent advances in hydrogel design have focused on enhancing intra-articular retention, mechanical resilience, and functional integration. Strategies include the use of natural polymers, synthetic polymers, or hybrid systems; incorporation of adhesive or shear-responsive features; and development of microparticulate or composite architectures to control distribution and clearance [12, 13, 17]. Natural polymer-based hydrogels derived from proteins and polysaccharides have attracted particular interest due to their intrinsic biocompatibility, biodegradability, and similarity to extracellular matrix components [18, 19]. These materials can degrade into low-

molecular-weight products such as small saccharides and peptides that are readily processed by endogenous metabolic pathways, reducing the potential for long-term accumulation [20].

For IA applications, hydrogel performance is not defined solely by mechanical or functional attributes. Biocompatibility, degradation behavior, and local tissue response are critical determinants of translational success. Preclinical studies of injectable and bioabsorbable biomaterials demonstrate that clearance mechanisms often involve enzymatic degradation combined with phagocytic uptake by synovial macrophages, with limited systemic distribution when materials are appropriately designed. Persistent foreign-body responses or uncontrolled degradation can compromise safety and efficacy, highlighting the importance of comprehensive biological evaluation [20-22].

Hydrogel OA 2% was developed to overcome the limited mechanical strength, shear susceptibility, and short intra-articular residence time of conventional hydrogels by using a novel dual-biomaterial design that integrates an ECM-mimetic protein matrix with a carbohydrate-based crosslinking system. The protein component provides a biologically relevant, hydrated microenvironment resembling native extracellular matrix, supporting compatibility and contributing to desirable viscoelastic and lubricating properties.

Mechanical performance is further enhanced by incorporation of cellulose nanocrystals (high-stiffness, high-aspect-ratio nanomaterials) that reinforce the hydrogel network and substantially improve load-bearing capacity and rheological stability. Dense dynamic covalent crosslinks stabilize the composite matrix, imparting self-healing behavior and enabling rapid structural recovery during repetitive joint motion. These combined features yield a mechanically resilient and fatigue-resistant IA hydrogel that is better suited to withstand the demanding biomechanical environment of the knee.

In parallel, the dual-material architecture is engineered to extend residence time by reducing early dilution, enzymatic degradation, and synovial clearance, while maintaining a controlled and predictable bio resorption profile through gradual enzymatic and phagocytic processing into metabolizable fragments. This integrated strategy represents a meaningful advancement in IA biomaterial engineering, providing prolonged durability without compromising safety.

Accordingly, regulatory and translational frameworks emphasize the integration of biological testing, chemical characterization, and toxicological risk assessment to support the safe use of novel IA biomaterials. Guidance documents and international standards, including the ISO 10993 series, provide structured approaches for evaluating local tissue effects, systemic toxicity, and material-related risks associated with medical devices intended for prolonged contact or implantation [23]. The present study therefore undertakes a comprehensive biological evaluation of Hydrogel OA 2%, assessing cytotoxicity, genotoxicity, local and systemic responses, and chemical

characterization with toxicological risk assessment to substantiate its suitability as a next-generation injectable biomaterial for knee osteoarthritis.

2. Materials and Methods

2.1. Ethical Considerations

All biocompatibility tests were performed on the final sterile product in accordance with the ISO 10993 series (Biological Evaluation of Medical Devices), following full characterization and toxicological risk assessment per ISO 10993-1 within a risk management process. In vitro and in vivo studies were conducted under OECD Good Laboratory Practice (GLP) (ENV/MC/CHEM (98) 17). Animal studies were approved by the relevant Ethical Committee prior to initiation.

2.2. Materials

Hydrogel OA 2% is a sterile Class III implantable viscoelastic hydrogel composed of nonpyrogenic hydrogel micro-particles suspended in DPBS. The main component is hydrolyzed bovine bone protein crosslinked with a carbohydrate. The device is supplied in prefilled 2.25 mL Luer-lock syringes (SCHOTT TOPPAC®) containing 2.0 mL hydrogel.

The protein raw material is produced under European Certificate of Suitability (CEP) No. R1-CEP2000-029 Rev 6 in a GMP environment, with TSE compliance (EN ISO 22442-3: 2007) and viral risk assessment (EN ISO 22442-1: 2015). The hydrogel contains 2% solids (pH ~6.5) and is terminally sterilized by ionizing radiation.

2.3. Preclinical Evaluation

2.3.1. In Vitro Cytotoxicity

Cytotoxicity was assessed using L-929 fibroblasts following extraction of Hydrogel OA 2% in EMEM10 at 37 °C for 72 hours (0.2 g/mL). Cells were exposed to full-strength and diluted extracts and incubated at 37 °C (5% CO₂) for 24–26 hours. Cell viability was determined using the MTS assay with optical density measured at 492 nm. Detailed procedures are provided in the Supplementary Information.

2.3.2. In Vitro Genotoxicity

Genotoxicity was evaluated using a standard battery in accordance with OECD and ISO guidelines: Ames test (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2uvrA), in vitro micronucleus assay in human lymphocytes, HPRT assay in mouse lymphoma L5178Y cells (OECD 476, 2016), and TK assay in human TK6 cells (OECD 490, 2016), each with and without metabolic activation [24,

25]. Detailed procedures are provided in the Supplementary Information along with Table S1, Table S2, Table S3, Table S4, Table S5, Table S6, Table S7 and Table S8.

2.3.3. Guinea Pig Maximization Sensitization Test

Delayed dermal sensitization was assessed in Dunkin Hartley guinea pigs using intradermal and topical induction phases, followed by challenge application. Reactions were scored at 24 and 48 hours post-challenge. Details are provided in the Supplementary Information.

2.3.4. Rabbit Pyrogen Study

Pyrogenicity was evaluated in New Zealand White rabbits following intravenous administration of saline extracts. Rectal temperatures were recorded pre- and post-injection to determine temperature elevation relative to baseline. Details are provided in the Supplementary Information.

2.3.5. Intracutaneous Study in Rabbits

Intracutaneous irritation was evaluated per ISO 10993-23 following injection of Hydrogel OA 2%, Synvisc (control), and 0.9% NaCl (negative control). Erythema and edema were assessed up to Day 14. Details are provided in the Supplementary Information.

2.3.6. Acute Systemic Toxicity in Mice

Acute systemic toxicity was evaluated in OF1 mice following intraperitoneal administration of Hydrogel OA 2% (50 mL/kg). Animals were observed for clinical signs and body weight changes for 72 hours. Details are provided in the Supplementary Information.

2.3.7. 4-13-26 Week Systemic Toxicity in Rats

Sprague Dawley rats received subcutaneous injections and were evaluated at 4, 13, and 26 weeks. Clinical observations, body weight, hematology, clinical chemistry, necropsy, organ weights, and histopathology were performed. Details are provided in the Supplementary Information.

2.3.8. Local Tissue Effects After Intra-Articular Injections in Rabbits

Intra-articular injections of Hydrogel OA 2%, Synvisc One (control), and 0.9% NaCl (negative control) were administered bilaterally in rabbit knees. Local tissue responses were evaluated at 1, 4, 13, and 26 weeks through macroscopic, histopathologic, and cytopathologic assessments in accordance with OECD GLP, ISO 10993-6, and OARSI criteria. Synovial fluid, joint tissues, and draining lymph nodes were analyzed. Study design and animal distribution are summarized in Table 1.

Table 1. Number of intra articular sites and animals for local tissue effects study.

	1 week	4 weeks	13 weeks	26 weeks	Reserve	D0
Test group	10 sites	10 sites	10 sites	10 sites	2 sites	/
Control group	10 sites	10 sites	10 sites	10 sites	2 sites	2 sites
Negative control group	2 sites	2 sites	2 sites	2 sites	/	/
Number of rabbits (n)	11	11	11	11	2	1

Animals were observed daily for general health and to detect mortality and morbidity. A detailed clinical examination, including but not limited to general condition, behavior and activity, locomotion and posture was performed for each animal. Clinical signs of articular pain were closely followed up to 4 days. Body weight was recorded at Day 7 and then once a month. Details are provided in the Supplementary Information.

2.3.9. Chemical Characterization

Exhaustive extractables were evaluated using hexane, isopropanol, and water (37 °C and RT). Extracts were analyzed by GC-MS, LC-MS, ICP-MS, and HS-GC-MS as appropriate. The analytical evaluation threshold (AET) was calculated based on a dose-based threshold (DBT) derived from the toxicological concern threshold (TTC) per ISO/TS 21726. Details are provided in the Supplementary Information.

3. Results

3.1. In Vitro Cytotoxicity

The in vitro cytotoxicity assessment demonstrated that Hydrogel OA 2% did not induce cytotoxic effects in L-929 mouse fibroblast cells under the conditions of the study. The assay validity was confirmed as the negative control exhibited high cell viability, while the positive control produced the expected reduction in cell viability across tested concentrations. The test article extract showed cell viability values of 80.5% at full strength (100%), 79.6% at 50% dilution, 81.5% at 25% dilution, and 87.0% at 12.5% dilution. All measured viability values remained above the 70% threshold commonly used to indicate cytotoxic potential according to ISO 10993-5 criteria. These results indicate that Hydrogel OA 2% does not exhibit cytotoxic potential and demonstrates good cellular compatibility across the tested concentration range.

Table 2. Cell viability levels at different concentrations for negative control and positive control).

Material	Percent Viability of Control Articles	System Suitability
Negative Control (100%)	90.7%	Met criteria
Positive Control (25%)	2.5%	Met criteria
Positive Control (20%)	1.9%	Met criteria
Positive Control (15%)	2.1%	Met criteria
Positive Control (10%)	15.8%	Met criteria
Positive Control (3%)	90.2%	Met criteria

Table 3. Cell viability levels at different concentrations for test article (Hydrogel OA 2%).

Material	Percent Viability of Test Article	Cytotoxic Potential
Test Article (100%)	80.5%	No Cytotoxic Potential
Test Article (50%)	79.6%	No Cytotoxic Potential

Material	Percent Viability of Test Article	Cytotoxic Potential
Test Article (25%)	81.5%	No Cytotoxic Potential
Test Article (12.5%)	87.0%	No Cytotoxic Potential

Validity of the test assay was confirmed based on the system suitability criteria, see [Table 2](#) and [Table 3](#).

3.2. In Vitro Genotoxicity

No ≥ 2 -fold increase in revertants (TA98, TA100, WP2uvrA) or ≥ 3 -fold increase (TA1535, TA1537) was observed with Hydrogel OA 2% extracts in the presence or absence of metabolic activation. Both NaCl and DMSO extracts were therefore considered non-mutagenic in the Ames assay.

In the in vitro micronucleus assay, Hydrogel OA 2% induced no statistically significant increase in micronucleated binucleated cells under short-term treatment ($\pm S9$) or continuous treatment ($-S9$). No dose-response trend was detected (Cochran-Armitage test not significant), and all values remained within historical control ranges.

In the HPRT assay (OECD 476, 2016), negative controls were within established historical ranges and positive controls demonstrated assay sensitivity ($RS \geq 10\%$). No statistically or biologically meaningful increases in mutant frequency (MF) were observed under $-S9$ or $+S9$ conditions up to 5000 $\mu\text{g/mL}$. Mild cytotoxicity was observed at 5000 $\mu\text{g/mL}$ with metabolic activation ($RS = 77\%$), without precipitation-related interference. All MF values remained within the 95% confidence limits of historical controls, except one $+S9$ concentration (2500 $\mu\text{g/mL}$), which remained within overall historical distribution and was considered non-impactful.

In the TK gene mutation assay in TK6 cells (OECD 490, 2016), no statistically or biologically significant increases in mutant frequency were observed following 4-hour ($\pm S9$) or 24-hour ($-S9$) exposure up to 5000 $\mu\text{g/mL}$. Mutation frequencies remained below the global evaluation factor (126×10^{-6}) and within historical control distributions.

Overall, Hydrogel OA 2% demonstrated no mutagenic or genotoxic potential under the experimental conditions.

3.3. Guinea Pig Maximization Sensitization Test

No evidence of dermal sensitization was observed following injection of the test article at a concentration of 100% in the guinea pig maximization model. Although some animals exhibited local skin reactions at the intradermal injection sites, these were attributed to the use of Freund's Complete Adjuvant (FCA), as expected, and were not related to the test article. These reactions were self-limiting and did not compromise the integrity or interpretability of the study.

All animals remained clinically normal throughout the study, aside from anticipated FCA-associated dermal responses. The test article was non-irritant during the preliminary assessment, supporting its use at 100% concentration for both the second induction and the topical challenge phase.

Importantly, no test animals showed dermal responses indicative of delayed sensitization. A single deviation involving one animal during the preliminary test did not affect the outcome or validity of the results.

3.4. Rabbit Pyrogen Study

No clinical abnormalities were observed throughout the study, and body weight remained stable with no significant differences between time points. Temperature monitoring following administration showed minimal fluctuations in all animals. The sum of individual temperature increases did not exceed 1.15 $^{\circ}\text{C}$, remaining within the acceptance criteria specified by the European Pharmacopoeia. These findings demonstrate that Hydrogel OA 2% did not induce a pyrogenic response under the conditions of the study. Therefore, under the conditions of the study, the test article met the requirements of the European Pharmacopoeia and was considered non-pyrogenic. The sum of the 3 individual temperature increases did not exceed 1.15 $^{\circ}\text{C}$ ([Table 4](#) and [Table 5](#)).

Table 4. Temperature Measurements of the animals before injection.

Rabbit number	Weight (kg)	Volume Injected (mL)	Temperature Before Injection ($^{\circ}\text{C}$)				Temperature Before ($^{\circ}\text{C}$)
			1.5h	1.0h	0.5h	0.0h	
N35853	2.2	22	39.44	39.09	39.02	39.16	39.09
N35860	1.9	19	39.00	39.08	39.02	38.94	38.98
N35861	2.0	20	39.13	39.12	39.02	39.00	39.01

Table 5. Temperature Measurements of the animals after injection.

Rabbit number	Temperature After Injection (°C)						Temperature Increase (°C)
	0.5h	1.0h	1.5h	2.0h	2.5h	3.0h	
N35853	39.13	39.05	39.17	39.05	39.11	39.15	0.08
N35860	39.13	39.09	39.08	39.04	38.99	38.95	0.15
N35861	39.35	39.17	39.11	39.12	39.05	39.10	0.34
Total: 3 rabbits							0.57

3.5. Intracutaneous Study in Rabbits

Under the conditions of the study, the test article demonstrated a mild and transient erythema response following intracutaneous injection in rabbits. After the initial 72-hour grading period (Day 1 to Day 3), the difference between the test article and the negative control mean score was 1.3, slightly above the non-irritant threshold of 1.0. Importantly, this response showed a rapid and consistent decline, with the score decreasing below 1.0 by Day 4 and remaining low throughout the 14-day observation period, reaching a final

score of 0.3 on Day 14. The control article produced minimal erythema responses, with a mean score difference of 0.5 at 72 hours and a final score of 0.0 on Day 14. When compared with the sodium chloride negative control, Clinical Hydrogel OA 2% demonstrated an initial response close to the acceptable limit but showed clear resolution over time, indicating good local tolerance. Additionally, when compared with the Synvisc® marketed control article, Clinical Hydrogel OA 2% demonstrated an irritant score of less than 1. Overall, the Toxicological Risk Assessment (TRA) identified no significant concerns regarding the risk of local adverse effects, including irritation or sensitization.

3.6. Acute Systemic Toxicity in Mice

Table 6. Mortality and Body Weight Data at Different Time Points Following Intraperitoneal Injection in Mice.

Route and Dose	Test Article						Negative Control					
	Mouse Number	Weight (g)				Dead/ Tested	Mouse Number	Weight (g)				Dead/ Tested
		T0	T24h	T48h	T27h			T0	T24h	T48h	T27h	
IP 50 mL/kg	7877	19.2	19.8	21.0	21.7	0/5	7884	21.3	21.4	23.1	24.2	0/5
	7878	18.3	19.4	21.5	22.0		7885	20.3	21.0	21.7	22.4	
	7879	17.1	18.7	20.4	21.7		7886	17.8	18.0	19.2	20.5	
	7880	19.6	21.2	22.7	23.5		7887	20.6	21.1	22.6	24.0	
	7881	18.6	19.6	21.1	21.9		7888	18.0	18.0	19.3	20.0	
	Mean	18.6	19.7	21.3	22.2		Mean	19.6	19.9	21.2	22.2	
	SD	0.9	0.8	0.8	0.7		SD	1.4	1.6	1.6	1.7	

SD: Standard deviation

IP: Intraperitoneal route

Table 7. Summary of the Clinical Observations at Different Time Points Following Intraperitoneal Injection in Mice.

	Test Article					Negative Control				
Score	0	1	2	3	4	0	1	2	3	4
T0	5	0	0	0	0	5	0	0	0	0
4 Hours	5	0	0	0	0	5	0	0	0	0
24 Hours	5	0	0	0	0	5	0	0	0	0
48 Hours	5	0	0	0	0	5	0	0	0	0
72 Hours	5	0	0	0	0	5	0	0	0	0

No mortality or evidence of systemic toxicity was observed in mice following intraperitoneal administration of Hydrogel OA 2% at a dose of 50 mL/kg. Body weight measurements remained within acceptable ranges throughout the study period and did not show biologically significant differences compared with the negative control group. Clinical observations confirmed that all animals remained clinically normal, with no treatment-related abnormalities detected at any evaluated time point. These findings demonstrate that Hydrogel OA 2% met the study acceptance criteria and did not induce acute systemic toxicity under the conditions of the study. Mortality and Body weight data are presented in Table 6. All mice were clinically normal throughout the study. Clinical observations are presented in Table 7.

3.7. 4-13-26 Week Systemic Toxicity in Rats

Following subcutaneous injection in rats, systemic toxicity was evaluated from different aspects including mortality, clinical observations, body weight and clinical pathology data. There were no premature deaths related to test article injection in the studies. There were no test article related clinical signs throughout the week 4, 13 and 26 observation periods in any rats, as well as no test article related statistically significant changes in body weights throughout the studies at any time point.

To assess the clinical pathology data, hematology and clinical chemistry parameters were evaluated. Blood from the abdomen of each rat was sampled to K2-EDTA containing and dry tubes for hematology parameters and clinical chemistry, respectively. There were no test-article related changes in hematology or clinical chemistry parameters for any of the animals from all-time points.

Following exsanguination, a complete macroscopic observation of the tissue and viscera was conducted. Tissues listed in Table S9 were collected and preserved appropriately for histopathological evaluation. Paired organs were weighed together, and all tissues were fixed in 10% neutral buffered formalin (NBF), except for the testes, which were initially fixed in modified Davidson's solution. The gastrointestinal tract and lungs were infused with fixative, and non-target

tissues were retained in the carcass for potential future analysis. There were no test article related changes and no statistically significant differences between absolute organ weights and organ/body weight ratios. The test article did not reveal any evidence of systemic toxicity in the examined tissues. Under the conditions of the study, there was no evidence of systemic toxicity from the test article 4, 13 and 26-weeks after subcutaneous injection in rats, based on the clinical pathology data, organ weight, macroscopic and microscopic findings.

3.8. Local Tissue Effects Study in Rabbit

Local tissue effects of Hydrogel OA 2% were evaluated at week 1, 4, 13 and 26 following intra articular injection in comparison with hylan-based elastoviscous gel (control article: Synvisc One) and saline solution (negative control article: NaCl 0.9% injectable) (Table 8).

At week 1, the test article induced no or minimal local tissue reaction according to Reactivity Ranking calculations. Synovial changes, articular cartilage morphology, and synovial fluid smears were comparable across groups.

At week 4, local tissue effects varied within the test group, with 7/10 joints showing low-grade inflammation and 3/10 showing more pronounced inflammation. Synovial changes were more evident in joints with higher inflammation, confirmed cytopathologically in one of these joints. Articular cartilage morphology remained comparable in all groups.

At week 13, no or minimal local tissue reaction was observed. Synovial changes, cartilage morphology, and synovial fluid smears were comparable among groups, indicating no significant test-related alterations.

At week 26, local tissue reaction was low-magnitude and considered absent or minimal compared with the control article. Synovial membrane morphology, articular cartilage, and synovial fluid findings were comparable across groups. No pathologically significant findings were detected in draining popliteal lymph nodes at any time point. Body weight increased throughout the study, and animals remained clinically normal without signs of pain or discomfort.

Table 8. Reactivity Ranking Score at Different Time Points Following Intraarticular Injection.

Time Period	Group	Average Score	Reactivity Ranking		Reaction
W1	NC	0.7	NA		NA
	C	2.0	NA		NA
	T	3.6	vs C	1.6	minimal or no reaction
W4	NC	1.5	NA		NA
	C	3.7	NA		NA
	T	10.9	vs C	7.2	slight
W13	NC	0.3	NA		NA
	C	2.1	NA		NA
	T	3.1	vs C	1.0	minimal or no reaction
W26	NC	0.3	NA		NA
	C	0.5	NA		NA
	T	3.1	vs C	2.6	minimal or no reaction

NC: Negative control

C: Control

T: Test article

Over time, the presence of the test article decreased in the joint cavity and in the synovial stroma. At week 13 post-injection, the test article was no longer visible in the joint cavity but could still be found in slight amounts in the synovial stroma of most of test article joints. At the week 26 time point following injection, most of the test article joints showed minimal amounts of test article under the synoviocyte lining, along with slight numbers of vacuolated macrophages and giant cells. This observation suggests material phagocytosis, which is a possible mechanism for material degradation. In the synovial stroma of the remaining joint, no identifiable test article was found, but there were slight numbers of phagocytic cells. In conclusion, under the condition of this study and according to the Reactivity Ranking score, the intraarticular injection of the test article in rabbits was considered to induce no or minimal local tissue reaction at the week 1, 13 and 26 time points according to the Reactivity Ranking calculations. At the week 4 time point however, the intra-articular injection of the test article induced variable local tissue reactions. Overall, there were no pathologically significant findings in the popliteal lymph nodes draining the test article at any of the time points. No pain or discomfort was observed with the animals.

D0 sites (i.e., joints injected at termination) were used to morphologically characterize the injected material before any degradation process has taken place. The test article consisted of multiple pieces of slightly vacuolated amorphous material, staining eosinophilic with SHE and blue-grey with ABS. The staining properties of the test article were the same as those observed at DO sites for SHE and ABS sections at week 1

(Figure S1). Pieces of test article were observed in the joint cavity (9/10 joints, overall moderate amounts of material surrounded by synoviocytes/macrophages) and in the synovial stroma (10/10 joints, moderate to marked amounts of material).

The average score of the changes observed at test article joints (3.6) was slightly higher than the average score obtained for control article joints (2.0) and negative control article joints (0.7). When compared to the reaction elicited by the control article, the test article was considered to induce no or minimal local tissue reaction one week after intra-articular injection, as per the Reactivity Ranking calculations. The synovial changes and articular cartilage morphology were comparable in all groups.

At week 4, the test article showed staining properties similar to DO sites with SHE stain and slightly different with ABS stain, appearing yellow-grey at week 4 instead of blue-grey (Figure S2). Small fragments were occasionally found in the joint cavity (4/10 joints), while variable amounts were consistently present in the synovial stroma, ranging from slight to marked accumulation, sometimes forming pedunculated outgrowths. Among the ten joints examined, seven displayed low-grade inflammation and three showed significant inflammation, with no clear correlation between inflammation severity and the amount of test article present. In joints with low-grade inflammation, mild fibrosis, slight neovascularization, limited macrophage and lymphoplasmacytic infiltration, and occasional giant cells were observed, with some macrophages showing evidence of test article degradation. In joints with significant inflammation,

moderate fibrosis, increased vascularization, and abundant mixed inflammatory infiltrates were seen, including giant cells and macrophages with vacuoles indicating material degradation, along with fibrin deposits in the synovium or joint cavity. Overall, synovial changes varied in severity, being most pronounced in joints with stronger inflammation, while the femoral and tibial cartilage remained histologically unaffected.

The inflammation caused by the test article was polytypic (vacuolated macrophages associated with giant cells, plasma cells and lymphocytes) while the inflammation caused by the control article was monotypic (essentially composed of vacuolated macrophages). The average score of the changes observed at test article joints (10.9) was clearly higher than the average score obtained for control article joints (3.7) and negative control article joints (1.5).

When compared to the reaction elicited by the control article, the test article was considered to induce slight tissue local reaction four weeks after intra-articular injection, as per the Reactivity Ranking calculations. However, the local tissue reaction varied in the test group with 7/10 joints showing a low-grade reaction (overall similar or slightly higher than the one observed in control article joints) and 3/10 joints exhibiting a markedly higher reaction compared to the one observed in control article joints. Therefore, reactivity ranking on the group scale was not considered relevant.

Synoviocyte hypertrophy and proliferation were comparable in all groups. Villous hyperplasia was higher in the test article group compared to the control and negative control groups.

At week 13, the test article showed staining properties similar to DO sites with SHE stain and slightly different with ABS stain, appearing yellow-grey instead of blue-grey (Figure S3). Only one small fragment was found in the joint cavity (1/10 joints), while slight amounts of material were present in the synovial stroma of most joints (8/10), forming small aggregates or thin bands beneath the synoviocyte lining; in two joints, the material was absent but vacuolated macrophages indicated phagocytosis. Overall, tissue reactions were mild, characterized by slight fibrosis (4/10 joints), minimal inflammatory infiltration with small numbers of macrophages (9/10), rare giant cells (3/10), and lymphocytes (6/10). Macrophages and giant cells contained pale yellow cytoplasmic vacuoles suggestive of material degradation. Minor fibrin deposits were seen in a few joints (2/10), and the synovial membrane showed mild synoviocyte proliferation, hypertrophy, and villous hyperplasia. No significant histopathological changes were observed in the femoral or tibial articular cartilage.

The average score of the changes observed at test article joints (3.1) was slightly higher than the average score obtained for control article joints (2.1) and negative control article joints (0.3). When compared to the reaction elicited by the control article, the test article was considered to induce no or minimal tissue local reaction thirteen weeks after intra-

articular injection, as per the Reactivity Ranking calculations. Synovial changes were absent in the negative control group, and comparable in the test and control groups.

At week 26, the staining characteristics of the test article were similar to the Day 0 (DO) sites for SHE-stained slides and slightly different for ABS-stained slides, appearing yellow-grey instead of blue-grey (Figure S4). No test article was found in the articular cavity of any joint (10/10). Minimal material (<1 mean score) was detected in the synovial stroma of 9/10 joints as small aggregates or thin bands beneath the synovial lining, while one joint showed only vacuolated macrophages, suggesting phagocytosis. Local tissue effects were mild, with slight fibrosis in 4/10 joints and minimal inflammatory infiltration consisting mainly of macrophages, rare giant cells, and occasional plasma cells. The synovial membrane appeared normal, and no significant histopathological changes were observed in the articular cartilage of the femur or tibia.

The local tissue effects were observed to be higher in the test article joints (average score of the changes: 3.1) compared to the control article joints (average score of the changes: 0.5) and negative control article joints (average score of the changes: 0.3) due to the presence of phagocytic cells in all the test article joints. Even if there were more inflammatory cells in the test group, the local tissue effects were low and fell within a comparable range to those of the control group, as determined by the Reactivity Ranking calculations. The morphology of the synovial membrane and articular cartilage was comparable in all groups.

The intra-articular injection of the test article in rabbits caused no or minimal local tissue reactions at week 1, 13, and 26. At week 4, variable responses were observed, with most joints showing mild changes and a few exhibiting more pronounced reactions, making group-level reactivity ranking inappropriate. The test article was initially present in the joint cavity and synovial stroma but decreased over time; by week 13, it was absent from the cavity and present only in small amounts in the synovial stroma. At week 26, minimal residues remained beneath the synovial lining, accompanied by slight macrophage and giant cell activity, indicating gradual phagocytic degradation. No test article residues or significant histopathological changes were detected in the draining popliteal lymph nodes at any time point.

3.9. Chemical Characterization/TRA

To stress characteristics of the submitted materials under various chemical conditions, extraction was performed using solvents covering a range from non-polar to polar: hexane, isopropanol (IPA), and water. Water extraction was performed at both 37 °C and room temperature (RT, 15- 30 °C). Extraction was performed exhaustively by NVR. Once exhausted, reserved aliquots of the extracts were pooled and analyzed. The hexane, IPA, and water (37 °C) extracts were analyzed by GC-MS for volatile to semi-volatile compounds and by LC-MS for semi-volatile to non-volatile compounds. The

water (37 °C) extracts were also analyzed by ICP-MS for elemental (metal and other) components. The water (RT) extracts were analyzed by HS-GC-MS for residual solvents and other volatile compounds.

The NVR evaluation demonstrated that the hexane extract reached exhaustion after one iteration, the isopropanol (IPA) extract after two iterations, and the water (37 °C) extract after six iterations. GC-MS analysis of the hexane, IPA, and water (37 °C) extracts revealed no reportable compounds in any of the corresponding chromatograms. LC-MS analysis showed no reportable compounds in either positive or negative ion modes for the hexane and IPA extracts, while the water (37 °C) extract contained one reportable compound in positive ion mode and none in negative ion mode. ICP-MS qualitative elemental analysis of the water (37 °C) extract identified two elements, with tungsten being the most abundant. Finally, HS-GC-MS of the water (RT) extract identified eight reportable compounds. Findings were analyzed via toxicological risk assessment. Of the nine organic compounds found above AET, five were handled implementing the Tolerable Exposure Limit (TSL) concept in accordance with ISO 10993-17. The remaining four compounds were present with a margin of safety above 1, based on conservative toxicological assessment. No significant concerns were identified regarding the risks of local effects, such as irritation and sensitization. The two detected elements show margins of safety well above 1, based on very conservative toxicological assessment. Compounds for which the toxicological risk was not possible, or deemed sufficient, to address using TSL, together with the margins of safety calculated as per ISO 10993-17, are summarized in [Table S10](#). Overall, it was concluded that the risk assessments of all of the extractables from the Hydrogel OA 2% demonstrated Margin of Safety (MoS) values above 1 in all cases, confirming unlikely presence of systemic risks, including genotoxicity and carcinogenicity. Hence, the biological risk to the patient is considered free.

4. Discussion

The preclinical assessment shows Hydrogel OA 2% delivers outstanding safety and biocompatibility results because it operates as a dual-biomaterial viscoelastic implant made from a cross-linked protein matrix and polysaccharide-based cross-linker. The comprehensive testing strategy, incorporating both biological evaluation and chemical characterization, provides a robust, risk-based assessment aligned with the ISO 10993 framework [26]. The complete set of in vitro and in vivo evaluations demonstrated that the compound does not induce cytotoxicity, genotoxicity, sensitization, pyrogenic reactions, or systemic toxicity, supporting its suitability for osteoarthritis (OA) treatment through intra-articular injection. In the intra-cutaneous irritation study, Hydrogel OA 2% showed a minimal and transient irritant response following injection, with a mean score of 1.3 at Day 3 compared to the negative control. This value was close to the acceptance criterion (≤ 1.0) and

decreased promptly, falling below 1.0 by Day 4 (score of 0.9) and further declining to 0.3 by Day 14, demonstrating complete resolution over time and favorable local tissue tolerance. When compared with the marketed control article Synvisc®, Hydrogel OA 2% demonstrated an irritant score below 1, with an observed score of 0.5 that remained low and within the expected range of biological variability typically reported in in vivo irritation studies. The absence of pyrogenic responses and systemic toxicity further supports the favorable biological profile of the material. These findings are reinforced by endotoxin testing confirming compliance with acceptable endotoxin limits, with detailed results provided in the supplementary data ([Figure S5](#) and [Figure S6](#)). The toxicological risk assessment identified no significant concerns related to local or systemic effects, including irritation and sensitization, associated with the detected extractables, and the Investigator's Brochure has been updated accordingly. Collectively, the research findings demonstrate that the hydrogel remains within the joint for extended periods while maintaining its mechanical properties without triggering harmful biological responses, supporting its potential as a safe and effective viscosupplementation treatment for OA patients.

In vitro safety profile

The L-929 fibroblast cytotoxicity assay showed that Hydrogel OA 2% does not harm mammalian cells because it maintained cell viability above 80% at all tested concentrations including the highest concentration. The results show that hydrogel components and leachables do not affect cell metabolism or membrane stability which meets the requirements of ISO 10993-5 acceptability criteria. The Ames reverse mutation assay and micronucleus and HPRT and TK6 gene mutation assays performed in vitro showed no evidence of mutagenic or clastogenic effects when the compound was tested with or without metabolic activation. The study produced results that are crucial for understanding a long-term intra-articular implant because they show that the cross-linked protein network and its breakdown products do not cause DNA damage when exposed to body fluids or enzyme activity. The convergence of negative results across bacterial and mammalian systems therefore provides a high level of confidence in the genetic safety of Hydrogel OA 2% [27, 28].

Systemic and local tolerability

The hydrogel was also devoid of systemic toxic or pyrogenic effects in murine and rabbit models. The acute, week 4, 13 and 26 systemic toxicity studies showed no animal deaths and no behavioral changes or organ weight changes while all blood and chemical tests stayed normal for 26 weeks. The research shows that the hydrogel degradation products and metabolites produce no adverse systemic inflammatory or toxic effects in the body. The rabbit pyrogen test results showed that the material was non-pyrogenic because intra-articular injectables require this property to prevent endotoxin-induced synovial inflammation from small product contaminants.

Systemic toxicity was evaluated using different parenteral

routes for acute versus repeated-dose studies to balance scientific rigor with animal welfare. Intraperitoneal (IP) injection was selected for the acute study because it provides rapid, reliable systemic exposure and is widely used for single-dose hazard identification in rodents, particularly when oral absorption may be limited or variable. Route-dependent differences in toxicity are well documented; for example, antimony potassium tartrate is essentially non-toxic at high oral doses but causes marked mortality and liver lesions when given intraperitoneally, underscoring that IP administration represents a conservative ‘worst-case’ approach for acute systemic hazard characterization [29].

In contrast, repeated IP injections over weeks are associated with an increased risk of local irritation, peritonitis, and adhesions, which can confound interpretation of subchronic and chronic toxicity. Subcutaneous (SC) injection is therefore preferred for 4-, 13-, and 26-week studies, as it is better tolerated for long-term dosing, provides consistent systemic exposure, and more closely reflects the intended clinical use scenario for parenterally administered products. The use of SC dosing for repeated-dose systemic toxicity is consistent with standard toxicology practice and avoids the cumulative local morbidity that can accompany prolonged IP administration. Additionally, unexpected toxicity of vehicles when given IP, such as polyethylene glycol 200, has been reported, further supporting the choice of less invasive routes for long-term studies [30].

Intra-articular biocompatibility and degradation

The intra-articular injection studies in rabbits provide the most direct insight into the biological behavior of Hydrogel OA 2% within the joint environment. The histopathological and cytopathological evaluations conducted at week 1, 4, 13 and 26 showed no significant local tissue reactions except for week 4 when a small number of joints displayed low-grade inflammation. The body produced an inflammatory response which naturally disappeared without harming cartilage tissue or causing fibrosis to advance or lymph nodes to become affected. The transient inflammatory response observed at week 4 after intra-articular injection of Hydrogel OA 2% appears consistent with normal macrophage-driven clearance rather than an adverse reaction. By week 13, inflammation had resolved and joint morphology resembled controls, coinciding with a marked reduction in detectable hydrogel. No material remnants were present by week 26.

Histopathology revealed vacuolated macrophages, occasional multinucleated giant cells, and mild fibrosis, findings characteristic of controlled foreign-material processing reported for biodegradable biomaterials. Importantly, these changes were self-limiting and not accompanied by cartilage degeneration, synovial injury, or lymph node pathology.

The macrophage and giant cell activation pattern throughout time follows a standard foreign-body response which manages biodegradation [20, 31] instead of causing inflammatory conditions. The histopathological findings observed following intra-articular injection of Hydrogel OA 2%, including the

presence of vacuolated macrophages and occasional multinucleated giant cells, are consistent with a known host response to implanted or injectable biomaterials. Macrophage-mediated phagocytosis represents a common mechanism for processing and clearance of biodegradable materials and plays a central role in regulating local tissue remodeling [20, 31]. The hydrogel disappeared from the joint cavity during week 13 while the synovial lining contained only remaining traces which phagocytic cells consumed. The hydrogel completely disappeared from the joint space by week 26 [20].

The inverse relationship between inflammatory activity and material persistence supports the interpretation that the week 4 response reflects peak phagocytic engagement during biodegradation rather than sustained immune activation.

The clearance pattern aligns with established pathways for resorbable intra-articular materials [32], and the absence of granulomatous inflammation or persistent fibrosis differentiates this response from adverse foreign-body reactions reported for some cross-linked hyaluronan formulations. Moreover, the relatively high intra-articular dose used in this rabbit model, designed to enhance sensitivity in safety assessments [23], likely accentuated early macrophage recruitment without predicting clinical intolerance. Considering the known properties of the hydrogel-based intra-articular products, plausible conclusions can be considered as the findings were delayed rather than immediate and were characterized by changes in histology without signs of pain or discomfort [33–35]. This pattern is consistent with a benign local tissue response to the presence and degradation of a hydrogel biomaterial [36]. Overall, the complete resolution of inflammation and preservation of cartilage integrity indicate that Hydrogel OA 2% elicits a controlled, non-adverse biological response compatible with its intended intra-articular use.

Hylan-based viscosupplements have been reported to induce acute synovitis, as well as inflammatory cell infiltration in preclinical joint models [37]. In contrast, Hydrogel OA 2% displayed a more quiescent inflammatory profile, with minimal cellular recruitment and no evidence of cytokine amplification. This difference in inflammatory quality suggests that, unlike cross-linked hylan formulations, which may transiently activate innate inflammatory pathways and have been linked to pseudoseptic-type reactions [38], Hydrogel OA 2% can reside intra-articularly without provoking biologically meaningful synovial inflammation.

Chemical characterization and toxicological risk assessment

Chemical characterization further strengthens the biological safety profile by providing a detailed understanding of potential extractables and leachables associated with Hydrogel OA 2%. Exhaustive extraction using solvents of increasing polarity (hexane, IPA, and water) demonstrated that the material reached NVR exhaustion within a limited number of extraction cycles, indicating a finite and controlled release profile. Analytical evaluation by GC-MS, LC-MS, HS-GC-MS, and ICP-MS revealed a limited number of organic compounds and trace elements above the analytical evaluation threshold.

Subsequent toxicological risk assessment, performed in accordance with ISO 10993-17 and supported by ISO 10993-18 chemical characterization principles, demonstrated margins of safety greater than one for all identified compounds, including Low Molecular Weight Chemicals. Importantly, no extractables were identified that raised concerns for systemic toxicity, genotoxicity, carcinogenicity, or local biological effects such as irritation or sensitization. These findings are consistent with, and complementary to, the negative outcomes observed in the in vitro and in vivo biological tests, reinforcing the conclusion that chemical constituents and degradation products of Hydrogel OA 2% are unlikely to pose clinically relevant risks.

Implications for osteoarthritis therapy

The preclinical safety data and degradation pattern of Hydrogel OA 2% shows promise for its application in treating osteoarthritis. The hydrogel structure contains viscoelastic properties and cross-linking elements which provide extended joint lubrication and mechanical support to maintain symptom relief beyond standard hyaluronic acid injection duration. The material shows no signs of ongoing inflammation or body-wide toxic reactions which allows it to stay inside the joint for long periods to provide mechanical support and protection without harming the joint structure. Moreover, the observed phagocytic clearance pathway suggests that repeat injection, if required, are unlikely to result in cumulative residue or adverse tissue remodeling.

Clinical research needs to confirm preclinical findings by conducting clinical studies in human osteoarthritic joints. The Hydrogel OA 2% dual-biomaterial structure functions as a flexible delivery system which releases disease-modifying or anti-inflammatory agents through controlled release mechanisms. The pre-clinical studies demonstrated a robust safety

profile which supports initiation of human clinical trials according to ISO and OECD guidelines.

5. Conclusion

The comprehensive ISO 10993-compliant preclinical program demonstrated that Hydrogel OA 2% exhibits an excellent biocompatibility and safety profile for intra-articular injection. The hydrogel was non-cytotoxic, non-genotoxic, non-pyrogenic, non-sensitizing, and free of systemic toxicity in both acute and settings week 4, 13 and 26 (Table 9). Intra articular injection showed preserved synovial and cartilage morphology at week 1, 13, and 26. Inflammation at week 4, disappeared on its own without harming cartilage or synovial tissue confirming high local tolerance and appropriate physiological interaction with joint tissues. The hydrogel underwent progressive phagocytic degradation, consistent with a resorbable biomaterial and without signs of adverse or persistent material buildup.

Importantly, exhaustive chemical characterization combined with toxicological risk assessment confirmed the absence of clinically relevant systemic or local risks, including genotoxicity and carcinogenicity.

Together, these findings establish Hydrogel OA 2% as a safe, biocompatible, and biodegradable viscoelastic implant capable of long-lasting residence within the joint space while maintaining tissue integrity. The demonstrated biological safety supports further clinical translation and evaluation of Hydrogel OA 2% as a next-generation hydrogel designed to enhance joint function in osteoarthritis.

Table 9. Biological safety summary of Hydrogel OA 2%.

Biological Safety	Test Model	Results
Cytotoxicity ISO10993-5	In vitro L-929 mouse fibroblast cells	Satisfied
Genotoxicity ISO10993-3, OECD 476 and 490	In vitro AMES - Salmonella typhimurium tester strains, Escherichia coli tester strain WP2uvrA In vitro Micronucleus - Human lymphocyte cell cultures In vitro HPRT - L5178Y cells In vitro mammalian cell mutation - Human lymphoblastoid TK6 cell line	Satisfied
Pyrogenicity European Pharmacopoeia (Chapter 2.6.8).	Rabbit pyrogen test	Satisfied
Sensitization ISO10993-10	Guinea pig maximization test	Satisfied
Intracutaneous Study in Rabbits ISO10993-23	Rabbit irritation test	Satisfied at day 14
Acute systemic toxicity ISO10993-11 4-13-26-week systemic toxicity ISO10993-11	Acute systemic toxicity at 4, 24, 48 and 72 hours after intraperitoneal injection Systemic toxicity at 4-13-26-week after intracutaneous injection	Satisfied
Local tissue effects ISO10993-6	4-13-26-week after intra articular injection	Satisfied
Chemical characterization ISO 10993-18 TRA ISO 10993-17	Exhaustive extractables study	Unlikely presence of systemic risks

This study provides an integrated preclinical evaluation of the biological safety, degradation behavior, and chemical risk profile of Hydrogel OA 2% under sensitivity-optimized conditions. However, several limitations should be noted: (i) all in vivo data were generated in animal models and may not fully reflect the biomechanical and inflammatory complexity of human osteoarthritic joints, even though the rabbit intra-articular model and extended follow-up to 26 weeks offer meaningful insight into biodegradation and tissue compatibility, differences in joint biomechanics, disease state, and synovial microenvironment between animal models and human osteoarthritis may influence material behavior; (ii) chemical risk assessment was based on identified extractables under controlled conditions and may not capture variability arising from patient factors, dosing strategies, or joint pathology; and (iii) comparative analysis was restricted to selected hyaluronan-based controls. Despite these considerations, the concordance of biological, histopathological, and chemical findings supports a controlled, non-adverse safety profile and justifies progression to clinical investigation.

Abbreviations

ABS	Alcian Blue Safranin
AET	Analytical Evaluation Threshold
AMES	Bacterial Reverse Mutation Assay
ANOVA	Analysis of Variance
CE	Cloning Efficiency
CEP	Certificate of Suitability
CO ₂	Carbon Dioxide
CPA	Cyclophosphamide
CRO	Contact Research Organization
DBT	Dose-Based Threshold
DMSO	Dimethyl Sulfoxide
D0	Day 0
DPBS	Dulbecco's Phosphate Buffered Saline
EtOH	Ethanol
FCA	Freund's Complete Adjuvant
GC-MS	Gas Chromatography–Mass Spectrometry
GLP	Good Laboratory Practice
HMP	Hydrogel Microparticles
HPRT	Hypoxanthine-Guanine Phosphoribosyl Transferase
HS-GC-MS	Headspace Gas Chromatography–Mass Spectrometry
ICP-MS	Inductively Coupled Plasma–Mass Spectrometry
IPA	Isopropanol
ISO	International Organization for Standardization
LC-MS	Liquid Chromatography–Mass Spectrometry
LMWC	Low Molecular Weight Chemicals
MF	Mutation Frequency
MMS	Methyl Methane sulfonate
MoS	Margin of Safety

MTS	[3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium]
NaCl	Sodium Chloride
NC	Negative Control
NVR	Non-Volatile Residue
OA	Osteoarthritis
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate Buffered Saline
PEG	Polyethylene Glycol
PMCID	PubMed Central Identifier
PMID	PubMed Identifier
PMS	phenazine methosulfate
RS	Relative Survival
RT	Room Temperature
S9	Metabolic Activation System (Rat Liver S9 Fraction)
SHE	Saffron-Hematoxylin-Eosin
SOFG	Safranin O Fast Green
TG	Thioguanine
TK	Thymidine Kinase
TK6	Human Lymphoblastoid Thymidine Kinase Cell Line
TRA	Toxicological Risk Assessment
TSL	Tolerable Exposure Limit
TTC	Threshold of Toxicological Concern
W	Week(s)

Supplementary Material

The supplementary material can be accessed at <https://doi.org/10.11648/j.jb.20261001.11>

Acknowledgments

The authors gratefully acknowledge NAMSA, Genotrix/Scantox and Institute Pasteur De Lille for performing the biocompatibility and preclinical safety studies in accordance with OECD Good Laboratory Practice (GLP) and ISO 10993 standards. The authors also thank both scientific and technical teams for their professional execution of the in vitro and in vivo testing programs.

Author Contributions

Burcu Gur: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing

Jorg Schelfhout: Conceptualization, Supervision, Funding acquisition, Writing – review & editing

Kris Van den Cruys: Writing – review & editing

Funding

This work is supported by Allegro NV/SA.

Data Availability Statement

The raw data and analytical reports are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] Steinmetz, Jaimie D et al.. Global, regional, and national burden of osteoarthritis, 1990–2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet Rheumatology*. n.d; 5(9): e508-e522. [https://doi.org/10.1016/S2665-9913\(23\)00163-7](https://doi.org/10.1016/S2665-9913(23)00163-7)
- [2] Hunter, David J, and Bierma-Zeinstra, Sita. Osteoarthritis. *The Lancet*. n.d; 393(10182): 1745-1759. [https://doi.org/10.1016/S0140-6736\(19\)30417-9](https://doi.org/10.1016/S0140-6736(19)30417-9)
- [3] Tang S, Zhang C, Oo WM, et al. Osteoarthritis. *Nat Rev Dis Primers*. 2025; 11: 10. <https://doi.org/10.1038/s41572-025-00594-6>
- [4] Tong L, Yu H, Huang X, et al. Current understanding of osteoarthritis pathogenesis and relevant new approaches. *Bone Res*. 2022; 10: 60. <https://doi.org/10.1038/s41413-022-00226-9>
- [5] Focsa MA, Florescu S, Gogulescu A. Emerging Strategies in Cartilage Repair and Joint Preservation. *Medicina*. 2025; 61(1): 24. <https://doi.org/10.3390/medicina61010024>
- [6] Testa G, Giardina SMC, Culmone A, et al. Intra-articular injections in knee osteoarthritis: a review of literature. *J Funct Morphol Kinesiol*. 2021; 6(1): 15. <https://doi.org/10.3390/jfmk6010015>
- [7] Kalairaj MS, Pradhan R, Saleem W, Smith MM, Gaharwar AK. Intra-Articular Injectable Biomaterials for Cartilage Repair and Regeneration. *Adv Healthc Mater*. 2024 Jul; 13(17): e2303794. <https://doi.org/10.1002/adhm.202303794>
- [8] Xue Y, Wang X, Wang X, et al. A comparative study of the efficacy of intra-articular injection of different drugs in the treatment of mild to moderate knee osteoarthritis: a network meta-analysis. *Medicine (Baltimore)*. 2023; 102(12): e33339. <https://doi.org/10.1097/MD.0000000000003339>
- [9] Glinkowski WM, Tomaszewski W. Intra-Articular Hyaluronic Acid for Knee Osteoarthritis: A Systematic Umbrella Review. *Journal of Clinical Medicine*. 2025; 14(4): 1272. <https://doi.org/10.3390/jcm14041272>
- [10] Evans, Christopher H., Kraus, Virginia B., and Setton, Lori A.. Progress in intra-articular therapy. *Nature Reviews Rheumatology*. n.d.; 10(1): 11-22. <https://doi.org/10.1038/nrrheum.2013.159>
- [11] Larsen, N. E., Dursema, H. D., Pollak, C. T., & Skrabut, E. M. (2012). Clearance kinetics of a hylan-based viscosupplement after intra-articular and intravenous administration in animal models. *Journal of biomedical materials research. Part B, Applied biomaterials*, 100(2), 457–462. <https://doi.org/10.1002/jbm.b.31971>
- [12] Kalairaj, M. S., Pradhan, R., Saleem, W., Smith, M. M., & Gaharwar, A. K. (2024). Intra-Articular Injectable Biomaterials for Cartilage Repair and Regeneration. *Advanced healthcare materials*, 13(17), e2303794. <https://doi.org/10.1002/adhm.202303794>
- [13] Chen, J., Deng, M., Wang, J., Liu, Y., Hu, Z., Luan, F., Zhu, H., & Zheng, C. (2025). Recent advances in injectable hydrogels for osteoarthritis treatments. *Frontiers in bioengineering and biotechnology*, 13, 1644222. <https://doi.org/10.3389/fbioe.2025.1644222>
- [14] Wang, S., Qiu, Y., Qu, L., Wang, Q., & Zhou, Q. (2022). Hydrogels for Treatment of Different Degrees of Osteoarthritis. *Frontiers in bioengineering and biotechnology*, 10, 858656. <https://doi.org/10.3389/fbioe.2022.858656>
- [15] Hashemi-Afzal, F., Fallahi, H., Bagheri, F., Collins, M. N., Es-laminejad, M. B., & Seitz, H. (2024). Advancements in hydrogel design for articular cartilage regeneration: A comprehensive review. *Bioactive materials*, 43, 1–31. <https://doi.org/10.1016/j.bioactmat.2024.09.005>
- [16] Gan, X., Wang, X., Huang, Y., Li, G., & Kang, H. (2024). Applications of Hydrogels in Osteoarthritis Treatment. *Biomedicines*, 12(4), 923. <https://doi.org/10.3390/biomedicines12040923>
- [17] Duan, W. L., Zhang, L. N., Bohara, R., Martin-Saldaña, S., Yang, F., Zhao, Y. Y., Xie, Y., Bu, Y. Z., & Pandit, A. (2023). Adhesive hydrogels in osteoarthritis: from design to application. *Military Medical Research*, 10(1), 4. <https://doi.org/10.1186/s40779-022-00439-3>
- [18] Bierbrauer, K. L., Alasino, R. V., Barclay, F. E., Belotti, E. M., Ortega, H. H., & Beltramo, D. M. (2021). Biocompatible Hydrogel for Intra-Articular Implantation Comprising Cationic and Anionic Polymers of Natural Origin: In Vivo Evaluation in a Rabbit Model. *Polymers*, 13(24), 4426. <https://doi.org/10.3390/polym13244426>
- [19] Tsubosaka, M., Kihara, S., Hayashi, S., Nagata, J., Kuwahara, T., Fujita, M., Kikuchi, K., Takashima, Y., Kamenaga, T., Kuroda, Y., Takeuchi, K., Fukuda, K., Takayama, K., Hashimoto, S., Matsumoto, T., Niikura, T., Tabata, Y., & Kuroda, R. (2020). Gelatin hydrogels with eicosapentaenoic acid can prevent osteoarthritis progression in vivo in a mouse model. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*, 38(10), 2157–2169. <https://doi.org/10.1002/jor.24688>
- [20] Rousselle, S. D., Ramot, Y., Nyska, A., & Jackson, N. D. (2019). Pathology of Bioabsorbable Implants in Preclinical Studies. *Toxicologic pathology*, 47(3), 358–378. <https://doi.org/10.1177/0192623318816681>

- [21] Sandker, M. J., Duque, L. F., Redout, E. M., Chan, A., Que, I., Löwik, C. W. G. M., Klijnsma, E. C., Kops, N., Steendam, R., van Weeren, R., Hennink, W. E., & Weinans, H. (2017). Degradation, intra-articular retention and biocompatibility of monospheres composed of [PDLLA-PEG-PDLLA]-b-PLLA multi-block copolymers. *Acta biomaterialia*, 48, 401–414. <https://doi.org/10.1016/j.actbio.2016.11.003>
- [22] Karami, P., Stampoultzis, T., Guo, Y., & Pioletti, D. P. (2023). A guide to preclinical evaluation of hydrogel-based devices for treatment of cartilage lesions. *Acta biomaterialia*, 158, 12–31. <https://doi.org/10.1016/j.actbio.2023.01.015>
- [23] International Organization for Standardization. ISO 10993-1: 2018. Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process. Geneva: ISO; 2018. <https://doi.org/10.3403/BSENISO10993>
- [24] Platel, A., Nesslany, F., Gervais, V., Claude, N., & Marzin, D. (2011). Study of oxidative DNA damage in TK6 human lymphoblastoid cells by use of the thymidine kinase gene-mutation assay and the in vitro modified comet assay: determination of No-Observed-Genotoxic-Effect-Levels. *Mutation research*, 726(2), 151–159. <https://doi.org/10.1016/j.mrgentox.2011.09.003>
- [25] Schwartz, J. L., Jordan, R., Evans, H. H., Lenarczyk, M., & Liber, H. L. (2004). Baseline levels of chromosome instability in the human lymphoblastoid cell TK6. *Mutagenesis*, 19(6), 477–482. <https://doi.org/10.1093/mutage/geh060>
- [26] O'Brien, M. T., Schuh, J. C. L., Wancket, L. M., Cramer, S. D., Funk, K. A., Jackson, N. D., Kannan, K., Keane, K., Nyska, A., Rousselle, S. D., Schucker, A., Thomas, V. S., & Tunev, S. (2022). Scientific and Regulatory Policy Committee Points to Consider for Medical Device Implant Site Evaluation in Non-clinical Studies. *Toxicologic pathology*, 50(4), 512–530. <https://doi.org/10.1177/01926233221103202>
- [27] International Organization for Standardization. Biological evaluation of medical devices—Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity. ISO 10993-3: 2014. Geneva: ISO; 2014 <https://doi.org/10.3403/BSENISO10993>
- [28] International Organization for Standardization. Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity. ISO 10993-5: 2009. Geneva: ISO; 2009. <https://doi.org/10.3403/BSENISO10993>
- [29] Dieter M. (1992). NTP technical report on the toxicity studies of Toxicity Studies of Antimony Potassium Tartrate (CAS No. 28300-74-5) in F344/N Rats And B6C3F1 Mice (Drinking Water and Intraperitoneal Injection Studies). Toxicity report series, 11, 1–D2. <https://doi.org/10.3403/BSENISO10993>
- [30] Thiele, W., Kyjacova, L., Köhler, A., & Sleeman, J. P. (2020). A cautionary note: Toxicity of polyethylene glycol 200 injected intraperitoneally into mice. *Laboratory animals*, 54(4), 391–396. <https://doi.org/10.1177/0023677219873684>
- [31] Xia, Z., & Triffitt, J. T. (2006). A review on macrophage responses to biomaterials. *Biomedical materials* (Bristol, England), 1(1), R1–R9. <https://doi.org/10.1088/1748-6041/1/1/R01>
- [32] Jackson, D. W., & Simon, T. M. (2006). Intra-articular distribution and residence time of Hylan A and B: a study in the goat knee. *Osteoarthritis and cartilage*, 14(12), 1248–1257. <https://doi.org/10.1016/j.joca.2006.05.015>
- [33] Tnibar A. (2022). Intra-articular 2.5% polyacrylamide hydrogel, a new concept in the medication of equine osteoarthritis: A review. *Journal of equine veterinary science*, 119, 104143. <https://doi.org/10.1016/j.jevs.2022.104143>
- [34] da Silva Xavier, A. A., da Rosa, P. P., de Brum Mackmill, L., & Roll, V. F. B. (2021). An assessment of the effectiveness of hyaluronic acid and polyacrylamide hydrogel in horses with osteoarthritis: Systematic review and network meta-analysis. *Research in veterinary science*, 134, 42–50. <https://doi.org/10.1016/j.rvsc.2020.11.013>
- [35] Aykaç B., Dinç M., Nar, Ö. O., Karasu, R., & Bayrak, H. Ç. (2025). Comparative efficacy of polyacrylamide hydrogel versus hyaluronic acid and corticosteroids in knee osteoarthritis: A retrospective cohort study. *Medicine*, 104(38), e44655. <https://doi.org/10.1097/MD.00000000000044655>
- [36] Watkins, A., Fasanello, D., Stefanovski, D., Schurer, S., Caracappa, K., D'Agostino, A., Costello, E., Freer, H., Rollins, A., Read, C., Su, J., Colville, M., Paszek, M., Wagner, B., & Reesink, H. (2021). Investigation of synovial fluid lubricants and inflammatory cytokines in the horse: a comparison of recombinant equine interleukin 1 beta-induced synovitis and joint lavage models. *BMC veterinary research*, 17(1), 189. <https://doi.org/10.1186/s12917-021-02873-2>
- [37] de Melo Nunes, R., Cunha, P. L. R., Pinto, A. C. M. D., Girão, V. C. C., de Andrade Feitosa, J. P., & Rocha, F. A. C. (2020). Hylan G-F20 and galactomannan joint flares are associated to acute synovitis and release of inflammatory cytokines. *Advances in rheumatology* (London, England), 60(1), 26. <https://doi.org/10.1186/s42358-020-00127-7>
- [38] Yoshioka, K., Katayama, M., Nishiyama, T., Harada, K., Takeshita, S., & Kawamata, Y. (2019). Biocompatibility study of different hyaluronan products for intra-articular treatment of knee osteoarthritis. *BMC musculoskeletal disorders*, 20(1), 424. <https://doi.org/10.1186/s12891-019-2815-6>

Biography

Burcu Gur is a Pre-Clinical Trial Manager at Allegro NV/SA, within the Pre-Clinical Department, where she supports the development and biological safety evaluation of medical devices. She holds a PhD in Biotechnology and completed an academic postdoctoral position in Molecular Physiology at Johannes Gutenberg University. During her doctoral studies, Dr. Gur participated in two government-funded research projects, contributing to translational research activities in the field of biotechnology. She is a member of the Osteoarthritis Research Society International (OARSI). Her primary areas of expertise include biotechnology, stem cell research, and medical device biocompatibility assessment in accordance with ISO 10993 standards. In her current role, she also acts as a scientific interface between pre-clinical and clinical teams, supporting clinical development through integrated translational and preclinical strategies.

Research Field

Burcu Gur: Biotechnology, Preclinical and Translational Science, Medical Device Biocompatibility and Safety Assessment, Stem Cell Biology, Biomaterials and Injectable Medical Devices, Osteoarthritis Research.