MEDICINE AND MATERIALS
Volume 6, Issue 1, 2026: 35-42 | ISSN: 2784 – 1499 & e-ISSN: 2784 – 1537
DOI: 10.36868/MEDMATER.2026.06.01.05 | www.medicineandmaterials.com |

COMPARATIVE IMMUNOMODULATORY EFFECTS OF HA-, CHITOSAN-, COLLAGEN-, AND PEG-BASED MATRICES ON INFLAMED CHONDROCYTES (IL-1B MODEL) IN VITRO

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Abstract

Osteoarthritis is characterized by progressive cartilage degradation driven by inflammatory mediators that disrupt chondrocyte homeostasis and extracellular matrix integrity. Biomaterial-based strategies have gained increasing attention as potential tools to modulate inflammatory responses and support cartilage repair. In our study, we performed a comparative in vitro evaluation of hyaluronic acid (HA)-, chitosan-, collagen-, and polyethylene glycol (PEG)-based matrices using an IL-1β-induced inflammatory human chondrocyte model. Physicochemical properties of the matrices were characterized and correlated with cytocompatibility, cell morphology, and inflammatory and catabolic responses. All matrices exhibited high cytocompatibility under both basal and inflammatory conditions. IL-1β stimulation induced robust secretion of pro-inflammatory mediators and upregulation of catabolic genes in control cultures. HA-based matrices showed the strongest attenuation of inflammatory cytokine release, while collagen matrices best preserved chondrocyte morphology and cartilage-specific gene expression. Chitosan matrices exerted moderate immunomodulatory effects, whereas PEG matrices demonstrated limited biological activity, consistent with their bioinert nature. Overall, our findings highlight the material-dependent modulation of chondrocyte behavior under inflammatory conditions and support the importance of rational biomaterial selection for cartilage repair strategies in osteoarthritis.

Keywords: osteoarthritis, chondrocytes, biomaterials, inflammation, hydrogels, cartilage repair.

Introduction

Articular cartilage degeneration represents a central pathological feature of osteoarthritis, a condition characterized by progressive matrix breakdown, chronic inflammation, and impaired chondrocyte function. Due to the avascular and aneural nature of cartilage, its intrinsic regenerative capacity is extremely limited, making tissue repair a major clinical challenge [1]. In this context, biomaterial-based strategies have emerged as promising tools to modulate the cellular microenvironment, support chondrocyte viability, and counteract inflammatory catabolic cascades [2].

Inflammatory cytokines, particularly interleukin-1β (IL-1β), play a pivotal role in osteoarthritic cartilage degradation by inducing the overexpression of matrix metalloproteinases

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and aggrecanases, suppressing extracellular matrix synthesis, and promoting phenotypic instability of chondrocytes [3-6]. Consequently, in vitro IL-1β-stimulated chondrocyte models are widely employed to investigate biomaterial-mediated immunomodulatory effects under controlled inflammatory conditions [2].

Among natural biomaterials, hyaluronic acid (HA) has received considerable attention due to its intrinsic bioactivity, interaction with CD44 receptors, and capacity to attenuate inflammatory signaling while supporting cartilage-specific matrix production [1,8-11]. Chitosan-based biomaterials, derived from chitin, offer favorable biocompatibility and tunable physicochemical properties, with emerging evidence supporting their ability to modulate inflammatory responses and enhance tissue regeneration [4]. Collagen scaffolds, as extracellular matrix–mimetic systems, provide bioactive ligands that promote cell adhesion and phenotype maintenance, and are already applied clinically in cartilage repair strategies [5,6].

In contrast, synthetic hydrogels such as polyethylene glycol (PEG) offer high reproducibility and controllable mechanical properties, although their bioinert nature may limit direct cell–matrix signaling unless functionalized [3,12-14]. Nonetheless, PEG-based systems are valuable comparators for dissecting the relative contribution of biochemical versus mechanical cues in chondrocyte responses to inflammation [8].

Recent advances emphasize the importance of immunomodulatory biomaterials capable of simultaneously attenuating inflammatory mediator release and preserving chondrocyte phenotype [7-9]. Injectable and hydrogel-based platforms have been extensively explored to achieve these objectives, highlighting the need for systematic comparative studies across different matrix classes [10,12]. Moreover, bioactive hybrid systems incorporating anti-inflammatory agents further underscore the relevance of matrix-driven modulation of IL-1β–induced pathways [13].

In our study, we performed a comparative in vitro evaluation of HA-, chitosan-, collagen-, and PEG-based matrices using an IL-1β–stimulated human chondrocyte model. By integrating physicochemical characterization with cytocompatibility, inflammatory, and catabolic readouts, our aim was to elucidate how matrix composition influences chondrocyte behavior under inflammatory conditions relevant to osteoarthritis.

Materials and Methods

Study design

We comparatively evaluated the immunomodulatory effects of four biomaterial matrices, hyaluronic acid (HA), chitosan, collagen, and polyethylene glycol (PEG), on human chondrocytes under inflammatory conditions induced by IL-1β. Their results included physicochemical matrix characterization, cytocompatibility, and cell morphology, as well as inflammatory and catabolic responses.

Biomaterial matrices preparation

We prepared the hydrogels based on HA at 2% (v/v) using biocompatible cross-linking. Chitosan matrices (2% w/v; degree of deacetylation >80%) were solubilized in dilute acetic acid and neutralized before gelation. Type I collagen hydrogels were prepared at 3 mg/mL and polymerized at 37 °C. PEG-based hydrogels (5% w/v) were synthesized by controlled cross-linking to obtain stable synthetic networks. All matrices were poured into standardized molds, hydrated overnight in phosphate-buffered physiological solution (PBS), and sterilized under aseptic conditions before cell seeding.

Physicochemical characterization

The swelling behavior was evaluated after 24-hour incubation in PBS at 37 °C by calculating the swelling ratio (Q). The compression modulus has been determined under unlimited compression. The surface wettability of the film-type matrices was evaluated by static measurements of the contact angle. The microstructural characteristics were qualitatively evaluated using scanning or confocal electron microscopy.

Chondrocyte culture

We cultured the primary human articular chondrocytes (passage P0–P2) in DMEM/F12 supplemented with 10% fetal bovine serum and antibiotics. We seeded the cells on the matrices at a density of $2–5 \times 10^4$ cells/cm^2 and allowed them to attach for 24 hours before inflammatory stimulation.

Inflammatory stimulation

Inflammation was induced by treatment with recombinant human interleukin-1β (IL-1β, 10 ng/mL) for 24 h. Control cultures were maintained under basal conditions without cytokine exposure. Culture supernatants were collected and stored at -80 °C for subsequent analyses.

Cytocompatibility and cell morphology

Cell metabolic activity was evaluated using the AlamarBlue assay and normalized to tissue culture plastic (TCP) basal controls. Cytotoxicity was assessed by lactate dehydrogenase (LDH) release. Cell viability was determined by Live/Dead fluorescence staining. Cytoskeletal organization and cell spreading were visualized using phalloidin staining, and the spreading area was quantified by image analysis.

Inflammatory and catabolic markers

The levels of IL-6, IL-8, and prostaglandin E2 (PGE2) in culture supernatants were quantified by the enzyme-linked immunosorbent assay (ELISA). The gene expression of inflammatory phenotypic markers (PTGS2), catabolic (MMP13, ADAMTS5), and cartilage (COL2A1, ACAN) was analyzed by quantitative real-time PCR. The relative expression was calculated using the $2^{-}\Delta\Delta Ct$ method after normalization to GAPDH.

Statistical analysis

Our experiments were carried out in three independent biological replicas with technical triplicates. The data are presented as mean ± standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. We analyzed the differences between the groups using the unidirectional or bidirectional analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. The statistical significance was set at $p < 0.05$. The analyses were performed using the GraphPad Prism software (version 9.5.1; GraphPad Software, San Diego, CA, USA).

Results

Physicochemical characterization of matrices

All matrices were successfully fabricated in standardized dimensions and remained structurally stable during culture. Swelling behavior and mechanical profiles differed across

compositions, with HA and PEG showing higher hydration capacity, while collagen and chitosan displayed comparatively lower swelling (Table 1). Microstructural assessment indicated distinct network architectures, potentially impacting nutrient diffusion and cell–matrix interaction.

Table 1. Physicochemical properties of HA, chitosan, collagen, and PEG matrices

Parameter	**HA**	**Chitosan**	**Collagen**	**PEG**
Polymer concentration	2% w/v	2% w/v	3 mg/mL	5% w/v
Gelation time (min)	5.2 ± 0.6	9.4 ± 1.1	21.6 ± 2.3	3.1 ± 0.4
Swelling ratio Q (24 h)	18.7 ± 2.1	9.3 ± 1.4	7.8 ± 1.2	21.4 ± 2.6
Compressive modulus (kPa)	18.2 ± 3.4	32.6 ± 4.1	11.5 ± 2.8	45.8 ± 6.3
Contact angle (°)	38 ± 4	62 ± 6	45 ± 5	78 ± 7
Qualitative microstructure	Homogeneous, highly porous	Dense, fibrillar	Loose fibrillar ECM-like	Uniform, fine mesh

Data is presented as mean ± SD from three independent experiments (Table 1). The swelling ratio (Q) was calculated after 24 h incubation in PBS at 37°C. Compressive modulus was determined under unconfined compression. Contact angle measurements were performed on film-type matrices. Microstructural features were assessed by SEM or confocal microscopy.

Cytocompatibility and chondrocyte morphology on matrices

Data are presented as mean ± SD from three independent experiments performed in technical triplicate. Metabolic activity was assessed using the AlamarBlue assay and normalized to TCP basal control (Table 2). LDH release is expressed as a percentage of maximal lysis control. Cell viability was quantified by Live/Dead fluorescence staining. Spreading area was calculated from phalloidin-stained actin cytoskeleton images using image analysis software. IL-1β stimulation was applied at 10 ng/mL for 24 h.

Table 2. Cytocompatibility and morphology metrics (mean ± SD)

Outcome	**Condition**	**TCP**	**HA**	**Chitosan**	**Collagen**	**PEG**
Metabolic activity (% of TCP basal)	Basal	100 ± 5	104 ± 6	98 ± 7	112 ± 8	95 ± 6
	+IL-1β	92 ± 6	96 ± 7	90 ± 8	105 ± 9	88 ± 7
LDH release (% max)	Basal	6.2 ± 1.1	5.8 ± 1.0	6.9 ± 1.3	5.1 ± 0.9	7.4 ± 1.2
	+IL-1β	7.5 ± 1.4	6.9 ± 1.2	8.1 ± 1.5	6.2 ± 1.1	8.6 ± 1.6
Live cells (%)	Basal	95.6 ± 2.3	96.8 ± 2.1	94.2 ± 2.8	97.9 ± 1.9	93.5 ± 3.0
	+IL-1β	93.1 ± 2.9	94.6 ± 2.6	92.4 ± 3.1	96.2 ± 2.4	91.8 ± 3.4
Spreading area (μm²)	Basal	820 ± 110	960 ± 130	880 ± 120	1180 ± 150	620 ± 95
	+IL-1β	760 ± 105	890 ± 125	810 ± 115	1090 ± 140	580 ± 90

Data is presented as mean ± SD from three independent experiments performed in technical triplicate (Table 3). Inflammation was induced using recombinant human IL-1β (10 ng/mL, 24 h). Cytokine and PGE2 levels were quantified in culture supernatants by ELISA and expressed as pg/mL. Gene expression was assessed by quantitative PCR and reported as fold-change relative to TCP basal control using the 2^-ΔΔCt method after normalization to GAPDH.

Reduced inflammatory and catabolic marker expression indicates immunomodulatory effects of specific matrices.

Immunomodulatory effects under IL-1β: secreted mediators and gene expression

IL-1β stimulation induced robust IL-6 secretion in TCP controls. All biomaterial matrices reduced IL-6 levels to varying extents, with HA-based matrices exhibiting the most pronounced anti-inflammatory effect (Figure 1).

Table 3. Inflammatory and catabolic markers in inflamed chondrocytes

Marker	Readout	TCP +IL-1β	HA +IL-1β	Chitosan +IL-1β	Collagen +IL-1β	PEG +IL-1β
IL-6	ELISA (pg/mL)	820 ± 95	410 ± 60	480 ± 70	520 ± 75	690 ± 90
IL-8	ELISA (pg/mL)	1250 ± 140	620 ± 85	710 ± 95	780 ± 110	980 ± 130
PGE2	ELISA (pg/mL)	960 ± 120	460 ± 65	520 ± 80	590 ± 85	810 ± 105
PTGS2 (COX-2)	qPCR (fold)	6.8 ± 0.9	3.2 ± 0.6	3.9 ± 0.7	4.4 ± 0.8	5.9 ± 1.0
MMP13	qPCR (fold)	7.5 ± 1.1	3.6 ± 0.8	4.2 ± 0.9	4.8 ± 1.0	6.3 ± 1.2
ADAMTS5	qPCR (fold)	6.2 ± 0.8	3.1 ± 0.6	3.7 ± 0.7	4.1 ± 0.8	5.4 ± 0.9
COL2A1	qPCR (fold)	0.42 ± 0.09	0.78 ± 0.12	0.70 ± 0.11	0.92 ± 0.14	0.55 ± 0.10
ACAN	qPCR (fold)	0.38 ± 0.08	0.74 ± 0.10	0.68 ± 0.09	0.88 ± 0.13	0.50 ± 0.09

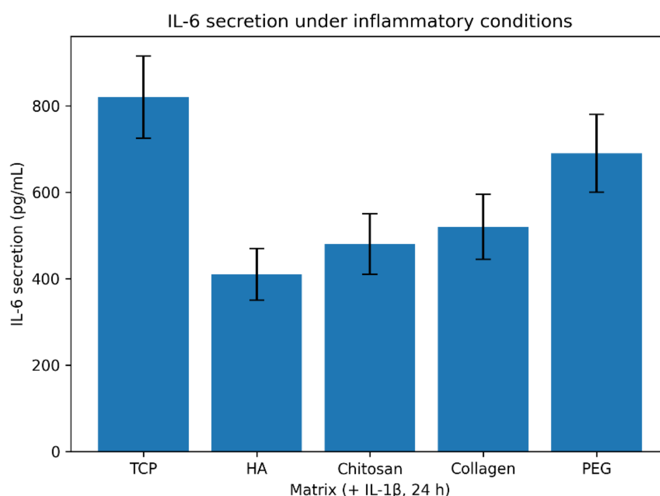

Figure 1. Modulation of IL-6 secretion by biomaterial matrices under IL-1β-induced inflammatory conditions

Discussion

Our current in vitro study investigated the immunomodulatory effects of four distinct biomaterial matrices, hyaluronic acid, chitosan, collagen, and polyethylene glycol, on human chondrocytes exposed to IL-1β-induced inflammatory conditions. By integrating physicochemical characterization with biological and molecular results, our study provides a

comparative framework for understanding how matrix composition influences chondrocyte behavior in an environment relevant to osteoarthritis.

In our experimental model, IL-1β stimulation successfully induced a pronounced inflammatory and catabolic phenotype in chondrocytes cultured on tissue culture plastic, as evidenced by elevated secretion of IL-6, IL-8, and PGE2, along with upregulation of PTGS2, MMP13, and ADAMTS5. These findings are consistent with previous reports describing IL-1β as a central mediator of cartilage degradation and inflammatory amplification in osteoarthritic conditions [2,3]. Importantly, cell viability remained high across all experimental groups, confirming that the observed changes were not confounded by cytotoxicity but rather reflected functional modulation of inflammatory pathways.

Among the investigated matrices, hyaluronic acid-based hydrogels demonstrated the most pronounced attenuation of inflammatory mediator release. In our study, HA significantly reduced IL-6 and PGE2 levels compared to TCP controls under IL-1β stimulation. This effect may be attributed to HA-CD44 interactions, which are known to interfere with NF-κB signaling and downregulate pro-inflammatory gene expression in chondrocytes [1,11]. These observations support previous evidence highlighting the intrinsic immunomodulatory potential of HA-based biomaterials beyond their mechanical or lubricating roles.

Chitosan matrices also exhibited a moderate but consistent anti-inflammatory effect, reducing cytokine secretion and catabolic gene expression relative to TCP. The cationic nature of chitosan, along with its capacity to interact electrostatically with inflammatory mediators, may partially explain this response [4]. While chitosan did not outperform HA in suppressing inflammatory markers, its favorable cytocompatibility and tunable physicochemical properties suggest potential value in composite or functionalized systems aimed at cartilage repair.

Collagen-based matrices displayed a distinct biological profile. Although their ability to suppress inflammatory mediators was less pronounced than that of HA, collagen scaffolds best preserved chondrocyte morphology and cartilage-specific gene expression, including COL2A1 and ACAN. In our study, chondrocytes cultured on collagen exhibited enhanced spreading and maintained a more differentiated phenotype under inflammatory challenge. These findings align with previous studies emphasizing the role of collagen as an extracellular matrix-mimetic scaffold that supports cell adhesion and phenotypic stability [5,6]. Thus, collagen may primarily contribute to structural and phenotypic support rather than direct immunosuppression.

In contrast, polyethylene glycol–based matrices demonstrated limited immunomodulatory capacity in our model. While PEG hydrogels provided mechanical stability and high reproducibility, their bioinert nature likely restricted direct cell-matrix signaling, resulting in weaker attenuation of IL-1β-induced inflammatory responses. Similar observations have been reported in previous studies, where PEG-based systems required biofunctionalization or incorporation of bioactive cues to effectively modulate cellular behavior [3,14]. Nonetheless, PEG matrices served as an important reference material in our comparative design, highlighting the contribution of biochemical interactions over purely mechanical effects.

Collectively, our findings underscore the importance of matrix composition in shaping chondrocyte responses under inflammatory conditions. The differential effects observed across biomaterials suggest that optimal cartilage repair strategies may require a balance between immunomodulatory capacity, mechanical support, and preservation of chondrocyte phenotype [7-10,12]. In this context, comparative in vitro studies such as ours provide valuable insights for the rational design of next-generation biomaterials targeting osteoarthritis.

Several limitations should be acknowledged. Our study was conducted in a simplified in vitro environment that does not fully recapitulate the complex cellular interactions present in

native cartilage tissue. Additionally, long-term effects and in vivo validation were beyond the scope of this work. Nevertheless, the standardized comparative approach employed here offers a robust platform for preclinical screening of biomaterials before more complex experimental models.

Conclusions

In our study, we demonstrated that biomaterial matrix composition plays a decisive role in modulating chondrocyte responses under IL-1β-induced inflammatory conditions. Hyaluronic acid-based matrices exhibited the most pronounced anti-inflammatory effects, significantly reducing pro-inflammatory mediator release, while collagen matrices best preserved chondrocyte morphology and cartilage-specific gene expression. Chitosan matrices provided intermediate immunomodulatory benefits, whereas polyethylene glycol matrices displayed limited biological activity despite favorable mechanical properties. Importantly, all investigated matrices maintained high cytocompatibility, confirming that the observed effects were driven by functional modulation rather than cytotoxicity. These findings highlight the importance of tailoring biomaterial design to achieve a balanced combination of immunomodulation, phenotypic support, and mechanical stability in cartilage repair strategies. Our comparative in vitro approach offers a valuable framework for preclinical evaluation of biomaterials and supports the rational selection of matrix systems for osteoarthritis-related applications. Future studies should focus on long-term and in vivo validation, as well as on the development of hybrid or functionalized matrices that integrate the complementary advantages of natural and synthetic biomaterials.

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Received: November 29, 2025
Accepted: February 25, 2026