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Bifidobacterium longum CBi0703 lysate modulates oxidative stress induced apoptosis and cartilage related gene expression in SW1353 chondrocytes: in vitro insights into the gut joint axis in Osteoarthritis

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Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage degradation, inflammation, and impaired joint function. This in vitro study evaluates the effects of *Bifidobacterium longum* CBi0703 lysate, alone and in combination with clinically used OA nutraceuticals, on viability, apoptosis and cartilage related gene expression in human SW1353 chondrocytes exposed to hydrogen peroxide induced oxidative stress, an OA like condition. SW1353 chondrocytes were exposed to H_2O_2 (50 µM, 2 h) to induce an OA-like state, followed by a 22-h treatment with *B. longum* CBi0703 lysate individual nutraceuticals (vitamin C, collagen, chondroitin sulphate, glucosamine sulphate, Chondro Mix, natural eggshell membrane (NEM)), or their combinations. Cell viability, proliferation, apoptosis, and expression of catabolic and anabolic genes were assessed.*B. longum* **CBi0703 and selected combinations enhanced chondrocyte proliferation, reduced caspase activation and modulated key catabolic (*MMP1*, *MMP13*, *ECM1*, *GBL1*) and anabolic (*COL2A1*, *SOX9*, *AGC1*, *TIMP1*) markers compared with the OA-induced vehicle. The combination with vitamin C upregulated *SOX9* and *TIMP1* while downregulating *COL1A1* and *ECM1*; the combination with chondroitin sulphate increased *COL2A1* expression; and the combination with glucosamine sulphate reduced late apoptosis. These results provide mechanistic insight into the potential chondroprotective actions of *B. longum* CBi0703 in an OA like in vitro model and support further preclinical and clinical studies to assess its role as an adjunct to established OA treatments.

Keywords Osteoarthritis, *Bifidobacterium longum* CBi0703, Cartilage degradation, Matrix metalloproteinases, Nutraceuticals, SW1353 chondrocytes, In vitro osteoarthritis model, Oxidative stress, Apoptosis, Gut joint axis

Osteoarthritis (OA) is a chronic, degenerative joint disease whose pathophysiology involves an imbalance between cartilage anabolism and catabolism, leading to extracellular matrix (ECM) degradation[1,2]. As the global population ages, the societal and economic burden of OA is expected to escalate, underscoring the urgent need for preventive strategies and effective therapeutic interventions. The clinical management of OA predominantly aims to alleviate pain, improve joint function, and slow disease progression[3,4]. Current therapeutic protocols are multifaceted, encompassing pharmacological and non-pharmacological approaches. Among pharmacological

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treatments, anti-inflammatory drugs, such as painkillers[5] and non-steroidal anti-inflammatory drugs (NSAIDs), dominate the market and are commonly prescribed for OA to reduce inflammation and provide symptomatic relief[6]. Despite their efficacy in managing pain, long-term NSAID use is associated with gastrointestinal, cardiovascular, and renal adverse effects[7], limiting their utility in chronic conditions like OA. In addition, disease-modifying OA drugs (DMOADs) modify the underlying disease process rather than merely addressing symptoms[8]. While DMOADs represent a promising approach, their clinical adoption has been constrained by poor therapeutic compliance, inconsistent efficacy, high costs, and limited availability. As their benefits are only perceived on the long-term, compliance represents a challenge and impacts overall efficacy. Finally, monoclonal antibodies, such as those targeting pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) or interleukin-1β (IL-1β), are gaining traction as advanced OA treatments[9]. These biologics effectively mitigate inflammation and slow structural degradation. However, their use is often restricted to severe cases due to their high cost and potential for immunogenicity. Despite these advancements, no current pharmacological therapy fully addresses the multifactorial nature of OA, particularly the imbalance between cartilage degradation and repair, requiring novel and complementary treatment strategies.

Today, OA management strategies often focus on stimulating cartilage anabolism to restore structural integrity[10]. Glucosamine (GS) and chondroitin sulphate (CS), for example, are widely utilized nutraceuticals that promote cartilage synthesis by enhancing glycosaminoglycan production[11]. The term nutraceuticals refer to bioactive food-derived substances that are intended to provide health benefits beyond basic nutrition, including support of joint structure and function in OA[12]. We selected vitamin C, collagen, CS, GS, a multi-ingredient formulation (Chondro Mix) and natural eggshell membrane (NEM) because they are widely used in OA management or joint-health supplements and have been previously reported to exert chondroprotective, anti-inflammatory and/or cartilage-anabolic effects. Vitamin C contributes to collagen synthesis and exerts antioxidant activity[13]; collagen and Chondro Mix provide structural amino acids and glycosaminoglycan precursors[14]; chondroitin sulphate and glucosamine are well-established agents that support extracellular matrix production[15]; and NEM has been associated with improvements in joint pain and function in OA populations[16]. In parallel, hyaluronic acid injections aim to restore synovial fluid viscosity, protecting joints and alleviating pain[17]. While these approaches show varying degrees of efficacy, their reliance on anabolic pathways limits their utility in addressing the overarching pathological hallmark of OA: excessive cartilage degradation. This gap highlights the potential significance of alternative strategies that emphasize reducing catabolic activity, such as interventions targeting the gut-joint axis. *Bifidobacterium longum* (*B. longum*), a well-characterized probiotic strain, has demonstrated considerable potential in promoting health through its anti-inflammatory and immunomodulatory properties[18,19]. Preclinical and clinical studies have revealed that *B. longum* modulates pro-inflammatory cytokine levels, thereby reducing systemic inflammation[20]. While several probiotics have shown its ability to enhance gut barrier function[21] only a few like *B. longum* CBi0703 demonstrated its capability to mitigate the translocation of endotoxins that contribute to systemic and joint inflammation. Concretely, in silico studies have shown its potential anti-inflammatory effect and immune modulation[22], and in vivo studies have demonstrated *B. longum* CBi0703's ability to reduce cartilage degradation[23]. This unique mechanism contrasts with that of current market leaders, which primarily focus on anabolic processes. By targeting catabolic pathways, *B. longum* CBi0703 lysate offers a complementary approach that could synergistically enhance existing OA treatments. However, the specific cellular mechanisms by which *B. longum* CBi0703 may protect chondrocytes are not fully understood. *B. longum* CBi0703 is a specific *B. longum* strain formulated as a lyophilized, inactivated preparation (postbiotic). Unlike other *B. longum* strains, it has shown efficacy in reducing cartilage lesions in an animal OA model and improving clinical scores in patients when combined with vitamin C[23,24]. This study therefore aimed to evaluate the in vitro effects of *B. longum* CBi0703 lysate, alone and in combination with established joint health nutraceuticals, on human chondrocyte viability, apoptosis, and the expression of key catabolic and anabolic genes under conditions of oxidative stress mimicking an OA-like environment.

Methods

Cell culture conditions and experimental model

The human chondrosarcoma cell line SW1353 was selected for this study as it provides a robust and reproducible in vitro model for investigating chondrocyte responses to OA-related stimuli, such as oxidative stress[25,26]. SW1353 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Leibovitz's L-15 Medium (L15) containing 10% (v/v) fetal bovine serum (FBS) and antibiotics 1% (v/v; penicillin–streptomycin, final concentrations 100 U/mL penicillin and 100 µg/mL streptomycin). Cells were maintained at 37 °C in a humidified 0% CO_2 atmosphere.

B. longum CBi0703 lysate is a product obtained from the freeze-dried culture of the strain, followed by its grinding. The result of this process is a mixture of *B. longum* metabolites and cell fractions, together with components of the culture medium. *B. longum* CBi0703 lysate was sourced from Biose Industrie, Aurillac, France. In this context, the term nutraceuticals refer to bioactive food-derived substances intended to provide health benefits for joint structure and function. The selected compounds—vitamin C (Vit C), collagen (Col), chondroitin sulphate (CS), glucosamine sulphate (GS), a multi-ingredient Chondro Mix (CM), and natural eggshell membrane (NEM)—are widely used in OA management and were chosen based on prior evidence of chondroprotective or anti-inflammatory effects[27–31]. Table 1 includes the ingredients and combinations evaluated in this work.

The working concentrations of *B. longum* CBi0703 lysate, Col, GS, CS and Vit C were selected a priori based on their frequent use in previous in vitro studies with SW1353 or related models and on internal feasibility tests under our culture conditions. These tests confirmed that the chosen doses did not reduce cell viability below approximately 80% in the absence of H_2O_2, a threshold commonly considered acceptable for mechanistic studies.

Name of combination	Experimental dose	References
B. longum CBi0703 lysate	200 µg/mL	[27]
Vitamin C (Vit C)	200 µg/mL	[32]
B. longum CBi0703 + Vitamin C (Vit C)	200 µg/mL + 200 µg/mL	
Collagen (Col)	1 mg/mL	[28]
B. longum CBi0703 + Collagen (Col)	200 µg/mL + 1 mg/mL	
Chondroitin sulphate (CS)	1 mg/mL	[29]
B. longum CBi0703 + Chondroitin sulphate (CS)	200 µg/mL + 1 mg/mL	
Glucosamine sulphate (GS)	1 mg/mL	[30]
B. longum CBi0703 + Glucosamine sulphate (GS)	200 µg/mL + 1 mg/mL	
B. longum CBi0703 + combination of chicken hydrolyzed collagen type II, chondroitin sulphate and hyaluronic acid (CM)	200 µg/mL + 1 mg/mL	
B. longum CBi0703 + Egg-shell membrane (NEM)	200 µg/mL + 100 µg/mL	[31]

Table 1. Ingredients evaluated in osteoarthritis in vitro model.

SW1353 cells were seeded 1×10^4 cells/well in 96-well plates to reach approximately 70–80% confluence at the time of H_2O_2 exposure and subsequent treatment with the different combinations, in order to ensure assay sensitivity and reproducibility and to avoid potential artefacts associated with over- or under-confluent cultures. To induce oxidative stress mimicking the OA environment, cells were treated with 50 µM hydrogen peroxide (H_2O_2) for 2 h. This concentration and duration have previously been demonstrated to result in concentration-dependent cytotoxicity and to trigger apoptosis and oxidative damage in SW1353 cells, consistent with chondrocyte behaviour under OA pathophysiological stress[33]. Then, cells were rinsed with PBS and treated for 22 h with the combinations as shown in Table 1. This H_2O_2-based model induces acute oxidative stress and apoptosis, capturing key aspects of OA-related chondrocyte damage[26].

Neutral red viability assay

Cell viability was assessed using the Neutral Red uptake assay. After H_2O_2 exposure and 22-h treatment, cells were incubated with Neutral Red solution (final concentration 50 µg/mL) for 3 h at 37°C[34]. The cells were then washed, and the incorporated dye was extracted with a decolorization solution (ethanol:glacial acetic acid:water, 50:1:49). Absorbance was measured at 540 nm using a microplate spectrophotometer (Infinite 200 PRO, Tecan Group Ltd., Männedorf, Switzerland). Dimethyl sulfoxide (DMSO, 50%) was used only as a cytotoxic positive control. The OA-induced condition (H_2O_2-treated cells without added treatments) served as the reference for evaluating treatment effects. For all assays, a minimum of three independent biological experiments were performed, with each condition measured in technical triplicate.

Cell proliferation assay

Cell proliferation was determined by measuring of the incorporation 5-Bromo-2'-deoxyuridine (BrdU), a synthetic thymidine analogue, into newly synthesized DNA of proliferating cells[35] using a colorimetric ELISA assay according to the manufacturer's instructions (BrdU ELISA kit, Abcam, UK). BrdU was added to the culture medium at a final concentration of 10 µM for the last 4 h of the 22-h treatment period. Cells were then fixed, and DNA was denatured. Incorporated BrdU was detected using a peroxidase-conjugated anti-BrdU antibody and a colorimetric substrate. Absorbance was measured at 450 nm using a microplate spectrophotometer (Infinite 200 PRO, Tecan).

Annexin V-FITC / propidium iodide (PI) apoptosis assay

Apoptosis was evaluated by flow cytometry using an Annexin V-FITC Apoptosis Detection Kit (Annexin V-FITC Kit, Miltenyi Biotec, Germany)[36]. Annexin V staining is primarily used to capture early apoptotic events, and the combined use of Annexin V with PI enables the identification of late apoptotic or necrotic cells. After treatments, cells were harvested, washed with cold phosphate-buffered saline (PBS), and resuspended in binding buffer. Cells were stained with Annexin V-FITC (5 µL per 100 µL cell suspension) and propidium iodide (PI, final concentration 10 µg/mL) for 15 min at room temperature in the dark. Data were acquired on a CytoFLEX flow cytometer (Beckman Coulter Life Sciences, IN, USA), collecting at least 10,000 events per sample acquired and analyzed using CytExpert software (v2.4) (Beckman Coulter Life Sciences). Early apoptotic cells were defined as Annexin V-positive/PI-negative (Annexin V^+/PI^-), and late apoptotic/necrotic cells as Annexin V-positive/PI-positive (Annexin V^+/PI^+)[37].

Caspase activity

Caspase activation was determined using a fluorogenic Apostat Intracellular Caspase Detection Kit (Catalog # 60191, Bio-Techne/R&D Systems, MN, USA)[38]. After treatments, cells were harvested, resuspended in assay buffer, and incubated with the Apostat reagent (1 × final concentration) for 30 min at 37 °C in the dark. After washing, cells were analyzed by flow cytometry on a CytoFLEX (Beckman Coulter Life Sciences). Caspase-positive cells were quantified based on fluorescence intensity compared to controls.

RNA isolation and RT-qPCR

Total RNA was extracted using the RNeasy Mini Kit (Catalog # 74,104, Qiagen, Hilden, Germany). RNA concentration and purity were assessed by spectrophotometry. Subsequently, 1 µg of total RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Catalog # 4,368,814, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Quantitative PCR was performed on a LightCycler 480 system (Roche Diagnostics GmbH, Basel, Switzerland) using the LightCycler 480 SYBR Green I Master mix (Roche Diagnostics GmbH). The cycling protocol consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Melt curve analysis was performed to verify amplicon specificity. GAPDH was used as the housekeeping gene for normalization. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method[39] with the OA-induced vehicle group as the calibrator. Three independent biological experiments were performed, with each sample analysed in technical triplicate. Primer sequences are listed in Table 2.

The primers used for the different genes were obtained from Biomers.net (Ulm, Germany).

Statistical analyses

For each determination, an initial exploration was carried out to rule out discrepant points within the groups using the Grubbs statistical test in GraphPad Prism 9 (GraphPad Software, Inc., La Jolla, CA, USA). For the Neutral Red viability assay and the BrdU proliferation assay, each experimental condition was evaluated in at least three independent biological experiments, with each condition measured in triplicate wells (technical replicates) per experiment. For flow cytometry analyses (Annexin V/PI and Apostat), a minimum of three independent biological experiments were performed, and for each experiment a single measurement per condition was acquired from a pooled cell suspension. For RT-qPCR, three independent biological experiments were carried out, and each sample was analysed in technical triplicate reactions for every target gene. All data are expressed as means ± standard error of the mean (SEM). For comparisons of multiple treatments, to reduce the risk of Type I error associated with repeated *t*-tests, statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test for multiple comparisons against the OA-induced vehicle group. This approach ensures rigorous control over familywise error rates while identifying significant differences between experimental groups. A probability level of $p < 0.05$ was defined as statistically significant and a probability below 0.1 was defined as trending toward significance.

Results

Evaluation of the properties of ingredient combinations on chondrocytes cartilage cell viability and proliferation

In the viability assay, none of the treatments reduced viability below 80% compared to the OA-induced vehicle (Fig. 1A). In OA conditions, human SW1353 chondrocytes treated with Col, *B. longum* CBi0703 and combinations of *B. longum* CBi0703 with Col, CM and NEM, promoted cell proliferation compared with the OA vehicle (Fig. 1B).

Evaluation of the properties of ingredient combinations on chondrocytes apoptosis

Caspase activation, a general marker of apoptotic signalling, was significantly reduced by treatment with GS and the combination of *B. longum* CBi0703 with GS compared to the OA vehicle (Fig. 2A). Annexin V/PI flow cytometry was used to distinguish early and late apoptotic stages. The percentage of early apoptotic (Annexin V^+/PI^-) cells was reduced by single treatment with *B. longum* CBi0703 or Col (Fig. 2B). The combination of *B. longum* CBi0703 with NEM increased early apoptosis. The percentage of late apoptotic (Annexin V^+/PI^+) cells was significantly reduced by the combinations of *B. longum* CBi0703 with GS and NEM (Fig. 2C).

Evaluation of *B. longum* CBi0703 and combinations on chondrogenesis-related gene expression

Expression of the dedifferentiation marker *COL1A1* was elevated in OA-induced cells, particularly after Vit C or GS treatment (Fig. 3A). This was counteracted by the combination of *B. longum* CBi0703 with Vit C. Expression

Target Gene	Forward sequence	Reverse sequence
COL1A1	CTCCTCGCTTTCCTTCCTCT	AATGGTGCTCCTGGTATTGC
COL2A1	CAACACTGCCAACGTCCAGAT	CTGCTTCGTCCAGATAGGCAAT
COL10A1	GCTTCAGGGAGTGCCATCA	CGGCATTGGGAAGCTGG
SOX9	ACACACAGCTCACTCGACCTTG	GGAATTCTGGTTGGTCCTCTCTT
AGC1	ACTTCCGCTGGTCAGATGGA	TCTCGTGCCAGATCATCACC
TIMP1	AGATAGCCTGAATCCTGCC	CTGGGTGGTAACTCTTTATTTC
ECM1	GTAACAGCCACCAGACAAGC	TCTGTCCTGTAGCCGTGAAG
MMP1	CTGTTCAGGGACAGAATGTGCT	TCGATATGCTTCACAGTTCTAGGG
MMP13	TCCTCTTCTTGAGCTGGACTCATT	CGCTCTGCAAACTGGAGGTC
GBL1	ATACTGGCTGGCTAGATCACTG	GGCAAAATTGGTCCCACCTATAA
GAPDH	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT

Table 2. Primer sequences for this study.

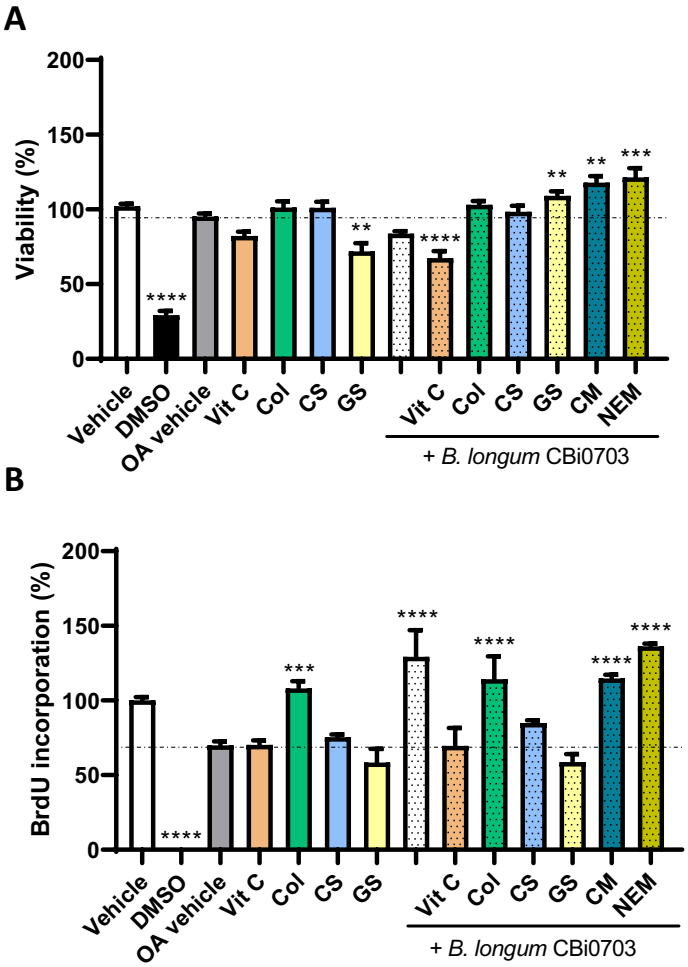

Fig. 1. Effects of the different treatments and combinations in SW1353 viability (**A**) and proliferation, 5-Bromo-2'-deoxyuridine (BrDU) assay (**B**) in conditions of osteoarthritis in the presence of the different treatments and combinations. Data are mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ *vs*. osteoarthritis (OA) vehicle. Statistical analyses were conducted using one-way ANOVA followed by Dunnett's post-hoc test with the OA-induced group as the reference. Abbreviations: OA, osteoarthritis; Vit C, vitamin C; Col, collagen; CS, chondroitin sulphate; GS, glucosamine; CM, Chondro Mix (Chondroitin Sulphate + glucosamine); NEM, egg-shell membrane.

of the cartilage integrity marker *COL2A1* tended to reduce in OA, but increased by combinations of *B. longum* CBi0703 with CS, CM, and NEM (Fig. 3B). The hypertrophic marker *COL10A1* was downregulated by GS and the combination of *B. longum* CBi0703 with GS (Fig. 3C). Expression of the chondrogenic transcription factor *SOX9* was reduced in OA, an effect prevented by *B. longum* CBi0703 alone and its combination with Vit C (Fig. 3D).

Evaluation of *B. longum* CBi0703 and combinations on catabolic gene expression

Expression of *ECM1* was increased in OA-induced cells, particularly after Vit C or GS treatment, a trend attenuated by the combination of *B. longum* CBi0703 with Vit C (Fig. 4A). *MMP1* expression was elevated by Vit C, GS, and the combinations of *B. longum* CBi0703 with Col or GS, but reduced by CS treatment (Fig. 4B). *MMP13* expression was increased in OA and further elevated by GS. This was counteracted by Col, CS, *B. longum* CBi0703 alone, and its combination with NEM (Fig. 4C). *TIMP1* expression was downregulated in OA as well in human chondrocytes treated with *B. longum* CBi0703 lysate, Col and CS (Fig. 4D). Treatment with GS and *B. longum* CBi0703 in combination with Vit C, Col, GS and CM prevented this process, upregulating the *TIMP1* expression.

Evaluation of *B. longum* CBi0703 on markers of cartilage integrity and articular senescence

Expression of the cartilage matrix component *AGC1* was reduced in OA-induced cells, particularly after Vit C or GS treatment (Fig. 5A). This reduction was counteracted by Col, CS, and *B. longum* CBi0703 alone. Expression of *GBL1* (β-galactosidase), a marker associated with cellular senescence, was increased in OA. This increase was attenuated by Col, CS, *B. longum* CBi0703 alone, and its combinations with Vit C, Col, CS, and CM (Fig. 5B).

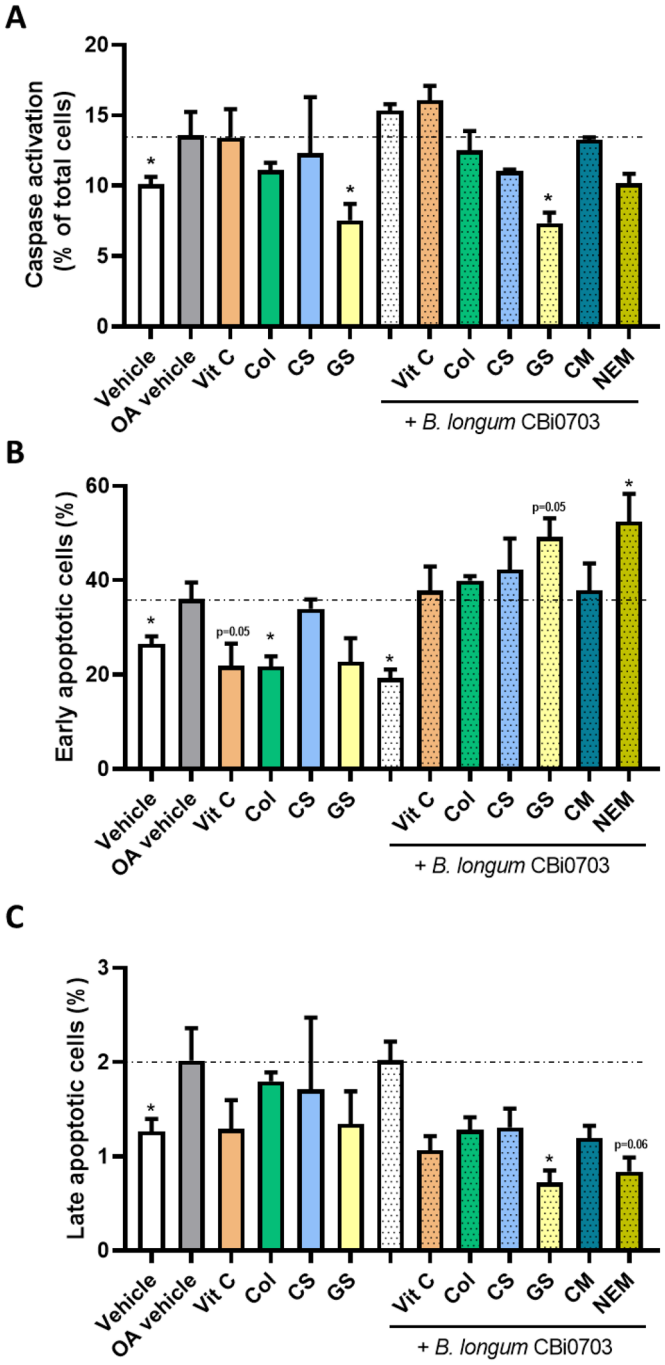

Fig. 2. Evaluation of caspase activation (**A**) and the percentage of early (**B**) and late apoptotic (**C**) chondrocytes in conditions of osteoarthritis in the presence of the different treatments. Data are mean ± SEM. $^{*}p < 0.05$ *vs*. osteoarthritis (OA) vehicle. Statistical analyses were conducted using one-way ANOVA followed by Dunnett's post-hoc test with the OA-induced group as the reference. Abbreviations: OA, osteoarthritic; Vit C, vitamin C; Col, collagen; CS, chondroitin sulphate; GS, glucosamine; CM, Chondro Mix (Chondroitin Sulphate + glucosamine); NEM, egg-shell membrane.

Discussion

The in vitro model employed in this study is based on a 2-hour exposure of SW1353 chondrocytes to H_2O_2, followed by treatment with *B. longum* CBi0703 and nutraceuticals, and is therefore focused on acute oxidative stress and apoptosis as key components of OA-related damage. While this approach is useful to dissect cytoprotective and molecular responses in a controlled setting, it does not fully recapitulate the multifactorial pathophysiology of osteoarthritis, which also involves chronic low-grade inflammation, altered mechanobiology, synovial and subchondral bone remodeling and interactions among multiple joint tissues. Consequently, our findings should be interpreted as mechanistic insights obtained in an OA-like oxidative stress model rather

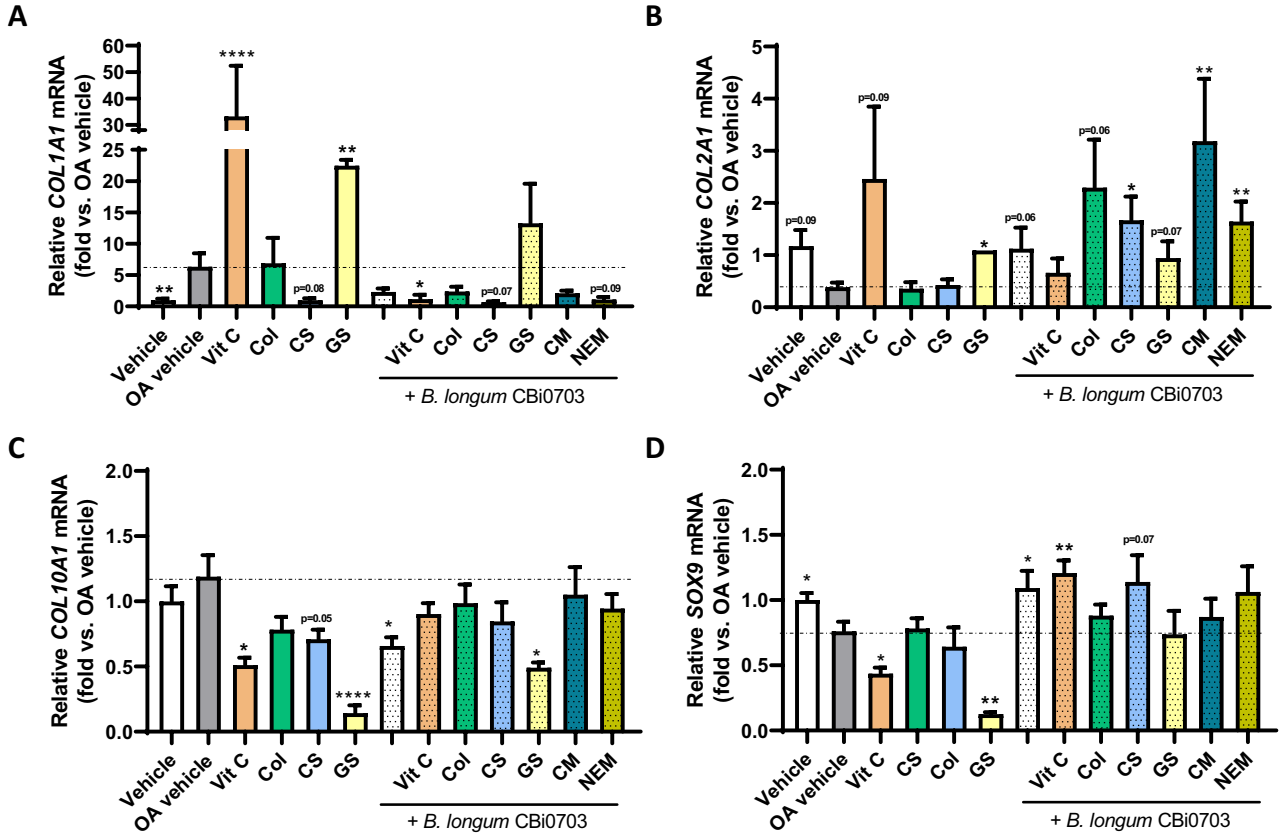

Fig. 3. Evaluation of chondrocyte *COL1A1* (**A**); *COL2A1* (**B**); *COL10A1* (**C**) and *SOX9* (**D**) gene expression in conditions of osteoarthritis in the presence of the different treatments and combinations. Data are mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$ *vs.* osteoarthritis (OA) vehicle. Statistical analyses were conducted using one-way ANOVA followed by Dunnett's post-hoc test with the OA-induced group as the reference. Abbreviations: OA, osteoarthritic; Vit C, vitamin C; Col, collagen; CS, chondroitin sulphate; GS, glucosamine; CM, Chondro Mix (Chondroitin Sulphate + glucosamine); NEM, egg-shell membrane.

than as a comprehensive representation of disease biology, and future studies in more complex in vitro systems, animal models and clinical settings will be necessary to validate and extend these observations. The results of this study provide crucial insights into the potential role of *B. longum* CBi0703 as an innovative therapeutic approach for OA. By focusing on the prevention of cartilage catabolism, this strain presents a unique mechanism of action compared to current available options, which predominantly target anabolic processes. The present results, obtained in an H_2O_2 induced OA like in vitro model, suggest that *B. longum* CBi0703 may complement existing OA strategies by targeting catabolic pathways, but confirmation in appropriate animal models and randomized clinical trials is still required. In this study, we quantified the mRNA expression of *MMP1*[40]and *MMP13*[41] as key catabolic enzymes, together with *AGC1* as a marker related to cartilage matrix integrity[42]. These results indicate that specific treatments modulate *MMP1* and *MMP13* expression and help preserve *AGC1* levels under OA like oxidative stress, which is compatible with a more favorable balance of matrix turnover. However, aggrecanases were not directly measured in our experiments, and any involvement of these enzymes in the observed effects remains hypothetical and should be addressed in future studies.

Furthermore, *B. longum* CBi0703 alone and in combination with certain nutraceuticals enhanced chondrocyte proliferation and reduced markers of apoptosis and senescence. For example, the combination with vitamin C upregulated *SOX9* and *TIMP1* while downregulating *COL1A1* and *ECM1*; the combination with chondroitin sulphate increased *COL2A1* expression; and the combination with glucosamine sulphate reduced late apoptosis. This suggests that the postbiotic may boost the chondroprotective action of established OA supplements[43]. The modulation of catabolic markers (*MMP1*, *MMP13*, *ECM1*) and anabolic or matrix-integrity markers (*AGC1*, *TIMP1*) suggest a potential protective effect of specific treatments on cartilage matrix homeostasis under OA-like oxidative stress; however, these data are based on mRNA expression and should be interpreted as mechanistic rather than direct evidence of reduced cartilage degradation. *B. longum* CBi0703 may potentially complement the anabolic effects of glucosamine and chondroitin sulphate, given the observed in vitro effects on catabolic markers and apoptosis, but this requires confirmation in appropriate animal models and clinical trials. We explicitly note that functional joint outcomes and symptom relief were not assessed in this study. While these in vitro findings provide valuable mechanistic insights, they may not fully capture the complexity of biological interactions in vivo. Following oral intake, digestion and metabolism may alter the compounds'

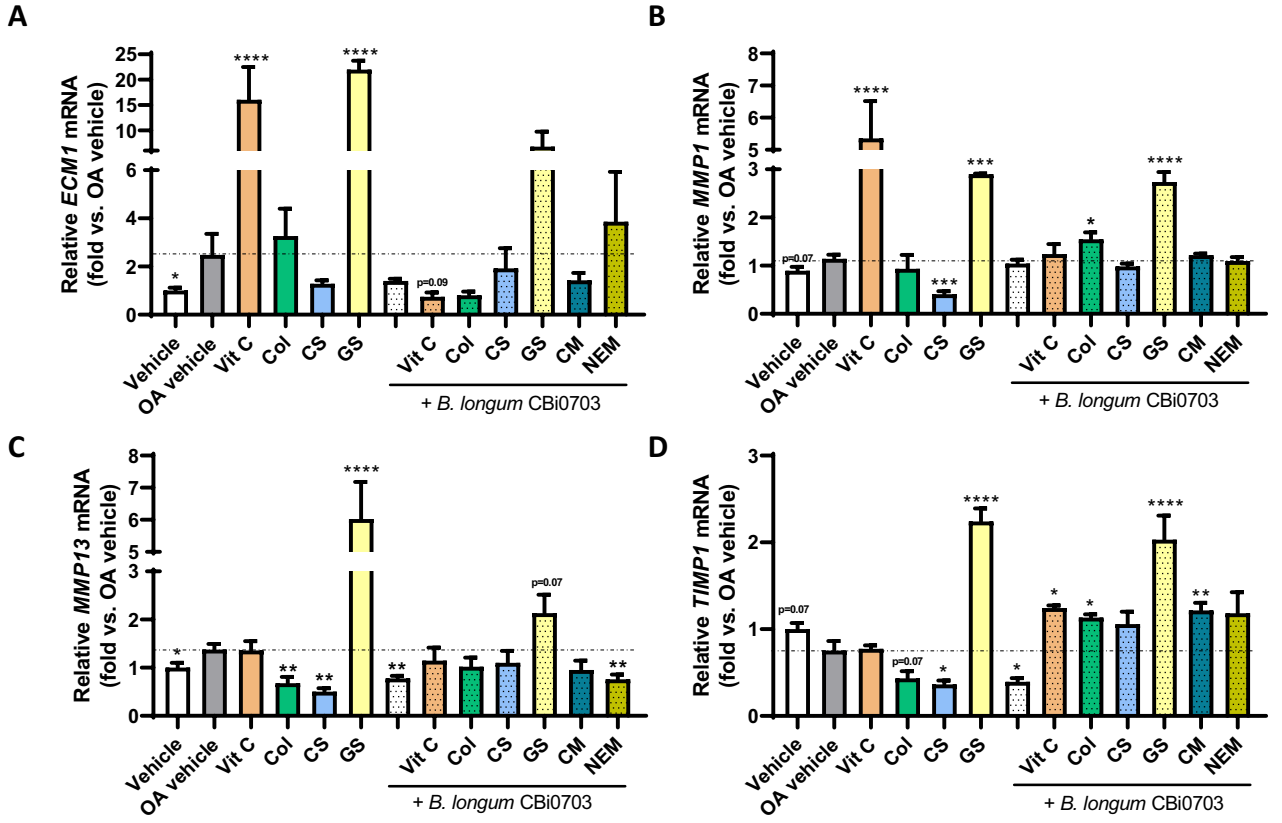

Fig. 4. Evaluation of *ECM1* (**A**); *MMP1* (**B**); *MMP13* (**C**) and *TIMP1* (**D**) gene expression in conditions of osteoarthritis in the presence of the different treatments and combinations. Data are mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ *vs.* osteoarthritis (OA) vehicle. Statistical analyses were conducted using one-way ANOVA followed by Dunnett's post-hoc test with the OA-induced group as the reference. Abbreviations: OA, osteoarthritic; Vit C, vitamin C; Col, collagen; CS, chondroitin sulphate; GS, glucosamine; CM, Chondro Mix (Chondroitin Sulphate + glucosamine); NEM, egg-shell membrane.

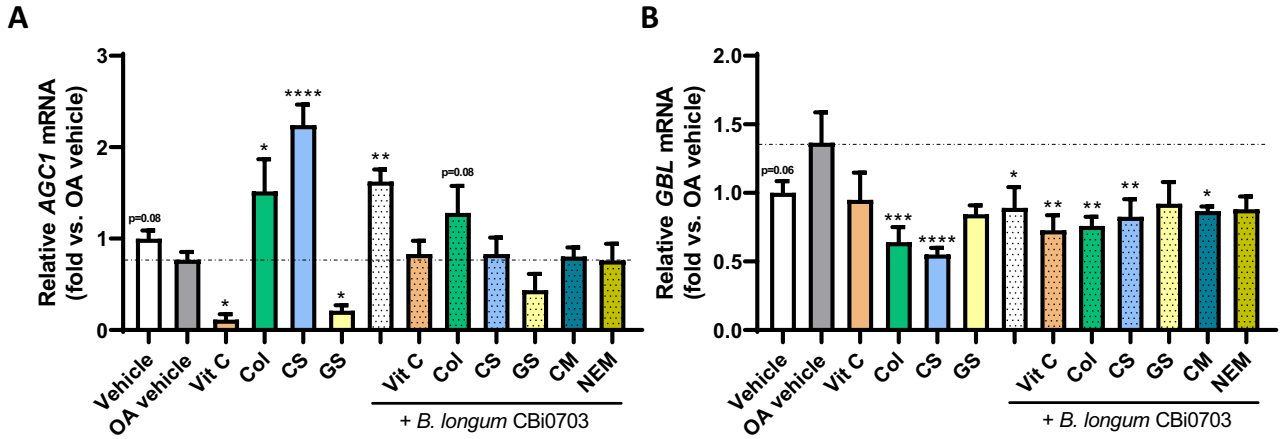

Fig. 5. Evaluation of *AGC1* (**A**) and *GBL* (**B**) gene expression in conditions of osteoarthritis in the presence of the different treatments and combinations. Data are mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ *vs.* osteoarthritis (OA) vehicle. Statistical analyses were conducted using one-way ANOVA followed by Dunnett's post-hoc test with the OA-induced group as the reference. Abbreviations: OA, osteoarthritic; Vit C, vitamin C; Col, collagen; CS, chondroitin sulphate; GS, glucosamine; CM, Chondro Mix (Chondroitin Sulphate + glucosamine); NEM, egg-shell membrane.

bioavailability and activity at target tissues[44]. Nevertheless, the observed protective effects across the in vitro model support their potential relevance for human health. Indeed, numerous nutraceutical ingredients are first studied through these approaches to screen for bioactive effects[45–48]. Future studies should incorporate simulated digestion, hepatic metabolism, and other models that better approximate physiological conditions[49]. Finally, it should be emphasized that the present study evaluated only mRNA expression levels of key chondrocyte metabolism related genes, together with functional readouts of viability, proliferation and apoptosis, and did not include protein level measurements or matrix staining. As a result, the observed changes should be interpreted as mechanistic evidence rather than direct proof of altered cartilage degradation at the tissue level. The observed changes in proliferation and gene expression are compatible with a chondroprotective profile, but our in vitro data do not directly demonstrate reduced cartilage degradation at the tissue level, and protein-level as well as matrix-deposition analyses will be required to confirm these effects. The in vitro model employed in this study is based on oxidative stress and does not fully recapitulate the multifactorial pathophysiology of OA, which involves chronic inflammation and tissue remodeling[50]. The use of the SW1353 chondrosarcoma cell line, while practical, cannot fully replicate primary chondrocyte biology. The long-term efficacy and sustainability of *B. longum* CBi0703's effects on joint health remain unknown, requiring extended studies to evaluate its impact over time. While synergistic effects with market leaders are hypothesized, potential interactions between *B. longum* CBi0703 and other OA treatments require careful investigation. Preclinical models of OA can provide insights into the in vivo efficacy and safety of *B. longum* CBi0703[51]. These studies should focus on cartilage preservation, inflammation reduction, and gut microbiota modulation. Ultimately, randomized controlled trials will be essential to evaluate the therapeutic potential of *B. longum* CBi0703 in OA patients and identify optimal combinations for enhanced efficacy.

Conclusions

The findings of this study underscore the potential of *B. longum* CBi0703 as an innovative and complementary approach to OA management and validate previous research conducted with this strain. By focusing on reducing cartilage catabolism, *B. longum* CBi0703 offers a unique mechanism of action that complements the anabolic effects of current nutraceuticals approaches. These in vitro results provide a mechanistic rationale for the observed in vivo and clinical effects of *B. longum* CBi0703 summarizing the main effects of each treatment. Supplementary Table S1 summarizes the main effects of each ingredient and combination on cell viability, proliferation, apoptosis and key cartilage-related genes in the OA-like in vitro model. Future studies should incorporate protein-level analyses, primary chondrocytes, and more complex in vivo models are warranted to confirm these findings and further establish its translational relevance for OA management.

Data availability

The data presented in this study are available on request from the corresponding author.

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References

1. Chen, D. et al. Osteoarthritis: toward a comprehensive understanding of pathological mechanism. *Bone Res* **5**, 16044 (2017).
2. Tang, S. et al. *Osteoarthritis. Nat Rev Dis Primers* **11**, 10 (2025).
3. Grässel, S. & Muschter, D. Recent advances in the treatment of osteoarthritis. *F1000Res* 9, (2020).
4. Maqbool, M. *et al.* An up to date on clinical prospects and management of osteoarthritis. *Ann Med Surg (Lond)* 72, 103077 (2021).
5. Richard, M. J., Driban, J. B. & McAlindon, T. E. Pharmaceutical treatment of osteoarthritis. *Osteoarthritis Cartilage* **31**, 458–466 (2023).
6. Lee, J. et al. Topical nonsteroidal anti-inflammatory drugs for management of osteoarthritis pain: A consensus recommendation. *J. Orthop. Res.* **41**, 1916–1924 (2023).
7. Bindu, S., Mazumder, S. & Bandyopadhyay, U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochem Pharmacol* **180**, 114147 (2020).
8. Rodriguez-Merchan, E. C. The Current Role of Disease-modifying Osteoarthritis Drugs. *Arch Bone Jt Surg* **11**, 11–22 (2023).
9. Smakaj, A. *et al.* Exploring Intra-Articular Administration of Monoclonal Antibodies as a Novel Approach to Osteoarthritis Treatment: A Systematic Review. *Biomedicines* 12, (2024).
10. Tiku, M. L. & Sabaawy, H. E. Cartilage regeneration for treatment of osteoarthritis: a paradigm for nonsurgical intervention. *Ther Adv Musculoskelet Dis* **7**, 76–87 (2015).
11. Jerosch, J. Effects of Glucosamine and Chondroitin Sulfate on Cartilage Metabolism in OA: Outlook on Other Nutrient Partners Especially Omega-3 Fatty Acids. *Int J Rheumatol* **2011**, 969012 (2011).
12. Ameye, L. G. & Chee, W. S. S. Osteoarthritis and nutrition. From nutraceuticals to functional foods: a systematic review of the scientific evidence. *Arthritis Res Ther* 8, R127 (2006).
13. DePhillipo, N. N. et al. Efficacy of Vitamin C Supplementation on Collagen Synthesis and Oxidative Stress After Musculoskeletal Injuries: A Systematic Review. *Orthop J Sports Med* **6**, 2325967118804544 (2018).
14. Sodhi, H. & Panitch, A. Glycosaminoglycans in Tissue Engineering: A Review. *Biomolecules* **11**, 29 (2020).
15. Baden, K. E. R. *et al.* The Safety and Efficacy of Glucosamine and/or Chondroitin in Humans: A Systematic Review. *Nutrients* 17, (2025).
16. Park, S. et al. Efficacy of natural eggshell membrane for knee osteoarthritis: A randomized, double-blind, placebo-controlled clinical trial. *J Funct Foods* **121**, 106449 (2024).
17. Altman, R. D., Manjoo, A., Fierlinger, A., Niazi, F. & Nicholls, M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: a systematic review. *BMC Musculoskelet Disord* **16**, 321 (2015).
18. Dong, J. et al. Immunomodulatory effects of the Bifidobacterium longum BL-10 on lipopolysaccharide-induced intestinal mucosal immune injury. *Front Immunol* **13**, 947755 (2022).
19. Yao, S., Zhao, Z., Wang, W. & Liu, X. Bifidobacterium Longum: Protection against Inflammatory Bowel Disease. *J Immunol Res* **2021**, 8030297 (2021).
20. Gavzy, S. J. et al. Bifidobacterium mechanisms of immune modulation and tolerance. *Gut Microbes* **15**, 2291164 (2023).

21. Ohland, C. L. & Macnaughton, W. K. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* **298**, G807–G819 (2010).
22. Meng, D. et al. Indole-3-lactic acid, a metabolite of tryptophan, secreted by Bifidobacterium longum subspecies infantis is anti-inflammatory in the immature intestine. *Pediatr Res* **88**, 209–217 (2020).
23. Henrotin, Y., Patrier, S., Pralus, A., Roche, M. & Nivoliez, A. Protective Actions of Oral Administration of Bifidobacterium longum CBi0703 in Spontaneous Osteoarthritis in Dunkin Hartley Guinea Pig Model. *Cartilage* **13**, 1204S-1213S (2021).
24. Adami. Studio osservazionale prospettico sull'utilizzo di Bifidobacterium longum CBi0703® e vitamina C in pazienti con gonartrosi e coxartrosi. *Giornale Italiano di Ortopedia e Traumatologia* 49, 195–202 (2023).
25. Gebauer, M. et al. Comparison of the chondrosarcoma cell line SW1353 with primary human adult articular chondrocytes with regard to their gene expression profile and reactivity to IL-1beta. *Osteoarthritis Cartilage* **13**, 697–708 (2005).
26. Pang, K. L. et al. Establishing sw1353 chondrocytes as a cellular model of chondrolysis. *Life* **11**, 1–17 (2021).
27. Ikeda, K. et al. Effects of heat stimulation and l-ascorbic acid 2-phosphate supplementation on myogenic differentiation of artificial skeletal muscle tissue constructs. *J Tissue Eng Regen Med* **11**, 1322–1331 (2017).
28. Honvo, G., Lengelé, L., Charles, A., Reginster, J.-Y. & Bruyère, O. Role of Collagen Derivatives in Osteoarthritis and Cartilage Repair: A Systematic Scoping Review With Evidence Mapping. *Rheumatol Ther* **7**, 703–740 (2020).
29. Tat, S. K., Pelletier, J.-P., Mineau, F., Duval, N. & Martel-Pelletier, J. Variable effects of 3 different chondroitin sulfate compounds on human osteoarthritic cartilage/chondrocytes: relevance of purity and production process. *J Rheumatol* **37**, 656–664 (2010).
30. Mattiuzzo, E. *et al.* In Vitro Effects of Low Doses of β-Caryophyllene, Ascorbic Acid and d-Glucosamine on Human Chondrocyte Viability and Inflammation. *Pharmaceuticals (Basel)* 14, (2021).
31. Ruff, K. J., Endres, J. R., Clewell, A. E., Szabo, J. R. & Schauss, A. G. Safety evaluation of a natural eggshell membrane-derived product. *Food Chem Toxicol* **50**, 604–611 (2012).
32. Lindsey, R. C., Cheng, S. & Mohan, S. Vitamin C effects on 5-hydroxymethylcytosine and gene expression in osteoblasts and chondrocytes: Potential involvement of PHD2. *PLoS ONE* **14**, e0220653 (2019).
33. Pang, K.-L. *et al.* Establishing SW1353 Chondrocytes as a Cellular Model of Chondrolysis. *Life (Basel)* 11, (2021).
34. Repetto, G., del Peso, A. & Zurita, J. L. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nat Protoc* **3**, 1125–1131 (2008).
35. Flomerfelt, F. A. & Gress, R. E. Analysis of Cell Proliferation and Homeostasis Using EdU Labeling. *Methods Mol Biol* **1323**, 211–220 (2016).
36. Telford, W. G. Multiparametric Analysis of Apoptosis by Flow Cytometry. *Methods Mol Biol* **1678**, 167–202 (2018).
37. Kay, J. G. & Grinstein, S. *Sensing phosphatidylserine in cellular membranes. Sensors (Basel)* **11**, 1744–1755 (2011).
38. Köhler, C., Orrenius, S. & Zhivotovsky, B. Evaluation of caspase activity in apoptotic cells. *J Immunol Methods* **265**, 97–110 (2002).
39. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–408 (2001).
40. Blasioli, D. J. & Kaplan, D. L. The roles of catabolic factors in the development of osteoarthritis. *Tissue Eng Part B Rev* **20**, 355–363 (2014).
41. Hu, Q. & Ecker, M. Overview of MMP-13 as a Promising Target for the Treatment of Osteoarthritis. *Int J Mol Sci* 22, (2021).
42. Smith, M. M. & Melrose, J. COMP Is a Biomarker of Cartilage Destruction, Extracellular Matrix and Vascular Remodeling and Tissue Repair. *Int J Mol Sci* **26**, 9182 (2025).
43. Gallagher, B. et al. Chondroprotection and the prevention of osteoarthritis progression of the knee: a systematic review of treatment agents. *Am J Sports Med* **43**, 734–744 (2015).
44. Rein, M. J. et al. Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. *Br J Clin Pharmacol* **75**, 588–602 (2013).
45. Avci, G. A., Yilmaz, Ü. İ. & Avci, E. Efficacy of probiotics, paraprobiotics, and postbiotics in colorectal cancer cell line and their role in immune response. *Rev Assoc Med Bras (1992)* 70, e20240226 (2024).
46. Prajapati, N. et al. Postbiotic production: harnessing the power of microbial metabolites for health applications. *Front Microbiol* **14**, 1306192 (2023).
47. Forero-Doria, O. et al. An In Vitro and In Silico Study of Antioxidant Properties of Curcuminoid N-alkylpyridinium Salts: Initial Assessment of Their Antitumoral Properties. *Antioxidants* **11**, 1104 (2022).
48. Allam-Ndoul, B., Guénard, F., Barbier, O. & Vohl, M.-C. Effect of different concentrations of omega-3 fatty acids on stimulated THP-1 macrophages. *Genes Nutr* **12**, 7 (2017).
49. Sensoy, I. A review on the food digestion in the digestive tract and the used in vitro models. *Curr Res Food Sci* **4**, 308–319 (2021).
50. Jenei-Lanzl, Z. et al. Emerging concepts and challenges in the development of disease-modifying osteoarthritis drugs – a more refined perspective. *Arch Pharm Res* **48**, 467–494 (2025).
51. Malfait, A.-M. & Little, C. B. On the predictive utility of animal models of osteoarthritis. *Arthritis Res Ther* **17**, 225 (2015).

Author contributions

Conceptualization, A.M.-C.; L.C-M.; M.T.; J.C.-C., C.d.L.; R.M.-C.; and X.E.; methodology, investigation, and data curation, A.M.-C.; J.B.-T.; C.D.-C; J.R.-G.; and Y.T.; writing, review and editing, L.C-M.; M.T.; C.d.L.; R.M.-C and X.E. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

L.C.-M.; M.A.-F.; M.T.; J.C.-C.; and C.d.L. are members of ABbiotek Health. The remaining authors declare no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Additional information

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