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Comprehensive in vitro and in vivo investigations of the therapeutic potential of Jeju lava seawater salt in osteoarthritis

Mangeun Kim¹, Min Hye Kim², Jinho Kim³, Kyungpil Kang³, Junsu Lee³, Mrinmoy Ghosh^{2,4} and Young-Ok Son^{1,2,5,6*} 

Abstract

Salts play a crucial role in maintaining human health by regulating fluid levels and supporting various physiological processes. However, conventional seawater-derived salts are associated with microplastic pollution and pose potential health risks. Jeju lava seawater (JLS), sourced exclusively from Jeju Island, has emerged as a unique alternative, free of microplastics and enriched with essential minerals such as magnesium, calcium, zinc, and iron. In this study, we investigated the effects of JLS on osteoarthritis (OA) pathogenesis, focusing on chondrocyte metabolism and OA development. We performed surgical destabilization of the medial meniscus to establish a murine model of OA. We examined the expression of catabolic and anabolic factors in JLS-treated chondrocytes. Our cell viability assay revealed that JLS treatment was not cytotoxic to chondrocytes at concentrations $\leq 0.5\%$. Additionally, JLS treatment resulted in a concentration-dependent increase in the expression of anabolic factors like aggrecan, SOX9, and COL2A1 while decreasing the expression of catabolic factors such as MMP3, MMP13, ADAMTS4, and ADAMTS5 in the chondrocytes stimulated with pro-inflammatory cytokines. Although not statistically significant compared to the control group, JLS intake slightly attenuated the OARSI score, osteophyte score, synovitis score, subchondral bone thickness, and osteophyte size in the mouse model of OA. Conclusively, these results suggest that JLS ameliorates OA by positively influencing chondrocyte metabolism, making it a promising therapeutic candidate for OA management.

Keywords Jeju lava seawater Salt, Osteoarthritis, Chondrocytes

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Introduction

Arthritis, associated with severe joint pain, has no effective treatment strategy. Natural remedies such as salt therapy have shown potential for treating arthritis and joint pain [1]. Salt therapy targets inflammation, the primary cause of pain, by introducing natural salt into the joints and providing calcium for enhancing bone strength. Importantly, the natural and organic salts used in this therapy not only relieve pain but also contribute to overall mental and physical well-being, without possessing side effects [2].

Salt therapy penetrates deep into the cells, allowing negative ions and properties to be inhaled or absorbed through the skin. These elements specifically target the inflamed joints and dehydrate the cells responsible for inflammation at the molecular level. Unlike prescription medications, which often simply mask pain, salt therapy directly addresses the underlying inflammation, leading to consistent improvements with regular sessions [3]. In addition to relieving arthritic pain, salt therapy promotes bone health by enhancing calcium deposition [4]. These dual benefits position salt therapy as a preferred alternative to prescription medications. The simplicity and flexibility of salt therapy sessions make them accessible and safer than prescription drugs, which possess potential side effects. Additionally, salt therapy

offers various health benefits, including skin cleansing, stress reduction, enhanced sports performance, and an improved immune system. Moreover, the positive effects of salt therapy are beneficial in osteoarthritis (OA), rheumatoid arthritis, asthma, and chronic obstructive pulmonary disease, leveraging its natural properties for effective pain and inflammation relief [3].

Traditionally, salt is derived from common seawater; however, it may be contaminated with microplastics, which are minute particles smaller than 5 mm in size. Recent studies have highlighted the presence of microplastics in dietary salts, raising concerns about human exposure to these particles [5]. On absorption in the human body, microplastics pose various detrimental health effects, including oxidative stress, cellular damage, inflammation, immune reactions, and neurotoxic effects [6]. Therefore, addressing and mitigating potential health implications associated with the presence of microplastics in salt sources is crucial.

Jeju lava seawater (JLS), a unique resource found exclusively on Jeju Island, undergoes specialized filtration as it traverses volcanic rock masses [7]. This unique filtration ensures the absence of microplastics, bestowing JLS salts with obvious advantages over conventional seawater salts. Additionally, the JLS undergoes meticulous sterilization and contains essential functional

Table 1 List of primers used for RT-PCR

Primer	Sequence	Tm value (°C)	Length (bp)
Mo GAPDH forward	TCAGTCCACCCAGAAGAC	58	450
Mo GAPDH reverse	TGTAGGCCATGAGGTCCAC		
Mo SOX9 forward	ATGCTATCTTCAAGGCGCTG	60	272
Mo SOX9 reverse	GACGTCTGAAGGTCTCAATGT		
Mo COL2A1 forward	CACACTGGTAAGTGGGCAAGACCG	58	173
Mo COL2A1 reverse	GGATTGTGTTGTTTCAGGGTTCGGG		
Mo aggrecan forward	GAAGACGACATCACCATCCAG	60	581
Mo aggrecan reverse	CTGTCTTTGTCACCCACACAT		
Mo MMP3 forward	AGGGATGATGATGCTGGTATGG	58	434
Mo MMP3 reverse	CCATGTTCTCCAAGTCAAAGG		
Mo MMP13 forward	TGATGGACCTTCTGGTCTTCTGG	58	474
Mo MMP13 reverse	CATCCACATGGTTGGGAAGTTCT		
Mo ADMTs4 forward	CATCCGAAACCTGTCAACTTG	58	287
Mo ADMTs4 reverse	GCCCATCATCTTCCACAATAGC		
Mo ADMTs5 forward	GCCATTGTAATAACCTGCACC	58	292
Mo ADMTs5 reverse	TCAGTCCCATCCGTAACCTTTG		
Mo MMP9 forward	TGCACTGGGCTTAGATCATTC	58	450
Mo MMP9 reverse	CCGTCTTGAAGAAATGCAGAG		
Mo Col10a1 forward	GCCAGGTCTCAATGGTCCTA	58	484
Mo Col10a1 reverse	AAAAGCAGACACGGGCATAC		
Mo Runx2 forward	GCCACCTTTACCTACACCCC	60	117
Mo Runx2 reverse	ACTCTGGCTTGGGAAGAGC		

minerals such as magnesium and calcium, as well as less common minerals like zinc, iron, and vanadium [8]. Notably, JLS possess anti-inflammatory properties [9], making it promising for treating inflammations. Furthermore, the application of JLS in balneotherapy has been clinically explored, particularly in the context of OA [10]. This underscores the distinctive qualities and potential therapeutic benefits of JLS.

Although the dietary intake of JLS has been linked to mineral-related benefits, such as anti-inflammatory and antioxidant effects, the specific effects of JLS on OA remain unexplored. Therefore, this study investigated the anti-inflammatory effects and mechanisms of JLS in OA using *in vitro* and *in vivo* experiments. Particularly, we compared the therapeutic effects of Korean natural sea salt, deep-ocean seawater salt, and JLS in a mouse model induced with OA via destabilization of medial meniscus (DMM). Additionally, we examined the effects of JLS on the viability of primary cultured chondrocytes.

Materials and methods

Ethical statement and preparation of salt samples

The JLS, supplied by Jeju mineral salt (Jeju-si, Jeju, South Korea), was obtained from lava seawater containing 3.5% salt. The original concentration of JLS was increased to 17% through evaporation using spray drying. Natural Korean sea salt (KS) and deep ocean seawater salt (DS) were purchased from Shinhan Corporation Co., Ltd. (Hanam, Gyeonggi Province, South Korea) and OCIAD (Goseong County, Gangwon Province, South Korea), respectively. All animal experiments were conducted in accordance with the guidelines and regulations approved by the Jeju National University Institutional Animal Care and Use Committee (approval numbers: 2020-0002, 2022-0059).

Inductively coupled plasma-mass spectrometry (ICP-MS)

Salts were prepared using a high-performance microwave digestion system (ETHOS UP; Milestone Srl, Sorisole, BG, Italy). The procedure involved melting the samples at a temperature of 80 °C for 48 h. Thereafter, the mineral contents of the samples were analyzed using the NexION®2000 ICP-MS (PerkinElmer, Shelton, Connecticut, USA). This approach ensured accurate and comprehensive determination of the mineral contents of the salts [11].

Chondrocyte culture

To obtain chondrocytes for *in vitro* culture, ICR mice (4–5 days old) were euthanized and the whole knee joints were removed and stored in phosphate-buffered saline (PBS) containing antibiotics. After thoroughly washing the knee joints with antibiotic-containing

Table 2 ICP-MS analysis of Jeju lava seawater (JLS), Korean natural sea salt (KS), and deep ocean sea water salt (DS)

	JLS	Korean natural sea salt	Deep ocean sea water salt
Ba	650 ppb	228 ppb	168 ppb
Ga	N.D	N.D	N.D
Ge	N.D	N.D	N.D
Rb	1,161 ppb	752 ppb	2,614 ppb
Li	1,745 ppb	1,519 ppb	4,196 ppb
Mo	3,717 ppb	1,104 ppb	11,825 ppb
V	2,625 ppb	2,526 ppb	2,849 ppb
B	37,581 ppb	36,827 ppb	94,031 ppb
Cs	N.D	N.D	N.D
Se	N.D	N.D	N.D
Sr	231,017 ppb	55,232 ppb	190,351 ppb
U	N.D	N.D	N.D
As	24 ppb	9 ppb	48 ppb
Mn	259 ppb	331 ppb	221 ppb
Co	99 ppb	21 ppb	21 ppb
Ni	1,404 ppb	1959 ppb	904 ppb
Cu	441 ppb	789 ppb	574 ppb
Cd	13 ppb	6 ppb	8 ppb
Pb	613 ppb	414 ppb	534 ppb
Zn	1,953 ppb	1,773 ppb	2,273 ppb
Fe	N.D	315 ppb	908 ppb
Na	286,828 ppm	245,257 ppm	223,075 ppm
Ca	7,692 ppm	517 ppm	8,662 ppm
K	7,725 ppm	7,140 ppm	12,939 ppm
Mg	14,256 ppm	13,539 ppm	38,159 ppm

N.D, Not Detected

PBS (2–3 times), the joints were treated with trypsin-ethylenediaminetetraacetic acid solution containing type 2 collagenase, followed by incubation at 37 °C for 3 h in a 5% CO₂ atmosphere under intermittent vortexing. Thereafter, the cartilage tissue was extracted from the knee joints, washed three times with PBS, and incubated in DMEM containing type 2 collagenase for 1.5 h. After incubation, the isolated chondrocytes were centrifuged at 200g for 3 min, washed twice with PBS, and seeded in either 6-well plates (at a density of 3×10^5 cells) or 96-well plates (at a density of 3×10^4 cells) for subsequent experimental procedures.

MTT assay and reverse or quantitative transcription polymerase chain reaction (RT-PCR/qRT-PCR)

Chondrocytes were seeded in 96-well plates (3×10^4 cells/well) and incubated at 37 °C for 12–16 h under a 5% CO₂ atmosphere. The culture medium was replaced with solutions containing 0.1, 0.2, and 0.5% salts, followed by incubation for an additional 24 h. After removing

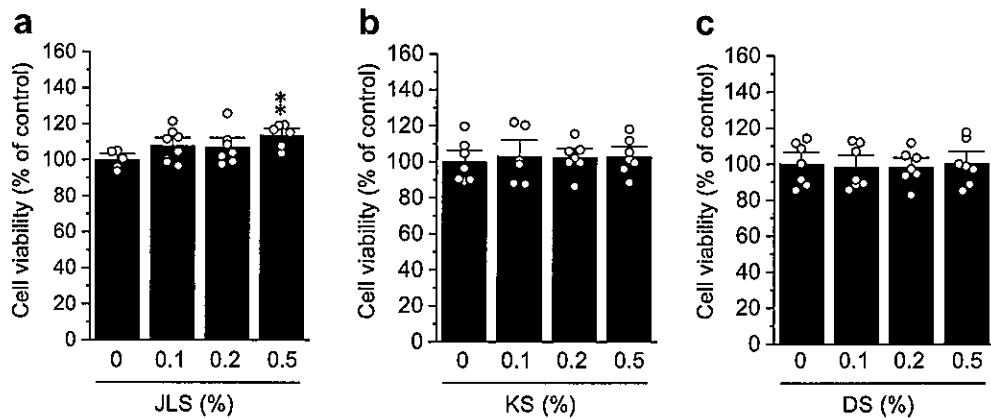


Fig. 1 The effect of salts on the viability of chondrocytes were evaluated using MTT assay. Chondrocytes were treated with Jeju lava seawater (JLS) salt, Korean sea salt (KS), and deep ocean seawater salt (DS) at concentrations of 0.1, 0.2, and 0.5% for 24 h, followed by the MTT assay. ** $p < 0.01$

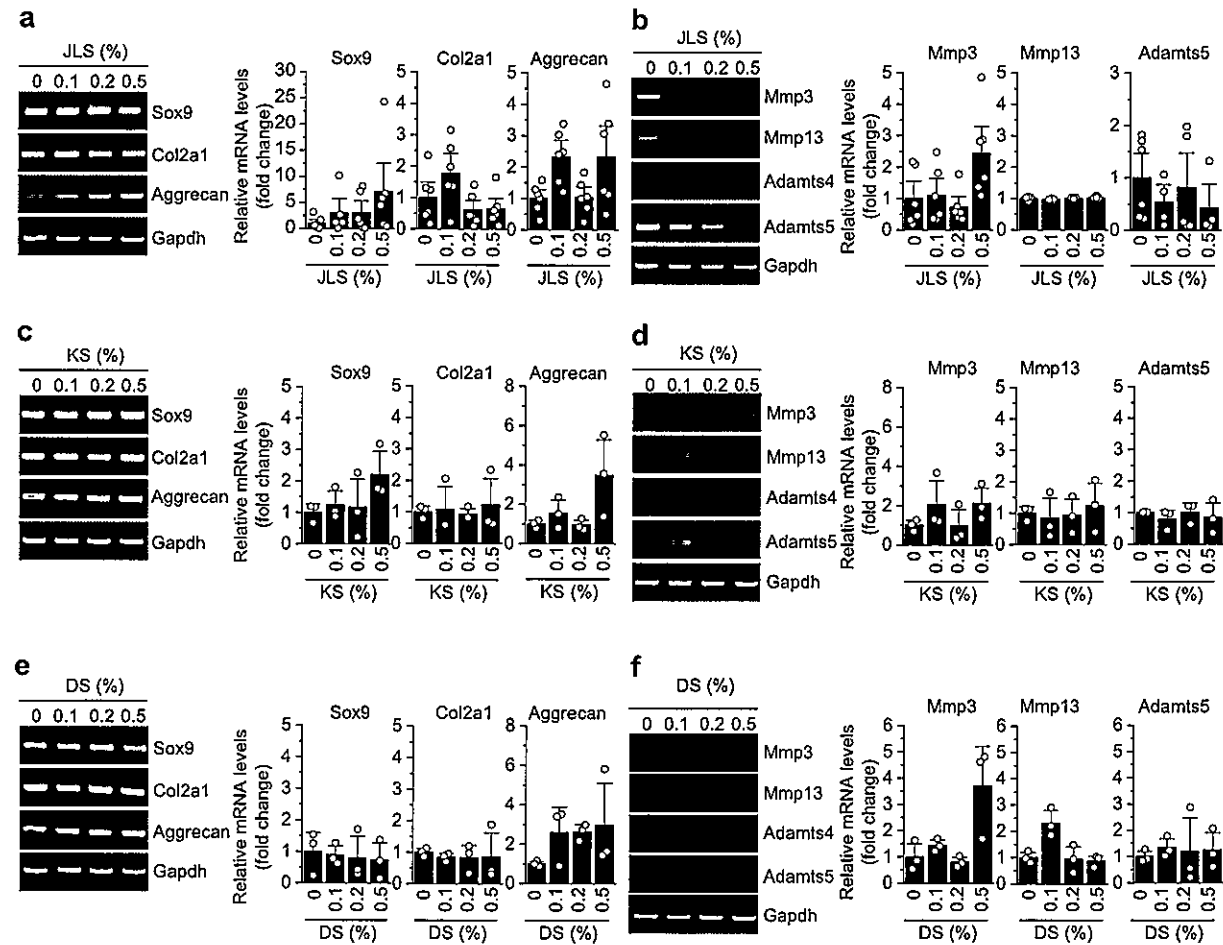


Fig. 2 The effect of salts on the expression of anabolic and catabolic factors in chondrocytes. Chondrocytes were treated with 0.1, 0.2, and 0.5% of Jeju lava seawater (JLS) salt, Korean sea salt (KS), and deep ocean seawater salt (DS) for 24 h. Conventional RT-PCR and qRT-PCR were used to analyze the mRNA expression of Sox9, Col2a1, Aggrecan, Mmp3, Mmp13, Adamts4, and Adamts5. Gapdh was utilized as an internal control

the culture medium, the MTT solution was added and incubated for 4 h. Finally, 50 μ L of isopropanol was added to the wells, and the optical density was measured at 450 nm using a SpectraMaxi3X spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at the Bio-Health Materials Core-Facility, Jeju National University.

For RT-PCR, chondrocytes were harvested after a 24-h incubation period, and total RNA was extracted from the cells using TRIzol reagent. Total RNA was reverse-transcribed to generate cDNA using the PrimeScript RT kit. PCR amplification of anabolic and catabolic genes was performed on the Veriti Thermal Cycler PCR

system (Thermo Fisher Scientific, Applied Biosciences) or BIO-RAD Real-Time PCR system (CFX96™ Real-Time System, Bio-Health Materials Core-Facility, Jeju National University) using SYBR premixed ExTaq reagent (Takara Bio, Mountain View, CA, USA) and specific primers. The primers used in this study are listed in Table 1.

Destabilization of the medial meniscus (DMM)

C57BL/6 mice (4-week-old) were randomly divided into two groups (n=20 mice/group: tap water (TAP) and JLS groups. Mice in the TAP and JLS groups received tap water and 1% JLS, respectively, for 4 weeks.

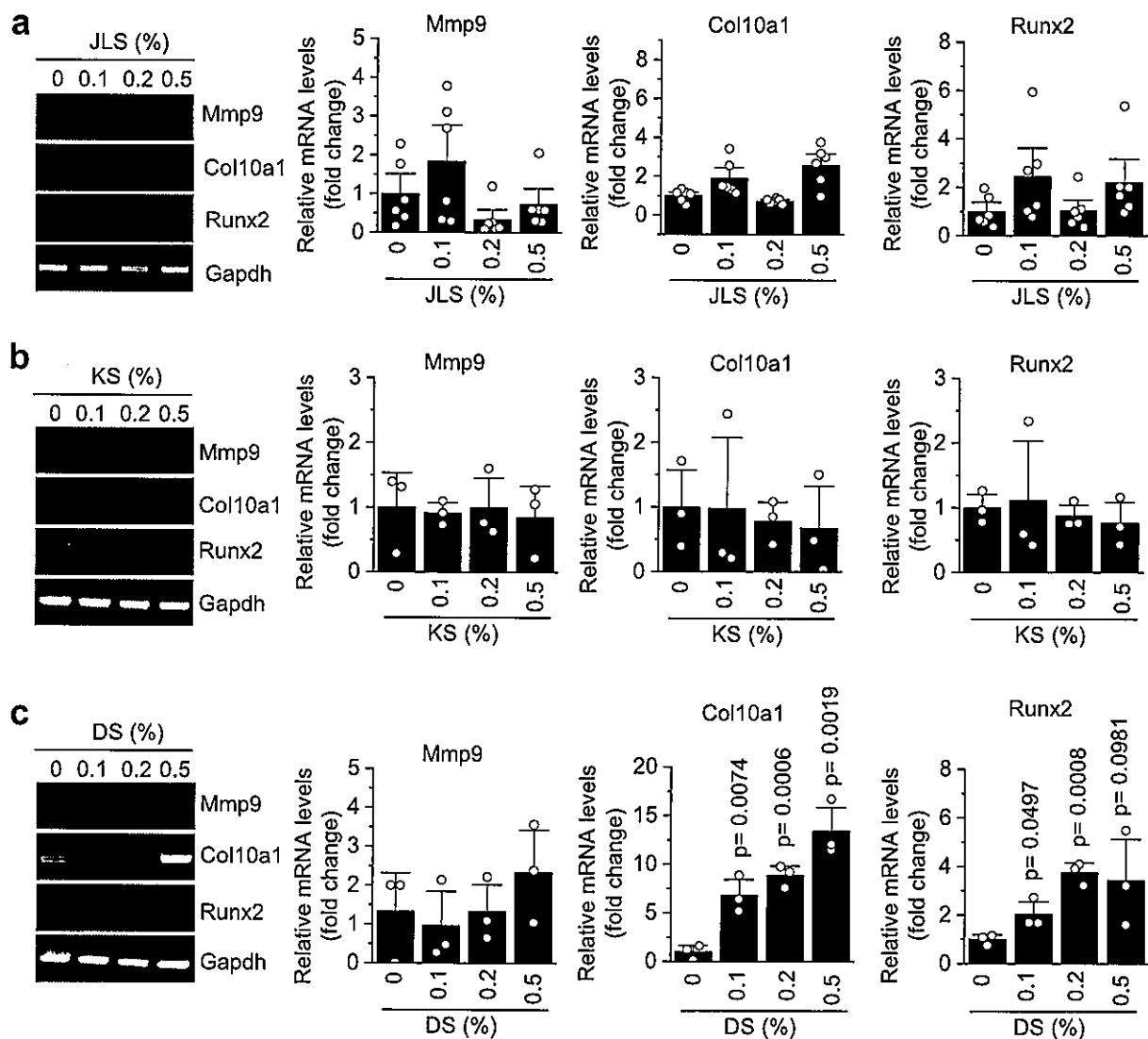


Fig. 3 The effect of Jeju lava seawater (JLS) salt, Korean sea salt (KS), and deep ocean seawater salt (DS) on the expression of hypertrophic marker genes in chondrocytes. Chondrocytes were treated with JLS, KS, or DS at the concentrations of 0.1, 0.2, and 0.5% for 24 h. Conventional RT-PCR and qRT-PCR were used to analyze the mRNA expression of Mmp9, Col10a1, and Runx2, with Gapdh serving as the internal control

Later, surgical destabilization of the medial meniscus (DMM) was performed to establish a murine model of OA, as previously described [12, 13]. Thereafter, the mice continued their respective drinking regimens for an additional 8 weeks. After 8 weeks post-surgery, the knee joints of the mice were collected, fixed with 4% paraformaldehyde, and decalcified with 0.5 M of ethylenediaminetetraacetic acid (EDTA) for 2 weeks. This experimental design aimed to assess the effects of JLS on DMM-induced OA in mice.

Histological analysis

The knee samples were dehydrated, paraffin-embedded, cut into 5- μ m thick sections, and stained with safranin-O to assess the extent of cartilage degeneration. Four experienced researchers individually assessed and scored the samples to evaluate parameters, such as osteophyte formation and synovitis, using the Osteoarthritis Research Society International (OARSI) scoring system. Subchondral bone thickness and osteophyte size were quantified using Adobe Photoshop 2021 (Adobe Systems, Inc., San Jose, CA, USA). This analysis aimed to provide a comprehensive evaluation of knee joint conditions, including cartilage health, bone morphology, and pathological changes.

Results

Differences in the mineral ratio between JLS and other salts

The mineral compositions and concentrations of JLS, KS, and DS were analyzed using ICP-MS, and the concentrations of the minerals were expressed as parts per billion (ppb) or parts per million (ppm) (Table 2).

The concentrations of barium (Ba) and rubidium in JLS, KS, and DS were 650; 228; and 168 ppb; respectively, and 1,161; 752; and 2,614 ppb; respectively. Additionally, the lithium (Li) and molybdenum (Mo) concentrations of JLS, KS, and DS were 1,745; 1,519; and 4,196 ppb, respectively, and 3,717; 1,104; and 1,1825 ppb, respectively. Moreover, the concentrations of vanadium (V) and boron (B) in JLS, KS, and DS were 2,625; 2,526; and 2,849 ppb, respectively, and 37,581; 36,827; and 94,031 ppb; respectively. However, selenium (Se) and uranium (U) were not detected in JLS, KS, and DS. The

concentrations of strontium (Sr) in JLS, KS, and DS were 231,017; 55,232; and 190,351 ppb; respectively.

Effects of salts on the viability of primary cultured chondrocytes

Treatment with 0.1–0.5% of JLS, KS, and DS exhibited no cytotoxic effects on the chondrocytes (Fig. 1). Notably, treatment with 0.5% of JLS significantly increased chondrocyte viability (Fig. 1a). However, treatment with the salts at concentrations >0.5% showed evidence of cytotoxicity (data not shown). These results suggest that salt treatment at concentrations of 0.1–0.5% did not adversely affect chondrocyte viability, whereas higher salt concentrations may be cytotoxic to chondrocytes. Based on these results, we decided to use 0.5% as maximum salt concentration in subsequent experiments.

Effects of salts on anabolic and catabolic factors of chondrocytes

To elucidate the mechanism of JLS on chondrocytes, we examined the effects of the salts on the expression of anabolic and catabolic factors in cultured primary chondrocytes. Salt treatments did not significantly affect Sox9 and Col2a1 expression in chondrocytes, suggesting that the investigated salts did not affect the crucial anabolic factors associated with chondrocyte function (Fig. 2a, c, e). In contrast, JLS treatment led to a concentration-dependent increase in the Aggrecan expression while decreasing the Mmp3, Mmp13, Adamts4, and Adamts5 expression in chondrocytes (Fig. 2b, d, f). Collectively, these results indicate that JLS may suppress the degradation of extracellular matrix (ECM) components by inhibiting the activities of Mmp3, Mmp13, and Adamts. Additionally, the decrease in the expression of catabolic factors suggests that JLS may prevent cartilage degradation by maintaining ECM integrity.

Effects of salts on hypertrophic marker genes in primary cultured chondrocytes

RT-PCR and qRT-PCR were used to examine the mRNA expression of the hypertrophic markers Mmp9, Col10a1, and Runx2. JLS treatment did not significantly affect the expression of Mmp9, Col10a1, and Runx2 in chondrocytes (Fig. 3a), indicating that JLS did

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Fig. 4 The effect of Jeju lava seawater (JLS) salt, Korean sea salt (KS), and deep ocean seawater salt (DS) on the proinflammatory cytokine-induced expression of anabolic factors in articular chondrocytes. Cells were treated with 1 ng/mL of IL-1 β (a, e, i), 10 ng/mL of TNF- α (b, f, j), 100 ng/mL of IL-6 (c, g, k), and 10 ng/mL of LPS (d, h, l) in the absence or presence of JLS, KS, and DS at concentrations of 0.1, 0.2, and 0.5% for 24 h. Conventional RT-PCR and qRT-PCR were used to analyze the mRNA expression of Sox9, Col2a1, and Aggrecan, with Gapdh serving as the internal control. * p < 0.05, ** p < 0.01, and *** p < 0.001 vs. cytokine-only treated group

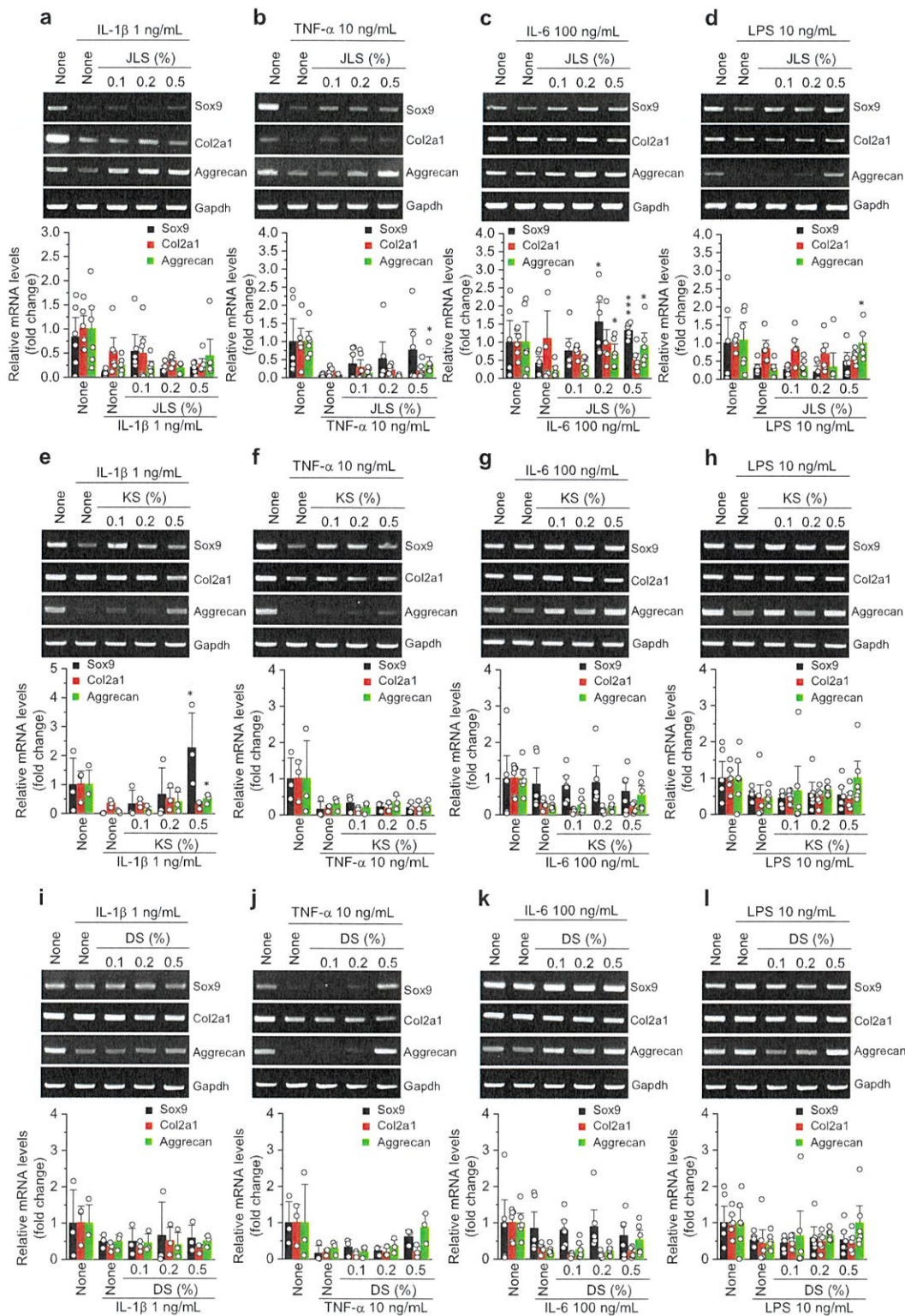


Fig. 4 (See legend on previous page.)

not induce hypertrophic changes in chondrocytes. Chondrocyte hypertrophic differentiation is linked with the transformation of chondrocytes into a hypertrophic phenotype, which plays a role in endochondral ossification. Notably, DS treatment significantly increased the expression of Col10a1 and Runx2 in a dose-dependent manner (Fig. 3c). Col10a1 and Runx2 are well-established markers of chondrocyte hypertrophic differentiation, and an increase in their expression indicates that DS may promote chondrocyte hypertrophic differentiation. Hypertrophic differentiation is a critical stage in cartilage development, characterized by the enlargement and mineralization of chondrocytes, and its dysregulation contributes to pathological conditions such as OA.

Effects of salts on proinflammatory cytokine-induced expression of anabolic and catabolic factors

We further examined the effects of JLS on proinflammatory cytokine-induced changes in the expression of anabolic and catabolic factors in chondrocytes. JLS and KS treatment reversed the IL-1 β (1 ng/mL)-induced decrease in aggrecan expression in chondrocytes (Fig. 4a, e). Similarly, treatment with the salts reversed the TNF- α (10 ng/mL)-induced decrease in the expression of Sox9, Col2a1, and aggrecan in a concentration-dependent manner (Fig. 4b, f, j). Additionally, JLS treatment significantly reversed the IL-6 (100 ng/mL)- and LPS (10 ng/mL)-induced alterations in Sox9 and aggrecan expression in primary chondrocytes (Fig. 4c, d).

Furthermore, JLS treatment significantly inhibited the expression of Mmp3, Mmp13, Adamts4, and Adamts5 induced by IL-1 β (1 ng/mL), TNF- α (10 ng/mL), IL-6 (100 ng/mL), and LPS (10 ng/mL) in primary chondrocytes (Fig. 5a–d). These results highlight the anti-inflammatory role of JLS in modulating the expression of crucial factors associated with cartilage homeostasis. KS treatment significantly suppressed the IL-1 β (1 ng/mL)- and TNF- α (10 ng/mL)-induced expression of Mmp13 and Adamts5 in chondrocytes (Fig. 5e, f). Additionally, DS treatment significantly suppressed the IL-1 β (1 ng/mL)- and TNF- α (10 ng/mL)-induced increase in Mmp13 and Adamts5 expression (Fig. 5i, j). Moreover, DS treatment slightly decreased IL-6 (100 ng/mL)-induced Adamts4 expression and significantly suppressed the

LPS (10 ng/mL)-induced increase in Adamts5 expression in chondrocytes (Fig. 5k, l).

JLS may attenuate DMM-induced osteoarthritis

Our in vitro experiments indicated that JLS has the most pronounced effect against osteoarthritis among the three salts. Therefore, we investigated the impact of JLS in a mice DMM model. To examine the anti-osteoarthritis effects of JLS in vivo, mice with DMM-induced OA were administered JLS. Compared with the TAP group, JLS treatment decreased cartilage erosion in the mice with DMM-induced OA (Fig. 6a). Although not statistically significant, JLS treatment reduced the OARSI score, osteophyte maturity, and subchondral bone plate thickness in mice with DMM-induced OA (Fig. 6b–d). Additionally, JLS treatment did not significantly affect osteophyte size or ameliorate synovitis (Fig. 6e–g).

Discussion

OA is characterized by morphological changes in the joints, including degeneration, fibrillation, and erosion of the cartilage surface. Osteophyte formation, characterized by bony outgrowth at the joint margins, often occurs in response to cartilage degeneration [14]. Hypertrophic alterations in chondrocytes indicate an increase in cell size and metabolic activity, with chondrocytes being the primary cellular components of cartilage [15]. Maintaining cartilage homeostasis relies on a delicate balance between anabolic (SOX9, Aggrecan, COL2A1) and catabolic (MMPs and ADAMTs) factors. Anabolic factors contribute to the synthesis and maintenance of the ECM in cartilage, ensuring structural integrity [16]. In contrast, catabolic factors are involved in ECM degradation and turnover. Notably, increased catabolism or decreased anabolism contributes to the degradation of cartilage components and promotes the development and progression of OA [17, 18]. Therefore, a comprehensive understanding of these molecular and cellular processes is essential for development of effective treatments for OA.

In the present study, JLS treatment upregulated the expression of aggrecan, a crucial protein for cartilage organization and ECM stabilization, in chondrocytes [19]. Additionally, JLS treatment led to a decrease in the expression of MMP13 and ADAMTS5. MMP13, a

(See figure on next page.)

Fig. 5 The effect of Jeju lava seawater (JLS) salt, Korean sea salt (KS), and deep ocean seawater salt (DS) on the proinflammatory cytokine-induced expression of catabolic factors in articular chondrocytes. Cells were treated with 1 ng/mL of IL-1 β (a, e, i), 10 ng/mL of TNF- α (b, f, j), 100 ng/mL of IL-6 (c, g, k), and 10 ng/mL of LPS (d, h, l) in the absence or presence of JLS, KS, and DS at the concentrations of 0.1, 0.2, and 0.5% for 24 h. Conventional RT-PCR and qRT-PCR were used to analyze the mRNA expression of Mmp3, Mmp13, Adamts4, and Adamts5, with Gapdh serving as the internal control. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. cytokine-only treated group

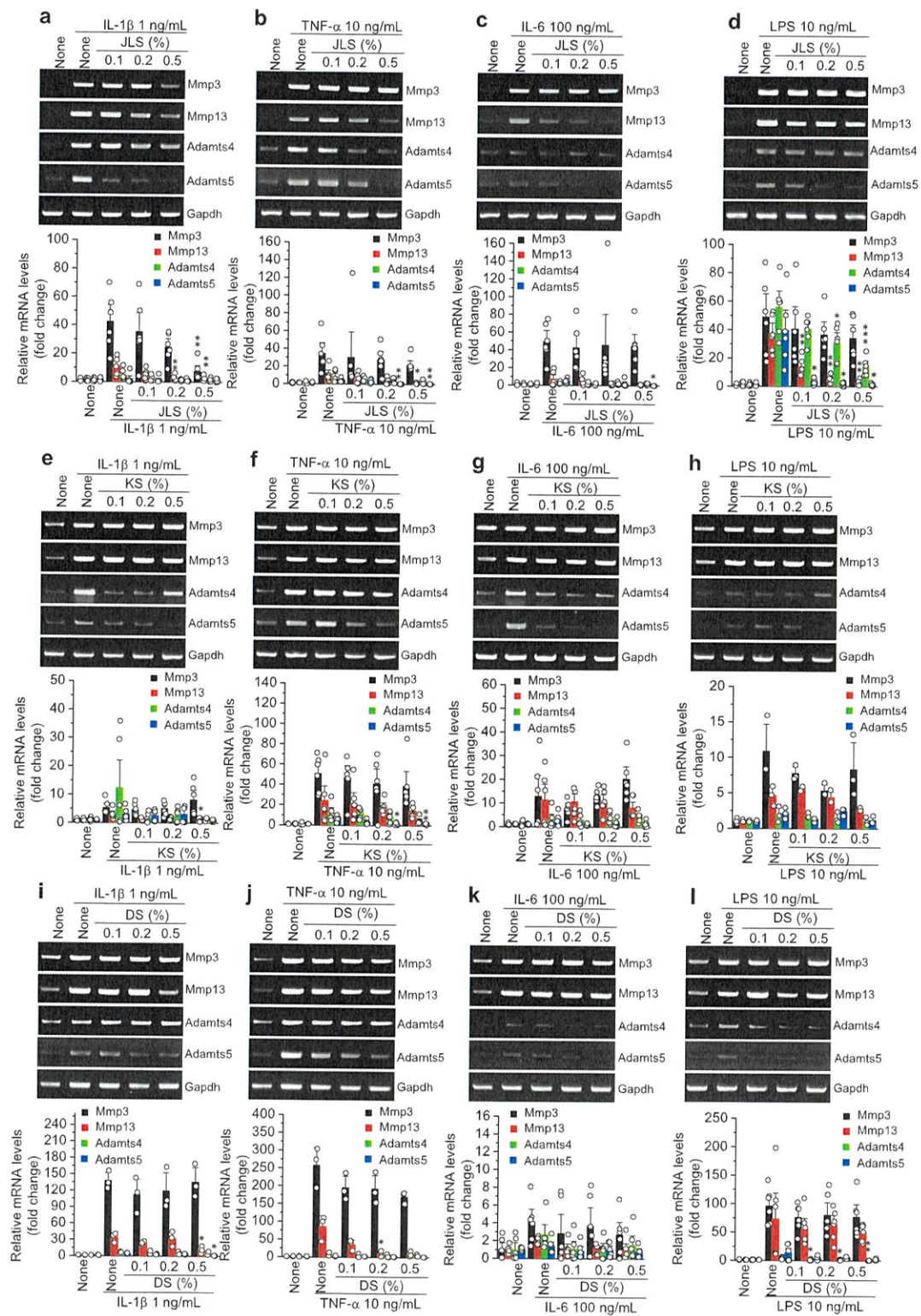


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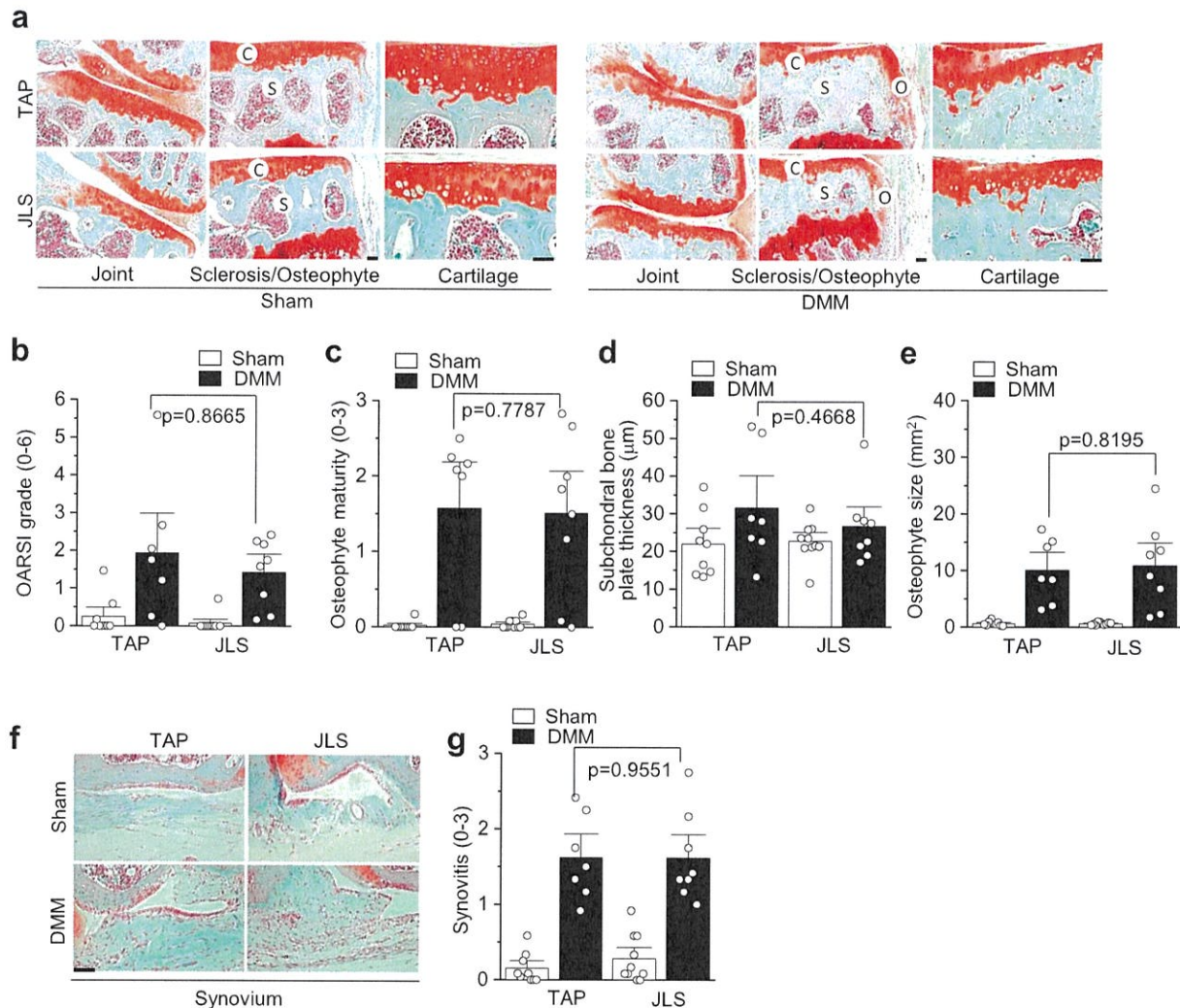


Fig. 6 The effect of Jeju lava seawater (JLS) salt on the pathogenesis of osteoarthritis (OA) in a murine model of DMM-induced OA. Mice in the tap water (TAP, $n=7$) and JLS ($n=7$) groups underwent DMM surgery, followed by further breeding for 8 weeks. **a** Joint, osteophyte, and cartilage tissue were visualized using Safranin O staining. The OARSI score (**b**), osteophyte maturity (**c**), subchondral bone thickness (**d**), and osteophyte size (**e**) were quantified. The synovium (**f**) and synovitis (**g**) were represented. Statistical analysis was performed using Mann-Whitney U test. Scale bar: 100 μm

member of the matrix metalloproteinase family, plays a critical role in cartilage degeneration, and a decrease in its expression inhibits OA progression [20]. ADAMTS5, a member of the ADAMTS family, is involved in the cleavage of the aggrecan core protein and contributes to ECM degradation [21]. Collectively, these findings suggest that JLS exerts positive effects on normal chondrocyte function, contributing to the maintenance of a healthy cartilage.

To elucidate the mechanism of JLS in OA, we examined the effects of JLS on the expression of anabolic and catabolic factors in chondrocytes. JLS treatment did not significantly affect the expression of key anabolic

factors (SOX9 and COL2A1), indicating the maintenance of cellular stability. However, JLS treatment led to a concentration-dependent increase in aggrecan expression, suggesting that JLS may positively regulate the synthesis of aggrecan, an essential ECM component associated with cartilage health. Additionally, JLS treatment suppressed the expression the catabolic factors Mmp3 and Adamts5, indicating that JLS may prevent the degradation of ECM components. In contrast, JLS treatment did not significantly affect the expression of hypertrophy markers, such as Mmp9, Col10a1, and Runx2, indicating the absence of hypertrophic changes in chondrocytes. Contrarily, DS treatment increased the

expression of Col10a1, suggesting that DS may induce chondrocyte hypertrophic differentiation. Overall, these results suggest that JLS does not induce chondrocyte hypertrophic differentiation.

In the present study, the decision to choose 1% JLS concentration in the in vivo experiment was based on the recommended daily salt intake for mice and the WHO recommended daily salt intake for humans [22]. Although JLS treatment did not induce any significant effect in the mouse model, further in vivo studies are necessary to elucidate the therapeutic effects of long-term JLS administration in OA. Future studies should refine the conditions and concentrations of JLS to optimize its efficacy for therapeutic purposes in live organisms.

Cell viability assay demonstrated that JLS, KS, and DS were not cytotoxic to chondrocytes at concentrations of 0.1–0.5%; however, all salts were cytotoxic at concentration > 0.5%. These results indicate that low concentrations of JLS are safe and well tolerated by the chondrocytes. Therefore, salt concentration was set at a maximum of 0.5% in subsequent experiments to preserve cellular health while exploring the potential impacts of the salts on chondrocyte function and behavior.

To evaluate the translational potential of these findings, an in vivo experiment was performed using a mouse model of DMM-induced OA. Several parameters, including the OARSI score, osteophyte size, synovitis score, subchondral bone thickness, and osteophyte size, were assessed. Despite the promising results of the in vitro experiments, JLS treatment did not significantly affect the examined parameters in the in vivo experiment. Overall, these results suggest that while JLS demonstrated positive effects on chondrocytes in a controlled in vitro environment, the complex in vivo conditions may introduce additional variables that influence the overall effectiveness of JLS. However, speculation that long-term treatment with JLS may exert promising effects in in vivo models. Several factors may contribute to the disparity between the in vitro and in vivo results, including the dosage of JLS, duration of exposure, and intricate interactions between the various elements present in the salt. Therefore, further exploration and refinement of the experimental conditions are necessary to comprehensively investigate the in vivo effects of JLS.

To gain a comprehensive understanding of the therapeutic potential of JLS in OA, future studies should explore JLS's interactions in vivo. This may involve adjusting experimental parameters, exploring various concentrations, and considering the dynamic nature of OA progression. Collectively, these findings underscore the complexity of translating in vitro observations

into in vivo outcomes and highlight the importance of continued research to fully understand the potential of JLS in OA treatment.

Abbreviations

DS	Deep ocean seawater salt
DMM	Destabilization of the medial meniscus
ICP-MS	Inductively coupled plasma-mass spectrometry
JLS	Jeju lava seawater
KS	Korean sea salt
OA	Osteoarthritis
OARSI	Osteoarthritis research society international

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Author contributions

MK performed the experiments and wrote the original draft. YM and YH conceived the experiments and analyzed the data. YOS, MK, and MG analyzed and interpreted the data. YOS executed funding acquisition, study design, data interpretation, and manuscript preparation. All authors were involved in writing the paper and provided final approval for the submitted and published versions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declared no conflict of interest.

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