공학석사 학위논문

Cartilage repair in temporomandibular joint osteoarthritis mediated by human umbilical cord stem cells stimulated with IFN-γ via immunomodulating activation of M2 macrophages

M2 대식세포의 면역조절 활성화를 통한 IFN-γ 자극 인간 제대 줄기세포의 턱관절 골관절염에서의 연골 치료

울산대학교 대학원

의 과 학 과

김 예 린

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이 논문을 공학석사학위 논문으로 제출함

2024 년 2 월

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English Abstract

Temporomandibular joint osteoarthritis (TMJOA) is a degenerative condition of the temporomandibular joint (TMJ) characterized by chronic inflammation and damage to joint structures. Only symptomatic treatment is available for managing TMJOA. Human umbilical cord mesenchymal stem cells (hUC-MSCs) show potential for treating TMJOA via their immune modulating actions in the disease area. Additionally, stimulation of inflammatory cytokine such as interferon-gamma in hUC-MSCs improves the therapeutic activity of naïve stem cells. Increasing evidence suggests that macrophages play important roles in modulating joint inflammation via various secreted mediators in the pathogenesis of TMJOA. This study was conducted to evaluate the effects of inflammatory cytokine-stimulated hUC-MSCs in repairing TMJOA-induced cartilage lesions and the role of macrophages in the disease. Our *in vitro* data showed that stimulated hUC-MSCs induce M2 polarization of macrophages and activate macrophages to express anti-inflammatory molecules. The *in vitro* effects of stimulated hUC-MSCs were confirmed *in vivo*. In a rat model of TMJOA, stimulated hUC-MSCs ameliorated inflammation and increased M2 macrophages ratio. Our findings show that inflammatory cytokines-stimulated hUC-MSCs immunomodulate M2 activation of macrophages to skew the local OA microenvironment towards a pro-chondrogenic atmosphere and promote cartilage repair under inflammatory conditions. Inflammatory cytokine-stimulated hUC-MSCs may be an effective treatment option for TMJOA, and macrophages play a key role in immune modulation.

Keywords: temporomandibular joint osteoarthritis, umbilical cord mesenchymal stem cells, stem cell priming, macrophage, M2

Contents

List of figures and tables

Introduction

Temporomandibular disorders (TMD) are a complex and challenging disease. Among the various diseases classified under TMD, temporomandibular joint osteoarthritis (TMJOA) is particularly serious, characterized by chronic progressive degeneration of the articular cartilage and inflammation of the joint [1,2]. Common symptoms of TMJOA include chronic pain, joint noise, and dysfunction, which often lead patients to seek medical care at orofacial pain clinics [3]. Clinical treatment options for TMJOA are currently limited to surgical interventions or symptomatic approaches, such as therapy with nonsteroidal anti-inflammatory drugs [4]. However, these treatments only provide temporary control of inflammation and pain relief without preventing cartilage destruction in TMJOA, as observed in osteoarthritis (OA) in other joints [5,6]. A key strategy for successfully treating degenerative TMJOA depends on progressive repair of the damaged articular cartilage. The development of TMJOA involves various pathological changes, including chondrocyte apoptosis and hypertrophy, as well as activation of innate immune cells, primarily macrophages [7]. These changes progressively alter the articular microenvironment, which is critical for cartilage tissue repair. Therefore, to effectively treat TMJOA, it is crucial to alter the inflammatory microenvironment to promote an immunomodulatory effect that shifts towards a pro-chondrogenic state while reducing cell damage [8].

Multipotent mesenchymal stem cells (MSCs) have been extensively investigated for their potential as cell-based therapies targeting immune-mediated, inflammatory, and degenerative diseases. These cells exhibit inherent immunosuppression, immunomodulation, and tissue regeneration capabilities. Human umbilical cord MSCs (hUC-MSCs) have several advantages over other MSCs, including easy availability, minimal immune rejection, and excellent immunomodulatory effects. Additionally, these cells can be harvested from discarded tissue [9,10]. We previously reported the potential immunomodulatory and anti-inflammatory effects of hUC-MSCs for treating TMJOA [11]. To improve therapeutic effect of MSCs in cartilage recovery, priming MSCs with inflammatory cytokines has been evaluated in numerous recent studies [12] Cell priming involves the preparation of cells to provide a specific cell function or systematic differentiation. In this study, we primed hUC-MSCs with interferon-gamma (IFN-

γ), which enhances the immunosuppressive properties of MSCs [13]. This strategy was used to improve the immunosuppressive function of hUC-MSCs and increase their secretion of anti-inflammatory and immunomodulatory factors.

Recent literature suggests that polarized macrophages have strong potential in OA therapeutics. The ability to suppress M1 macrophages or induce M2 polarization can substantially alleviate OA symptoms. Macrophages, as key players in innate immunity, possess a remarkable degree of plasticity [14]. hUC-MSCs can also promote the polarization of M2-type macrophages and the expression of anti-inflammation-related cytokines [15,16].

In this study, we explored whether inflammatory cytokine-stimulated hUC-MSCs (primed hUC-MSCs) have therapeutic effects on TMJOA [17] and whether these MSCs can induce the conversion of macrophages to the anti-inflammatory M2 phenotype. Additionally, we investigated whether MSCs can shift to the M2 phenotype even in an inflammatory environment by stimulating macrophages with lipopolysaccharide (LPS) [18,19]. We compared the effects of macrophage polarization between MSCs primed with IFN-γ and naïve MSCs. A TMJOA rat model and primed hUC-MSC injections were used to confirm these results *in vivo*.

Materials and methods

Cell culture and treatment

RAW264.7 murine macrophages were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). These cells were cultured at 5% CO_2 at 37 °C in Dulbecco's Modified Eagle Medium/F12 supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. RAW264.7 cells were activated with IFN-γ (2.5 ng/mL) and LPS (200 ng/mL) to polarize the cells to M1-like macrophages.

hUC-MSCs were obtained from CELLnLIFE, Inc. (Seoul, Korea). hUC-MSCs expressed CD73, CD90 and CD105 (supplementary data). These cells were cultured with 5% CO_2 at 37 °C in minimum essential medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. hUC-MSCs were stimulated with IFN-γ for 24 h. IFN-γ was diluted to a final concentration of 200 U/mL in the medium. hUC-MSCs from passages 3–6 were used in all experiments.

Co-culture of macrophages and hUC-MSCs

RAW264.7 cells were seeded into a 24-well plate at a density of 5×10^4 cells/well. LPS (200 ng/mL) was used to activate the RAW264.7 cells in Dulbecco's Modified Eagle Medium. Next, naïve hUC-MSCs and primed hUC-MSCs seeded in the trasnswell with activated RAW264.7 cells at a cell ratio of 1:1. After 24, 48, or 72 h, the cells were collected for RNA and protein extraction and analysis. Monocultures of activated-M1-like macrophages were used as controls.

Quantitative real-time polymerase chain reaction

Total RNA was extracted using an RNeasy® Mini Kit (QIAGEN, Hilden, Germany). RNA was converted to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative reverse transcription-polymerase chain reaction (PCR) was performed using Applied Biosystems™ *Power* SYBR™ Green Master Mix on an Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System (Foster City, CA,

USA). PCR was performed using gene-specific primers for the M1-related genes inducible nitric oxide synthase (*iNOS*), interleukin 6 (*Il-6*), interleukin 12 beta (*Il-12β*), tumor necrosis factor alpha (*Tnf-α*), and interleukin 1 beta (*Il-1β*) and M2-related genes arginase 1 (*Arg1*) and interleukin 10 (*Il-10*), as well as for glyceraldehyde 3-phosphate dehydrogenase (Table 1). The expression of all genes was normalized to that of GAPDH.

Enzyme-linked immunosorbent assay

RAW264.7 cells were placed in the transwell, whereas primed/naïve hUC-MSCs were placed in the 24-well plate. After co-culture, the supernatants of primed/naïve hUC-MSCs were collected and quantitatively analyzed for indoleamine 2,3-dieoxygenase (IDO) expression. IDO levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

Experimental animals

Sprague–Dawley rats (female, weighing 230 ± 13 g) were purchased from the Orient Bio, Inc. (Seongnam, Korea). All rats were randomly divided into four groups: (1) normal group; (2) OA group; (3) naïve hUC-MSC injection (NM) group; and (4) primed hUC-MSC injection (PM) group. The OA model was induced using monoiodoacetate (MIA). The TMJs of rats were injected intra-articularly with 0.05 mL (0.5 mg/50 μL) of MIA once [20]. At 2 weeks after inducing TMJOA, the rats were injected intra-articularly with naïve or primed hUC-MSCs (10^5 cells/0.05 mL). All rats were sacrificed at 2, 4, and 8 weeks after MSC injection.

In vivo micro-computed tomography images of the subchondral bone

Micro-computed tomography (CT) images of condyle were obtained using an *in vivo* animal scanner (SkyScan 1176; Bruker-microCT, Kontich, Belgium). Scanning was performed using the following settings: X-ray source voltage of 50 kVp, current of 500 μA, pixel size of 12.64 μm, and exposure time of 60 ms. Cross-sectional images were reconstructed using a filtered

back-projection algorithm (NRecon, Skyscan, Bruker). To compare anatomical changes in the articular structures among groups, three-dimensional images were acquired using a CTvox (Bruker).

Head withdrawal threshold measurement

The mechanical sensitivity of the rats was assessed using the von Frey microfilament method [21] following established procedures with the use of Aesthesio® precisie tactile sensory evaluators (Danmic Global, LCC, CA, USA). This technique gauges the minimal force intensity required to trigger a reflexive response when applied to the TMJ of the rat. Starting from the lowest filament gauge, a sequence of filaments was applied to the pre-auricular area to stimulate the TMJ position. The experiment was performed twice by two researchers in a blinded group designations of the rats.

Histology staining and immunohistochemical analysis

The repaired temporomandibular cartilage tissues of rat were collected and subjected to histopathological examination. The collected specimens were fixed with 10% neutral buffered formalin and then decalcified with 0.5 M EDTA for 28 days. The decalcified samples were embedded using paraffin wax. The cartilage was sectioned at 5 μm and stained with hematoxylin and eosin and safranin O/fast green. The alteration in TMJOA cartilage tissue was evaluated using a modified Mankin score. The histological score of the specimens was calculated by two researchers who were blinded to the experimental conditions. For immunohistochemical analysis, 5 μm thick sections of cartilage tissue were treated with primary antibodies against CD68 (ab125212, Abcam, Cambridge, UK), CD163 (ab182422, Abcam), and CD206 (ab64693, Abcam).

Ethics statement

This work was approved by the Public Institutional Bioethics Committee designated by the MOHW (IRB No.2020-1825-011). All animal experiments were performed following relevant

guidelines and regulations of the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences, Asan Medical Center (IACUC, Approval No. 2021-12-208).

Statistical analysis

All data are presented as the mean ± standard deviation. The statistical significance of the differences was evaluated using the *t*-test. Correlation analysis and coefficient calculation were performed with GraphPad Prism (GraphPad Software, CA, USA). ImageJ software (version 1.53t, NIH, Bethesda, MD, USA) was used to analyze and statistically process the images. A p-value < 0.05 was considered to indicate a significant difference.

Results

Primed hUC-MSCs promote polarization to M2 macrophages *in vitro*

To determine an appropriate cell model for further experiments, we initially characterized M1-like macrophages by assessing the expression of typical markers. We induced RAW264.7 polarization to the M1 phenotype using LPS and IFN-γ, resulting in upregulation of the mRNA expression of *Il-1β*, *Tnf-α*, *iNOS*, *Il-6*, and *Il-12β*. In subsequent co-culture experiments, we used activated-M1-like macrophages to mimic inflammatory conditions (Fig. 1a). Polarization of macrophages has been linked to OA progression [22]. Therefore, we investigated the influence of naïve hUC-MSCs and primed hUC-MSCs on macrophage polarization using a co-culture system with RAW264.7 cells (Fig. 1b). As shown in Fig. 1c, stimulation by naïve and primed hUC-MSCs led to upregulation of *Arg1* and *Il-10* mRNA expression, which are markers of M2 macrophages. These results indicate that both hUC-MSC types promote the polarization of macrophages towards the M2-type. Primed hUC-MSCs more effectively induced M0 to M2 macrophage polarization than naïve hUC-MSCs, as indicated by stronger upregulation of *Arg1*.

In the OA environment, M1 macrophages secrete pro-inflammatory factors. To investigate whether naïve and primed hUC-MSCs affect the polarization of M1 to M2 macrophages, we separately co-cultured these cells with M1-like macrophages. MSCs secrete the immunoregulatory factor IDO, which suppresses T-cell responses. IDO secretion was quantified using ELISA, revealing that IDO secretion from primed hUC-MSCs was approximately eight-fold higher than that from naïve hUC-MSCs (Fig. 1d). To predict the responses of primed/naïve hUC-MSCs in an inflammatory environment, we utilized a co-culture system with RAW264.7 cells. Both naïve and primed hUC-MSC exhibited a two-fold increase in IDO secretion compared to that under monoculture conditions. As shown in Fig. 1e, stimulation by both naïve and primed hUC-MSCs upregulated the expression of the M2 markers *Arg1* and *IL-10* mRNA while noticeably downregulating the mRNA expression of the M1 markers *Il-1β*, *iNOS*, and *Il-6*. These results indicate that both naïve and primed hUC-MSCs can promote the polarization of M1 macrophages towards the M2-type. Notably, macrophages co-cultured with primed hUC-MSCs exhibited higher upregulation *Arg-1* mRNA expression (M2 related gene) and greater downregulation of *Il-1b* and *iNOS* mRNA expression

(M1 related gene) compared to those co-cultured with naïve hUC-MSCs. This result demonstrates that primed hUC-MSCs are more efficient in polarizing macrophage from the M1 to the M2-type. There was no significant difference in *Il-10* and *Il-6* mRNA expression.

Primed hUC-MSCs increased the ratio of M2 marker-positive cells *in vivo*

M2 macrophages exert anti-inflammatory effects that contribute to tissue repair and alleviate inflammation [23]. Immunostaining was conducted to analyze the immunomodulation ability of primed hUC-MSCs and naïve hUC-MSCs on macrophage polarization to M2 in the MIA-induced TMJOA model. After inducing TMJOA by injecting MIA, in the OA, NM, and PM groups, the total number of macrophages increased compared to the normal group (Fig.2a). As shown in Fig. 2b, injection of both hUC-MSCs remarkably promoted M2 macrophage polarization in the MIA-induced TMJOA model in contrast to in the OA group. This effect was substantiated by the elevated expression of CD163 and CD206 (supplementary data), which were both evident in the synovial tissue of the primed and naïve hUC-MSC treatment groups. Notably, compared to in the naïve hUC-MSC group, the primed hUC-MSC group exhibited greater enhancement of M2 macrophage ratio (Fig.2c). Immunostaining showed that primed hUC-MSCs mitigated inflammation and protected against cartilage degradation in the TMJOA model by inducing M2 macrophage polarization.

Primed hUC-MSCs and naïve hUC-MSCs effectively alleviated pain in the TMJOA rat model

To investigate the effects of primed and naïve hUC-MSCs on TMJOA *in vivo*, we established a TMJOA rat model using MIA for an observation period of 10 weeks (Fig. 3a). Measurement of the head withdrawal threshold (Fig. 3b) showed that in the OA group, pain responses gradually eased over time. Notably, the groups injected with primed and naïve hUC-MSCs experienced faster pain relief compared to that in the OA group. However, there was no significant difference between the NM and PM groups.

Primed hUC-MSCs promote TMJ recovery in a TMJOA rat cartilage

Safranin O/fast green staining revealed extensive proteoglycan loss in the OA group (Fig. 4a). In contrast, proteoglycans in the naïve and primed hUC-MSC groups were increased at 2, 4 and 8 weeks compared to those in the OA groups. Importantly, the primed hUC-MSC group exhibited higher proteoglycan production than the naïve hUC-MSC group, suggesting that primed hUC-MSCs ameliorated TMJOA progression caused by MIA, resulting in a superior therapeutic effect compared to that of naïve hUC-MSCs. Additionally, hematoxylin and eosin staining confirmed severe cartilage erosion in the OA group, whereas both the naïve and primed hUC-MSC groups showed smooth and intact cartilage surfaces. Notably, the PM group exhibited a more improved cartilage surface compared to that in the NM group (Fig. 4b). Morphological changes in the TMJ articular cartilage were assessed based on the modified Mankin score (Table 2) [24]. As shown in Fig. 4c, the PM group showed the lowest scores among all groups, except for the normal group. These results indicate that primed hUC-MSCs strongly alleviate OA *in vivo*.

As shown in Fig. 5a, MIA-injected rats exhibited characteristic morphological changes of TMJOA, including cartilage surface erosion and subchondral bone damage, compared to normal rats. However, in the PM group, the TMJ cartilage was significantly recovered. In contrast, the OA group displayed limited improvement in the articular cartilage surfaces of TMJOA rats.

Discussion

TMJOA is characterized by cartilage degradation, subchondral bone remodeling, and persistent synovial inflammation. Inflammatory cytokines disrupt the metabolic balance of articular chondrocytes as OA progresses, leading to cell death and extracellular matrix breakdown [25]. In addition, chronic inflammation can compromise the adaptive capacity of the TMJ [26]. Therefore, counteracting inflammation is crucial in preventing OA development, with the synovial macrophage phenotype closely linked to inflammation [27,28]. Macrophages are immune cells present in nearly all tissues. Their principal function is maintaining tissue homeostasis and safeguarding against infections. Macrophages can polarize into two phenotypes: M1 (pro-inflammatory) and M2 (anti-inflammatory) types. Fahy et al. [29] demonstrated that M1 macrophages in OA synovial tissue hinder the chondrogenic differentiation of MSCs *in vitro* via IL-6. Ding et al. [30] indicated that M2 macrophages counteract inflammation by releasing IL-10, promoting cartilage graft viability. Strategies aimed at curbing M1 macrophages or promoting M2 polarization show considerable potential for alleviating OA symptoms. hUC-MSCs have the potential to induce M2-type macrophage activation and the expression of anti-inflammatory cytokines. We primed hUC-MSCs with IFN-γ to enhance their anti-inflammatory capabilities and promote a more effective M2 shift, after which we compared the therapeutic effects of primed and naïve hUC-MSCs on TMJOA.

To achieve a therapeutic effect by alleviating inflammation in TMJOA, we verified the relationship of this finding and M2 transition through *in vitro* experiments using a co-culture system with hUC-MSCs. Our results showed that hUC-MSCs can shift from the M0 and M1 states to the M2 state. Following co-culture, M2 markers such as *Arg-1* and *Il-10* were increased. ARG-1 functions in wound healing, and IL-10 has a pro-chondrogenic effect, which may have therapeutic implications for TMJOA. Moreover, in activated RAW264.7 cells (M1), the M1 state was inhibited, as evidenced by the reduction in inflammatory molecules (M1 markers), including *Il-6*, *Il-1β*, and *iNOS*. This shift resulted in suppression of inflammatory cytokine expression and augmentation of anti-inflammatory cytokine expression. Furthermore, primed hUC-MSCs had a more pronounced effect on macrophage polarization compared to that of naïve MSCs. Additionally, in the *in vivo* experiment, the number of M2 marker-positive

cells increased more significantly in the PM group compared to that in the NM group. This observation confirms that primed hUC-MSCs are more effective in driving the macrophage polarization towards the M2 type.

Kanglu et al. [31] demonstrated that extracellular vesicles derived from hUC-MSCs can promote the polarization of M2 macrophages *in vitro*. In our *in vivo* study, we injected naïve or primed hUC-MSCs rather than extracellular vesicles. Directly injecting customized and primed hUC-MSCs into an inflammatory environment may be the most practical and effective approaches compared to using extracellular vesicles. According to our results, these predictions were supported by the secretion of IDO. The expression intensity of IDO modulates macrophage differentiation [32]. After stimulating hUC-MSCs with IFN-γ, secretion of the immunoregulatory molecule IDO was increased. When we co-cultured naïve or primed hUC-MSCs with RAW264.7 cells activated into M1, the secretion of IDO was increased. This result indicates that primed hUC-MSCs exhibited an even more pronounced boost, and even naïve hUC-MSCs influenced the inflammatory environment. Elevated IDO secretion may enhance the immunosuppressive capacity of hUC-MSCs, which in turn may impact the suppression of M1 macrophages and differentiation into M2 macrophages.

In the *in vivo* TMJOA rat model, the pain response in the OA group was gradually alleviated over time, indicating a self-healing process. Rats injected with naïve and primed hUC-MSCs exhibited faster pain relief compared to that in the OA group. These results confirm the effectiveness of primed and naïve hUC-MSCs injection in treating TMJOA. Although there was no significant difference in the pain response between groups, notable distinctions were apparent in the morphological changes of the TMJ. Remarkably, the PM group showed accelerated TMJ recovery compared to that in the NM group, as demonstrated by faster and more effective recovery of the cartilage and subchondral bone and the production of proteoglycans in the primed hUC-MSC group compared to that in the naïve hUC-MSC group. These findings support that primed hUC-MSCs promoted TMJ recovery and facilitated the conversion of macrophages into the M2-type. We demonstrated that primed hUC-MSCs may have therapeutic effects on TMJOA.

Conclusion

hUC-MSCs stimulated with IFN-γ modulated macrophage polarization, creating an anti-inflammatory microenvironment that supported cartilage repair. These results demonstrate the promising role of these cells in TMJOA treatment, offering a novel avenue for OA management.

Figure

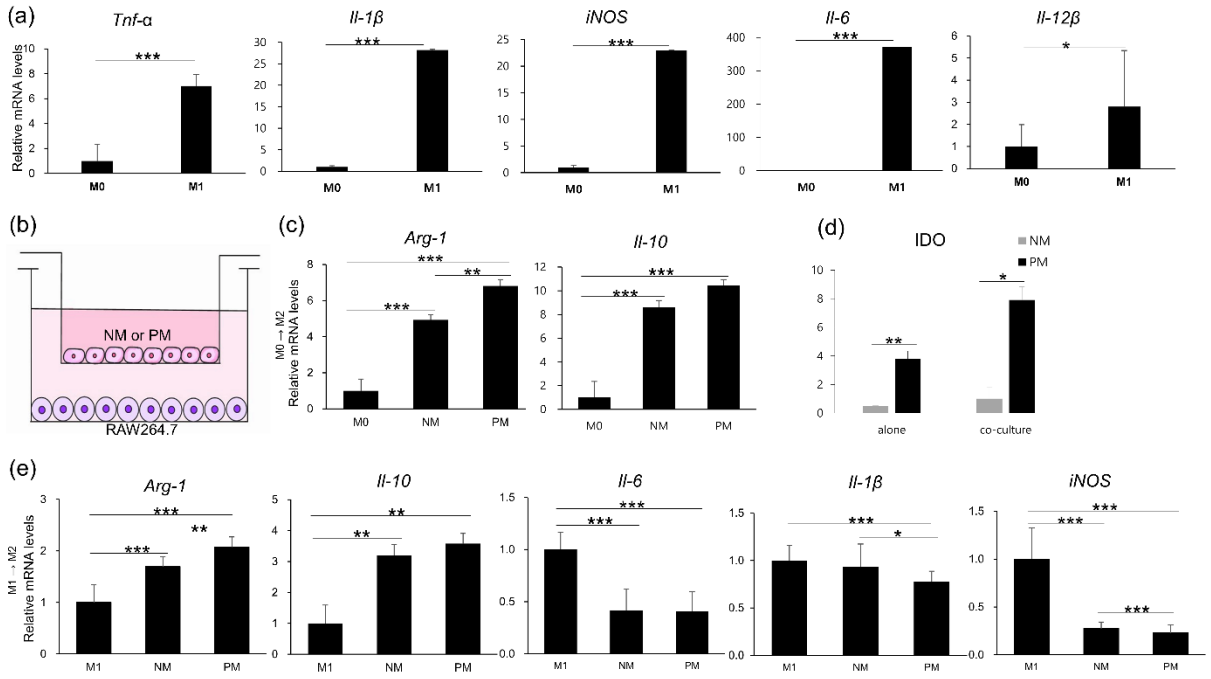


Fig. 1. hUC-MSCs promote the transformation of macrophages from M0 and M1 to M2 type

(a) RAW264.7 stimulated by LPS polarize to M1-like macrophages. Relative mRNA expression of the M1-related genes *Tnf- α*, *Il-1β*, *iNOS*, *Il-6*, and *Il-12 β* was determined using quantitative RT-PCR. (b) Schematic representation of the *in vitro* co-culture system. hUC-MSCs were cultured on the upper transwell inserts in multi-well plates containing RAW264.7 cells. (c) RAW264.7 were shifted from the M0 state to the M2 state. Relative mRNA expression of the critical genes *Arg-1* and *Il-10* was determined in polarized macrophages using quantitative RT-PCR. (d) ELISA. Determination of IDO in supernatants. (e) RAW264.7 cells polarized from M1 to M2 types. Relative mRNA expression of the critical genes *Arg1*, *Il-10*, *Il-6*, *Il-1β*, and *iNOS* was determined in polarized macrophages using quantitative RT-PCR; data from triplicate experiments are presented as the mean ± S.D. *p < 0.05, **p < 0.01, ***p < 0.005. ELISA, enzyme-linked immunosorbent assay; hUC-MSC, human umbilical cord mesenchymal stem cell; IDO, indoleamine 2,3-dieoxygenase; LPS, lipopolysaccharide; RT-PCR, reverse transcription polymerase chain reaction.

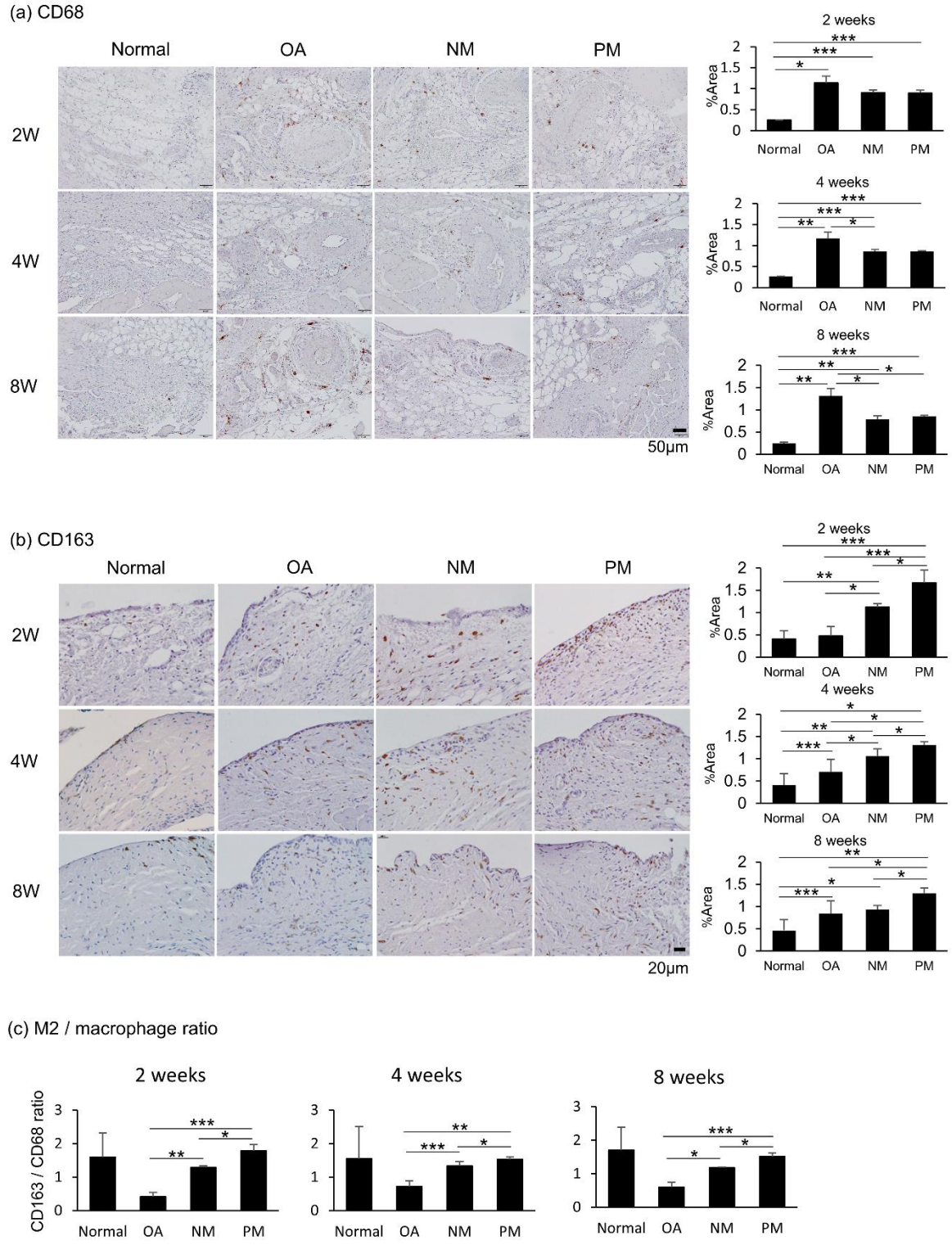


Fig. 2. M2 / macrophage ratio increased in the PM group
(a) Detection of CD68 (macrophage marker) using immunohistochemistry in RAW264.7 cells and statistical results. Scale bar: 50 μm. ImageJ analysis for CD68. (b) Detection of CD163 (M2 marker) using immunohistochemistry in RAW264.7 cells and statistical results. Scale bar:

20 μm. ImageJ analysis for CD163. (c) M2 / macrophage (M1+M2) ratio; average values were obtained by analyzing photos of three samples per group using ImageJ software. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.

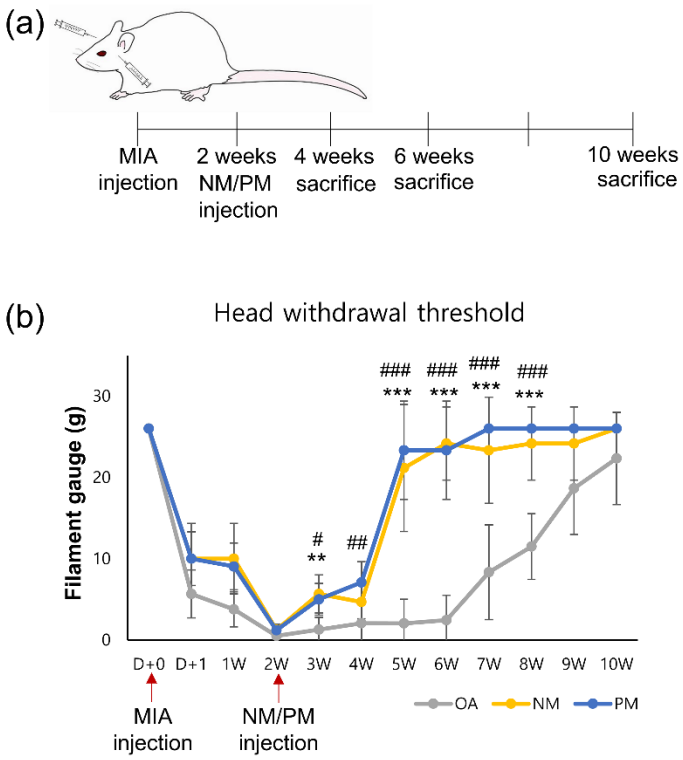


Fig. 3. Pain response over time in the TMJOA rat model

(a) *In vivo* schematic diagram. (b) TMJ HWT measurement graph. NM and PM groups experienced faster pain relief compared to that in the OA group; Biological replicates: n = 4; technical replicates: n = 2. Data are represented as the mean ± S.D.*p < 0.05, **p < 0.01, ***p < 0.005 between OA and NM groups. #p < 0.05; ##p < 0.01; ###p < 0.005 compared between OA group and PM group. HWT, head withdrawal threshold; NM, naïve MSC injection; OA, osteoarthritis; PM, primed MSC injection; TMJ, temporomandibular joint; TMJOA, temporomandibular joint osteoarthritis.

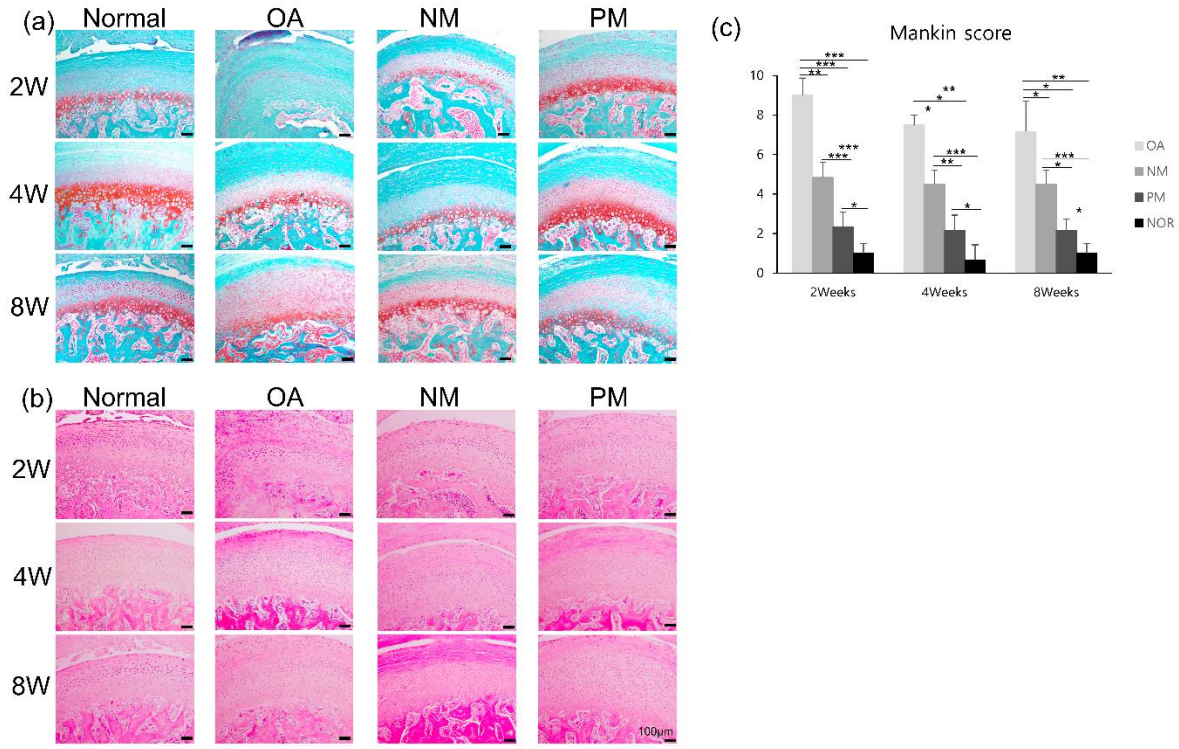


Fig. 4. Cartilage regeneration after injection of primed hUC-MSCs

(a) Representative images of Safranin O and Fast green staining in synovial tissue. Scale bar: 100 μm. (b) Representative images of H&E staining in synovial tissue. Scale bar: 100 μm. (c) Cartilage destruction evaluated using the modified Mankin score. Average values were obtained by analyzing safranin O-stained images of three samples per group; technical replicates: n = 2. *p < 0.05, **p < 0.01, ***p < 0.005. H&E, hematoxylin and eosin; hUC-MSC, human umbilical cord mesenchymal stem cell.

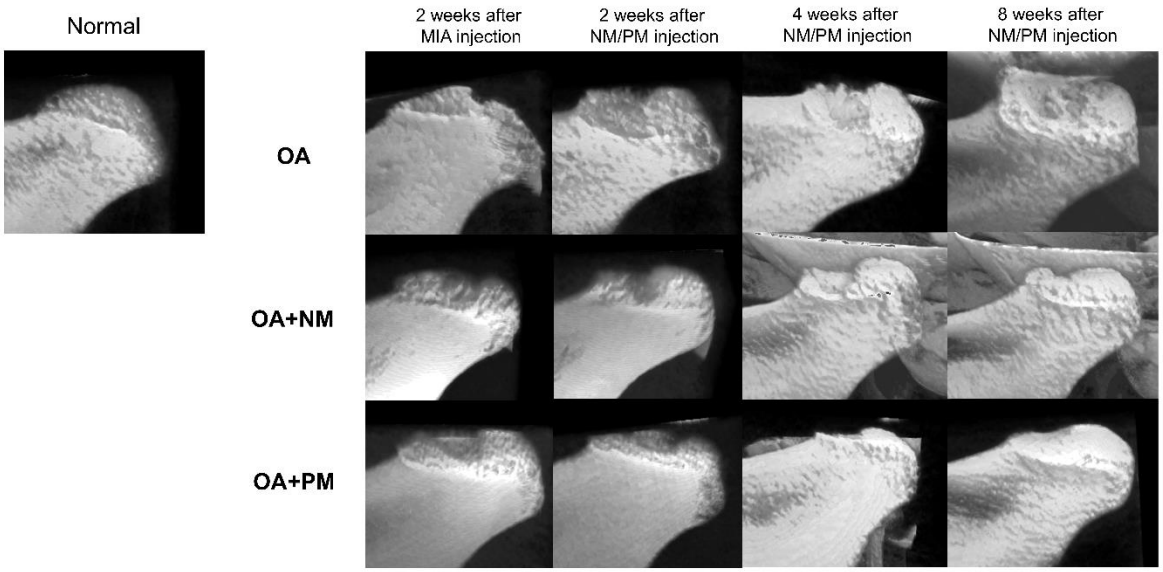


Fig. 5. *In vivo* micro-computed tomography three-dimensional images of rat TMJ condylar head

Normal TMJ of rat image, MIA-induced TMJOA of rat model image at 2, 4, and 8 weeks in NM or PM groups. hUC-MSC, human umbilical cord mesenchymal stem cell; MIA, monoiodoacetate; NM, NM, naïve MSC injection; PM, primed MSC injection; TMJ, temporomandibular joint; TMJOA, temporomandibular joint osteoarthritis.

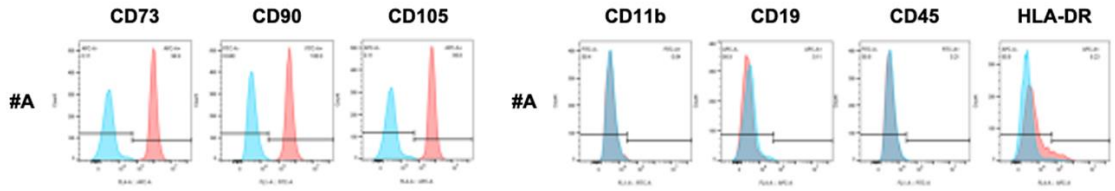
Table 1. Murine primers used for PCR amplification.

Marker		Forward	Reverse	Product size
M1 related genes	*Tnf-α*	CTCAGCGAGGACAGCAAGG	AGGGACAGAACCTGCCTGG	108 bp
	Il-1β	TGAAGCAGCTATGGCAACTG	GGGTCCGTCAACTTCAAAGA	94 bp
	Il-6	AACGATGATGCACTTGCAGA	GGTACTCCAGAAGACCAGAGGA	127 bp
	Il-12β	GTGGAATGGCGTCTCTGTCT	GGTCTGGTTTGATGATGTCCCT	204 bp
	iNOS	AACGGAGAACGTTGGATTTG	CAGCACAAGGGGTTTTCTTC	147 bp
M2 related genes	*Arg-1*	GACAGGGCTCCTTTCAGGAC	GCCAAGGTTAAAGCCACTGC	108 bp
	Il-10	TGAATTCCCTGGGTGAGAAG	TGGCCTTGTAGACACCTTGG	147 bp
House keeping gene	GAPDH	TGGCAAAGTGGAGATTGTTGCC	AAGATGGTGATGGGCTTCCCG	156 bp

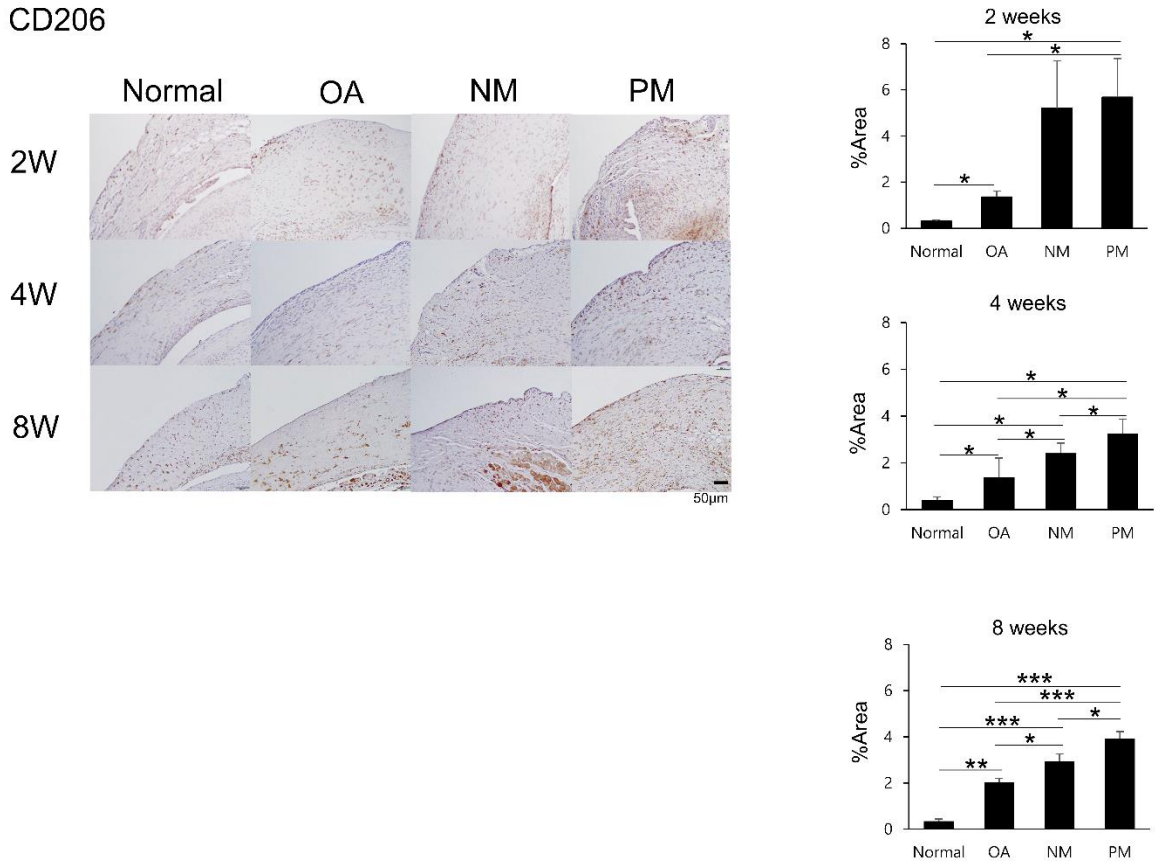
Table 2. Modified Mankin score.

Parameters	Grade
Pericellular Safranin O staining	
Normal	**0**
Slightly enhanced	**1**
Intensely enhanced	**2**
Background Safranin O staining	
Normal	**0**
Slightly increased or decreased	**1**
Severe increase or decrease	**2**
No staining	**3**
Arrangement of chondrocytes	
Normal	**0**
Appearance of clustering	**1**
Hypocellularity	**2**
Cartilage structure	
Normal	**0**
Fibrillation in the superficial layer	**1**
Fibrillation beyond the superficial layer	**2**
Missing articular cartilage	**3**

Supplementary data



Supplemental figure 1. hUC-MSCs phenotyping examination showed CD73, CD90 and CD105 expression. There are no expression of CD11b, CD19, CD45 and HLA-DR.



Supplemental figure 2. Detection of CD206 (M2 marker) using immunohistochemistry in RAW264.7 cells and statistical results. Scale bar: 50 μm. ImageJ analysis for CD206.

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국문 초록

Temporomandibular joint osteoarthritis (TMJOA)는 턱관절 골관절염으로 만성 염증과 관절 구조 손상을 특징으로 하는 턱관절(TMJ)의 퇴행적인 상태입니다. 현재 TMJOA 는 증상적인 치료만 가능합니다. 인간 제대조직유래 중간엽 줄기세포(hUC-MSCs)는 질병 부위에서의 면역 조절 효과를 통해 TMJOA 를 치료하는 잠재력이 있다고 알려져 있습니다. 게다가, 인간 제대조직유래 중간엽 줄기세포를 interferon-gamma 와 같은 염증성 사이토카인으로 자극함으로써, 줄기세포의 치료 효과를 향상시킬 수 있습니다. 마크로파지들이 TMJOA 의 진행 과정에서 다양한 분비 매개체를 통해 관절 염증을 조절하는 데 중요한 역할을 한다는 것을 점점 더 많은 연구들이 증명하고 있습니다. 이 논문에서는 interferon-gamma 자극을 받은 hUC-MSCs 가 TMJOA 에 의한 연골 손상을 치료하고 질병 내에서 마크로파지의 역할을 평가하기 위한 연구를 하였습니다. in vitro 실험을 통해 interferon-gamma 자극을 받은 hUC-MSCs 가 마크로파지를 M2 극성으로 유도하여 항염증 분자를 발현하도록 한 것을 확인했습니다. 또한 interferon-gamma 자극을 받은 hUC-MSCs 의 in vitro 결과는 in vivo 실험에서도 확인되었습니다. TMJOA 랫드 모델에서 염증성 사이토카인 자극을 받은 hUC-MSCs 는 염증을 완화시키고 M2 타입 마크로파지의 수를 증가시켰습니다. 이러한 결과는 염증성 사이토카인 자극을 받은 hUC-MSCs 가 연골 재생을 촉진하며, 염증성 환경에서 연골 치료를 촉진하는 데 마크로파지가 중요한 역할을 한다는 점을 보여주었습니다. 결국, 염증성 사이토카인 자극을 받은 hUC-MSCs 가 TMJOA 의 효과적인 치료를 위한 선택지가 될 수 있으며, 마크로파지가 면역 조절에서 핵심적인 역할을 한다고 생각됩니다.