Extracorporeal Shockwave Therapy Accelerates the Healing of a Meniscal Tear in the Avascular Region in a Rat Model

ABSTRACT

BACKGROUND: The treatment of meniscal tears in the avascular region remains a clinical challenge. Extracorporeal shock wave therapy (ESWT) is a minimally invasive, safe, and effective therapy for various orthopedic disorders. However, the therapeutic effect of ESWT on meniscal tears has not been reported.

PURPOSE: The purpose of the present study is to evaluate the therapeutic effect of ESWT in the treatment of meniscal tears.

STUDY DESIGN: Controlled laboratory study.

METHODS: Twelve-week-old male Wister rats were divided into three groups (Normal, ESWT [-], and ESWT [+]). We made a full-thickness 2-mm longitudinal tear in the avascular region in the latter 2 groups. At one week after surgery, the ESWT (+) group received 800 impulses of shockwave at 0.22 mJ/mm^2 energy flux density in a single session. We performed a pathological examination to evaluate meniscal healing (n=10 for each group), and immunohistochemistry to analyze the expression of bromodeoxyuridine (BrdU) and CCN family member 2 (CCN2) at 2, 4, and 8 weeks after ESWT (n=5 for each group). The CCN2, Sry-type high mobility-group box 9 (SOX 9), Vascular Endothelial Growth Factor (VEGF-a), Aggrecan, collagen type 1 alpha 2 (Col1a2) and collagen type 2 alpha 1 (Col2a1) levels at the site of the meniscal tear at 4 weeks after ESWT were quantitatively evaluated by a real-time PCR (n=5 for each group).

RESULTS: The meniscus healing scores in the ESWT (+) group were significantly higher than those in the ESWT (-) group at 4 and 8 weeks. The ratio of BrdU-positive cells and CCN2-positive cells were the highest in the ESWT (+) group among the three groups. In the ESWT (+) group, the real-time PCR revealed that the levels of CCN2, SOX9, Aggrecan and Col2a1 were upregulated. All significant data were $p < 0.05$.

CONCLUSION: ESWT promoted the healing of meniscal tears in the avascular area. ESWT stimulated proliferation of meniscus cells and the upregulation of cartilage-repairing factors such as CCN2, with the upregulation of the cartilage-specific extracellular matrix expression.

Clinical Relevance: ESWT may be an effective therapeutic option that promotes meniscal healing in the avascular region.

Keywords: extracorporeal shockwave therapy (ESWT), meniscal healing, meniscal tear, CCN2, SOX9

What is known about this subject: In general, a meniscus tear in the avascular region does not heal well even if meniscus suture is performed. Recently, biological augmentative treatment including fibrin clots and PRP is performed to improve meniscal healing in the avascular region. However, meniscal repairs with these therapies do not always have good results. Therefore, additional treatment options that improve meniscal healing are required.

Extracorporeal shock wave therapy (ESWT) is a minimally invasive, safe, and effective therapy for various orthopedic disorders including tendinopathy, calcification and OA. However, the therapeutic effect of ESWT on meniscal tears is unknown.

What this study adds to existing knowledge: ESWT promoted the healing of meniscal tears in the avascular area. Although this is still a hypothesis, this study indicated that ESWT provided a therapeutic effect on meniscal tears via upregulation of the mRNA expression of CCN2 and SOX9, thus suggesting that ESWT is beneficial as a useful therapy for meniscal tears.

INTRODUCTION

The meniscus plays a crucial role in the knee load motion, stability, and shock absorption of the knee[7, 21]. Meniscus injury causes a significant loss in the function of the knee and promotes progression to osteoarthritis (OA) [5, 22]. In humans, the perimeniscal capillary plexus supplies blood flow to approximately 25% of the outside of the meniscus; the remaining 75% of the meniscus forms the avascular region[1, 27, 28]. Several studies have shown that meniscal tears in the avascular region have the poor restorative ability, and successful healing is not achieved with suture repair alone[6, 29]. Thus, augmentative treatments, such as fibrin clots[16] and platelet-rich plasma[11] are used to improve meniscal healing; however, meniscal repairs with these therapies do not always have good results.

Previous studies showed that neovascularization and the blood supply are crucial factors in the process of meniscal healing.[20, 28] This healing process, which is similar to that of general wound healing, is considered to be an extrinsic process and has been observed in the early phase.[12] In addition, recent studies reported that inner meniscal cells express CCN family member 2 (CCN2) and Sry-type high mobility-group box 9 (SOX9), indicating that the avascular region of the meniscus has characteristics similar to articular cartilage.[9, 10] For meniscal healing in the avascular region, the importance of stimulating these intrinsic factors has been suggested.[13]

Extracorporeal shockwave therapy (ESWT) has been used for the treatment of various orthopedic disorders, including tendinopathies[24, 25, 30, 32]. Low-energy ESWT exerts its function through promoting biological processes, including tissue regeneration, bone remodeling, anti-inflammation and cartilage protection[4, 34-36, 38]. The efficacy of the ESWT is caused by the direct stimulation of the cells and can be ascribed to the transduction of the acoustic shockwave signal into biological signals that result in cell proliferation or differentiation through a mechano-transduction process[8, 37].

The purpose of the present study is to evaluate the therapeutic effect of ESWT in meniscal tears. In addition, this study focused on several genes that are known to play a crucial role in the healing process of the meniscus. We hypothesized that ESWT would accelerate the healing of meniscal tears in the avascular region in a rat model.

MATERIALS AND METHODS

Animals

This study was approved by the Animal Care and Experimentation Committee, Gunma University (No. 16-041). All efforts were made to minimize the number of animals used and the suffering of all animals. Twelve-week-old male Wister rats (body weight, 240–260 g) were purchased from SLC Japan (Hamamatsu, Japan). All rats were housed at the Biological Resource Center of our institution under controlled temperature (24°C) of light/dark and were fed a standard commercial diet with *ad libitum* access to tap water.

Surgical procedure

Ninety Wister rats were divided into three groups (Normal [untreated], ESWT [-], and ESWT [+]). Seventy-five rats were used for pathological examination and 15 were analyzed by a real-time PCR. The Normal group was left without surgery. A meniscal tear was created in the two other groups. Rats in the surgical groups were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (12 mg/kg). According to the method of a previous study[17], the right knee was exposed using the medial parapatellar approach with the patella laterally dislocated, and the medial meniscus was identified at the knee joint in full flexion. A full-thickness 2-mm longitudinal tear was made in the avascular region (white–white zone) of the anterior horn of the right medial meniscus of the rat using a scalpel. The capsule and skin were

then closed. The rats in both groups were allowed to move and feed themselves freely in the cages. Rats with significantly restricted activity after surgery were not identified. None of the rats used for experiments had any wound infections.

ESWT protocol

According to the method of a previous study, [35] the ESWT (+) group received 800 impulses of shockwave at 0.22 mJ/mm^2 energy flux density under general anesthesia in a single session at one week after the surgery (Dornier MedTech.; Dornier ARIES Vet, Germany). The ESWT probe was applied slightly forward of the medial joint line in full flexion under anesthesia with an intraperitoneal injection of ketamine and xylazine.

Sample collection and preparation

The rats were euthanized at 2, 4, and 8 weeks following ESWT. Then, the medial meniscus was taken from the knee joint and the surrounding synovial membrane was removed. The collected meniscus was immediately soaked in formalin for 24 hours for histological evaluation. After that, specimens were immersed in neutral decalcification solution (Yuaikasei, Hyogo, Japan) for 5 days and embedded in paraffin. The meniscus specimens for the real-time polymerase chain reaction were frozen in liquid nitrogen immediately after collection and stored at -80ºC until use.

120

121 **Histologic evaluation of meniscal healing**

122 We used specimens obtained at 2, 4, and 8 weeks after ESWT for evaluation in the ESWT (-)
123 group and the ESWT (+) group (n=10 for each group). The paraffin blocks were cut at 2.5 μm,
124 and serial sections perpendicular to the defect were stained with hematoxylin-eosin (HE) and
125 Safranin O/ Fast green. We determined the meniscus healing score, which evaluates the
126 existence of connective tissue and its amount at the site of the meniscus tear. The score ranges
127 from 0 to 3 points (0 points, no noticeable reaction at all; 1 point, no bridge linking the two
128 components; 2 points, connective tissue between the two components; 3 points, explants, with
129 fibrous continuity between both sides of the gap)[19]. All specimens were assessed independently
130 by two orthopedic surgeons (O.T. and S.H.). The inter-observer intraclass correlation
131 coefficient was 0.85, and the intra-observer intraclass correlation coefficient was 0.91.

132

133 **Immunohistochemical analyses**

134 We used specimens obtained at 2, 4, and 8 weeks after ESWT for the evaluation in the Normal,
135 ESWT (-) and ESWT (+) groups (n=5 for each group). Immunohistochemical staining of 2.5-
136 μm-thick sections was performed using a streptavidin–biotin–peroxidase system kit (Histofine,
137 Nichirei, Japan) and chromogen (diaminobenzidine). Specimens were stained with rabbit
138 monoclonal anti-bromodeoxyuridine (BrdU) antibody (concentration 1:200; Abcam,

Cambridge, MA, USA) to analyze the proliferation rate of meniscal cells, anti-CCN2 antibody (concentration 1:400; Abcam) to analyze the change in the ratio of cells expressing cartilage-repairing factor, and mouse polyclonal anti-collagen type 2 antibody (concentration 1:100; Abcam) to analyze the progress of meniscal healing. We administered BrdU diluted to 0.8 mg/mL in drinking water for 9 days from the day after ESWT and changed the water daily (drinking water method)[33]. The ratios of BrdU- and CCN2-positive cells to all cells were calculated using an automated cell counter plugin software program (GunmaLI as a plug-in for ImageJ)[31] at ×10 magnification.

Real-time PCR

For the PCR, a 2 mm × 2 mm piece of medial meniscus, including the injured site, was resected from the ESWT (-), and ESWT (+) groups at 4 weeks after ESWT. In the Normal group, the same size piece of the medial meniscus at the same site was harvested from the rats of the same age (n = 5 for each group). For this experiment, a Minilys homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) was used to homogenize the piece of the meniscus (5000 rpm, 5 cycles of 30 seconds). Total RNA was isolated from the meniscus using an RNeasy Mini Kit (Qiagen, Hilden, Germany), and cDNA was synthesized from isolated total RNA using a ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). SYBR® Green Realtime PCR Master Mix (Toyobo) and a StepOne™ Real-Time PCR System (Applied Biosystems,

158 Carlsbad, CA, USA) were used to perform the real-time PCR. The quantified relative
159 expression of the gene of interest was normalized to the GAPDH housekeeping gene by the
160 ΔCT method. The nucleotide sequences of the primers are shown in Table 1.
161

Table 1. Primers

Gene		Primer sequence
CCN2	Forward	5’-CCACCCGAGTTACCAATGAC-3’
	Reverse	5’-GTGCAGCCAGAAAGCTCA-3’
SOX9	Forward	5’-AGACCAGTACCCGCATCT-3’
	Reverse	5’-CGCTCCGCCTCCTCCAC-3’
VEGF-a	Forward	5’-TTCAGAGCGGAGAAAGCATT-3’
	Reverse	5’-GAGGAGGCTCCTTCCTGC-3’
Acan	Forward	5’-TTGGAGCCGGAGACGACAGA-3’
	Reverse	5’-AGAGGCAGAGGGACTTTCGGT-3’
Col1a2	Forward	5’-CCGTGCTTCTCAGAACATCA-3’
	Reverse	5’-CTTGCCCCATTCATTTGTCT-3’
Col2a1	Forward	5’-TTCCTCCGTCTACTGTCCACTGA-3’
	Reverse	5’-CTACATCATTGGAGCCCTGGAT-3’
GAPDH	Forward	5’-GTCTTCACTACCATGGAGAAGG-3’
	Reverse	5’-TCATGGATGACCTTGGCCAG-3’

Statistical analysis

All statistical analyses were performed using the SPSS 25.0 software program (IBM Corp, Armonk, NY, USA). Data were analyzed by a one-way analysis of variance (ANOVA), as applicable, followed by Tukey's post hoc analysis for the detection of differences across the three groups. The Mann–Whitney U test was used for the detection of differences between two groups. P values of <0.05 were considered to indicate statistical significance.

RESULTS

Meniscal healing after ESWT

The pathological findings of the normal meniscus with HE staining and Safranin O staining are shown in Fig. 1A. The pathological findings of the site of the tear with HE staining are shown in Fig. 1B. At 2 weeks, meniscal healing was not completed in any of the rats. At 8 weeks, partial bridge linking was observed in 6 rats in the ESWT (-) group, but complete bridge linking was not observed. However, bridge linking was observed in all rats in the ESWT (+) group (partial, n=6; complete, n=4).

The pathological findings of the site of the tear with Safranin O staining and immunostaining of type 2 collagen are shown in Fig. 1C. In the ESWT (+) group, deep staining of Safranin O was observed around the injured site of the meniscus compared to the ESWT (-) group. Type 2 collagen expression was observed around the injured site of the meniscus in the ESWT (+) group compared with the ESWT (-) group.

The meniscus healing scores are shown in Fig. 1D. The scores gradually increased in both groups. In the ESWT (+) group, there was a significant difference from 2 weeks to 4 weeks ($p = 0.019$). However, this score did not change in the ESWT (-) group at any of the observation time points. At 2 weeks, the healing scores of the ESWT (+) and ESWT (-) groups did not differ to a statistically significant extent. However, at 4 weeks, the score of the ESWT (+) group was

189 significantly higher in comparison to the ESWT (-) group at 4 weeks (1.4±0.3 vs. 1.0±0.2,

190 p=0.009), and 8 weeks (2.4±0.2 vs. 2.0±0.2, p=0.015).

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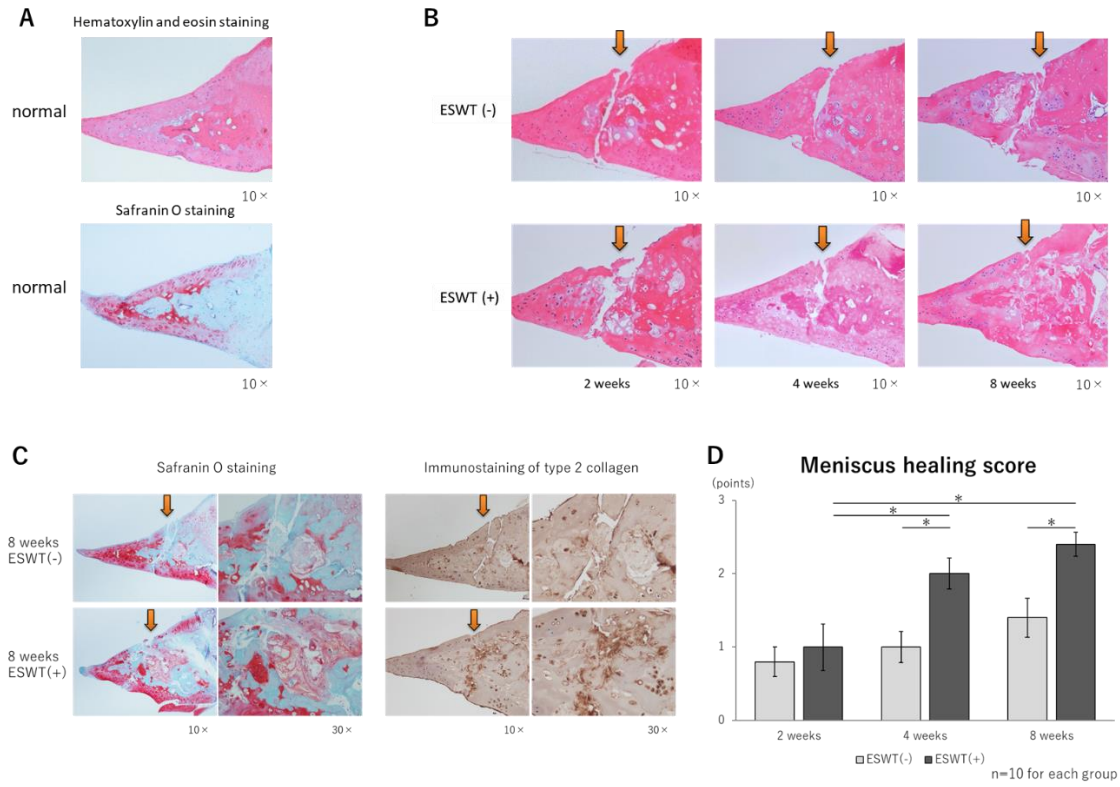

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193 **Figure 1. Histological findings of meniscus tear and the evaluation of meniscal healing.**

194 (A) Pathological findings of normal meniscus with HE staining and Safranin O staining. (B)

195 HE staining at the site of the meniscus tear in the ESWT (-) group and the ESWT (+) group at

196 2, 4, and 8 weeks after ESWT. Arrow, injured site. (C) Safranin O staining and immunostaining

197 of type 2 collagen at the site of the meniscus tear in the ESWT (-) group and the ESWT (+)

198 group at 8 weeks after ESWT. Arrow, injured site. (D) The meniscus healing score at 2, 4, and

199 8 weeks after ESWT. This score evaluated the extent of the healing of the meniscal tear (0-3

200 points) based on the findings of HE staining. Data were expressed as the mean and the error

201 bar represents the SE. P values were determined using the Mann–Whitney U test. An asterisk

202 indicates a significant difference (n=10 for each group). HE, hematoxylin and eosin; ESWT,

203 extracorporeal shockwave therapy.

204

The ratio of BrdU- and CCN2-positive cells

The ratio of BrdU-positive cells to all cells is shown in Fig. 2E. This ratio did not change at any of the observation time points in the Normal, ESWT (-), or ESWT (+) groups (approximately 50%, 50%, and 65%, respectively). This ratio was significantly higher in the ESWT (+) group than in the Normal and ESWT (-) groups at 2 weeks ($p=0.008$, $p=0.046$), 4 weeks ($p=0.025$, $p=0.008$), and 8 weeks ($p<0.001$, $p=0.009$) However, there were no significant differences between the Normal and ESWT (-) group at 2, 4, and 8 weeks.

The ratio of CCN2-positive cells to all cells is shown in Fig. 3E. This ratio was approximately 40% in all groups at 2 weeks. In the ESWT (+) group, this ratio significantly increased to 60.8% at 4 weeks ($p=0.001$), and then significantly increased to 64.5% at 8 weeks ($p=0.001$). However, in the ESWT (-) group, this ratio significantly increased to 50.7% at 8 weeks ($p<0.001$). In the Normal group, this ratio did not change at any of the observation time points. In the ESWT (+) group, this ratio was significantly higher than that in the Normal and the ESWT (-) groups at 4 weeks ($p<0.001$, $p<0.001$), and 8 weeks ($p<0.001$, $p=0.001$).

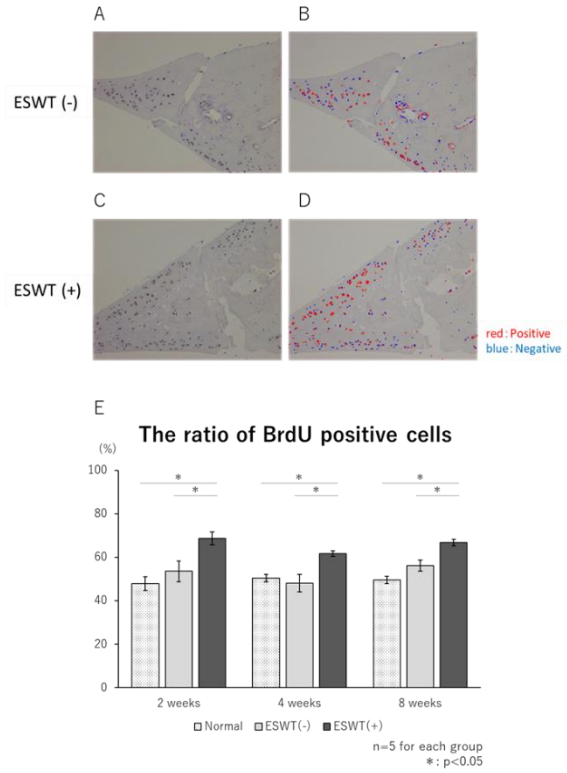

Figure 2. Immunostaining with anti-BrdU antibody

(A, B) A representative measurement of the ratio of BrdU-positive cells to all cells in the ESWT (-) group at 4 weeks. The analysis was performed with Gunma LI (A), and positive or negative cells were automatically calculated as shown in (B). The ratio, in this case, was 50.2%. (C, D) A representative measurement of the ratio of BrdU-positive cells to all cells in the ESWT (+) group at 4 weeks. The ratio, in this case, was 63.3%. (E) Comparison of the ratios of BrdU positive cells to all cells at 2, 4, and 8 weeks after ESWT. In the Normal, ESWT (-), and ESWT (+) groups, this ratio was 47.9±3.1%, 53.6±4.7%, and 68.8±3.0%, respectively, at 2 weeks; 50.5±1.7%, 48.1±4.0%, and 61.7±1.2% at 4 weeks; and 49.6±1.6%, 56.2±2.6%, and 66.9±1.5% at 8 weeks. The data are expressed as the mean and error bar represents the SE. The

231 p values were obtained using ANOVA and post hoc test with Tukey's analysis. An asterisk
232 indicates a significant difference (n=5, for each group). BrdU, bromodeoxyuridine; ESWT,
233 extracorporeal shockwave therapy.
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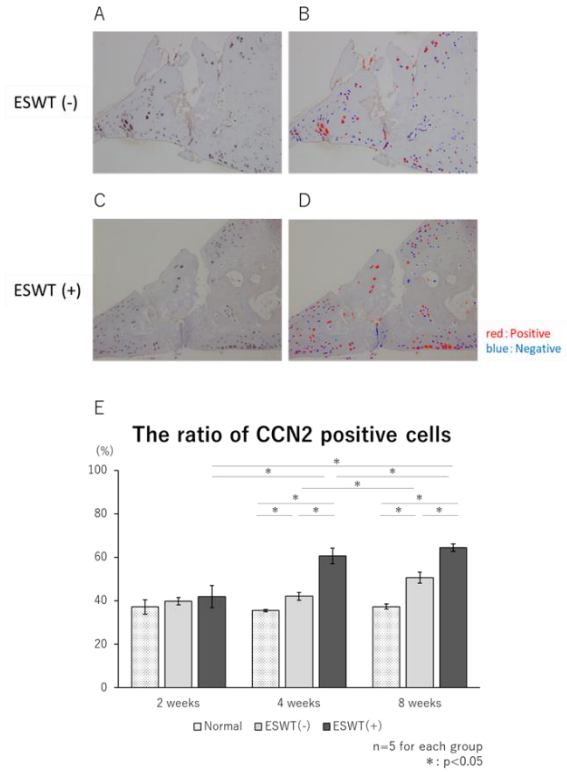

Figure 3. Immunostaining with anti-CCN2 antibody

(A, B) A representative measurement of the ratio of CCN2-positive cells to all cells in the ESWT (-) group at 4 weeks. The ratio, in this case, was 46.2% (C, D) A representative measurement of the ratio of CCN2-positive cells to all cells in the ESWT (+) group at 4 weeks. The ratio, in this case, was 59.4%. (E) Comparison of the ratio of CCN2-positive cells to all cells at 2, 4, and 8 weeks after ESWT. In the Normal, ESWT (-), and ESWT (+) groups, this ratio was 37.2±3.2%, 39.8±1.7%, and 41.9±5.1%, respectively, at 2 weeks; 35.5±0.6%, 42.1±1.8%, and 60.8±3.7% at 4 weeks; and 37.4±1.2%, 50.7±2.5%, and 64.5±1.7% at 8 weeks. The data are expressed as the mean and error bar represents the SE. The p values were obtained using ANOVA and post hoc test with Tukey's analysis. An asterisk indicates a significant

247 difference (n=5, for each group). CCN2, CCN family member 2; ESWT, extracorporeal

248 shockwave therapy.

249

The gene expression in the meniscus

The expression of CCN2, SOX9, Vascular Endothelial Growth Factor (VEGF-a), aggrecan, collagen type 2 alpha 1 (Col1a2), and collagen type 2 alpha 1 (Col2a1) was examined at four weeks after the ESWT by real-time PCR (Fig. 4). Regarding the mRNA expression of factors related to meniscal healing, CCN2 was significantly upregulated in the ESWT (+) group to a level approximately 2.5-fold higher than that in the Normal and ESWT (-) groups (Fig. 4A, $p=0.003$ and $p=0.005$, respectively). SOX9 was also significantly upregulated in the ESWT (+) group, to a level approximately 3.5-fold higher than that in the Normal and ESWT (-) groups (Fig. 4B, $p=0.002$ and $p=0.003$, respectively). However, the mRNA expression of VEGF-a in the three groups did not differ to a statistically significant extent (Fig. 4C).

Regarding the mRNA expression of cartilage-specific extracellular matrix (ECM) components, the mRNA expression of Aggrecan and Col2a1 in the ESWT (+) group were significantly increased to levels approximately 4- and 3.5-fold higher than those in the ESWT (-) group, respectively (Fig. 4D and 4F, $p=0.042$ and $p=0.039$). However, the difference between the Normal and ESWT (-) groups was not significant. The mRNA expression of Col1a2 in the three groups did not differ to a statistically significant extent (Fig. 4E).

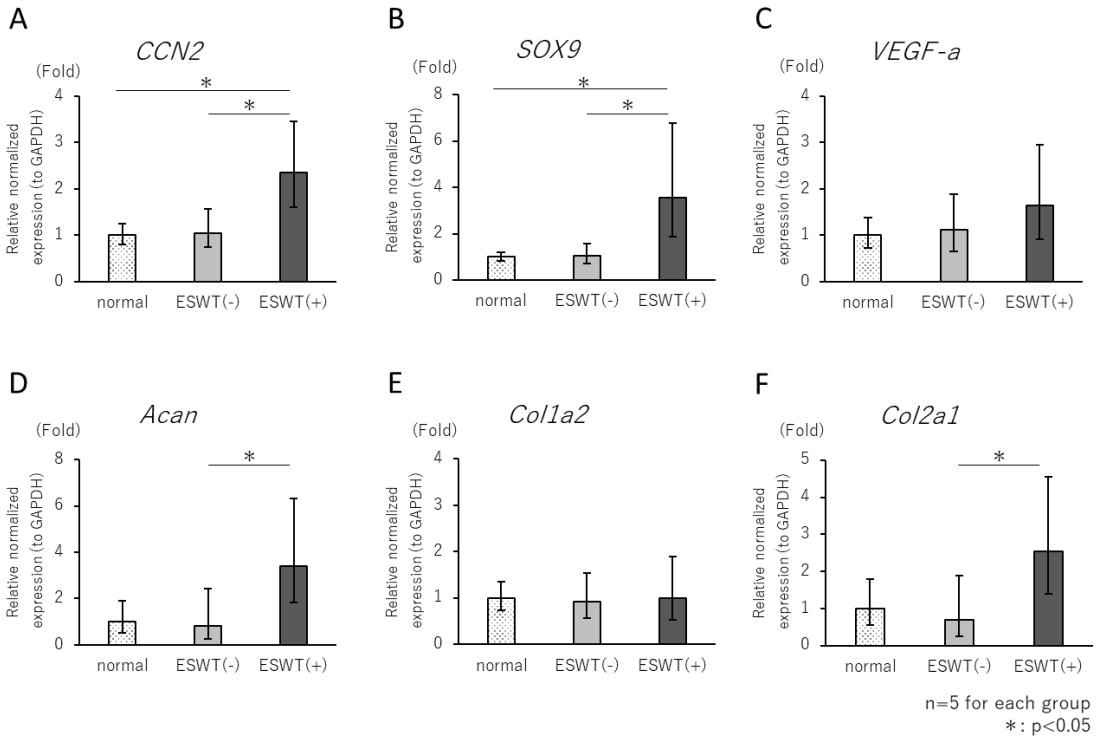

Figure 4.

The mRNA levels of CCN2 (A), SOX9 (B), VEGF-a (C), Aggrecan (D), Col1a2 (E), and Col2a1 (F) were analyzed by a real-time PCR. The amounts of these mRNAs were normalized to the amount of GAPDH mRNA. In all graphs, the ordinate indicates the relative ratio to the Normal group. The error bar represents the SD. The p values were obtained using an ANOVA and post hoc test with Tukey's analysis. An asterisk indicates a significant difference (n=5, for each group). CCN2, CCN family member 2; SOX9, Sry-type high mobility-group box 9; VEGF-a, Vascular Endothelial Growth Factor; Acan, Aggrecan; Col1a2, collagen type 1 alpha 2; Co2a1, collagen type 2 alpha 1; ESWT, extracorporeal shockwave therapy.

DISCUSSION

This study has two main findings. First, ESWT can accelerate meniscal healing in the avascular region and stimulates the proliferation of meniscus cells. Second, ESWT induces the upregulation of cartilage-repairing factors such as CCN2 with the ECM gene expression. To our knowledge, this is the first report of a beneficial effect of ESWT on meniscal healing.

In the present study, we have shown that ESWT promotes the healing of meniscal tears in the avascular region and stimulates the proliferation of meniscus cells, as confirmed by the improved meniscus healing score and the increased ratio of BrdU-positive cells. A previous study reported that the progression of meniscal healing in the avascular region proceeds in parallel with cell proliferation[17]. In the drinking water method, BrdU is taken up in DNA during the synthesis phase of the cell cycle during a 48 h period, and it remains in the cell for 70 days after the 9-day administration period[33]. Since we started the administration on the day after ESWT, we could accurately evaluate the effect of single-session ESWT on the early phase after injury.

In the current study, ESWT caused the upregulation of the mRNA expression of CCN2, SOX9 and cartilage-specific ECM components, such as Aggrecan and Col2a1, in the ESWT (+) group comparison to the ESWT (-) group. CCN2 is a cysteine-rich protein that strongly promotes the production of cartilaginous matrix proteins and stimulates the proliferation of chondrocytes and the hypertrophic differentiation of growth plate chondrocytes[41]. SOX9 is a

298 chondrogenic transcription factor that is expressed in chondrocytes, and this factor is essential
299 to chondrocyte differentiation and cartilage formation[2]. Oh et al. reported that SOX9 directly
300 regulates the CCN2 transcription in growth plate chondrocytes and suggested that CCN2 is
301 located downstream of SOX9[26]. Furumatsu et al. reported that human meniscus cells in the
302 avascular region have a chondrocytic morphology expressing CCN2 and SOX9, and an ability
303 to produce cartilage-specific ECM components, similar to articular cartilage[9, 10]. He et al.
304 demonstrated that CCN2 was able to promote ECM deposition (types I and II collagen) within
305 the meniscal avascular region, and further enhance meniscal healing in this region[13].
306 The authors of a recent study reported that ESWT is considered to induce cellular
307 mechanotransduction process and modulate cellular metabolism and tissue homeostasis via the
308 mechanosensory units integrated into the cell membrane[18]. The stimulation can also influence
309 conformational changes in membrane proteins and activate ion channels and transporters to
310 send messages to cells in signaling pathways. ESWT promotes tissue regeneration via the
311 upregulation of the mRNA expression of VEGF-a, proliferating cell nuclear antigen (PCNA),
312 transforming growth factor-beta (TGF-b1), and bone morphogenetic proteins (BMPs)[14, 37, 39,
313 40]. In summary, ESWT can upregulate various growth factors by directly stimulating cellular
314 tissue or cells. In the present study, ESWT promoted the mRNA expression of CCN2 and SOX9
315 at four weeks after this therapy, although activation of the mRNA expression of VEGF-a was
316 not demonstrated. Furthermore, ESWT enhanced the mRNA expression of ECM. These results

led us to hypothesize that ESWT promotes meniscal healing via signal transduction of the stimulated meniscal cell and the activation of these factors.

Recently, a similar therapeutic effect on meniscal tears by external stimulation other than ESWT has been reported. Kumatsuki et al. demonstrated that low-intensity pulsed ultrasound (LIPUS) treatment might protect the meniscus from degenerative change and exert a reparative effect on the meniscal tear via the upregulation of the mRNA expression of CCN2 and SOX9[15]. Yilmaz et al. reported that ESWT and LIPUS have systemic proliferative and regenerative effects on cartilage[42]. They also reported that the therapeutic effects of the two treatments on cartilage do not differ to a statistically significant extent. Although the mechanism of meniscal healing is still incompletely understood, inducing factors related to cartilage repair and chondrogenesis by external stimulation including ESWT will have a positive effect for meniscal tears in the avascular region.

The present study was associated with some limitations. First, since the whole rat right knees were exposed to ESWT, this therapy might have affected the tissue surrounding the knee other than meniscus tissue. The delivery of ESWT into the knee joint may have several pleiotropic effects on several tissues. Other factors affecting the tissue healing response in the knee joint include biomechanical forces, blood supply, nutrient delivery, synovial fluid and the supply of various growth factors[3, 23]. Further studies are needed to elucidate the detailed cellular mechanism through which ESWT accelerates meniscal healing. Second, the genes assessed

were not numerous or diverse. In this study, we focused on several genes that are known to play a crucial role in the healing process of the meniscus. An exhaustive analysis of the gene expression might provide further information. Third, we performed assessments for up to eight weeks after ESWT, which is still relatively early in the healing process, as we wanted to determine whether or not ESWT accelerates meniscal healing. Therefore, the healing process through completion has not been confirmed. Fourth, functional improvements in the healing outcomes were not quantitatively assessed in this study. The final limitation is our use of rat models with different motor characteristics and weight bearing from humans. These results must be carefully interpreted before our findings can be applied to clinical practice.

CONCLUSION

ESWT accelerated meniscal healing in a rat model of meniscal tear in the avascular region. ESWT promoted cellular proliferation at the site of the meniscus tear, which was represented by an increasing ratio of BrdU-positive cells. ESWT was found to upregulate the mRNA expression of CCN2 and SOX9, as assessed by both immunohistochemical staining and a real-time PCR. ESWT also upregulated the mRNA expression of ECM components at the site of the meniscal tear. The present results suggest that ESWT may be applicable as a healing promotion therapy for meniscal tears in the avascular region.

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