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Does extracorporeal shockwave therapy treat leg length discrepancy? an experimental animal study

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Abstract

Background Extracorporeal shockwave therapy (ESWT) is widely used to treat musculoskeletal diseases, but its impact on adolescents with unhealed epiphyseal plates is concerning. It remains unclear whether ESWT applied to growth plates promotes or inhibits bone growth. Low energy ESWT does not cause damage of articular cartilage and promotes the growth of articular cartilage. Therefore, the application of ESWT to treat the leg length discrepancy is a possibleoption.

Methods Here, the 96 adolescent rats were used to demonstrate that different levels of ESWT developed different effects on the epiphyseal plate and bone growth. The effects and safety of ESWT on the epiphyseal plate were measured at different energy levels of 0.1, 0.25, and 0.5 mJ/mm^2 with 800 impulses, 4 Hz at the 7, 13, and 25 weeks.

Results Additionally, the treatments promoted the growth and length of the tibia bone as the ESWT application by compared with Sham group. Notably, ESWT stimulated the expression of IL-1β at the 7 week, which then decreased by the 25 week. However, no apoptosis signals and cell death were detected, and there was no tissue damage to the epiphyseal plate. The expression of SOX9, BMP2, and BMP4 was observed in the epiphyseal plate following ESWT, suggesting a role in promoting bone growth.

Conclusion Our results suggest that ESWT is a safe therapeutic modality for stimulating bone growth at the epiphyseal plate in adolescents, leading to increased bone length. This approach holds potential for future treatment of patients with leg length discrepancies.

Keywords Extracorporeal shockwave therapy, Epiphyseal growth plate, Leg length discrepancy, BMP pathway

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Background

Physeal injury is a common musculoskeletal disorder in pediatric patients, encompassing conditions such as apophysitis, transphyseal fractures, and physeal arrests, often leading to joint angular deformities and leg length discrepancies [1]. Apophysitis, commonly associated with sports activities, is a self-limited condition resulting from repeated mechanical stress at the apophysis, leading to chronic inflammation at the affected sites. It can present as conditions such as Osgood-Schlatter disease, little league shoulder, little league elbow, Sever's disease, or Iselin's disease [2]. In the acute phase, treatment typically focuses on conservative approaches, including pain control, rehabilitation, and rest [3]. However, managing chronic apophysitis remains challenging, with no universally effective treatment identified [4]. Physeal fractures are another concern, as the structure of the physis represents a relatively weak point in long bones, making it susceptible to trauma. The force of the injury often passes through the physis, leading to fractures that typically require surgical internal fixation [5]. During the healing process, complications such as angular deformities may arise due to overgrowth, undergrowth, or physeal arrest. Physeal arrest, often a consequence of trauma or infection, can result in significant joint deformities and leg length discrepancies. Treatment options for such deformities vary based on patient age and the severity of the condition. For younger patients, corrective orthoses are initially considered, while more advanced deformities necessitate surgical interventions, such as corrective osteotomy [6]. For patients with leg length discrepancies (LLD), treatment methods consist of physeal bar removal, chondrodiastasis, epiphysiodesis, and the use of circular fixation for limb lengthening or deformity correction [1, 7]. Early management is crucial in these cases to prevent further complications and to improve patient outcomes.

In light of this, extracorporeal shockwave therapy (ESWT) has emerged as a promising non-surgical alternative in musculoskeletal treatments [8]. Although traditionally used for conditions like chronic tendinitis and fracture nonunion, ESWT offers potential advantages in the treatment of conditions affecting the epiphyseal plate, despite some caution in pediatric cases. It provides the physicians various effective and non-surgical alternative in treating the recalcitrant situations such as chronic tendinitis and fasciitis, calcified tendinitis and fracture nonunion [8]. However, the 2016 International Society for Medial Shockwave Treatment (ISMST) guideline lists that high energy focused ESWT is contraindicated in the treatment of area around the epiphysis, which restrict the application of ESWT in the population of pediatrics and adolescents. Almost all of the studies from the literature concerning ESWT at the epiphysis are based on animal models. Dr. Yeaman suggests that high energy ESWT causes extensive dysplastic lesion in the growth plate with shortening of the limb in a rat model [9]. Bussy reports the use of ESWT at the convex side of the angular deformity for the treatment of carpal joint valgus deformities in young foals [10].

Contrary to previous concerns, histological examinations have shown no damage to the rabbit epiphysis following ESWT treatment [11]. Additionally, high-energy ESWT has been reported to stimulate growth in the rabbit epiphysis, with overgrowth observed in the femur shaft of immature rabbits [12, 13]. Long-term studies have indicated increased cellularity and basophilia of the extracellular matrix in the adolescent rat epiphysis without negative effects on extremity measurements [14]. Further research has demonstrated dose-dependent effects of ESWT in promoting new bone formation in the femur, while other studies have shown accelerated healing of osteochondritis dissecans in rabbit models [15, 16]. These findings collectively support the potential of ESWT as a beneficial therapeutic approach for bone growth and repair.

Previous studies have demonstrated the chondroprotective effects of ESWT in the treatment of knee osteoarthritis, showing promising results in preserving cartilage [17–19]. In addition to these therapeutic benefits, histological examinations revealed thickening of the physeal plate and hypercellularity of the epiphysis following ESWT. This study further highlights the dose-dependent effects of ESWT on the epiphyseal plate, with varying energy flux densities producing distinct outcomes on bone growth. The ESWT induced undergrowth might be utilized as a technique for epiphysiodesis in patients with leg length discrepancies or angular deformities.

Materials and methods

Animals

All rats (96 rats, aged 4 weeks) were handled with care and in compliance with the ethical standards following the Guide for the Care and Use of Laboratory Animals, as published by the National Institutes of Health. All animals were housed and two rats in a cage under standard conditions which was room temperture at 23 ± 1 °C, humidity at 50 ± 20%, and with a 12-h light and dark cycle. The Center for Laboratory Animals at Hospital, administered veterinary care to the rats. In this study, adolescent rats were included based on their age and health status to ensure uniformity in the development of their epiphyseal plates. Animals exhibiting signs of illness or abnormal growth patterns prior to the experiment were excluded to avoid skewing the results. The study was performed following the ARRIVE guidelines [20] and received approval from the Institutional Animal Care and Use Committee (IACUC) at the hospital.

Study design

The experiments were performed with 96 Sprague-Dawley rats (4 weeks old). The sample size was determined using G*Power statistical analysis (version 3.1.9.7), which indicated that eight rats per group would provide sufficient statistical power (0.8) to detect a 10% difference in the experiment as the reference from the previous study [14]. All rats were randomly divided into four groups with different time points (8 rats for each group and each time point): Sham, 0.1, 0.25, and 0.5 mJ/mm^2 groups (Fig. 1A). The rats in the Sham group (zero energy) did not receive surgery or treatment (0 mJ/mm^2). In the 0.1 mJ/mm^2 group, the epiphyseal plate of left rat knee received ESWT with 0.1 mJ/mm^2, 800 impulses, 4 Hz. In the 0.25 mJ/mm^2 group, rats received ESWT with 0.25 mJ/mm^2, 800 impulses, 4 Hz. In the 0.5 mJ/mm^2 group, rats received ESWT with 0.5 mJ/mm^2, 800 impulses, 4 Hz. All rats were treatment at the first week

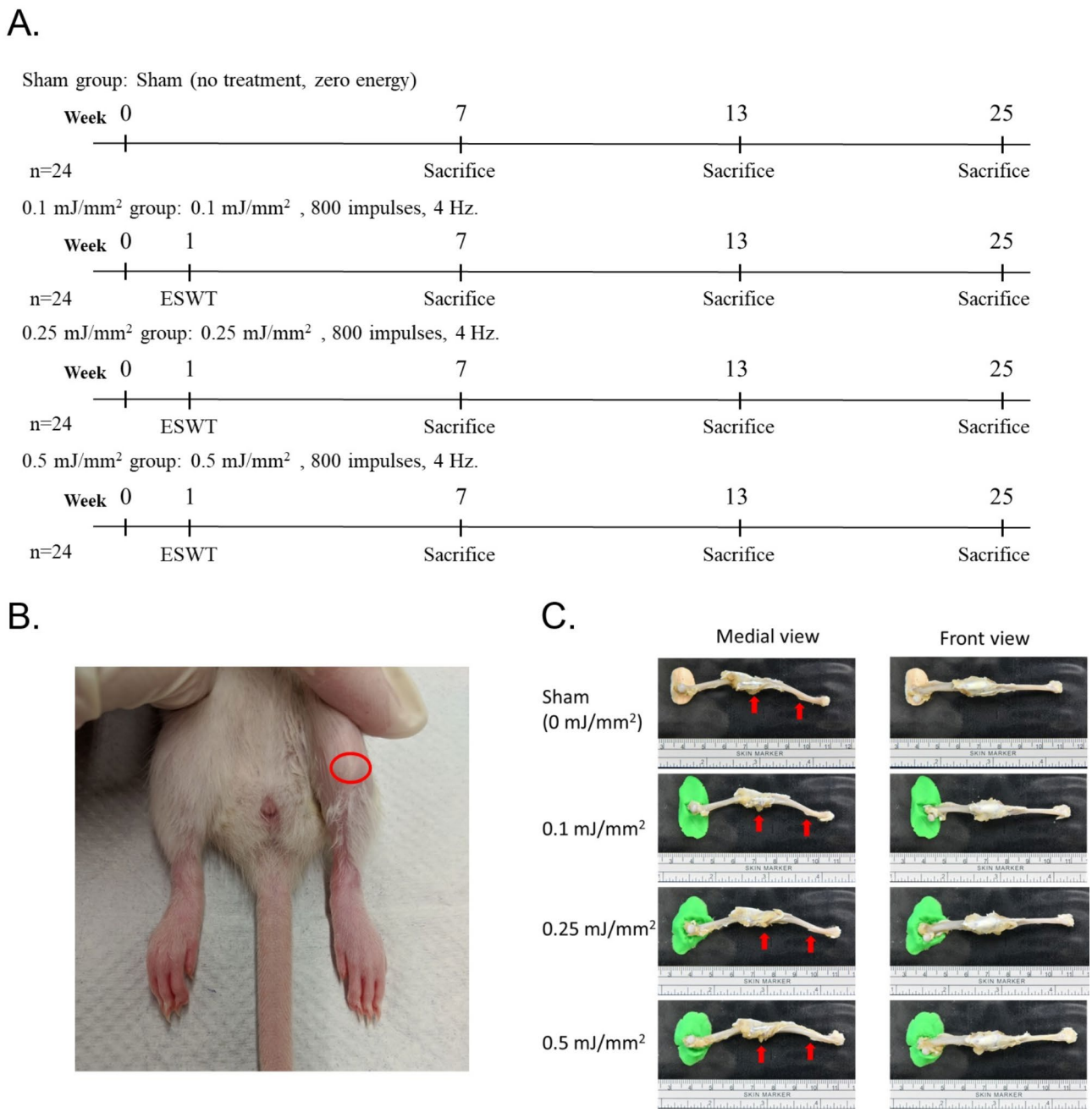

Fig. 1 (**A**) The experimental design. (**B**) The application of ESWT on epiphyseal plate of left knee of rat. The red circle is indicated the epiphyseal plate of rat and focused ESWT application by different energy. (**C**) The measurement of the length of tibia after ESWT on epiphyseal plate of rats in Sham (0), 0.1, 0.25 and 0.5 mJ/mm^2 groups. $N = 8$. ESWT = extracorporeal shockwave therapy

and postoperative care included administering prophylactic antibiotics, specifically ampicillin at 25 mg/kg, and ketorolac at 1 mg/kg/day for pain relief, both given for five days following the surgery. The rats were sacrificed at 7, 13 and 25 weeks for the experiments.

ESWT application

Shockwaves were generated using the DUOLITHR SD1 ultra shockwave machine (Storz Medical AG, Tägerwilen, Switzerland). The shockwaves were focused on the epiphyseal plate of the left tibial knee in rats, positioned 0.5 cm below the joint line and 0.5 cm from the medial skin surface. Three experimental groups received 800 shockwave impulses with energy flux densities of 0.1, 0.25, and 0.5 mJ/mm², at a frequency of 4 Hz, in a single treatment session. Following ESWT, the animals were returned to their housing cages for routine care and monitoring.

Specimen processing

The animals were euthanized at 7, 13, and 25 weeks for each group, and rat knees were harvested for histological and immunohistochemical analysis. The samples were then subjected to decalcification using a 10% PBS-buffered EDTA solution for a duration of 4 weeks at 4 °C, with the decalcification solution replaced every 3 days. After decalcification, the tissues were embedded in paraffin. Longitudinal sections of 5 μm thickness were cut and placed on slides (Thermo Fisher Scientific, USA) for subsequent analysis.

Histological analysis

The specimens were stained using both the traditional haematoxylin-eosin (H&E) method and safranin O. To quantify the results, five random areas from three sections of each specimen were analyzed with a Zeiss Axioskop 2 Plus microscope (Carl Zeiss, Germany). Images from each specimen were captured using a cool CCD camera (Media Cybernetics, USA). Manual counting was performed for image analysis, with results verified through the use of Image-Pro Plus Image Analysis software (Media Cybernetics). The histological evaluation was used a modified version of the guidelines for the growth plate established by Quintana [21].

TUNEL assay

Apoptosis in the specimens from poly-lysine-coated slides was assessed using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, following the procedure presented in the study [22]. TUNEL activity was performed using in situ cell death detection kits (Merck KGaA, USA) to identify apoptotic cells in the epiphyseal plate. The specimens were treated with a peroxidase-conjugated anti-digoxigenin antibody (R&D Systems, USA). After staining, a peroxidase substrate (Sigma-Aldrich, USA) was applied to visualize the TUNEL reaction by developing its color.

Immunohistochemistry

The IL-1β, SOX9, BMP2 and BMP4 antibodies were purchased. The specimens mounted on poly-lysine-coated slides were initially deparaffinized using Pro-Par Clearant (Anatech Ltd., USA), a xylene substitute, followed by ethanol treatment before being rehydrated in water. After rinsing with PBS, the sections were incubated with a blocking solution containing 0.1% Tween 20 and 3% normal goat serum for 30 min at room temperature. Primary antibodies of IL-1β (1:50; ab9787, Abcam, USA), SOX9 (1:50; ab26414, Abcam, USA) BMP2 (1:50; PA5-85956, Invitrogen, USA) and BMP4 (1:100; ab39973, Abcam, USA) were applied and incubated overnight at 4 °C. The immunoreactivity of the specimens was assessed using a horseradish peroxidase (HRP)-3′,3′-diaminobenzidine (DAB) staining kit for cells and tissues (R&D Systems, USA), following the procedures outlined in the study [22]. Positive immunolabeled cells were counted in five regions across three sections of each specimen using a Zeiss Axioskop 2 plus microscope (Carl Zeiss, Germany). Image analysis was performed with Image-Pro® Plus software (Media Cybernetics, USA) to process all images and data.

Statistical analysis

Statistical analysis was used SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean ± standard deviation (SD). To assess normality, the Kolmogorov-Smirnov test was applied to each variable. For variables following a normal distribution, comparisons were made using the paired Student's t-test. In cases where the data were nonparametric, the Wilcoxon Signed Ranks test was utilized for within-group analysis, while the Mann-Whitney U test was employed to compare differences between groups. Statistical significance was defined at p-values of less than 0.05, 0.01, and 0.001.

Results

ESWT on the epiphyseal plate promotes bone growth

The treatment groups of ESWT was applied at 0.1 mJ/mm², 0.25 mJ/mm² and 0.5 mJ/mm² with 800 impulses, 4 Hz, on the epiphyseal plate of left tibia (Fig. 1A and B). After sacrifice of rats, the right and left lower limbs were measured the length first. The length of tibia was measured as indication of the red arrow in the Fig. 1C. The data analysis and results showed no statistically significant changes in the length of the left tibia (ESWT applied) at the 7, 13, and 25 weeks (Fig. 2A and suppelmental Table 1). However, there was an improvement in the length of the left tibia treated with ESWT (0.1, 0.25,

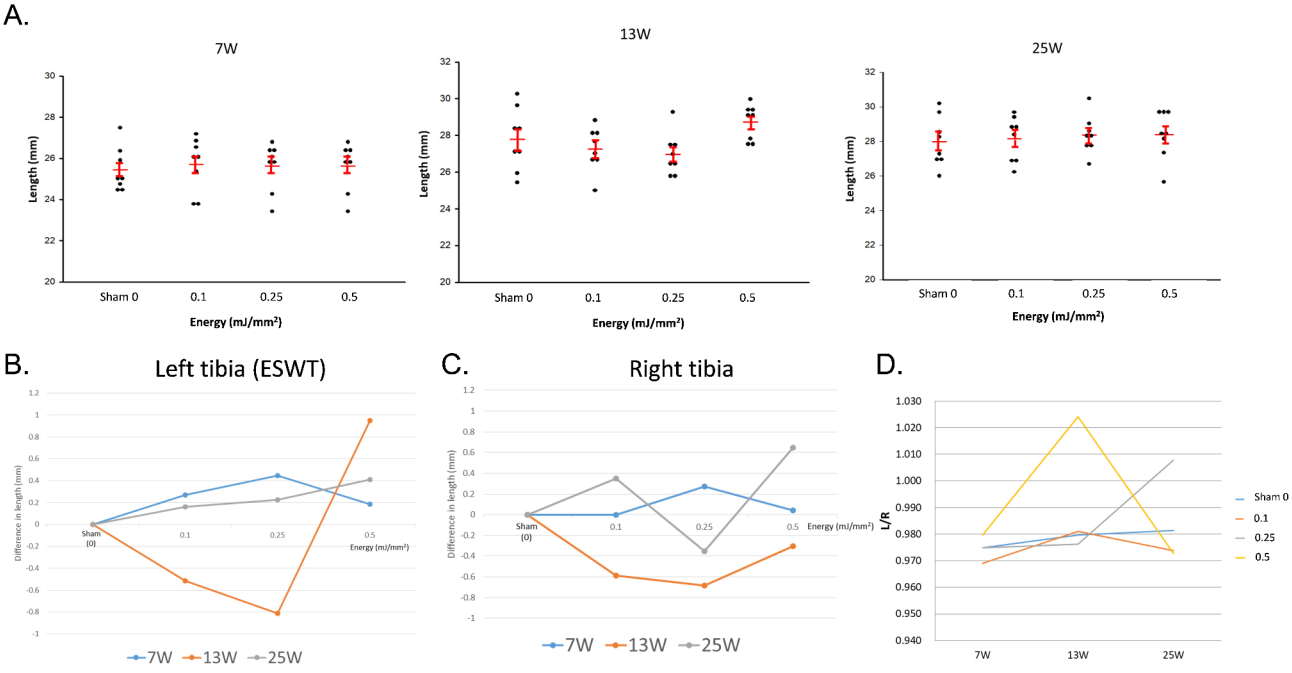

Fig. 2 The length of both tibias after ESWT on the epiphyseal plate of left tibia. (**A**) The length of left tibia after ESWT at 7, 13 and 25 weeks. (**B**) The increasing length of left tibia after ESWT as comapred with Sham group at 7, 13 and 25 weeks. (**C**) The increasing length of right tibia without ESWT as comapred with Sham group at 7, 13 and 25 weeks. (**D**) the length ratio of left tibia over right tibia at 7, 13, and 25 weeks. ESWT = extracorporeal shockwave therapy. R = right tibia and L = left tibia. $N = 8$

Table 1 The markers of the Sham, 0.1 mJ/mm^2, 0.25 mJ/mm^2 and 0.5 mJ/mm^2 groups on epiphyseal growth plate of left rat knee

IL-1β	**7 week**	***P*-value***	**13 week**	***P*-value**	**25 week**	***P*-value**
Sham	11.053		8.403		11.41	
0.1	19.19979	< 0.001	12.45667	< 0.001	14.95063	< 0.05
0.25	18.44438	< 0.001	12.28875	< 0.001	15.46125	< 0.001
0.5	16.07979	< 0.001	12.38521	< 0.05	14.3775	< 0.001
TUNEL	**7 week**	***P*-value**	**13 week**	***P*-value**	**25 week**	***P*-value**
Sham	undetectable	None	undetectable	None	undetectable	None
0.1	undetectable	None	undetectable	None	undetectable	None
0.25	undetectable	None	undetectable	None	undetectable	None
0.5	undetectable	None	undetectable	None	undetectable	None
SOX9	**7 week**	***P*-value**	**13 week**	***P*-value**	**25 week**	***P*-value**
Sham	14.537		14.589		11.144	
0.1	17.38083	< 0.01	18.32188	< 0.001	14.25625	> 0.05
0.25	18.70688	< 0.001	19.46354	< 0.001	14.74688	< 0.05
0.5	17.32229	< 0.05	13.84354	> 0.05	15.43042	< 0.001
BMP2	**7 week**	***P*-value**	**13 week**	***P*-value**	**25 week**	***P*-value**
Sham	4.1325		15.5535		11.2075	
0.1	5.955	< 0.05	20.99	< 0.05	16.62333	< 0.001
0.25	6.854375	< 0.001	17.17	> 0.05	17.48125	< 0.001
0.5	12.29313	< 0.001	18.748	> 0.05	16.80833	< 0.001
BMP4	**7 week**	***P*-value**	**13 week**	***P*-value**	**25 week**	***P*-value**
Sham	11.6065		13.6295		8.4745	
0.1	16.26	< 0.01	17.15429	< 0.001	11.48813	< 0.05
0.25	18.22625	< 0.001	17.32521	< 0.001	12.18938	< 0.05
0.5	17.52792	< 0.001	16.16729	> 0.05	10.25229	> 0.05

*The *P* value of 0.1, 0.25 and 0.5 mJ/mm^2 groups are compared with Sham group

and 0.5 mJ/mm²) compared to the Sham group (Fig. 2B and suppelmental Table 1). The length of the right tibia (no ESWT) did not improve compared to the Sham group (Fig. 2C and suppelmental Table 1). Additionally, the ratio of the length of the left to the right tibia showed that the ratio for the 0.25 mJ/mm² group at the 25 week was higher than that of the other treatment groups (Fig. 2D and suppelmental Table 1). These results indicate that ESWT could promote bone regeneration when applied to the epiphyseal plate.

Pathological analysis of epiphyseal plate after ESWT

Next, the pathological analysis of epiphyseal plate was surveyed by hematoxylin and eosin (HE) as well as Safarine O staining. In the HE staining, the epiphyseal plate and structure of the primary spongiosum were no difference after different level of ESWT at 7, 13 and 25 weeks as compared with Sham group (Fig. 3A). In the Safarine O staining, the organization of chondrocytes of epiphyseal plate was further observation and there was no any damage in the reserve zone, proliferative zone, hypertrophic zone and primary spongiosum in the 0.1, 0.25 and 0.5 mJ/mm^2 groups as compared with Sham group (Fig. 3B).

The short term microinflammation and safety of ESWT on epiphyseal plate of rats

The expression of pro-inflammatory and apoptosis markers were measured on epiphyseal plate after ESWT at different levels with 800 impulses, 4 Hz. The expression of pro-inflammatory cytokine, IL1-β, was measured and there were significant difference after ESWT at 0.1, 0.25 and 0.5 mJ/mm^2 with 800 impulses, 4 Hz on epiphyseal plate of left tibia (Fig. 4A; Table 1). In addition, the apoptosis marker, TUNEL activity, was also measured and no signals were detected in the Sham and treatment groups (Fig. 4B; Table 1). The results indicated that ESWT at 0.1, 0.25 and 0.5 mJ/mm^2 with 800 impulses, 4 Hz increased the low level expression of IL1-β but no cell apoptosis was observed. The levels of ESWT on the epiphyseal plate are safety in this experiments.

ESWT promotes the expression of SOX9, BMP2, and BMP4 on epiphyseal plate

The expression of SOX9 can prevent epiphyseal plate closure and keeps the epiphyseal plate healthy. The immunohistochemistry was performed to detect the expression of SOX9 after ESWT at 0.1, 0.25 and 0.5 mJ/mm^2 with 800 impulses, 4 Hz at 7, 13 and 25 weeks on epiphyseal plate (Fig. 5A; Table 1). The results indicated that the expression of SOX9 was significant higher at 0.25 mJ/

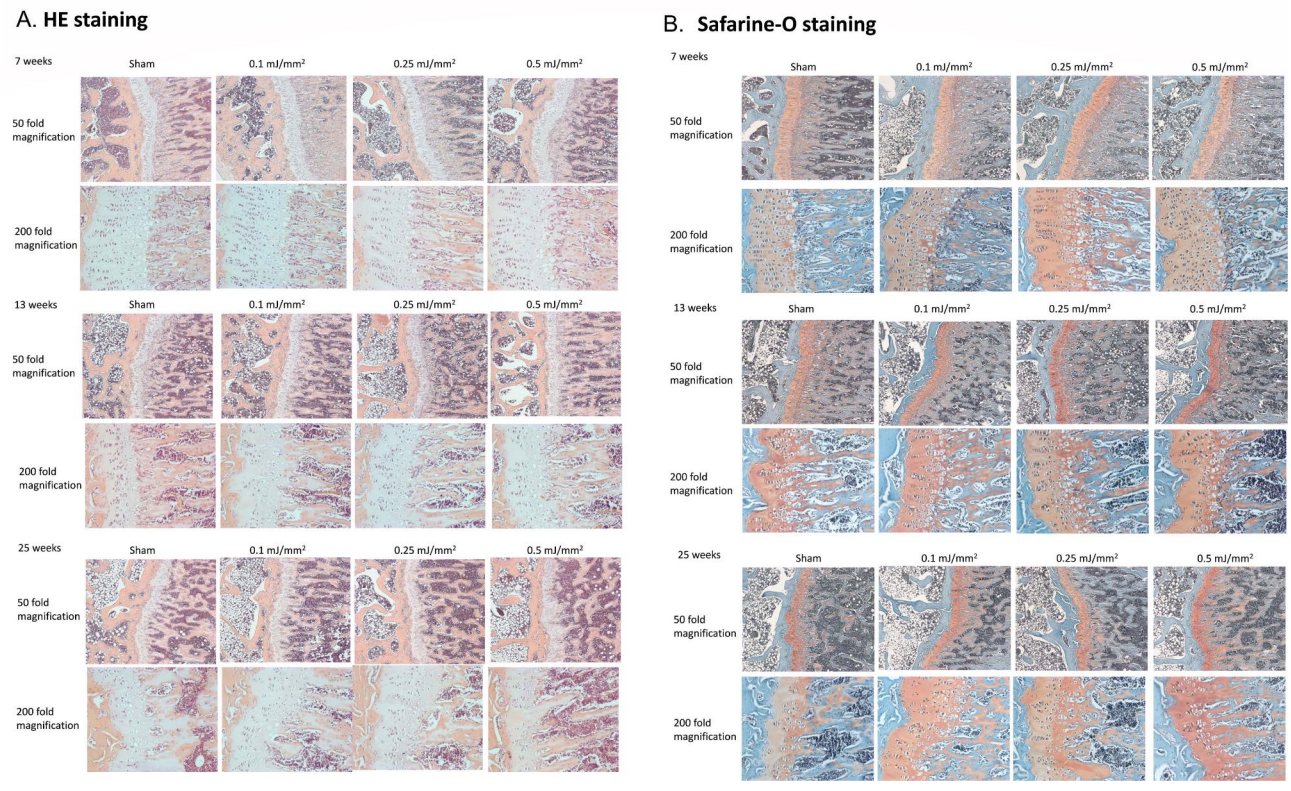

Fig. 3 The hematoxylin and eosin stain (**A**) as well as safranin O staining (**B**) of epiphyseal plate of rats. The figures displayed the 50 × and 200 ×magnification pictures

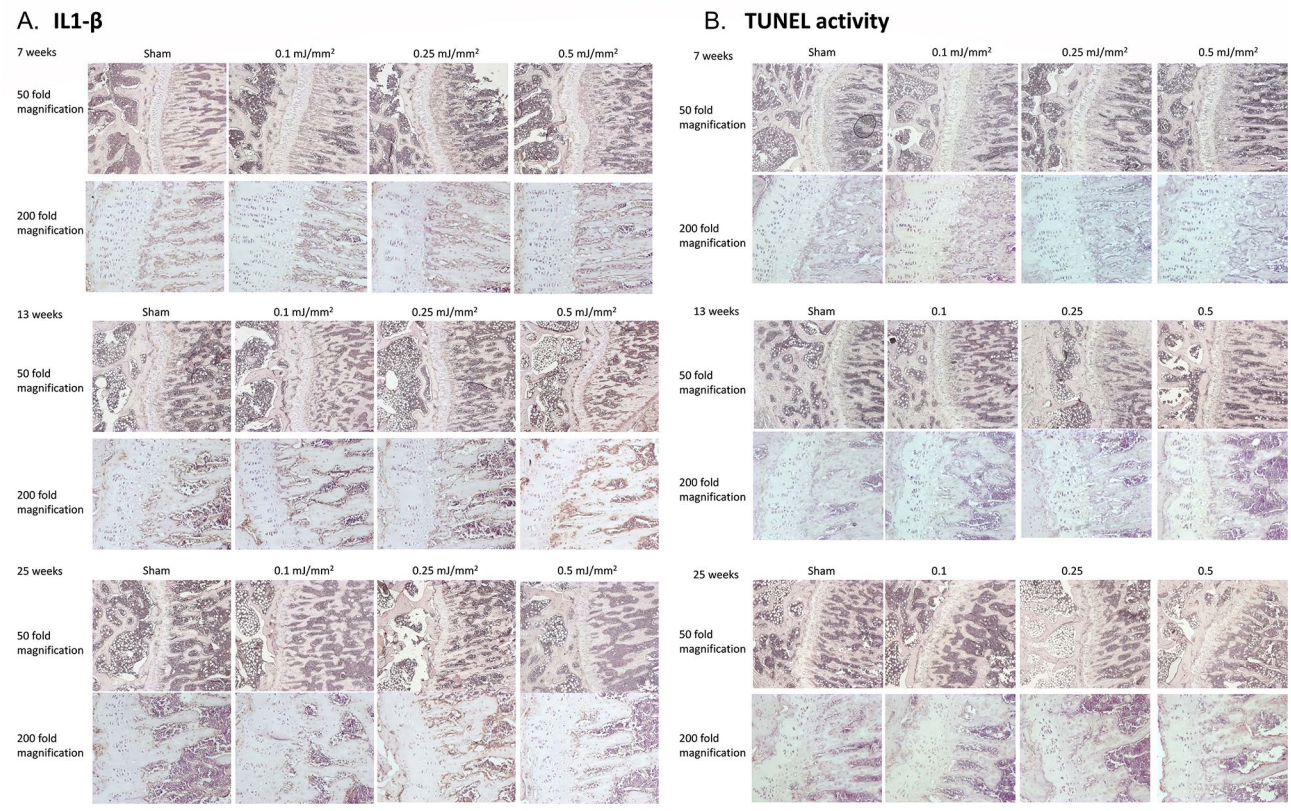

Fig. 4 The immunohistochemistry stainings of IL1-β (**A**) and TUNEL activity (**B**) on epiphyseal plate of rats after different levels of ESWT. The figures displayed the 50 × and 200 ×magnification pictures

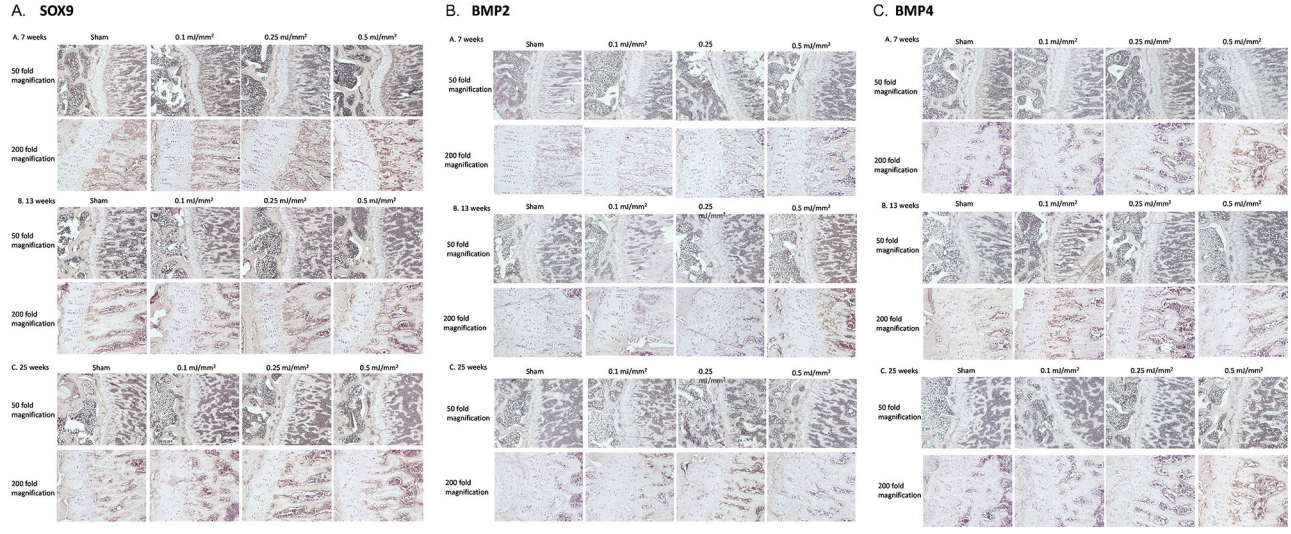

Fig. 5 The immunohistochemistry stainings of SOX9 (**A**), BMP2 (**B**) and BMP4 (**C**) on epiphyseal plate of rats after different levels of ESWT. The figures displayed the 50 × and 200 ×magnification pictures

mm^2 group than 0.1 mJ/mm^2 and 0.5 mJ/mm^2 groups at different time points.

In addition, the growth factors, BMP2 and BMP4 were measured after ESWT treatments. BMP2 was significantly stimulated at 7 week and continues to 25 week in the epiphyseal plate (Fig. 5B; Table 1). BMP4 was significantly stimulated in all treatment groups at 7 week and continous significantly expressed in 0.1 and 0.25 mJ/mm^2 groups at 13 and 25 weeks but not in 0.5 mJ/mm^2 group in the epiphyseal plate (Fig. 5C; Table 1). The results

indicated that ESWT stimulated BMP2 and BMP4 pathway to promote bone growth after application on epiphyseal plate and low-energy ESWT (≤ 0.25 mJ/mm^2, 800 impulses) was more suitable for use on the epiphyseal plate.

Discussion

In the study, the results demonstrated that different energy flux density of ESWT developed different effects on the epiphyseal plate. ESWT was safe for the epiphyseal plate at energy levels of 0.1, 0.25, and 0.5 mJ/mm^2 with 800 impulses, 4 Hz and it promoted the growth and lengthening of the tibia bone post-treatment. Although short term microinflammation may occur, no cell apoptosis or tissue damage was observed following ESWT. In addition, ESWT stimulated the expression of SOX9, BMP2 and BMP4 on the epiphyseal plate to promote bone growth by BMP pathway. The ESWT-induced undergrowth observed in this study suggests potential applicability as a technique for epiphysiodesis, which could be further explored in the context of managing leg length discrepancy or angular deformity in future.

Over the past decade, Overuse and throwing injuries in young athletes with developing skeletons have risen due to greater competition intensity and more frequent playing opportunities [23]. The treatment options for those overuse injuries included medication, physical therapy, and cessation of throwing. Extracorporeal shockwave had been proved effective in tendinitis. It has been reported that shockwave therapy induces neovascularization into the tendon-bone junction in rabbits [24]. ESWT has the potential to increase the formation of neo-vessels and the expression of angiogenesis-related markers in tendon-bone junction tissue. The neovascularization may play a role in tissue regeneration at the tendon-bone junction.

To date, there are still many concerns regarding the application of extracorporeal shockwave on the epiphyseal plate. The high-energy extracorporeal shock waves had no damage to the epiphysis in rabbits under histological examination [11]. In addition, the long-term effects of different dosage extracorporeal shockwaves on epiphysis of adolescent rat. After eight months follow-up, they found there was no significant difference in femoral length, tibial length and femoral supracondylar mediolateral width between different shockwaves dosage groups and control group [14]. Further, high-energy extracorporeal shockwaves stimulated the growth of immature rabbit epiphysis. The epiphyseal plaque thickness of tibia in higher dosage shockwave groups (14 kW, 0.6 mJ/mm^2, 3000 shots three times) was significantly higher than those in lower dosage group(14 kW, 0.6 mJ/mm^2, 1500 shots three times) and control group [13]. In current study, different levels of ESWT (0.1, 0.25, and 0.5 mJ/mm^2 with 800 impulses at 4 Hz) enhanced bone growth and lengthened the tibia without causing tissue damage (Figs. 3 and 4; Table 1). ESWT showed no negative effects on the epiphyseal plate of adolescent rats.

ESWT could increase bone growth by chondrogenesis in cultured fetal rat metatarsals [25]. Furthermore, radial shockwave treatment effects on cultured human growth plate and in vivo rabbit models. The results demonstrated that ESWT exposure upregulated SOX9 and collagen type II compared to control. In the rabbit models, increased the length of tibial was observed after application of high-energy ESWT. The results showed that ESWT is a non-invasive and safe therapy to stimulate bone growth [26]. The radial extracorporeal shock wave was ever used to treatment Osgood-Schlatter disease in adolescent patients. There were no side effects or long-term complications reported after 5.6 years follow up [27].

Microinflammation is an low-grade, chronic inflammatory condition that occurs at a microscopic level. It is characterized by the presence of low levels of inflammatory markers and immune cells in tissues, which can persist over long periods without causing overt symptoms. This type of inflammation is often associated with various chronic conditions, such as cardiovascular diseases, chronic kidney disease, diabetes, and obesity, and can contribute to the progression of these diseases by causing subtle but continuous damage to tissues and organs [28–30]. In the study, various levels of ESWT (0.1, 0.25, and 0.5 mJ/mm^2 with 800 impulses at 4 Hz) stimulated the expression of low-level IL-1β, detected at the 7, 13, and 25 weeks (Figs. 3 and 4; Table 1). The increased levels of IL-1β might induce short-term microinflammation, but no tissue damage was observed following ESWT. This short-term microinflammation may play a role in the tissue repair process by inducing the expression of BMP proteins, such as BMP2 and BMP4, which are involved in bone regeneration (Fig. 5; Table 1) [31]. However, the effects and mechanisms by which ESWT induces short-term microinflammation to promote tissue repair remain unclear and require further investigation.

There are several limitations to this study. Firstly, this is an in vivo study performed on small rodents, and the results may not directly translate to large animal or human clinical trials. The histological changes observed in the rat epiphyseal plate may differ from those in larger animals or humans, necessitating further investigation. The short-term microinflammatory effects induced by ESWT on the epiphyseal plate are still not well understood, and further investigation is needed to clarify this. Additionally, it is unclear whether ESWT would cause microinflammation in humans. Furthermore, the dosages of ESWT were optimized for this animal study and may vary when applied to the human epiphyseal plate. Lastly, there are various types of shockwave devices available on

the market, and their effects on the epiphyseal plate may differ.

Conclusion

The current animal study demonstrated the ESWT is safety and promote the growth and length of tibia bone. In addition, ESWT stimulates the expression of SOX9, BMP2 and BMP4 on the epiphyseal plate and may promote bone growth by BMP pathway. The results indicate that ESWT is a safe therapeutic modality in adolescents with open epiphyseal plate and may be applied to improve leg length discrepancy in rat model.

Abbreviations

ESWT	Extracorporeal shockwave therapy
IL-1β	Interleukin-1β
SOX9	SRY-Box transcription factor 9
BMP2	Bone morphogenetic protein 2
LLD	Leg length discrepancies
ISMST	International Society for Medial Shockwave Treatment
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
HE	Hematoxylin and eosin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-025-03519-6.

Supplementary Material 1

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Author contributions

Shun-Wun Jhan: Conceptualization, Data curation, Funding Acquisition, Investigation, Methodology, Supervision, Validation, Writing-original draft, Writing-review & editing.Kuan-Ting Wu: Formal Analysis, Investigation, Writing-original draft.Wen-Yi Chou: Formal Analysis, Data curation, Writing-original draft. Jeng-Wei Chen: Methodology, Validation, Writing-original draft.Ka-Kit Siu: Data curation, Investigation, MethodologyWen-Chiung, Huang: Data curation, Methodology, Validation.Ching-Jen Wang: Data curation, Methodology, Writing-original draft.Jai-Hong Cheng: Conceptualization, Validation, Writing-original draft, Writing-review & editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) at KCGMH (No. 2020073001).

Competing interests

The authors declare no competing interests.

Consent to publish

Not applicable.

ICMJE COI statement

The authors have declared that they did not receive any honoraria or consulting fees in writing this manuscript. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article..

Data sharing statement

All data relevant to the study are included in the article or are available as supplementary files.

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References

1. Dabash S, Prabhakar G, Potter E, Thabet AM, Abdelgawad A, Heinrich S. Management of growth arrest: current practice and future directions. J Clin Orthop Trauma. 2018;9:S58–66.
2. Longo UG, Ciuffreda M, Locher J, Maffulli N, Denaro V. Apophyseal injuries in children's and youth sports. Br Med Bull. 2016;120(1):139–59.
3. Arnold A, Thigpen CA, Beattie PF, Kissenberth MJ, Shanley E. Overuse physeal injuries in youth athletes. Sports Health: Multidisciplinary Approach. 2017;9(2):139–47.
4. Midtiby SL, Wedderkopp N, Larsen RT, Carlsen A-MF, Mavridis D, Shrier I. Effectiveness of interventions for treating apophysitis in children and adolescents: protocol for a systematic review and network meta-analysis. Chiropr Man Ther. 2018;26(1).
5. Cepela DJ, Tartaglione JP, Dooley TP, Patel PN. Classifications in brief: Salter-Harris classification of pediatric physeal fractures. Clin Orthop Relat Res. 2016;474(11):2531–7.
6. Ruzbarsky JJ, Goodbody C, Dodwell E. Closing the growth plate: a review of indications and surgical options. Curr Opin Pediatr. 2017;29(1):80–6.
7. Kumar S, Sonanis SV. Growth modulation for coronal deformity correction by using eight Plates—Systematic review. J Orthop. 2018;15(1):168–72.
8. Moya D, Ramón S, Schaden W, Wang C-J, Guiloff L, Cheng J-H. The role of extracorporeal shockwave treatment in musculoskeletal disorders. J Bone Joint Surg. 2018;100(3):251–63.
9. Yeaman LD, Jerome CP, McCullough DL. Effects of shock waves on the structure and growth of the immature rat epiphysis. J Urol. 1989;141(3 Part 1):670–4.
10. Bussy C, Auzas F, Muñoz JA. Clinical use of extracorporeal shockwave therapy (ESWT) for the treatment of carpus Valgus deformities in young foals: A retrospective study of 64 cases (2006–2009). Open J Veterinary Med. 2013;03(01):46–51.
11. Nassenstein K, Nassenstein I, Schleberger R. Wirkung hochenergetischer extrakorporaler Stoßwellen auf Wachstumsfugen - Eine histomorphologische studie. Z Orthop Grenzgeb. 2005;143(06):652–5.
12. Saisu T, Takahashi K, Kamegaya M, Mitsuhashi S, Wada Y, Moriya H. Effects of extracorporeal shock waves on immature rabbit femurs. J Pediatr Orthop Part B. 2004;13(3):176–83.
13. Ozturk H, Bulut O, Oztemur Z, Kaloglu C, Kol IO. Effect of high-energy extracorporeal shock waves on the immature epiphysis in a rabbit model. Arch Orthop Trauma Surg. 2007;128(6):627–31.
14. Oztemur Z, Ozturk H, Bulut O, Ozyurek S, Kaloglu C, Golge UH. The long-term effects of extracorporeal shock waves on the epiphysis of the adolescent rat. J Orthop Sci. 2013;18(1):159–64.
15. Lyon R, Liu XC, Kubin M, Schwab J. Does extracorporeal shock wave therapy enhance healing of osteochondritis dissecans of the rabbit knee?? A pilot study. Clin Orthop Relat Res. 2013;471(4):1159–65.
16. Tischer T, Milz S, Weiler C, Pautke C, Hausdorf J, Schmitz C, et al. Dose-Dependent new bone formation by extracorporeal shock wave application on the intact femur of rabbits. Eur Surg Res. 2008;41(1):44–53.
17. Wang CJ. An overview of shock wave therapy in musculoskeletal disorders. Chang Gung Med J. 2003;26(4):220–32.
18. Wang C-J. Extracorporeal shockwave therapy in musculoskeletal disorders. J Orthop Surg Res. 2012;7(1).
19. Wang C-J, Weng L-H, Ko J-Y, Sun Y-C, Yang Y-J, Wang F-S. Extracorporeal shockwave therapy shows chondroprotective effects in Osteoarthritic rat knee. Arch Orthop Trauma Surg. 2011;131(8):1153–8.

20. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010;8(6).
21. Quintana-Villamandos MB, Sánchez-Hernández JJ, Delgado-Martos MJ, Delgado-Baeza E. Evolutional patterns of articular cartilage following growth plate injury in rats. J Orthop Sci. 2009;14(5):646–51.
22. Cheng J-H, Hsu C-C, Hsu S-L, Chou W-Y, Wu Y-N, Kuo C-EA et al. Adipose-Derived mesenchymal stem Cells-Conditioned medium modulates the expression of inflammation induced bone morphogenetic Protein-2, -5 and– 6 as well as compared with shockwave therapy on rat knee osteoarthritis. Biomedicines. 2021;9(10).
23. Hutchinson MR, Ireland ML. Overuse and throwing injuries in the skeletally immature athlete. Instr Course Lect. 2003;52:25–36.
24. Wang C-J, Wang F-S, Yang KD, Weng L-H, Hsu C-C, Huang C-S, et al. Shock wave therapy induces neovascularization at the tendon–bone junction. A study in rabbits. J Orthop Res. 2003;21(6):984–9.
25. Ramesh S, Zaman F, Madhuri V, Savendahl L. Radial extracorporeal shock wave treatment promotes bone growth and chondrogenesis in cultured fetal rat metatarsal bones. Clin Orthop Relat Res. 2020;478(3):668–78.
26. Ramesh S, Zaman F, Sävendahl L, Madhuri V. Radial shockwave treatment promotes chondrogenesis in human growth plate and longitudinal bone growth in rabbits. Bone. 2022;154.
27. Lohrer H, Nauck T, Schöll J, Zwerver J, Malliaropoulos N. Einsatz der Extrakorporalen stoßwellentherapie Bei therapieresistentem M. Schlatter. Sportverletzung · Sportschaden. 2012;26(04):218–22.
28. Olivier V, Dunyach-Remy C, Corbeau P, Cristol J-P, Sutra T, Burtey S et al. Factors of microinflammation in non-diabetic chronic kidney disease: a pilot study. BMC Nephrol. 2020;21(1).
29. Shikata K, Makino H. Microinflammation in the pathogenesis of diabetic nephropathy. J Diabetes Invest. 2013;4(2):142–9.
30. Tasic D, Radenkovic S, Kocic G, Deljanin Ilic M, Ignjatovic A. Microinflammation factors in the common diseases of the heart and kidneys. Dis Markers. 2015;2015:1–7.
31. Colavite PM, Vieira AE, Palanch Repeke CE, de Araujo Linhari RP, De Andrade RGCS, Borrego A, et al. Alveolar bone healing in mice genetically selected in the maximum (AlRmax) or minimum (AlRmin) inflammatory reaction. Cytokine. 2019;114:47–60.

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