SIN-CHN SCIENTIFIC PRESS PTE.LTD.

Molecular & Cellular Biomechanics 2025, 22(5), 1240.
https://doi.org/10.62617/mcb1240

Article

In vitro study on the effects of mechanical load on the behavior and signal pathway regulation of pediatric hip cartilage cells

Yan Liu[1], Peng Yang[1], Zhijian Pan[2], Guangxi Chen[1], Hongliang Liu[1], Jiangfa Huang[1,*]

[1] Department of Sports Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China
[2] Department of Orthopedics, The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510000, China
*** Corresponding author:** Jiangfa Huang, huangjf008008@163.com

CITATION

Liu Y, Yang P, Pan Z, et al. In vitro study on the effects of mechanical load on the behavior and signal pathway regulation of pediatric hip cartilage cells. Molecular & Cellular Biomechanics. 2025; 22(5): 1240. https://doi.org/10.62617/mcb1240

ARTICLE INFO

Received: 21 December 2024
Accepted: 28 February 2025
Available online: 24 March 2025

Abstract: Objective: To explore the effects of mechanical load on the behavior and related signaling pathways of pediatric hip cartilage cells, providing experimental evidence for optimizing cartilage regeneration and repair strategies. **Methods:** Human-derived pediatric hip cartilage cells were cultured in vitro and subjected to uniaxial tensile stress at a specific frequency (1 Hz) with varying strain amplitudes (0%, 2%, 5%, 10%). Cell proliferation curves were assessed using the CCK-8 assay, while qPCR was used to detect changes in the expression of COL2A1, ACAN, and genes such as ITGB1, TGF-β1, WNT3A, MAPK1, among others. Western Blot analysis was conducted to measure protein levels of Collagen II, Aggrecan, MMP-13, as well as p-ERK, p-p38, β-catenin, MAPK1, and MAPK14. The relationship between strain amplitude and cellular biological effects was also analyzed. **Results:** After mechanical strain application, cell proliferation significantly increased on days 5 and 7 ($p < 0.05$), and COL2A1 and ACAN gene expression levels were upregulated ($p < 0.05$). Protein levels of Collagen II and Aggrecan significantly increased, while MMP-13 levels decreased ($p < 0.05$). Upstream signaling molecules such as Integrin β1, TGF-β1, and WNT3A were upregulated ($p < 0.05$), while TGFBR2 showed no significant changes ($p > 0.05$). Downstream molecules p-ERK, MAPK1, and MAPK14 were significantly upregulated ($p < 0.05$), whereas p-p38 and β-catenin showed no significant differences ($p > 0.05$). ACAN and p-ERK expression levels exhibited a dose-response relationship with increasing strain amplitude ($p < 0.05$). **Conclusion:** Mechanical load promotes the proliferation and matrix synthesis of pediatric hip cartilage cells through specific regulation of upstream and downstream signaling pathway molecules, showing strain intensity-dependent molecular responses. This study lays the foundation for precision regulation strategies in cartilage development and repair.

Keywords: pediatric hip cartilage cells; mechanical load; signaling pathways; dose-response relationship

1. Introduction

Articular cartilage is crucial in maintaining normal joint function and mobility, yet the development and maturation of hip joint cartilage in children are often influenced by multiple factors [1]. Recent epidemiological studies indicate that developmental dysplasia of the hip (DDH) and other pediatric hip disorders have a prevalence ranging from 1% to 3% in newborns worldwide, making them one of the most common musculoskeletal abnormalities in children [2]. If left untreated or inadequately managed, these conditions can lead to unstable hip joints, impaired weight-bearing capacity, and a high risk of early-onset osteoarthritis. In addition, the altered biomechanical forces in the immature hip joint can interfere with normal cartilage development, potentially resulting in chronic pain and reduced range of

motion later in life [3]. Despite progress in surgical interventions and conservative treatments, cartilage has a limited self-regenerative capacity, and there is still no consensus on standardized regenerative protocols for pediatric patients. The challenge lies not only in restoring the anatomical structure but also in promoting functional cartilage regeneration that can accommodate the continuing growth and development unique to children [4]. Clinical observations show that early abnormal stress distribution can lead to hip joint cartilage degeneration or dysplasia, causing joint pain, mobility disorders, and subsequent degenerative changes [5]. Existing studies mostly focus on adult chondrocytes or animal models, and a systematic understanding of cellular behavior and molecular mechanisms of pediatric hip cartilage under stress conditions is still lacking [6]. Mechanical regulation in chondrocytes not only affects proliferation and differentiation, but may also modulate matrix metabolism and signal transduction through specific molecular pathways, although the relevant mechanisms remain unclear [7]. The lack of in-depth understanding of the behavior of pediatric hip joint chondrocytes and the signaling regulatory pathways under mechanical loading leads to difficulties in formulating precise early clinical interventions. In orthopedic rehabilitation and joint regeneration therapy, if the regulatory patterns of specific mechanical parameters on chondrocyte function can be determined, it will help optimize rehabilitation strategies and improve the precision and effectiveness of tissue engineering and regenerative medicine [8]. This study utilized an in vitro model to apply controllable mechanical loading on pediatric hip joint chondrocytes, and employed molecular biology and cytological methods to comprehensively evaluate changes in cell proliferation, gene and protein expression. It further explored the dose-effect relationships between strain amplitude, mechanosensitive molecules, and downstream signaling molecules. The aim of this study is to elucidate the fine regulatory mechanisms of mechanical stimulation on chondrocyte biological behavior and key signaling pathways, thereby providing empirical evidence and theoretical references for the development of targeted interventions and the optimization of joint cartilage regeneration strategies in clinical practice.

2. Materials and methods

2.1. Cell source and culture

Human pediatric hip joint chondrocytes (catalog number HCH-001), provided by a certified biological cell bank, were taken out from liquid nitrogen storage and immediately thawed in a 37 ℃ water bath with continuous gentle shaking until the cell suspension in the cryopreservation tube was completely thawed. After thawing, the cell suspension was transferred into pre-warmed DMEM/F12 (Gibco, USA) medium at 37 ℃ and thoroughly mixed, followed by centrifugation at 300×g for 5 min. The supernatant was discarded, and fresh DMEM/F12 medium was added to resuspend the cells. The cells were seeded at a density of 1×10^5 cells/mL into culture flasks and incubated under conditions of 37 ℃ and 5% CO_2. The medium contained 10% fetal bovine serum (Gibco, USA), 100 U/mL penicillin (Sigma-Aldrich, USA), and 100 μg/mL streptomycin (Sigma-Aldrich, USA). The medium was changed every 2 days, and when the cell confluence reached 80%, the cells were digested with 0.25% trypsin (Beyotime, China) for passage. After expansion to the 3rd passage, the cells

were used for subsequent experiments.

The pediatric hip cartilage cells used in this study were selected based on the following criteria: (i) cells derived from donors aged between 2 and 10 years old, diagnosed with developmental hip conditions but without systemic metabolic diseases; (ii) certified by the cell bank to be free from contamination and mycoplasma infection; and (iii) demonstrating stable proliferation and cartilage-specific marker expression (e.g., collagen II, aggrecan) in preliminary tests. These selection criteria were established to ensure that the harvested chondrocytes accurately represent pediatric cartilage biology and to enhance the reproducibility and reliability of subsequent experiments.

2.2. Experimental grouping and mechanical loading parameter setting

The 3rd passage proliferating pediatric hip joint chondrocytes were seeded at a density of 2×10^4 cells/cm^2 onto elastic biomembrane culture plates (Flexcell BioFlex Culture Plates, Flexcell International, USA) uniformly coated with type I collagen (Sigma-Aldrich, USA). After the cells had adhered and reached approximately 70% confluence, they were randomly divided into a control group and a loading group. The control group was continuously cultured under the same conditions for 7 days without applying mechanical strain. The loading group was subjected to cyclic uniaxial stretching at 1 Hz and 5% strain using the FX-5000T cell mechanical stimulation system (Flexcell International, USA) for 2 h per day, continuously for 7 days.

To investigate the dose-effect relationship between strain amplitude and cellular biological effects, another set of experiments was conducted with a fixed frequency of 1 Hz and strain amplitudes set at 0%, 2%, 5%, and 10%. All other conditions were the same as those in the loading group. All groups were independently repeated 3 times, with 3 technical replicates each time. The selection of a 1 Hz frequency and strain amplitudes of 2%, 5%, and 10% was based on prior biomechanical studies indicating that these ranges approximate physiological loading conditions experienced by hip cartilage during normal gait and moderate exercise. Specifically, 1 Hz is commonly used to simulate the frequency of human walking, while strain amplitudes below 2% may be insufficient to elicit robust cellular responses, and amplitudes exceeding 10% could lead to excessive mechanical stress or cell damage. Pilot experiments conducted in our laboratory also confirmed that these parameters effectively induce changes in chondrocyte proliferation and matrix metabolism without compromising cell viability, thereby providing a balance between physiological relevance and experimental feasibility.

2.3. Cell proliferation detection

On days 1, 3, 5, and 7 of continuous culture under mechanical stimulation or control conditions, a CCK-8 kit (Dojindo, Japan) was used to evaluate cell proliferation. Three wells of samples were taken from each group at each time point. 10 μL of CCK-8 solution was added into 100 μL of culture medium in each well and incubated at 37 ℃ for 2 h. Absorbance was measured at 450 nm using a microplate reader (BioTek, USA). Data were expressed as mean ± standard deviation, and differences between groups were compared using an independent sample *t*-test, with

$P < 0.05$ indicating statistically significant differences.

2.4. Gene expression analysis

After the experiment, total RNA from the cells was extracted using TRIzol reagent (Invitrogen, USA), and the RNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). 1 μg of total RNA was reverse-transcribed into cDNA using a PrimeScript RT kit (Takara, Japan). Quantitative PCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA) using SYBR Green PCR Master Mix (Takara, Japan).

Cartilage-related genes COL2A1 and ACAN were detected, using GAPDH as the internal reference; mechanical-related and signaling pathway genes ITGB1, TGF-β1, TGFBR2, WNT3A, CTNNB1, MAPK1, and MAPK14 were also detected. Primers were synthesized and their amplification efficiency was verified by a professional company. The PCR cycling conditions were 95 ℃ for 30 s of pre-denaturation, followed by 40 cycles of 95 ℃ for 5 s and 60 ℃ for 30 s [9]. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Differences between groups were compared using an independent sample *t*-test, with $P < 0.05$ considered significant.

2.5. Protein level analysis (Western Blot)

Cells were lysed with ice-cold RIPA lysis buffer (Beyotime, China) containing protease inhibitors (Roche, Switzerland), then subjected to ultrasonic disruption at 4 ℃ and centrifuged at 12,000× *g* for 10 min to collect the supernatant. Protein concentration was measured using a BCA protein assay kit (Thermo Fisher Scientific, USA) [10]. Thirty micrograms of protein were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, USA).

After blocking the membrane with 5% skim milk for 1 h, the corresponding primary antibodies, including Collagen II, Aggrecan, and MMP-13, Integrin β1 (all purchased from Abcam, UK), TGF-β1, TGFBR2, WNT3A, p-ERK, p-p38, β-catenin, MAPK1, MAPK14 (all purchased from Cell Signaling Technology, USA), were added. The primary antibodies were diluted according to the manufacturer's instructions and incubated overnight at 4 ℃. On the following day, HRP-conjugated secondary antibodies (Cell Signaling Technology, USA) were incubated at room temperature for 1 h. The membranes were then developed with ECL substrate (Thermo Fisher Scientific, USA), and band images were obtained using a Bio-Rad ChemiDoc imaging system (Bio-Rad, USA).

β-actin (primary antibody from Cell Signaling Technology, USA) was used as an internal reference for normalization. The optical density values were measured using ImageJ (NIH, USA). Differences in protein expression between groups were evaluated by independent sample *t*-test ($P < 0.05$ was considered significant).

2.6. Association analysis between mechanical parameters and biological effects

With the frequency fixed at 1 Hz, stretch amplitudes of 0%, 2%, 5%, and 10% were set, and all other conditions remained the same. The aforementioned CCK-8 proliferation assay, qPCR gene expression analysis, and Western Blot protein detection

were repeated. The biological indices obtained were subjected to linear or polynomial regression analysis with strain amplitude to determine the dose-effect relationship between strain amplitude and changes in cell function. Statistical analysis was performed as described above, with $P < 0.05$ considered significant.

2.7. Data statistical analysis

All quantitative results were expressed as mean ± standard deviation. Data analysis was performed using SPSS 26.0 software (IBM, USA). For repeated measurements of the same cell group under different conditions (e.g., 0%, 2%, 5%, and 10% strain amplitudes), repeated measures ANOVA was used to determine the overall differences among conditions [11]. If the ANOVA showed statistical significance, a post hoc multiple comparison test was conducted. For comparisons between independent samples, an independent sample *t*-test was used; for multiple independent group comparisons, one-way ANOVA was used. $P < 0.05$ was considered statistically significant. All experiments were repeated 3 times, each with 3 technical replicates, to ensure the reliability and reproducibility of the results.

3. Results

3.1. Proliferation of pediatric hip joint chondrocytes and changes in cartilage-related gene expression

Using a quantitative PCR detection system designed and established with specific primers targeting COL2A1, ACAN, ITGB1, TGF-β1, TGFBR2, WNT3A, CTNNB1, MAPK1, MAPK14, and GAPDH, stable amplification of multiple genes was achieved, laying the foundation for subsequent studies on transcriptional changes in hip joint chondrocytes under mechanical strain conditions (**Table 1**). In the early stages of continuous culture (days 1 and 3), no statistically significant difference in cell proliferation was observed between the control and loading groups ($p > 0.05$). However, by days 5 and 7, the proliferation levels of the loading group were significantly higher than those of the control group ($p < 0.05$), indicating that mechanical strain significantly promotes cell proliferation in the later stages (**Figure 1A**). Moreover, after 7 days of mechanical loading, the expression of COL2A1 and ACAN genes was significantly upregulated in the loading group ($p < 0.05$), further demonstrating that mechanical strain enhances the expression of cartilage-specific genes and matrix synthesis (**Figure 1B**).

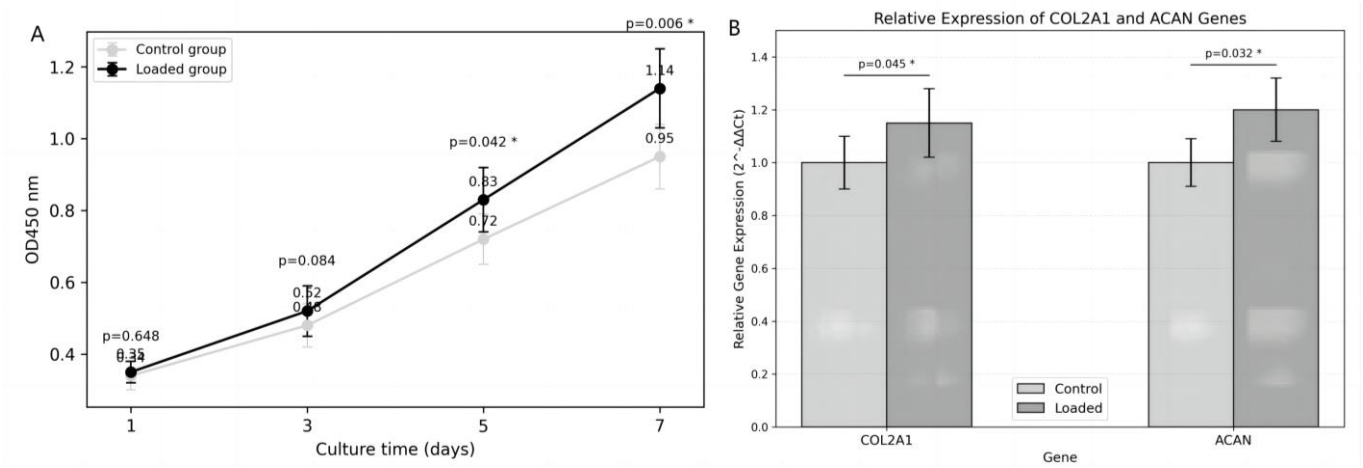

Figure 1. Effects of Mechanical Strain on Cell Proliferation and Gene Expression. **(A)** Cell proliferation curve for the control and loading groups (days 1, 3, 5, 7); **(B)** Bar chart of relative expression levels of COL2A1 and ACAN genes.
Note: Differences between the two groups were compared using an independent sample t-test, * $P < 0.05$ indicates statistically significant differences.

Table 1. Primer sequences for quantitative PCR.

Gene	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Product Length (bp)
COL2A1	TGCGACAAGTCTTGGTCCAG	CAGGTTGAAGAGTCCCTGGA	132
ACAN	AGCTCCCTTGTCTGGAGATG	GGAGACACGGGATCTTCATC	140
ITGB1	TACGCTCAGCATCAAGTCCG	CCAGGAAGATGAGACCAGCA	118
TGF-β1	GCTTCTGGGAAAGAGCAAGC	TCAGTTCAGGCAGGAGCGTA	102
TGFBR2	AAGGACGCTCAATGGCTTCC	TCCTCTTCACGTGCATTTCG	125
WNT3A	ACTTGACTGCAGTCCCAAGA	GGTTCACATGGGTTTGCATG	110
CTNNB1	AATTGCTGTGTGGGCTCCTA	TGAGTCCTTTTCGGCGAGTA	95
MAPK1	GCTGACCTACTTGGGGAAGA	CACTGTGTTCAATGTGCACG	108
MAPK14	CAGTTAGAGCTGGGAGTCCG	AGAGGGAAGAACTCTCGGGA	130
GAPDH	CGAGATCCCTCCAAAATCAA	GGTGAGGTCTCATGAGTCCT	115

3.2. Mechanical signal sensing and expression changes of signal pathway-related genes

In this study, the relative expression levels of seven signaling pathway-related genes were compared between the control group and the loading group. The results showed that the expression levels of ITGB1, TGF-β1, WNT3A, and MAPK1 genes in the loading group were significantly higher than those in the control group ($p < 0.05$), while there was no significant difference in the expression of TGFBR2, CTNNB1, and MAPK14 genes between the two groups ($p > 0.05$) (**Figure 2**). These findings suggest that mechanical strain selectively regulates specific signaling pathway genes, potentially affecting chondrocyte proliferation, differentiation, and signal transduction processes.

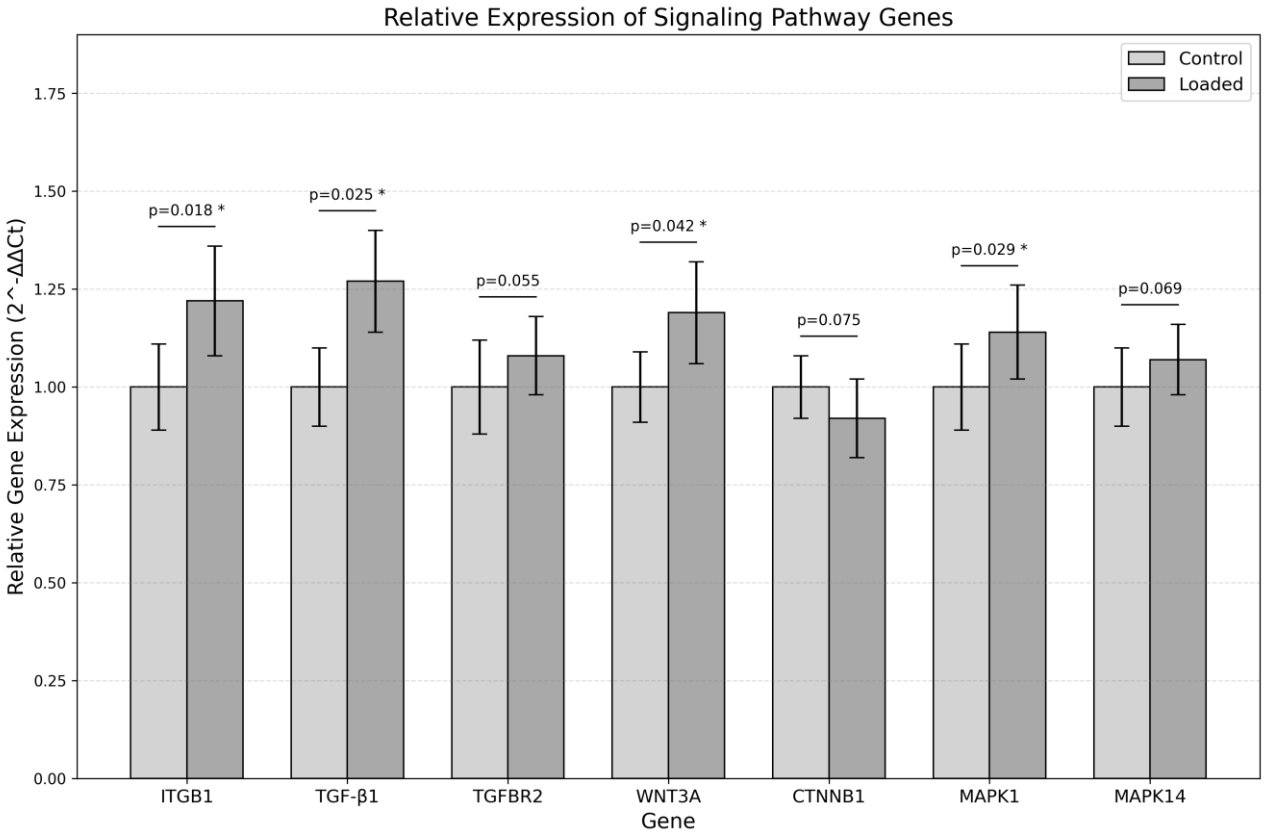

Figure 2. Bar charts of relative expression levels of ITGB1, TGF-β1, TGFBR2, WNT3A, CTNNB1, MAPK1, and MAPK14 genes.

Note: Differences between the two groups were compared using independent sample *t*-test, * $P < 0.05$.

3.3. Analysis of ECM and signal pathway-related protein expression

Under loading conditions, the relative expression levels of key ECM-related proteins Collagen II and Aggrecan in chondrocytes were significantly elevated, while MMP-13 was markedly reduced ($p < 0.05$), indicating that mechanical strain may promote cartilage matrix synthesis and inhibit its degradation, thus altering the dynamic balance of the cartilage microenvironment (**Figure 3A**). At the same time, the protein levels of upstream signaling molecules Integrin β1, TGF-β1, and WNT3A were significantly upregulated ($p < 0.05$), while TGFBR2 showed no significant change ($p > 0.05$), suggesting that mechanical strain exerts selective regulation on upstream signaling pathway molecules (**Figure 3B**). Among downstream signaling proteins, p-ERK, MAPK1, and MAPK14 were significantly upregulated ($p < 0.05$), while p-p38 and β-catenin showed changes that were not significant ($p > 0.05$), indicating that mechanical strain has a selective effect on downstream pathway activation (**Figure 3C**).

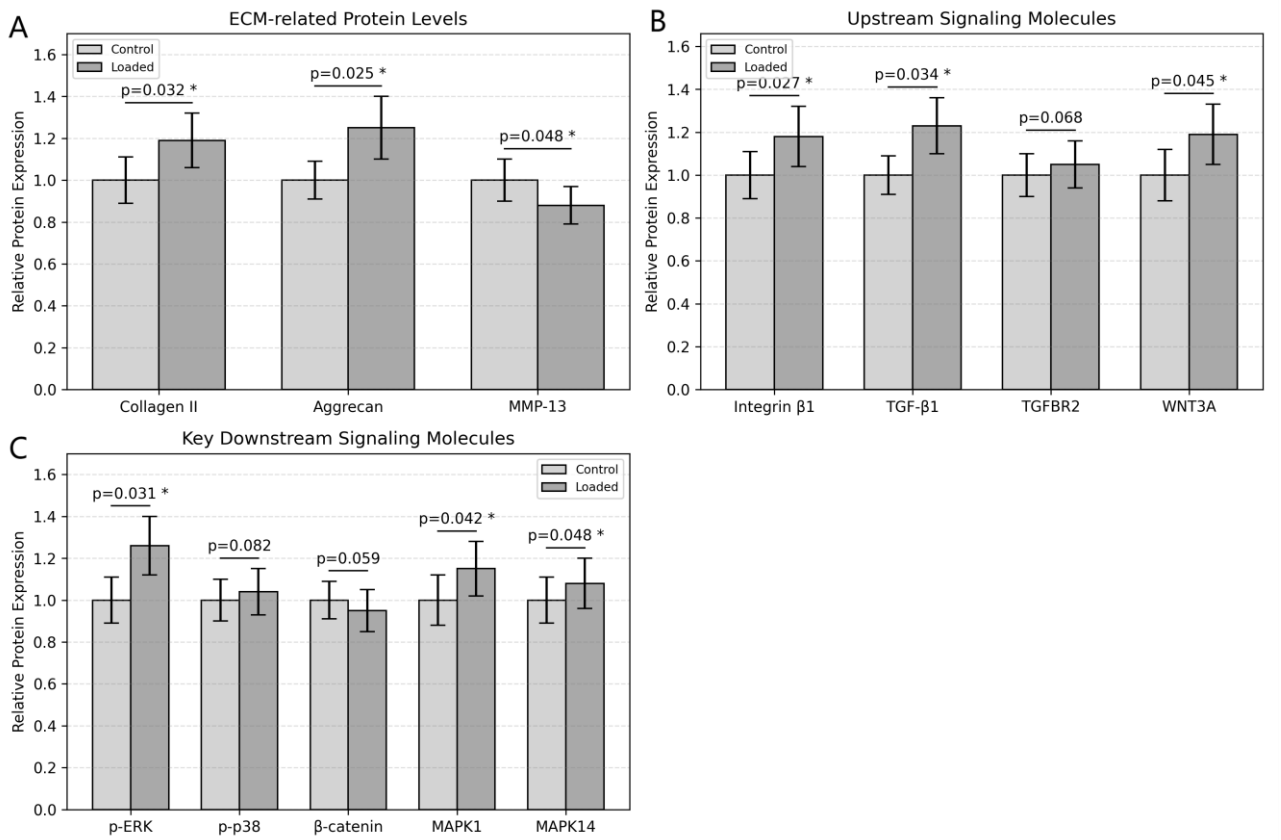

Figure 3. Regulatory Effects of Mechanical Strain on ECM and Key Signaling Pathway Proteins. **(A)** Western Blot results and quantitative analysis of ECM-related proteins (Collagen II, Aggrecan, MMP-13); **(B)** Western Blot results and quantitative analysis of mechanical sensing and key upstream signaling molecules (Integrin β1, TGF-β1, TGFBR2, WNT3A); **(C)** Western Blot results and quantitative analysis of key molecules and activation states in downstream signaling pathways (p-ERK, p-p38, β-catenin, MAPK1, MAPK14).

Note: Differences between groups were compared using independent sample *t*-test, * $P < 0.05$.

3.4. Quantitative correlation between different strain amplitudes and cellular biological responses

With increasing strain amplitude, both ACAN gene expression and p-ERK protein levels showed a linear increasing trend. Statistical analysis (such as linear regression or correlation analysis) indicated a significant positive correlation between their changes at different strain levels ($p < 0.05$). This result demonstrates a clear dose-effect relationship between the intensity of mechanical strain and the molecular responses of chondrocytes (including ACAN gene expression and p-ERK protein levels), providing empirical evidence for further elucidation of the mechanisms of mechanical stimulation (**Figure 4**).

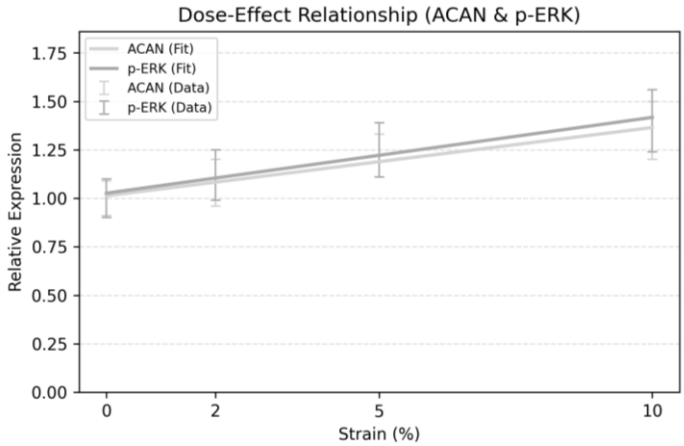

Figure 4. Dose-effect relationship curves of ACAN gene expression and p-ERK protein levels under strain amplitudes of 0%, 2%, 5%, and 10%.

Note: Repeated measures ANOVA was used to compare the changing trends under different strain amplitudes, $P < 0.05$ indicates significant differences.

4. Discussion

During growth, moderate mechanical strain can significantly increase the proliferation rate of chondrocytes and promote the upregulation of characteristic cartilage genes represented by COL2A1 and ACAN. This characteristic indicates that exogenous mechanical conditions provide a more favorable microenvironment for chondrocytes, enabling them to exhibit higher viability and metabolic capacity at later time points in the culture cycle [12]. The increase in cell number is not merely a simple proliferative action; more importantly, it implies that under appropriate strain stimulation, chondrocytes acquire the conditions necessary to maintain and reinforce their own functions, thereby creating possibilities for subsequent higher-level tissue repair. Along with changes in gene expression, key extracellular matrix components such as collagen and aggrecan are significantly upregulated. This not only represents accelerated synthesis of essential structural proteins by the cells but also reflects the high sensitivity of cells to mechanical stimulation [13]. Under strain conditions, the molecular pathways inside chondrocytes are precisely regulated to ensure a continuous supply of adequate matrix components during mechanical stimulation, thereby supporting normal tissue function and mechanical properties [14]. Moreover, this process is not merely a unidirectional enhancement of synthesis; it also involves active regulation of the degradation process. Under normal conditions, matrix proteins are continuously renewed and degraded. However, when cells are subjected to moderate strain stimulation, the expression levels of degradation-related enzymes (such as MMP-13) decrease, creating ideal conditions for matrix accumulation and the maintenance of homeostasis [15]. These findings bring to mind improvements in joint cartilage repair strategies in clinical and regenerative medicine. Knowing that exogenous strain can simultaneously upregulate anabolic processes and downregulate catabolic activities makes the rational control of mechanical conditions a feasible strategy. In tissue engineering, to promote the regeneration of damaged cartilage, it is usually necessary to create favorable biological and mechanical conditions so that newly formed cells can rapidly proliferate while continuously synthesizing the

necessary matrix components. If a certain degree of mechanical strain is applied at an appropriate time, it can provide more stable mechanical support and renewed impetus for the regenerating cartilage tissue, thereby potentially improving repair efficiency and long-term stability [16]. Integrating this information allows us to infer that under moderate mechanical stimulation, chondrocytes achieve synchronous improvements in both quantity and quality, providing data support for optimizing clinical repair strategies. Nevertheless, a broader survey of current literature suggests that the effects of mechanical load on cartilage cells can vary based on factors such as loading type (uniaxial stretch, compression, or shear), frequency, magnitude, and cellular origin. For instance, some studies utilizing dynamic compression models reported more pronounced anabolic responses, while others employing hydrostatic pressure or fluid shear stress observed weaker or even contradictory effects on matrix synthesis and catabolic enzyme expression [17]. Moreover, variations in cell phenotype (e.g., chondrocytes vs. chondroprogenitor cells) and culture systems (monolayer vs. 3D scaffolds) have also yielded inconsistent outcomes, partly due to methodological differences and the complex interplay of signaling pathways [18]. Recent investigations have highlighted the importance of mechanical loading regimes tailored to specific developmental stages, underscoring the possibility that pediatric chondrocytes may respond differently compared to adult cells under similar mechanical stimuli [19]. A comprehensive review of these studies reveals that while most findings support the notion that moderate, physiologically relevant loads enhance matrix synthesis, discrepancies remain regarding optimal loading parameters and potential threshold effects. Such inconsistencies warrant further research to clarify the nuanced relationships between mechanical cues, cell biology, and clinical outcomes in cartilage repair and regeneration. By controlling the duration and amplitude of strain, cells can adapt to the mechanical environment while enhancing their own functions, ultimately promoting the stable reconstruction of cartilage tissue under natural and artificial conditions. This logical chain effectively links basic biological phenomena with applied strategies, giving theoretical depth to the observations and providing a direction for practical operations.

Interestingly, when compared to adult cartilage cells, pediatric chondrocytes often exhibit distinct responses to mechanical load due to their higher proliferative capacity, more robust matrix production, and ongoing developmental signals. Studies on adult articular chondrocytes have shown that excessive or repetitive high-intensity loading can trigger catabolic pathways—elevating enzymes such as MMP-13—leading to cartilage breakdown rather than repair. In contrast, pediatric chondrocytes typically demonstrate greater resilience and stronger anabolic responses under moderate mechanical stimulation, as their extracellular matrix is still in the process of active growth and remodeling. These discrepancies highlight the importance of considering age-specific differences in mechanotransduction and cellular adaptation, especially when designing rehabilitation protocols or tissue-engineered constructs. By tailoring loading parameters to the developmental stage, it may be possible to harness the higher regenerative potential of pediatric cartilage, thus optimizing clinical outcomes and reducing the risk of degenerative changes later in life.

Under external stress conditions, chondrocytes do not passively accept all incoming stimuli. Instead, they accurately select inputs through specific receptors and

pathways. The integrin-mediated signaling, which is significantly enhanced under strain conditions, provides a stable channel for mechanical stimulation to enter the cell. As a sensing factor, integrin rapidly converts mechanical information into biochemical signals, constructing an efficient pathway for the cell to respond to external forces [20]. In this process, along with the enhanced expression of TGF-β1 and WNT3A, the upstream regulatory network of the cell no longer remains a mere quantitative change but tends toward qualitative optimization, gradually evolving into a more orderly, refined, and directional regulatory mode. When certain specific upstream molecules are selectively amplified under strain, while another portion of key nodes remain essentially unchanged, this reserved strengthening process helps the cell maintain a clear response target amid the intricate torrent of signals [21]. The lack of significant changes in TGFBR2 suggests that this pathway has not been fully activated, implying that the cell seems to be exploring how to keep certain regulatory pathways relatively silent under different stress loads, thereby achieving a more flexible and targeted response strategy. Mechanical stimulation is not a simple additive process; rather, it involves discrimination and selection, akin to a systematic input-output module that only opens or strengthens certain signaling pathways at the appropriate time to ensure that the ultimately manifested biological effects of the cell align with actual needs [22]. This selective amplification and stability maintenance pattern provides a basis for explaining how articular cartilage maintains function and homeostasis under long-term stress environments. Under loading conditions, cells constantly adjust the weighting of various pathways so that mechanical information can be rapidly and effectively converted into the biochemical signals required for life activities, while maintaining low or moderate responses in irrelevant pathways to conserve resources and reduce unnecessary disturbances. In this way, the chondrocyte's survival environment no longer depends on the passive provision of external conditions. Instead, driven by mechanical information, it actively shapes the distribution of internal activation and silence regions, thereby optimizing cellular responses, enhancing tissue homeostasis, and ensuring the functioning of the joint.

Under the existing mechanical signal input, chondrocytes do not treat all downstream molecules equally. Instead, they consciously select key pathways to optimize biochemical reactions. The significant upregulation of certain activating molecules indicates that the resources and energy of the entire network tend to be concentrated in these pathways. The enhanced expression of p-ERK and MAPK-related proteins suggests that the ERK/MAPK axis may occupy a central position in the linear stress response mechanism, providing a strong regulatory fulcrum for the cell to adjust proliferation, differentiation, and metabolic activities under dynamic load conditions [23]. This strategy enables the cell to more efficiently engage in synthesis, remodeling, and repair work under mechanical stimulation, reducing unnecessary energy consumption and prioritizing limited resources for more critical physiological processes. Not all pathways are amplified in equal proportion. Although β-catenin shows weak changes without reaching statistical significance, it hints that certain pathways may be finely limited under stress conditions to prevent potential adverse effects from excessive activation. When some pathways are significantly enhanced, others may be maintained under cautious regulation to preserve the overall harmony of the signaling network [24]. Through such reserved and focused scheduling, cells

achieve balance and integration along multiple signaling axes, refining mechanical information into precise molecular instructions. Effective resource allocation and pathway selection endow chondrocytes with better functional adaptability in a changing stress environment, and achieve improved homeostasis maintenance with minimal waste, thereby creating conditions for the joint's overall balance and continuous operation.

With the incremental gradient of strain amplitude, the molecular response of chondrocytes exhibits a clear dose-effect characteristic. Nevertheless, a more detailed exploration of this dose-response relationship could benefit from advanced analytical approaches, including mathematical or computational modeling. By quantifying the rate at which certain gene or protein expressions change in response to incremental strain levels, it may be possible to identify inflection points or threshold effects that standard statistical analyses overlook. Such modeling could also incorporate temporal factors, simulating how chondrocytes might adapt their matrix synthesis or signaling pathways over time under varying mechanical regimes. Ultimately, this integrative approach could offer deeper mechanistic insights and guide the design of more precisely targeted load-based interventions in both research and clinical contexts. As cells face increasing mechanical stimulation from low to high levels, their ability to synthesize key matrix molecules and activate specific signaling factors is enhanced. This trend is not accidental; rather, it reflects the quantitative adaptation cells make to the external environment under multidimensional regulation. By finely controlling external strain parameters within an appropriate range, cells can translate mechanical signals into precise and efficient biological outputs, thus creating conditions for cartilage tissue regeneration and the maintenance of homeostasis. Such regularity makes it possible to introduce measurable and predictable strain strategies in practice. In injury repair or tissue engineering, one need not rely on blindly applying loads; instead, evidence-based selection of strain amplitude and duration that match the target functional state can guide cells to grow, metabolize, and remodel under more appropriate mechanisms. Through this quantitative approach, one can not only reconstruct the basic morphology of healthy tissue, but also potentially enhance its load-bearing capacity and durability, enabling articular cartilage to maintain its optimal state during complex daily activities. This provides an effective pathway for clinical applications, converting the originally abstract concept of mechanical stimulation into operable parameter settings, and advancing from empirical models to data-driven precision interventions. As research continues to expand, optimizing strain parameters can further combine with pharmaceutical interventions, gene editing, or materials innovations to form a comprehensive, multi-level collaborative scheme. This would enable cartilage tissue not only to survive in complex mechanical environments, but also to present higher-level adaptation and stability, ultimately providing greater benefits for the restoration of individual function and quality of life.

The findings from this study have direct relevance for pediatric orthopedic and rehabilitation practice. By demonstrating that pediatric hip chondrocytes respond favorably to moderate mechanical loads—enhancing matrix synthesis and downregulating catabolic enzymes—our results suggest that strategically applied mechanical stimulation could be incorporated into early rehabilitation protocols for children with hip dysplasia or other developmental hip disorders. For instance,

targeted physiotherapy programs involving controlled weight-bearing exercises or specialized mechanical-loading regimens may help promote cartilage health and potentially prevent disease progression. From a surgical perspective, understanding the specific strain parameters that optimize chondrocyte proliferation and matrix production could guide postoperative rehabilitation strategies, including the timing and intensity of mobilization. Moreover, these insights may support the design of novel tissue-engineered constructs that harness the benefits of mechanical loading to encourage robust cartilage regeneration. Ultimately, translating these in vitro findings into clinical practice could offer more individualized, developmentally appropriate interventions, improving both the short-term recovery and long-term joint outcomes for pediatric patients.

Nevertheless, it should be acknowledged that this study was conducted under in vitro conditions, which may not fully recapitulate the complex biochemical and biomechanical milieu of the native hip joint. The absence of vascularization, immune components, and the three-dimensional architecture inherent to cartilage tissue in vivo can affect cell behavior and regulatory pathways. Additionally, pediatric hip cartilage may exhibit unique developmental dynamics and interactions within a growing organism, which are challenging to replicate in a simplified laboratory setup. Consequently, the direct translation of these findings to clinical scenarios should be approached with caution, and further in vivo or organotypic culture studies are needed to validate the mechanical loading parameters and their long-term effects on pediatric hip cartilage. A more comprehensive understanding of these limitations will guide future experimental designs and help ensure that the results are both biologically relevant and clinically translatable.

5. Conclusion

By precisely controlling strain parameters, this study elucidated the response characteristics of pediatric hip joint chondrocytes to mechanical loading from multiple perspectives, including proliferation rate, matrix synthesis, and activation of key signaling pathways. The results showed that under specific strain conditions, chondrocytes exhibit significantly enhanced proliferation and matrix synthesis, with selective regulation of related pathway molecules, demonstrating a clear dose-effect relationship. This refined response to mechanical information provides empirical references for subsequent clinical applications and engineering strategies, laying the foundation for improving the precision and efficiency of articular cartilage repair and functional reconstruction.

Author contributions: Conceptualization, YL and PY; methodology, YL; software, PY; validation, YL, PY and ZP; formal analysis, YL; investigation, PY; resources, GC; data curation, HL; writing—original draft preparation, YL; writing—review and editing, PY; visualization, GC; supervision, HL; project administration, JH; funding acquisition, JH. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge the Guangzhou Science and Technology Program — Joint Funding Project of Universities (Institutes) for Basic and Applied Basic Research

(Grant No. 202201020321). The authors acknowledge the Guangdong Provincial Hospital of Traditional Chinese Medicine Special Fund for Scientific and Technological Research in Traditional Chinese Medicine (Grant No. YN2024MS062).

Ethical approval: Not applicable.

Conflict of interest: The authors declare no conflict of interest.

References

1. Fu S, Thompson CL, Ali A, et al. Mechanical loading inhibits cartilage inflammatory signalling via an HDAC6 and IFT-dependent mechanism regulating primary cilia elongation. Osteoarthritis and Cartilage. 2019; 27(7): 1064-1074. doi: 10.1016/j.joca.2019.03.003
2. Fang T, Zhou X, Jin M, et al. Molecular mechanisms of mechanical load-induced osteoarthritis. International Orthopaedics. 2021; 45(5): 1125-1136. doi: 10.1007/s00264-021-04938-1
3. Cai X, Warburton C, Perez OF, et al. Hippo Signaling Modulates the Inflammatory Response of Chondrocytes to Mechanical Compressive Loading. bioRxiv. 2023. doi: 10.1101/2023.06.09.544419
4. Bloks NG, Harissa Z, Mazzini G, et al. A Damaging COL6A3 Variant Alters the MIR31HG-Regulated Response of Chondrocytes in Neocartilage Organoids to Hyperphysiologic Mechanical Loading. Advanced Science. 2024. doi: 10.1002/advs.202400720
5. He Z, Leong DJ, Xu L, et al. CITED2 mediates the cross-talk between mechanical loading and IL-4 to promote chondroprotection. Annals of the New York Academy of Sciences. 2019; 1442(1): 128-137. doi: 10.1111/nyas.14021
6. Gilbert SJ, Bonnet CS, Blain EJ. Mechanical Cues: Bidirectional Reciprocity in the Extracellular Matrix Drives Mechano-Signalling in Articular Cartilage. International Journal of Molecular Sciences. 2021; 22(24): 13595. doi: 10.3390/ijms222413595
7. Qi H, Zhang Y, Xu L, et al. Loss of RAP2A Aggravates Cartilage Degradation in TMJOA via YAP Signaling. Journal of Dental Research. 2022; 102(3): 302-312. doi: 10.1177/00220345221132213
8. Stampoultzis T, Guo Y, Nasrollahzadeh N, et al. Mimicking Loading-Induced Cartilage Self-Heating in Vitro Promotes Matrix Formation in Chondrocyte-Laden Constructs with Different Mechanical Properties. ACS Biomaterials Science & Engineering. 2023; 9(2): 651-661. doi: 10.1021/acsbiomaterials.2c00723
9. Chang SH, Mori D, Kobayashi H, et al. Excessive mechanical loading promotes osteoarthritis through the gremlin-1–NF-κB pathway. Nature Communications. 2019; 10(1). doi: 10.1038/s41467-019-09491-5
10. Savadipour A, Nims RJ, Katz DB, et al. Regulation of chondrocyte biosynthetic activity by dynamic hydrostatic pressure: the role of TRP channels. Connective Tissue Research. 2021; 63(1): 69-81. doi: 10.1080/03008207.2020.1871475
11. Li X, Han Y, Li G, et al. Role of Wnt signaling pathway in joint development and cartilage degeneration. Frontiers in Cell and Developmental Biology. 2023; 11. doi: 10.3389/fcell.2023.1181619
12. Jia S, Liu W, Zhang M, et al. Insufficient Mechanical Loading Downregulates Piezo1 in Chondrocytes and Impairs Fracture Healing Through ApoE-Induced Senescence. Advanced Science. 2024; 11(46). doi: 10.1002/advs.202400502
13. Jiang W, Liu H, Wan R, et al. Mechanisms linking mitochondrial mechanotransduction and chondrocyte biology in the pathogenesis of osteoarthritis. Ageing Research Reviews. 2021; 67: 101315. doi: 10.1016/j.arr.2021.101315
14. Zhao D, Li H, Liu S. TIMP3/TGF-β1 axis regulates mechanical loading-induced chondrocyte degeneration and angiogenesis. Molecular Medicine Reports. 2020. doi: 10.3892/mmr.2020.11386
15. Lee W, Nims RJ, Savadipour A, et al. Inflammatory signaling sensitizes Piezo1 mechanotransduction in articular chondrocytes as a pathogenic feed-forward mechanism in osteoarthritis. Proceedings of the National Academy of Sciences. 2021; 118(13). doi: 10.1073/pnas.2001611118
16. Zhang M, Xiong S, Gao D, et al. Tension regulates the cartilage phenotypic expression of endplate chondrocytes through the α-catenin/actin skeleton/Hippo pathway. Journal of Cellular and Molecular Medicine. 2024; 28(4). doi: 10.1111/jcmm.18133
17. Dietmar HF, Weidmann PA, Alberton P, et al. Load activated FGFR and beta1 integrins target distinct chondrocyte mechano-response genes. bioRxiv. 2024. doi: 10.1101/2024.10.11.617817
18. Aeppli T, Zhang Z, Zaman F, et al. SAT190 The Effect Of Mechanical Loading On Bone Growth Ex Vivo. Journal of the Endocrine Society. 2023; 7(Supplement_1). doi: 10.1210/jendso/bvad114.487

19. Somemura S, Kumai T, Yatabe K, et al. Physiologic Mechanical Stress Directly Induces Bone Formation by Activating Glucose Transporter 1 (Glut 1) in Osteoblasts, Inducing Signaling via NAD+-Dependent Deacetylase (Sirtuin 1) and Runt-Related Transcription Factor 2 (Runx2). International Journal of Molecular Sciences. 2021; 22(16): 9070. doi: 10.3390/ijms22169070
20. Song CX, Liu SY, Zhu WT, et al. Excessive mechanical stretch-mediated osteoblasts promote the catabolism and apoptosis of chondrocytes via the Wnt/β-catenin signaling pathway. Molecular Medicine Reports. 2021; 24(2). doi: 10.3892/mmr.2021.12232
21. Zhuang H, Ren X, Zhang Y, et al. Trimethylamine-N-oxide sensitizes chondrocytes to mechanical loading through the upregulation of Piezo1. Food and Chemical Toxicology. 2023; 175: 113726.
22. Higgins SM, Jones RC, Lawrence KM, et al. Investigation of the chondrocyte response to varying mechanical load: assessment of impact stress. Osteoarthritis and Cartilage. 2020; 28: S179. doi: 10.1016/j.joca.2020.02.290
23. Yan M, Sun Z, Wang J, et al. Single-cell RNA sequencing reveals distinct chondrocyte states in femoral cartilage under weight-bearing load in Rheumatoid arthritis. Frontiers in Immunology. 2023; 14. doi: 10.3389/fimmu.2023.1247355
24. Kotelsky A, Carrier JS, Buckley MR. Real-time Visualization and Analysis of Chondrocyte Injury Due to Mechanical Loading in Fully Intact Murine Cartilage Explants. Journal of Visualized Experiments. 2019; (143). doi: 10.3791/58487