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Original Article

Mechanism of static loading injury in human skeletal muscle cells

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Abstract: Objective: To establish a cellular-level mechanical injury model for human skeletal muscle cells and investigate changes in the mechanical effect mechanism after such injuries. Methods: The FX-5000™ Compression System was used to apply constant static mechanical pressure to human skeletal muscle cells. A factorial design analysis was conducted to discover the optimal injury model by evaluating the correlation between the amount of pressure, the duration of mechanical stimulation, and the number of days of observation. Skeletal muscle cell injury was evaluated by measuring cell metabolism, morphology, and calcium homeostasis. Results: Mechanical injury was modeled as continuous pressure of 1 MPa for 2 hours with observation for 3 days. The results show that mechanical injury increased creatine kinase, intracellular Ca^{2+} concentration, and malondialdehyde content, decreased superoxide dismutase, and caused cell swelling and severe cytoplasmic vacuolization (all $P < 0.05$). Conclusion: This model of mechanically-injured human skeletal muscle cells provides an experimental model for the clinically common skeletal muscle injury caused by static loading pressure. It may be used to study the mechanism of action of treatment methods for mechanically injured skeletal muscle.

Keywords: Mechanical injury, factorial design, FX-5000™, mechanical effects, mechanism study

Introduction

Experimental studies frequently utilize skeletal muscle injury models, including models of drug injury and mechanical injury [1]. The drug injury approach is suitable only for modeling skeletal muscle cell injury under the theory of free radical and lipid peroxidation injury, which differs somewhat from common clinical skeletal muscle injuries [2, 3]. Mechanical injury models can avoid this deficiency and are more appropriate for clinical practice. These models often simulate mechanical muscle injury using striking, pulling, and pressing methods [4]. The striking method uses blunt, non-penetrating effects as a clinical contusion simulation [5]. Modeling muscle strain caused by pulling involves experiments accompanied by electrical stimulation, although the result of this method differs significantly from that of strains in humans caused primarily by centrifugal forces resulting from movements [6, 7]. To simulate pressing, mechanical injury models use a continuous pressure load to cause chronic injury that is highly analogous to chronic injury to human skeletal muscle. However, the intensity and duration of the pressure required for this injury are stringent, and research on direct action at the cellular level has only begun.

The FX-5000™ compression system utilized in this study's experimental intervention can accurately regulate the cycle, magnitude, frequency, and duration of the loading force. This system operates directly at the cellular level, emulating the prolonged constant pressure that the human body endures, efficiently replicating static loading injury to human skeletal muscle. The action of external mechanical forces induces changes in the cellular biomolecular structure, converting force signals into physiological and biochemical signals and triggering subse-

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quent cascade reactions to exert biological effects [8]. Research has shown that drugs, ischemic perfusion, and other types of injury cause oxidative stress, Ca^{2+} overload, and other phenomena in skeletal muscle cells [9, 10]. However, it is unknown whether such changes also occur in mechanically stimulated injury of human skeletal muscle cells.

In this study, human skeletal muscle cells were pressurized with continuous static mechanical force using the FX-5000™ compression system. Injury measurements were screened based on the cell survival rate and then validated for cell metabolism, morphology, and calcium homeostasis to develop a model for mechanically-injured human skeletal muscle cells. This model offers an experimental reference for the common skeletal muscle injuries caused by static loading pressure in clinical settings. Additionally, it establishes a basis for understanding the mechanism of the mechanical effect of therapeutic methods for mechanically-injured skeletal muscle.

Materials and methods

Cultured human skeletal muscle cells

Human skeletal muscle cells were derived from primary cells from the ScienceCell Research Laboratory (ScienceCell Research Laboratory, Carlsbad, USA). The skeletal muscle cell medium contained 5% fetal bovine serum, 1% skeletal muscle cell growth supplement, 10,000 μg/ml streptomycin, and 10,000 U/ml penicillin. Cells were cultured in an incubator (Thermo Fisher Scientific, Shanghai, China) at 37°C with 5% CO_2. All the reagents were purchased as high-quality products from regular sources, and all the experiments were conducted using the above-mentioned contamination-free cells.

Culture of cells in a biomimetic three-dimensional environment

To build a model of mechanically injured human skeletal muscle cells, the cells needed to be cultured in a three dimensional (3D) environment to simulate the structure of skeletal muscle cell survival in the human body. For this purpose, a 3D Life Dextran-Peg hydrogel kit (Cellendes, Reutlingen, DE) was used to prepare the 3D culture of normal cells. The cell-containing hydrogel was then placed in a six-well plate (Shanghai Titan Technology Co., Ltd., Shanghai, China) and incubated for further culture.

Pressurization of cells by the FX-5000™ compression system

The mechanical injury modeling study was divided into three groups: a blank control group, a normal control group, and an injured group. The normal control and injured groups were incubated in a 3D environment, and the blank control group was incubated in a two-dimensional environment of normal cells and then compared to the normal control group to observe the effect of the 3D hydrogel on the cells. The cells were placed in six-well BioFlex® culture plates (Flexcell International Corporation, USA). Cells in the injury group underwent mechanical injury through static continuous pressure (static wave, frequency 0.1 Hz) produced by the FX-5000™ Compression System (Flexcell International Corporation, USA). **Figure 1** presents a schematic diagram of the mechanical injury modeling.

Factorial design

Two rounds of factorial design screening were conducted. The screening showed that the 3D hydrogel could withstand a maximum pressure of 1.2 MPa for 1 hour (h) and that cells could survive in the 3D hydrogel environment without any intervention for a maximum of 3 days (d) (**Table 1**). The first screening was done with pressures of 0.4, 0.8, and 1.2 MPa; pressure durations of 1, 2, 3, and 4 h; and experiment durations of 1, 2, and 3 d. The second screening was done with pressures of 0.8, 1.0, and 1.2 MPa; pressure durations of 1, 2, and 3 h; and experiment durations of 1, 2, and 3 d. The results of the study were measured by the cell survival rate.

Cell survival rate

Three groups of cells were extracted from the hydrogel at the time of the assay so that each group was kept in the same culture environment. All three groups were maintained in equal volumes of a cell and medium mixture. The quantity of the mixture was determined based on the blank control. Because the blank control group had the most comprehensive cell counts, changes in cell counts could be clearly observed

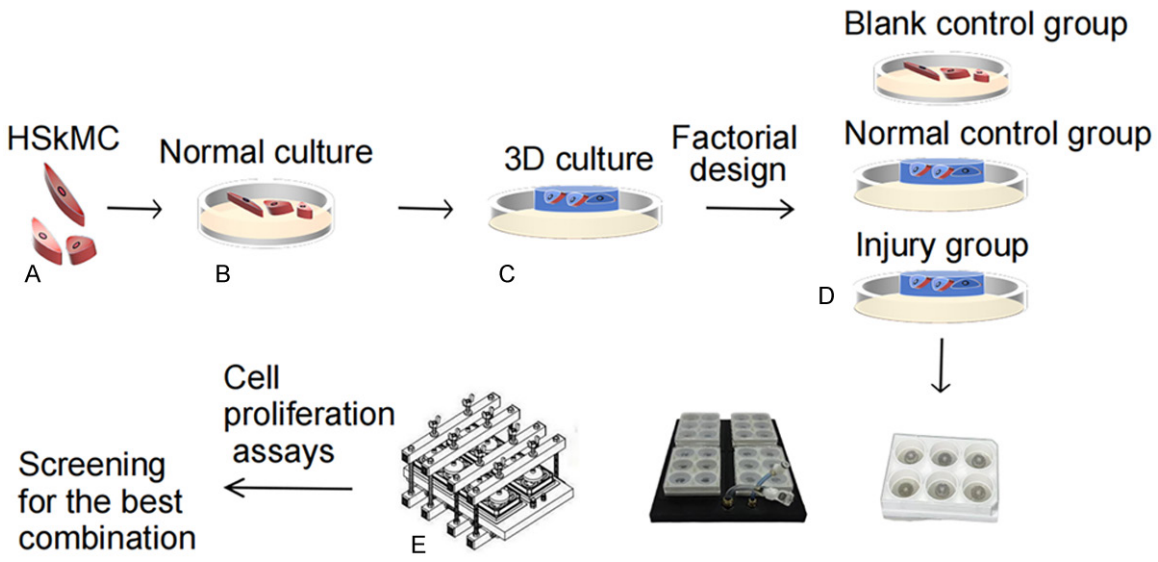

Figure 1. Schematic diagram of the operation of mechanically injured human skeletal muscle cells (HSkMC). A. Normal HSkMC. B. Normal HSkMC culture. C. Three-dimensional environment cell culture for observing the effect of the 3D environment on the cells. D. After the factorial design, the cells were grouped into a blank control group, a normal control group, and an injury group. E. The process of the intervention for the injury group (illustrated from right to left), continuous static pressurization, and, finally, preliminary screening by cell survival rate.

Table 1. Effect of the 3D environment on the active proliferation rate of skeletal muscle cells (n = 6)

	Day 1	Day 2	Day 3	Day 4	Day 5
Cell survival rate (%) (mean ± SD)	63.20 ± 0.17	56.36 ± 0.32*	46.43 ± 0.45$^{*,▲}$	39.13 ± 0.15$^{*,▲,\#}$	22.36 ± 0.11$^{*,▲,\#,■}$

Notes: *P < 0.05 compared to day 1, $^{▲}$P < 0.05 compared to day 2, $^{\#}$P < 0.05 compared to day 3, $^{■}$P < 0.05 compared to day 4.

when cells were injured. The mixtures were diluted to a density of 1×10^4 cells/well, uniformly inoculated in a 96-well plate (Thermo Fisher Scientific, Shanghai, China), and incubated for 12 h. The cell survival rate was then measured using an enhanced CCK-8 kit (Beyotime, Shanghai, China). The cells were incubated for an additional 4 h after adding CCK-8, and the absorbance was measured at a wavelength of 450 nm using a fully automated enzyme labeling instrument (ELX800, Biotek, Vermont, USA), which was operated while being protected from light. The cell survival rates of the normal control and injured groups were determined by comparing their optical density with that of the blank control group. Then, by subtracting the cell survival rate of the control group from that of the injured group, we calculated the impact of mechanical injury on the cell survival rate while eliminating the influence of the hydrogel.

Procedures for measuring creatine kinase activity, superoxide dismutase activity, and malondialdehyde content

After screening the parameters of the mechanically-injured human skeletal muscle cell model, the difference between the normal control group and the injured group was observed by cell metabolism, which was detected by creatine kinase (CK), superoxide dismutase (SOD), and malondialdehyde (MDA) assay kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China). CK was measured by the colorimetric method, SOD was measured by the WST-1 method, and MDA was measured by the thiobarbituric acid method.

Cell ultrastructure study method

Changes after cell injury were observed from a morphological perspective. (1) The groups of

cells were collected and fixed in 2.5% glutaraldehyde solution (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 3 hours. (2) The samples were washed and rinsed in phosphate buffer solution (Suzhou No. 3 Pharmaceutical Factory Co., Ltd., Suzhou, China) three times for 20 min each. (3) They were then fixed in 1% osmium tetroxide (Alfa Aesar Company, Shanghai, China) for 2 h and rinsed in buffer solution three more times for 15 min. (4) Next, the samples were dehydrated for 20 min at 4°C in 30%, 50%, 70%, and 80% ethanol successively and 20 min in a 90% ethanol:90% acetone (1:1) solution. (5) The samples were soaked through, rinsed three times with 100% acetone at room temperature for 20 min each, and placed sequentially in pure acetone and resin (1:1) solution (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 20 min at room temperature before being placed in pure resin in an oven for 1.5 h two times. (6) They were then embedded for 12 hours at 37°C and 48 hours at 60°C. (7) After the material preparation procedure was completed, 0.5-μm sections were obtained using a diamond knife in an ultra-thin sectioning machine (Leica, Wetzlar, DE). (8) Double-staining was carried out with uranyl acetate dioxide-lead citrate (Sinopharm Chemical Reagent Co., Ltd., China), and the samples were air-dried and prepared for use. (9) Transmission electron microscopy (FEI, Hillsboro, USA) was used for observation.

Changes in intracellular Ca^{2+} concentration

A Fluo-4 AM (calcium ion fluorescent probe, 2 mM) kit (Beyotime, Shanghai, China) was used for the assay. The cells were extracted from the hydrogel and transferred to a six-well dish for incubation; four sub-wells were prepared for each cell group, maintaining a uniform cell count. Subsequently, adherent cells were washed three times with phosphate-buffered saline (PBS) (Shanghai Zhongqiao Xinzhou Biotechnology Co., Ltd., Shanghai, China), and 200 μL Fluo-4 AM diluted with PBS to a concentration of 4 μM was added to each well. The dishes were incubated in the incubator for 30 min, protected from light. The Fluo-4 AM was discarded, washed with PBS, and observed for changes in Ca^{2+} fluorescence intensity with excitation wavelengths of 420-485 nm using a 10× fluorescence microscope (Olympus Co., Ltd., Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using SPSS 25.0 software (IBM, Armonk, USA). Normally distributed data underwent multifactorial analysis of variance (ANOVA) to analyze the main effects of pressure, pressure duration, and experiment duration as well as the interaction between the three factors. The SOD activity, MDA content, CK activity, and intracellular Ca^{2+} fluorescence intensity results were compared between the groups using one-way ANOVA. The results are expressed as mean ± standard deviation. Data that were not normally distributed or had uneven variances were tested using non-parametric tests.

Results

Factorial design screening results

Two factorial design rounds were conducted in this study. The cell survival rate following mild to moderate cell injury should differ from the normal control group by 20%-50% [11, 12]; this rate was selected by observing the changes in the cell survival rates. In the first round of factorial design, the pressure was set to 0.4, 0.8, and 1.2 MPa; the pressure duration was set to 1, 2, 3, and 4 h of continuous mechanical force injury; and the durations of observation were 1, 2, and 3 d. The results of screening showed that at 0.4 MPa, even when pressurized for 4 h and observed for 3 d, the difference in the cell survival rate compared to the normal control group was less than 10%, which did not meet the requirement of the experimental design. The decrease in the cell survival rate following 0.8 MPa and 1.2 MPa of pressure was more in accordance with the experimental design; in particular, the cell survival rate at 0.8 MPa pressurization for 3 h and 3 d of observation was reduced by approximately 20% compared with the normal control group (**Figure 2**). The statistical results showed a main effect of pressure, duration, and length of observation ($P < 0.05$) and an interaction between the three factors ($P < 0.05$). However, the difference between 0.8 MPa and 1.2 MPa was large, so a second round of factorial design was conducted to narrow the range.

The second round of factorial design included the following parameters: pressure of 0.8, 1, and 1.2 MPa; duration of continuous mechani-

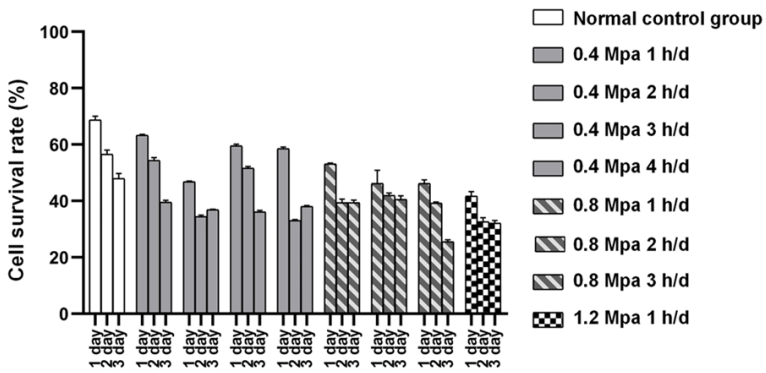

Figure 2. First round of survival rate screening of skeletal muscle cell activity by different pressure intensity, duration of pressurization, and days of pressurization. In the figure, 0.4 MPa 1 h refers to mechanical injury pressurization of 0.4 MPa for 1 hour; the hydrogel broke in the experiments with 0.8-MPa pressurization for 4 h and 1.2-MPa pressurization for 2-4 h and they could not be continued, so these experiments were omitted from the figure. In the blank control group, cells were cultured in a two-dimensional environment. The optical density of this group was mainly used to calculate the survival rate of the normal control group versus the injured group; therefore, it is not depicted in the graphs.

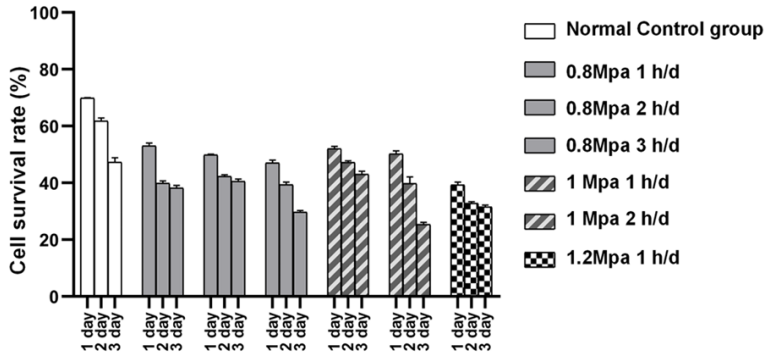

Figure 3. Second round of screening of the survival rate of skeletal muscle cell activity by different pressure intensity, duration of pressurization, and days of pressurization. In the figure, 0.8 MPa 1 h refers to mechanical injury pressurization of 0.8 MPa for 1 hour; the hydrogel broke in the experiments with 1-MPa pressurization for 3 h and 1.2-MPa pressurization for 2-3 h and they could not be continued, so these experiments were omitted from the figure.

cal force injury of 1, 2, and 3 h; and length of observation of 1, 2, and 3 d. The results showed that the parameter combination of 1 MPa pressure for 2 h observed over 3 d was most consistent with a 20%-50% reduction in the cellular proliferation rate compared to the normal control group (**Figure 3**). The main effects of all three factors were significant ($P < 0.05$) and all three interacted with each other ($P < 0.05$). The optimal parameter combination to establish the model of mechanically injured human skeletal muscle cells was 1 MPa of continuous pressurization for 2 h and observation of 3 d.

Procedures for measuring CK activity, SOD activity, and MDA content

The injured group showed increased CK activity ($P < 0.05$) (**Figure 4A**), decreased SOD activity ($P < 0.05$) (**Figure 4B**), and increased MDA content ($P < 0.05$) (**Figure 4C**). Moreover, prolonged static mechanical stimulation decreased the ability of the human skeletal muscle cells to resist oxidative damage and it increased oxygen radicals and the generation of lipid oxidation reactions, leading to cellular injury.

Cellular ultrastructure studies

In the normal control group, the cell morphology exhibited no abnormalities; the cell and nuclear membranes were intact, the cell nuclei were normal, and the organelles were evenly distributed (**Figure 5A**, **5B**). In the injured group, the cell membrane remained mostly intact, and the cell morphology was relatively normal. However, significant swelling was observed, and the organelles were unequally distributed. Although the mitochondria were not visibly affected, severe vacuolation of the cells was observed. These results show that mechanical force stimulation in a sustained static state significantly impacted the internal structure of the cells (**Figure 5C**, **5D**).

Changes in intracellular Ca^{2+} concentration

Fluo-4 AM was utilized to detect the concentration of intracellular Ca^{2+} in this study. The graph in **Figure 6A** shows that the green fluorescence intensity of cells in the injured group was significantly higher than that of cells in the normal

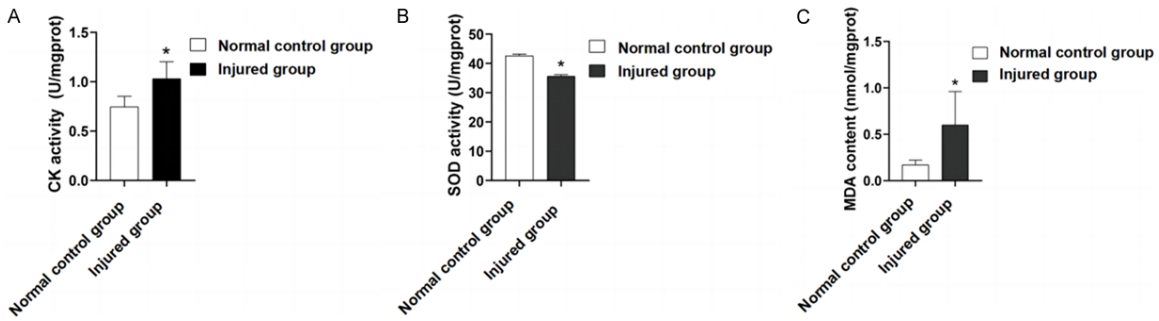

Figure 4. Effect of mechanical injury on metabolic indices of human skeletal muscle cells. Changes in (A) intracellular creatine kinase, (B) intracellular superoxide dismutase, and (C) intracellular malondialdehyde. *P < 0.05 compared to the normal control group.

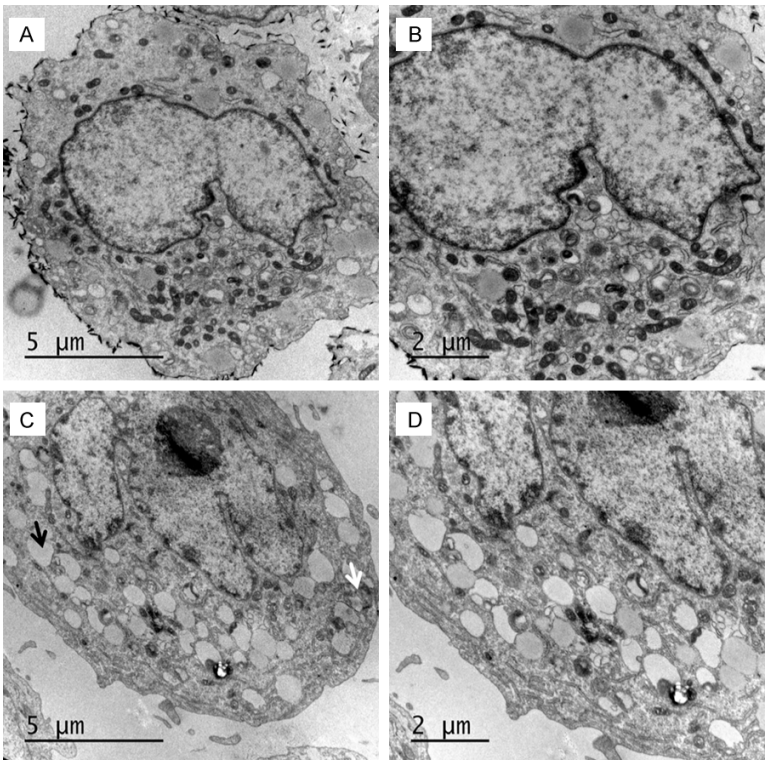

Figure 5. Ultrastructural changes in mechanically injured human skeletal muscle cells in (A, B) the normal control group and (C, D) the injury group. The cells are magnified 4,200 times in (A) and (C) and 6,000 times in (B) and (D). In (C), the black arrow indicates intracellular vacuoles, and the white arrow indicates uneven distribution of organelles. In (C) and (D), the overall whitish color of the cells in the injury group indicates cell swelling.

control group. The fluorescence intensity values were analyzed using ImageJ-Win64 software, which also indicated that the injured group had a higher fluorescence intensity than the normal control group (P < 0.05) (**Figure 6B**). These results show that the continuous static mechanical stimulation led to an increased intracellular Ca^{2+} concentration in human skeletal muscle cells.

Discussion

Most of the common experimental models of mechanically injured human skeletal muscle cells are animal models, but the controllability of animal experimental models is highly variable [13]. In vitro cell cultures can reduce and refine the shortcomings of animal models and be applied to experimental protocols in a highly controllable manner [14]. In this study, normal human skeletal muscle cells were cultured in vitro in 3D biomimetic hydrogels that perfectly mimic the natural microenvironment [15]. Mechanical pressure-induced cell death is related to the pressure duration, pressure intensity, and number of pressure cycles; therefore, the parameters of the mechanical injury model are influenced by multiple factors and must be studied using a multifactorial study design [16, 17]. In this study, a factorial design analysis was used to observe the relationship between pressure intensity and duration and the number of cycling days. The factorial design enables an analysis of the main effect of each factor and the interaction between factors, which can pro-

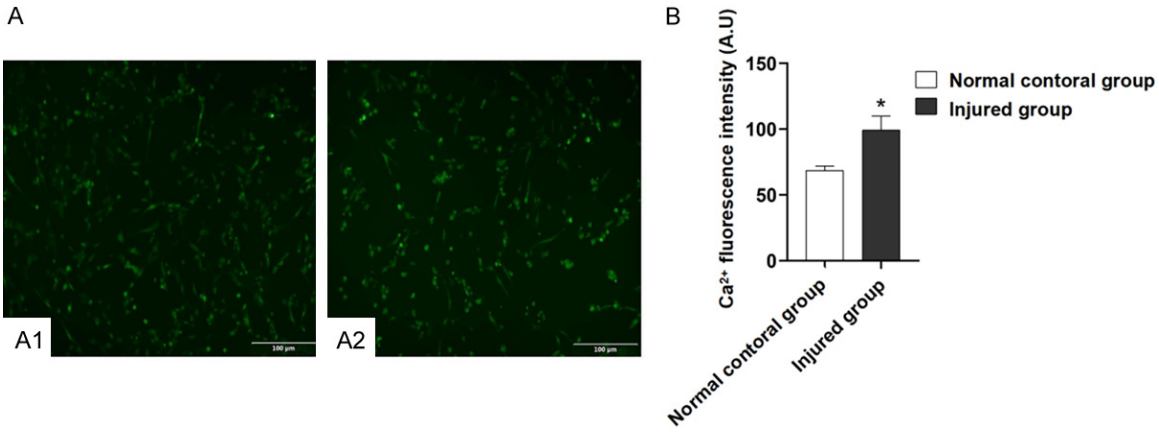

Figure 6. Effect of mechanical injury on the intracellular calcium ion concentration in human skeletal muscle cells. A. Visualization of calcium ion concentration fluorescence (10× microscope) in the normal control group (A1) and the injury group (A2). B. Result of the ImageJ-Win64 analysis. *P < 0.05.

vide more detailed information and is suitable for screening mechanical injury models [18].

Two rounds of screening were conducted in this study. The results of the first round of factorial design screening showed that the cell survival rate of the normal control group gradually decreased as the number of days of observation increased, indicating that the hydrogel had some effect on the cells. The hydrogel's inherent viscoelasticity mimics the viscoelastic characteristics of tissues and was, therefore, able to overcome the pressure limitations of the hydrogel itself, but the viscoelasticity still had some effect on the cells [15]. When observing one day, the cells were continuously subjected to a fixed pressure of 0.4 Mpa or 0.8 Mpa, and it was found that there was no too obvious fluctuating trend in the cell survival rate as the pressurization duration increased. However, when the cells were observed for 3 consecutive days, it was found that the cell proliferation rate gradually decreased with the increase in the number of experimental days. The cell survival rate was most obviously decreased in the case of large pressure and long experimental duration, which shows that prolonged days of pressurization had obvious effects on skeletal muscle injury, and the rest period during the injury did not play a role in repairing the micro-damage of skeletal muscle cells.

The results of the second round of screening showed that when the pressure intensity reached 1 MPa, the degree of reduction in the cell survival rate was proportional to the duration of pressurization and the number of days of pressurization. At continuous pressurization of 1 MPa for 2 h over 3 d, there was a difference of approximately 21.93% in the cell survival rate compared to that of the normal control group, whereas continuous pressurization of 0.8 MPa for 3 h over 3 d showed a difference of only 17.63% in the cell survival rate. Continuous pressurization of 1 MPa for 2 h over 3 d was more in line with the experimental design goal of mild to moderate injury of skeletal muscle cells. The results further show a strong relationship between the three factors of pressure, duration, and number of days such that they form an inseparable whole.

Skeletal muscle cell injury can result from disturbances in energy metabolism, dysregulation of intracellular calcium homeostasis, and other causes and can be verified by metabolic indices, morphology, and calcium homeostasis of the injury [4, 19]. The study results show that mechanical injury to human skeletal muscle cells resulted in increased CK activity and MDA content and decreased SOD activity. When skeletal muscle cells are subjected to prolonged mechanical pressure stimulation, the destruction of the sarcoplasmic reticulum leads to limited uptake of intracellular calcium ions. Moreover, the sarcoplasmic reticulum membrane Ca^{2+}-ATPase needs to hydrolyze a large amount of ATP to release energy to support the uptake of calcium ions, and a large amount of CK is released outside of the cell membrane, leading to an increase in the level of CK activity [20-22]. Prolonged mechanical

pressure stimulation also leads to an increase in intracellular oxygen free radicals, and a large number of free radicals produce lipid oxidation reactions on the cell membrane, generating a large amount of MDA [23, 24]. This leaves the cell in a state of imbalance between oxidation and antioxidants, which reduces the vitality of SOD, an antioxidant, so that it can no longer contribute to scavenging free radicals, reducing inflammation, and other antioxidant functions [25, 26]. In this study, the increased CK activity and MDA content and the decreased SOD activity together verify that intracellular metabolic disorders and oxidative stress occurred after mechanical injury to the human skeletal muscle cells, which confirms the success of the model of mechanically injured human skeletal muscle cells from the point of view of cellular metabolism.

The cellular ultrastructure detection results show that the normal control group had a basically normal cell morphology, complete cell membranes and nuclear membranes, normal cell nuclei, and evenly distributed organelles. In the injury group, the cell membranes were relatively complete and the cell morphology was relatively normal, but the cells were swollen, the organelles were unevenly distributed, and the cells were severely vacuolated, although the mitochondria were not obviously swollen. Cytoplasmic vacuolization is an abnormal manifestation of the cell mainly originating from the endoplasmic reticulum, lysosomes, mitochondria, and all other organelles with membrane structures [27]. Studies have shown that cytoplasmic vacuolization is mainly caused by osmotic interactions between the disturbed ionic homeostasis in the endoplasmic reticulum, mitochondria, and other organelles and is not a function of intracellular proteins that control cellular function [28]. Therefore, mechanical injury to human skeletal muscle cells may lead to disturbances in intracellular ionic homeostasis. Excessive accumulation of vacuoles causes metabolic abnormalities, endoplasmic reticulum stress, oxidative stress, and other responses that lead indirectly to cell death [29]. Of all the organelles, the mitochondria are most sensitive to cell injury: when skeletal muscle cells are injured, the mitochondria become swollen, and static loading leads to an increase in the concentration of calcium ions in the mitochondria [30-32]. The results of this study showed that the mitochondrial changes were not significant in the injury group, but it was inferred that the mitochondria were injured by the cellular metabolism assay. The study showed that mitochondrial function changes were not significant when mild to moderate stress stimuli were applied [33, 34]. Thus, the increase in the calcium ion concentration after the injury may not have reached the limit of the mitochondria, or the 3 days of mechanical stimulation may not have been sufficient to achieve mitochondrial swelling. The cell swelling and cytoplasmic vacuolization further verify the success of the modeling of mechanically injured human skeletal muscle cells from a morphological point of view, consistent with the conclusions from the metabolic indicators.

Ca^{2+} functions as a second messenger in skeletal muscle and plays a significant role in the processes of the excitation-contraction-relaxation cycle and skeletal muscle cell degradation. An imbalance in Ca^{2+} homeostasis can lead to direct skeletal muscle cell injury [35]. When the cells encounter mechanical stimulation, the pressure stimulates mechanosensitive ion channels present on the cell membrane, which in turn decreases the permeability of the cell membrane for ions, thereby promoting the entry of Ca^{2+} into the cytoplasm [36]. When the concentration of cytoplasmic Ca^{2+} increases, it inhibits the uptake and release function of Ca^{2+} in the mitochondria, resulting in an additional increase in the intracytoplasmic Ca^{2+} concentration [36, 37]. This subsequent increase causes calcium overloading, ultimately leading to cellular injury. The study results demonstrate a marked increase in Ca^{2+} fluorescence intensity following mechanical injury to human skeletal muscle cells, indicative of a significant elevation in the intracellular Ca^{2+} concentration after injury.

The calcium ion concentration assay results align with the trend observed in the earlier cellular metabolism assay. Mechanical injury leads to an increase in the intracellular oxidative stress metabolite MDA content, a decrease in the activity of the oxygen radical scavenger SOD, and the occurrence of oxidative stress in the cells; whereas an increase in the intracellular Ca^{2+} concentration leads to the occurrence of oxidative stress in the cells, which showed that the cellular metabolism assay indirectly responded to the fact that mechanical injury to human skeletal muscle cells leads to

overloading of the intracellular Ca^{2+} concentration, which in turn results in cellular injury. The cellular ultrastructure examination showed that mechanical injury caused severe vacuolization and cell swelling. Increased vacuolization leads to endoplasmic reticulum stress and oxidative stress responses, which are caused by Ca^{2+} overload. These responses corroborate the results of the intracellular calcium ion concentration assay. Successful modeling of mechanically injured human skeletal muscle cell models was demonstrated by three different aspects of cellular metabolism, morphological changes, and calcium ion concentration.

This study successfully established a model of mechanically injured human skeletal muscle cells with an optimal parameter combination of 1 MPa of pressure for 2 h over 3 d. Continuous static mechanical injury has a certain effect on the metabolism, morphology, and calcium homeostasis of human skeletal muscle cells. This study offers an experimental model for clinically common skeletal muscle injury caused by static load pressure and helps to further the study of the mechanism of action of treatment methods for such injuries.

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Disclosure of conflict of interest

None.

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