Journal of Biomechanics 49 (2016) 1305–1310

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Change in viability of C2C12 myoblasts under compression, shear and oxidative challenges

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ARTICLE INFO

Article history:
Accepted 10 March 2016

Keywords:
Shear
Damage mechanics
Myoblasts
Oxidative stress
Deep tissue injury

ABSTRACT

Skeletal and epidermal loadings can damage muscle cells and contribute to the development of deep tissue injury (DTI) – a severe kind of pressure ulcers affecting many people with disability. Important predisposing factors include the multiaxial stress and strain fields in the internal tissues, particularly the vulnerable muscles around bony prominences. A careful study of the mechanical damage thresholds for muscle cell death is critical not only to the understanding of the formation of DTI, but also to the design of various body support surfaces for prevention. In this paper, we measured the mechanical damage thresholds of C2C12 myoblasts under prescribed compressive strains (15% and 30%) and shear strains (from 0% to 100%), and studied how oxidative stress, as caused potentially by reperfusion or inflammation, may affect such damage thresholds. A flat plate was used to apply a uniform compressive strain and a radially increasing shear strain on disks of Gelatin–methacrylate (GelMA) hydrogel with myoblasts encapsulated within. The percentages of cell death were estimated with propidium iodide (PI) and calcein AM staining. Results suggested that cell death depended on both the level and duration of the applied strain. There seemed to be a non-linear coupling between compression and shear. Muscle cells often need to function biomechanically in challenging oxidative environments. To study how oxidative stress may affect the mechanical damage thresholds of myoblasts, cell viability under compressive and shear strains was also studied after the cells were pre-treated for different durations (1 h and 20 h) with different concentrations (0.1 mM and 0.5 mM) of hydrogen peroxide (H_2O_2). Oxidative stress can either compromise or enhance the cellular resistance to shear damage, depending on the level and duration of the oxidative exposure.

1. Introduction

Non-ambulatory people such as those with severe physical disabilities commonly suffer from pressure ulcers, which are defined by the National Pressure Ulcer Advisory Panel (NPUAP) (2009) as 'localized injuries to the skin and/or underlying tissues usually over a bony prominence, as a result of pressure, or pressure in combination with shear'. Pressure ulcers impair life quality, potentially lead to infection and other clinical complications, and can largely increase the cost of hospitalization as well as clinical risks (Allman et al., 1999; Agam and Gefen, 2007; Dealey et al., 2012).

Depending on the nature of the loading, pressure ulcers can develop from the superficial skin into the deep tissue layers or start deep and develop towards the skin. These two types of pressure ulcers are referred as superficial pressures ulcer and deep tissue injury (DTI) respectively (NPUAP, www.npuap.org; Ankrom et al., 2005). DTI, because of its deeper origin, can be challenging to detect early for timely management.

The generation and development of DTI include complex processes such as ischemic damage, damage due to direct cell deformation, reperfusion damage and blockage of lymphatic drainage (Kosiak, 1959; Daniel et al., 1981; Miller and Seale, 1981; Reddy et al., 1981; Gawlitta et al., 2007b; Mak et al., 2010). Among them, direct cell deformation has been reported contributing much to DTI (Gawlitta et al., 2007a, 2007b; Xiao et al., 2014). Experimental studies in different scales and models have been reported, including single cell experiments, experiments on cells monolayer, studies using bio-artificial muscles, as well as animal studies. (Bouten et al., 2001; Wang et al., 2005; Berlowitz and Wilking, 1989; Gefen et al., 2008; Ceelen et al., 2008; Yao et al., 2015). Results showed that the level of deformation/stress and the loading duration were two key factors determining the damaging effects. The tolerance of cells to strain/stress decreases as loading

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http://dx.doi.org/10.1016/j.jbiomech.2016.03.014
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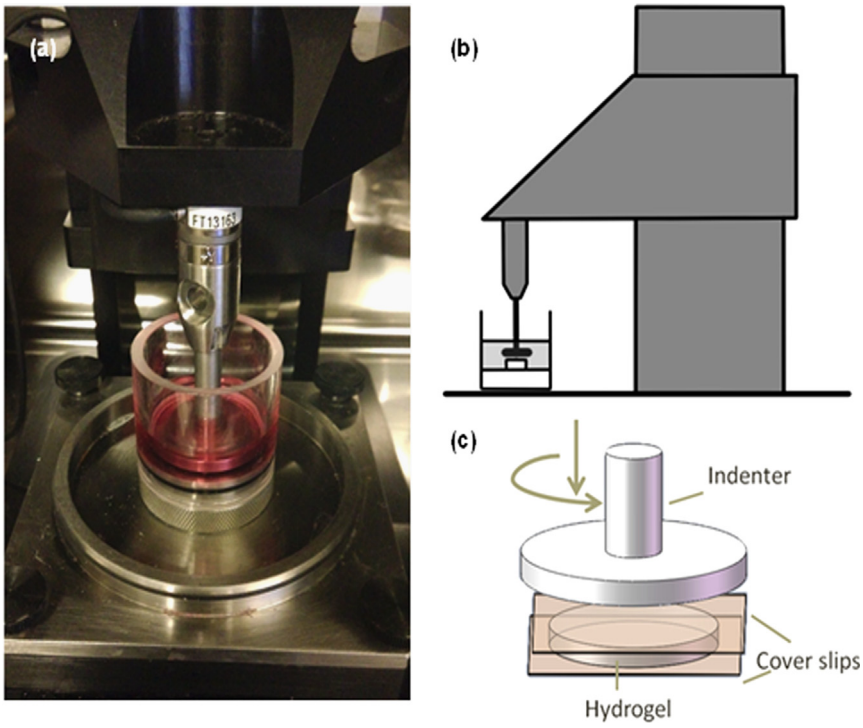

Fig. 1. Experimental setup for compressive and shear loading. (a) Actual setup for experiments. (b) A schematic diagram showing the experimental setup. (c) The close-up arrangement of the flat loading plate and the cells – embedded hydrogel. Twisting was performed to generate a radially increasing shear strain field inside the cylindrical hydrogel.

duration increases in a decreasing sigmoid relationship of the Boltzmann type (Gefen et al., 2008; Yao et al., 2015).

Apart from compressive damage, shear can also contribute in development of pressure ulcer (Bennett and Lee, 1985; Crenshaw and Vistnes, 1989; Curley et al., 2003; Bluestein and Javaheri, 2008). Frictional shear on skin could reportedly compromise skin blood perfusion, leading to ischemic damage (Bennett et al., 1979; Zhang and Roberts, 1993). Frictional shear may also generate heat or blister and accelerate the breaking down of skin (Dinsdale, 1974; Goldstein and Sanders, 1998). However, regarding injuries to the deeper tissues, the damaging effect of shear is still poorly understood.

It has been hypothesized that oxidative stress, as potentially caused by post-ischemic reperfusion or inflammation, might also contribute to the development of DTI (Choudhury et al., 1991; Peirce et al., 2000; Federico et al., 2007). On cellular level, oxidative stress is related to the release of cytochrome c, a key protein associated with triggering caspase activities and cell apoptosis (Ott et al., 2007; Siu et al., 2009). Oxidative stress may also affect the polymerization and assembly of the actin cytoskeleton through up or down regulation of different signaling pathways such as Rho signaling (Sun et al., 2014; Wong et al., 2015). Depending on oxidant concentration and exposure duration, oxidative stress exposure may have different effects on the tolerance of cells to physical insults (Yao et al., 2015; Duan et al., 2015).

In this paper, we reported for the first time the measurements of shear damage thresholds of C2C12 myoblasts for cell death under combined compressive and shear strains, and studied how oxidative stress may affect such damage thresholds.

2. Materials and methods

2.1. Gelatin methacrylate synthesis

At 50 °C, 10 g of gelatin (type A) was dissolved in 100 mL of Dulbecco's phosphate-buffered saline (PBS, 1X, Gibco, Life Technologies). A total of 12 mL methacrylate anhydrate was then added to this 10% gelatin solution and stirred for 4 h under the same temperature. The product solution was then placed in an envelope made of 6 KDa cut-off dialysis membrane and dialyzed against Deionized water (DI water) for one week at 45 °C, with DI water changed three times per day to remove the remaining extra methacrylate anhydrate. Lyophilization was then performed to freeze dry the liquid product for 4 days at −104 °C. The product was then stored in −20 °C until testing.

2.2. Cell preparation

C2C12 myoblasts (CRL-1772, ATCC Organization) at passage between 16 and 19 were used in this study. The cells were cultured at 37 °C with 5% carbon dioxide, in Dulbecco's Modified Eagle Medium (DMEM, Gibco®, Life Technologies Inc.) supplemented with 10% Fetal Bovine Serum (FBS, Gibco®, Life Technologies Inc.) and 1% Penicillin streptomycin (Pen Strep, Gibco®, Life Technologies Inc.).

2.3. Hydrogel formation and cell 3D culture

The C2C12 myoblasts were suspended after treated with 0.25% trypsin (Gibco®, Life Technologies Inc.). Cell suspension (1.5×10^4 cells/mL) was then mixed with the GelMA hydrogel precursor (GelMA solution in PBS) to form a 5% GelMA hydrogel precursor with cell concentration of 6.75×10^3 cells/mL. Photoinitiator Irgacure 2959 (ALDRICH®, Sigma-Aldrich Inc.) was added into the mixture at a concentration of 0.05% for gelation. 320 mL precursor was then injected into the PDMS mold for UV exposure and gelation. Two cover slips (one with 3-(Trimethoxysilyl) propyl methacrylate surface-treatment and the other without) were stuck onto either sides of the mold. The surface-treated cover slip was allowed to bind with the hydrogel. The untreated cover slip prevented the precursor from air exposure during gelation. After 12 min exposure to UV light (400–500 mW/cm^2), the cylindrical GelMA hydrogels with C2C12 myoblasts encapsulated within were soaked in culture media and placed into an incubator for 3 days at 37 °C with 5% carbon dioxide. After that, an additional 120 mL 5% GelMA precursor without cells was added into the mold for further gelation. Another surface-treated cover slip was used to bind with the other hydrogel surface. The cylindrical hydrogel so formed was 1.2 mm high and 16.4 mm in diameter.

2.4. Hydrogen peroxide (H_2O_2) pre-treatment

Hydrogen peroxide (H_2O_2, Unichem Inc.) was used as an extrinsic oxidant to pre-treat myoblasts before the second gelation. Two concentrations – 0.1 mM and 0.5 mM were selected to study the effects of H_2O_2 pre-treatment. Two treatment durations – 1 h and 20 h were adopted to study the differences between chronic