MECHANICALLY INDUCED DAMAGE IN TISSUE ENGINEERED SKELETAL MUSCLE

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INTRODUCTION

Engineered tissues offer strong possibilities as model systems for studying tissue responses to physical, chemical or biological stimuli. Moreover, they can be used to investigate specific pathologies or clinical treatments with less ethical considerations and better experimental control than animal models or human studies. We use the concept of tissue engineering to design in-vitro models for studying the etiology and prevention of mechanically induced damage in skeletal muscle, as is common in pressure sores.

Previous studies have demonstrated that skeletal muscle is highly susceptible to sustained compression, leading to tissue breakdown within 2 h of straining [1]. This breakdown starts at the cellular level with disintegration of contractile proteins and damage to the cell membrane and nucleus, followed by inflammatory reactions [2]. Although it is clear that both the duration and magnitude of compression affect cellular breakdown, the mechanobiological pathways whereby tissue compression leads to cell damage are poorly understood. Theories focusing on impaired oxygen transport and metabolism within the tissue can only partly explain the onset of pressure sores and have to date not been fully verified. Recently, we demonstrated that sustained deformation of the cells inside the tissue is an additional trigger for muscle breakdown [3]. Using a hierarchy of complementary model systems, ranging from single cells to animal models, we aim to elucidate the multi-factorial etiology of pressure sore related muscle damage. The present study employs tissue engineered skeletal muscle to investigate the relationship between gross tissue compression and cell damage.

MATERIALS AND METHODS

Engineered skeletal muscle

Reproducible skeletal muscle constructs were developed by modifying the protocols of Vandenburgh and colleagues [4]. In brief, C2C12 myoblasts were seeded within a suspension of growth medium, collagen gel and Matrigel (ICN Biomedicals). The suspension was then plated into 6-well culture dishes and allowed to attach for 6 hours in a humidified incubator. Resulting disc shaped constructs (~15 mm diameter, ~0.5 mm thick) were embedded in growth medium. From day 3 onwards the constructs were further cultured in a differentiation permissive medium to induce myoblast fusion and differentiation into elongated myofibers. By day 10 in culture the constructs contained a branched network of multinucleated, maturated and contractile myofibers with an overall viability > 95% [Fig. 1].

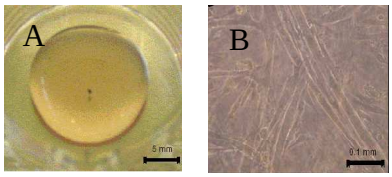

Figure 1. A: Engineered muscle tissue construct. B: Network of contractile, multinucleated myotubes.

Compressive straining

A specially designed loading device [Fig. 2] was used to subject tissue constructs to well-defined, clinically relevant straining regimens. The device, based on a micro-pipette manipulator, allows simultaneous compression of 6 constructs with circular glass indenters (diameter: 5 mm) that can be individually moved with an accuracy of 10 μm.

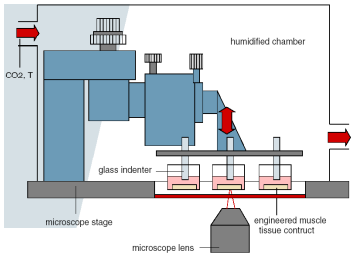

Figure 2. Compression device.

During experiments temperature (37°C) and CO_2 (5%) is controlled by a custom made incubator and a heating plate. The whole set-up is mounted to the stage of an inverted confocal laser scanning microscope (Zeiss, LSM 510) to monitor cellular responses to compression with time and to determine construct deformation from serial 3D scans.

At day 10 in culture six constructs were subjected to 50% gross compressive strain, while six more constructs served as unstrained controls. For the latter condition the indenters were gently placed on top of the constructs.

Damage assessment

The evolution of cellular breakdown was assessed with a newly developed quantitative, real-time, and non destructive viability assay [5]. The assay is based on discriminative dual fluorescent staining, as visualized with confocal microscopy. Living cells are stained with CellTracker™ Green, whereas nuclei of dead cells are stained with Propidium Iodide [Fig. 3]. To determine construct viability, tile-scan fluorescence images (5x5 tiles) were taken at predetermined intervals, covering an area of 5 mm^2 below each indenter. All images were taken from the central horizontal section of the compressed construct. Dead cell numbers were quantified using custom made image analysis software and the percentage damage was determined by normalizing for the average total cell number.

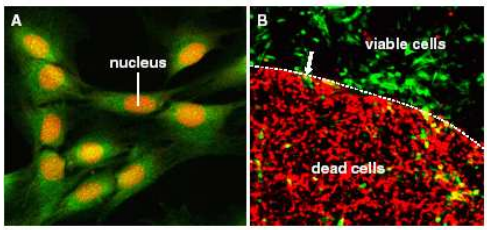

Figure 3. A: Fluorescent staining of dying muscle cells. B: Scan of indenter edge showing clear demarcation between life and dead cells.

RESULTS

Cell damage in strained constructs developed rapidly and increased dramatically with time of compression. For each time period it was significantly higher than in unstrained controls, where the percentage damage remained constant with time of compression [Fig. 4]. Furthermore, for each time point constructs showed highly localized and uniformly distributed cell damage underneath the indenter [Fig. 5].

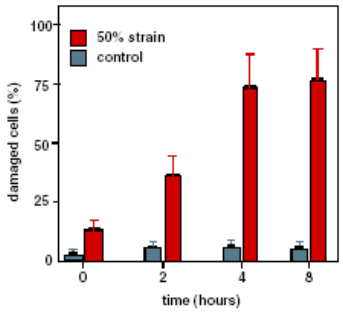

Figure 4. Percentage cell damage with time.

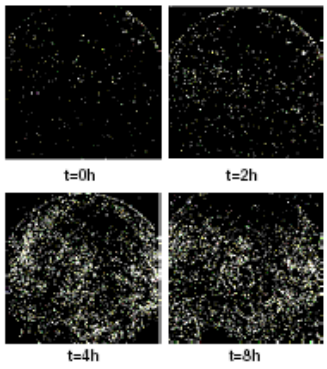

Figure 5. Damage location below indenter in strained constructs. White dots correspond with dead cells.

DISCUSSION

The in-vitro muscle tissue model is a promising tool to study the evolution of compression induced skeletal muscle damage and hence the etiology of pressure sores developing in this tissue under clinically relevant conditions. The present study indicates that cellular breakdown might indeed be triggered by sustained cell deformation, since impaired oxygen supply would have led to gradients in cell damage across the indented area or increased damage in unstrained controls with time. In-vitro measurements of tissue metabolism as well as comparisons with single cell studies and animal models will provide further insight into the relative and combined contribution of cell deformation and tissue metabolism in the onset of pressure sores. Multi-scale finite element models, incorporating individual tissue components, are employed to link the different length scales of these models and to understand and predict local cell damage from global tissue strains [6].

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