

A HIERARCHICAL APPROACH TO STUDY DAMAGE EVOLUTION IN SKELETAL MUSCLE – A CONTRIBUTION TO PRESSURE ULCER RESEARCH

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INTRODUCTION

Pressure ulcers are localized areas of tissue breakdown in skin and/or underlying tissues. They result from prolonged mechanical loading, typically common in subjects who are bedridden, wheelchair bound or wear a prosthesis. The sores are painful, difficult to treat and represent a burden to the community in terms of health care and money. Prevalence figures remain unacceptably high, ranging between 8 % and 23 %. The costs associated with the management of pressure ulcers in the U.S. exceed \$ 6.4 billion annually [1].

The high prevalence figures can partly be attributed to a limited understanding on how and why pressure ulcers develop. Although a lot is known about risk factors and prevention and treatment strategies a clear view on the basic pathways, whereby mechanical loading leads to soft tissue breakdown is still missing. Several hypotheses can be found in the literature on the causes of pressure sores. Local ischemia due to occlusion of blood vessels is generally accepted as a major risk factor. Several other risk factors have been implicated including reperfusion injury [2], impaired interstitial fluid flow [3], lymphatic drainage [4] and sustained deformation of cells [5]. What is certain is that in order to reduce the prevalence of pressure ulcers, it is essential to improve and expand the knowledge of the etiology in terms of basic science and clinical practice.

For this purpose, we have adopted a hierarchical approach, studying the effects of loading in distinct, yet complementary model systems with increasing length scales and complexity. This implies studies at the level of individual cells in culture, studies on model systems of tissue engineered biological tissues, animal studies and human studies. Experimental studies should aim at elucidating the relationships between mechanical loading, the pathophysiological response and tissue breakdown in testing hypotheses on the etiology. Computer models are used to predict the association between external and internal mechanical conditions and to assess the validity of extrapolating between different hierarchical model systems. The objective of the present study is to show how such an approach study might work. In particular, can results from the loading of tissue

engineered constructs be used to predict the outcome of animal experiments? Bosboom *et al.* [6,7] performed experiments, in which the tibialis anterior of anaesthetised rats was loaded for 2 hours with an external indenter at a pressure 250 kPa. After 2 hours the load was removed and the rats were allowed to recover from anaesthesia. After 24 hours the location and amount of tissue damage in the muscle were determined with a combination of T2-weighted Magnetic Resonance Imaging (Fig.1) and histological techniques.



In vitro studies on tissue engineered muscle cells were performed to examine a relationship between cell death and cell strain. A theoretical model must be employed to derive local stresses in the muscle tissue from the global externally applied mechanical load. A problem is the large difference in length scale of the individual cells (30 – 50 μm) and the muscle (4 – 6 mm). This was solved, using a multi-level finite element technique [8].

Figure 1: tissue damage detected by T2-weighted MRI. White spots within muscle are damaged cells

METHODS

Experiments on tissue engineered muscle constructs

Engineered muscle tissue constructs were developed, by suspending premature muscle cells in a collagen scaffold. The muscle cells fuse into a branched network of multinucleated, contractile myofibres by the application of appropriate biochemical and mechanical cues. A compression device enabled simultaneous indentation of six identical constructs. Constructs were compressed for 8 hours at 0 (control) and 30 and 50 percent strain. A real-time, non-destructive viability assay was used to monitor cell death [9]. This assay is based on fluorescent

staining of cells and provides quantitative data on location and number of dead cells below the indented area.

Damage evolution law for individual cells

Figure 2 indicates the percentage number of dead cells as a function of loading period for different construct strains. It is clear that at 50% strain, a marked cell death is initiated after 1 hour and reaches a value of 80% by 8 hours. At 30% strain, cell death is initiated after 2 hours and increases thereafter reaching 50% at 8 hours loading. A damage evolution parameter D is defined, which accumulates

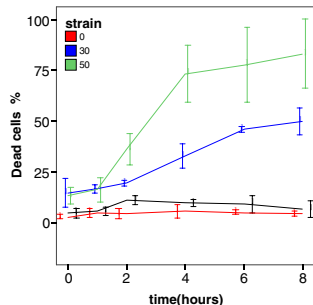


Figure 2: Cell viability as a function of time for different loading regimes

with time when the average strain energy density U in a cell is higher than a cell sensitivity parameter α . We propose the damage evolution equation:

$$D = \int_0^t \beta(U - \alpha) dt$$

, where α and β are material parameters which can be determined from the *in-vitro* experiment. When the damage evolution parameter D becomes higher than a certain threshold the cell dies.

Multi-level FEM analysis of animal experiment

A macroscopic plain strain mesh is used to model the cross-section of the muscle. In each integration point of the macro mesh a new finite element mesh is defined to model a representative volume element of the microstructure of the muscle. By fitting a representative volume element, with the tissue microstructure from the skeletal muscle on the data presented in fig. (2) a sensitivity law for individual cells was derived. By using microstructural periodicity constraints, the global deformation and stress field of the macro model is coupled to the microstructure [8]. A parallel processing approach was indicated for two reasons: a finite element model has to be solved in each RVE and there is little communication between the different processes. The simulation was performed on a Beowulf cluster with 64 PC's. Fig. 3 shows the damage evolution in the muscle at three different point in

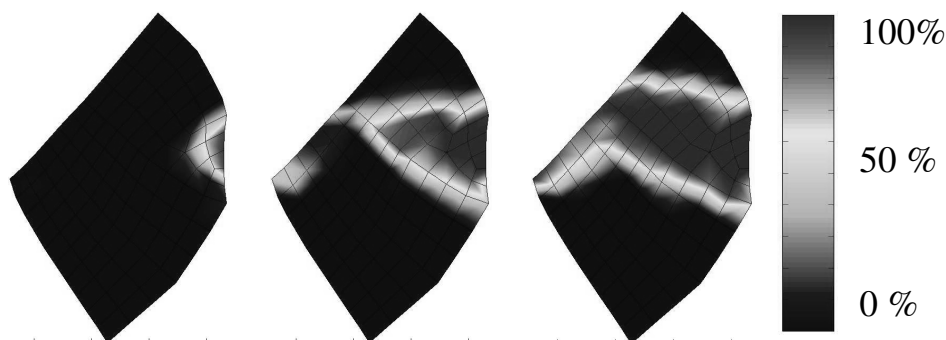


Figure 3 : The temporal progression of the percentage of damaged cells in tissue loaded with an external indenter

time. It is clear that the damage evolves from an area near to the indenter to deeper layers of muscle tissue. The location of the damage is similar to what is found in the animal experiments (6,7).

DISCUSSION

Experiments by Breuls showed that cells can be severely damaged within a two hour period of mechanical loading, a period which has been shown to be sufficient to initiate the development of pressure sores in a clinical setting. The animal experiments from Bosboom [6] confirm that it is possible to induce comparable damage in this time period, although the loading applied was in excess of that considered normal for human subjects. The multi-level finite element technique appears a useful technique to bridge the gap between different length scales and to test the hypothesis that results from experiments on tissue engineered constructs can be extrapolated within limits to in-vivo situations. However, to validate if this true, it is necessary to follow damage evolution as a function of time in the in-vivo situation. For this we are now developing a loading device that can be used with Magnetic Resonance Imaging equipment. By using T2-weighted images damage in muscle can be detected and its progress monitored in time .

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