# Tissue-engineered model for evaluating skeletal muscle damage in pressure ulcers

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## Introduction

The most adhered hypothesis for the development of (deep) pressure ulcers is that local anoxia (0% $O_2$) initiates tissue breakdown. Another, more recent hypothesis is that cellular deformation due to tissue compression causes tissue degeneration. A model system has been developed to investigate the relative contributions of anoxia and compression in the development of pressure ulcers in engineered muscle tissue. The effects of anoxia and/or compression are monitored in time using 'viable' markers for cell damage and cell death.

## Engineering of skeletal muscle

Myoblasts (muscle cells) were suspended in a collagen I solution [1]. The mixture was molded into shape in modified culture dishes [2] with pieces of Velcro as anchoring points (fig 1). As a result of cellular matrix remodeling a static pre-stress was created, causing myotube alignment.


Figure 1 *Macroscopic change of the gel appearance from the moment of molding (d0), to day 1 (d1) and day 2 (d2).*

After gelling in growth medium, the medium was shifted to two types of differentiation medium (2%HS and serum-free) to initiate maturation of the cells into myotubes. At days 2 and 6 after shifting F-actin staining of the constructs showed the beneficial effect of the serum-free medium on myotube development and alignment (fig 2).

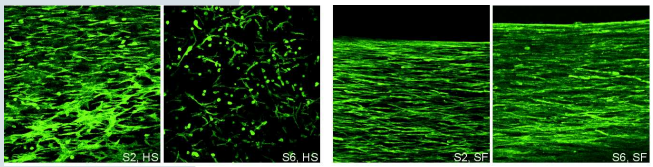


Figure 2 *F-actin staining of engineered muscle at the location indicated in figure 1. Left: Shift days 2 and 6, differentiated with serum (HS). Right: Shift days 2 and 6, differentiated without serum (SF).*

## Compression and anoxia

A compression device was assembled consisting of an indentor in a small incubator that can be mounted onto the stage of a confocal microscope (fig 3). The oxygen level in the device is adjustable between anoxic and normoxic (20%) conditions. Temperature (37°C) and humidity (100%) can also be controlled.

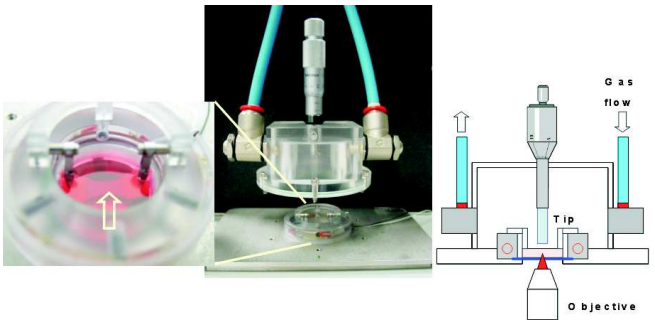


Figure 3 *Left: The incubation well is shown; arrow indicates engineered muscle. Center: the complete set-up is shown. Right: a schematic of the set-up located above the laser scanning objective for real-time monitoring of the tissue.*

## Markers of cell damage

A protocol was developed for real-time monitoring of cell death development (apoptotic and necrotic, figure 4). Irreversible cell damage can be quantified by measurement of NO, and creatine kinase release from the cells.

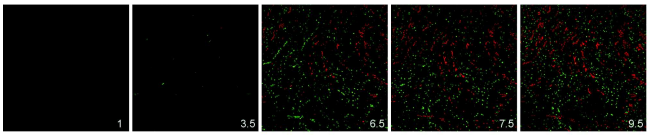


Figure 4 *Development of cell death in time. Some cell nuclei that stained apoptosis-positive (green) at 6.5 hours, were necrotic (red) at 7.5 and 9.5 hours.*

## Conclusions

Three methods have been developed, which can provide insight in cell survival during poor oxygenation and/or compression of skeletal muscle tissue. The amount of cell damage and cell death can be quantified and qualified in time. Together with results from numerical and animal studies the results can be translated into guidelines for clinical practice.

## Future

Experiments on the engineered skeletal muscle to monitor:

- damage from static and dynamic compression
- anoxic damage
- combined compressional and anoxic damage

*References:*

[1] Breuls R.G. et al.,: *Tissue Eng., 2003, 9(2): 269-81.*

[2] Dennis R.G. et al.,: *Am J Physiol Cell Physiol., 2001, 280(2): C288-95.*