Proceedings of the ASME 2008 Summer Bioengineering Conference (SBC2008)
June 25-29, Marriott Resort, Marco Island, Florida, USA

SBC2008-192322

A METHOD FOR DETERMINING THE STRAIN-TIME ENDURANCE OF CELLS IN PLANAR TISSUE-ENGINEERED CONSTRUCTS SUBJECTED TO LARGE COMPRESSIVE DEFORMATIONS

Amit Gefen,[*,a] Bastiaan van Nierop,[*] Dan L. Bader,[*,b] Cees W. Oomens[*]

*Department of Biomedical Engineering,
Eindhoven University of Technology, Eindhoven,
The Netherlands

[a]On sabbatical leave from the Department of
Biomedical Engineering, Faculty of Engineering,
Tel Aviv University, Israel

[b]School of Engineering and Materials Science,
Queen Mary University of London,
United Kingdom

INTRODUCTION

The mechanical environment of cells influences their normal growth and function, and may also affect the development of diseases and chronic injuries. Accordingly, there is substantial interest in determining the endurance of cells subjected to controlled mechanical strains for given time periods. A standardized, generic experimental method for determining strain-time thresholds for cell death is so far missing in the literature. In this study, a new experimental method was developed to measure strain-time thresholds of cells in planar tissue-engineered constructs subjected to large compressive strains. The method was applied to measure a strain-time threshold for differentiated C2C12 murine skeletal muscle cells in tissue-engineered bio-artificial muscle (BAM) constructs.

METHODS

The method is based on loading a viable planar tissue-engineered construct with a half-sphere-shaped indentor that produces a non-uniform, concentric strain distribution under the indentor (Fig. 1). The following assumptions are made: (i) the construct is planar, and rests on a rigid foundation, (ii) the inferior surface of the construct may slide freely on the foundation, and (iii) the contact between the indentor and superior surface of the construct is frictionless. Given these assumptions, the true compressive strain in the construct at a distance r from the indentor's central axis z is [1]:

$$E_{zz}(r)=\begin{cases}\ln\left\{1+\frac{1}{h}\left[\delta-R+R\sqrt{1-\left(\frac{r}{R}\right)^2}\right]\right\} & \forall r\in[0,a]\\ 0 & \forall r\in(a,\infty)\end{cases} \qquad (1)$$

where h is the thickness of the construct, R is the radius of the indentor, δ is the maximal depth of indentation, and $a = (2R\delta - \delta^2)^{1/2}$ is the contact radius between the indentor and construct [1].

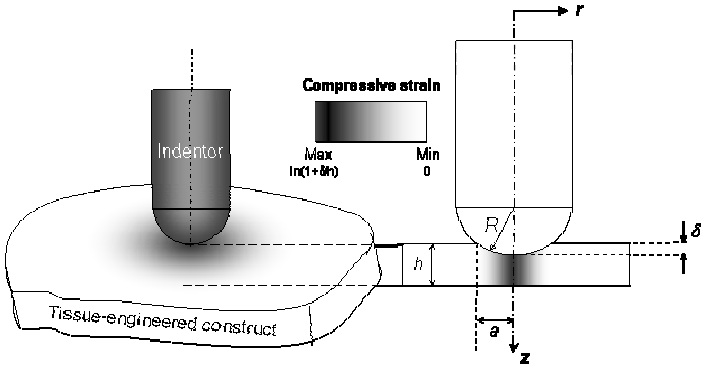

Figure 1. Theoretical distribution of compressive strains in a planar construct compressed by a half-spherical indentor

Hence, cells within the construct which lie at locations $r \geq a$ are theoretically subjected to zero compressive strain. However, cells located at r<a are subjected to compressive strains which increase nonlinearly as r decreases (eq. 1). A damage radius r_d<a, within which cells undergo necrosis resulting from exposure to excessive compressive strains is measurable by means of necrotic staining. In this study, propidium iodine (PI) was selected for staining necrotic cells. The PI dye enters necrotic cells due to their increased membrane permeability and intercalates with DNA and RNA, thus staining the nuclei of necrotic cells with red florescence. The damage radius r_d grows with time, as disintegration of contractile proteins and breakdown of the membranes progresses in the loaded construct. As implied by eq. (1), damage radii $r_d(t)$ correspond with critical strains $E^c(t)$ above which cell death occurs in the construct. Hence, fitting a

closed-form function to experimental $E^c(t)$ data points allows explicit determination of the strain-time threshold for the cells in the construct.

In order to demonstrate the utility of the above method, we employed it for determining strain-time thresholds in BAMs. BAMs with size of approximately 12×4×1mm were produced from C2C12 murine skeletal muscle cells cultured in three-dimensions (3D) within an extracellular matrix substitute, as previously described by Gawlitta and colleagues [2]. When matured, on day 7-11 post-molding, BAMs were incubated with 10 μM PI dye for 10min. Within a test chamber filled with medium and controlled at 37ºC, a hemispherical indentor with radius of 1.5mm was slowly lowered on the BAMs, thereby producing maximal compressive strains of ~80%. The BAMs were then monitored for cell necrosis using a confocal laser scanning microscope that excited the PI at a wavelength of 543nm and monitored reflections at a wavelength of 617nm. The region of necrotic cells at a central horizontal plane under the indentor was photographed unloaded and every 15min thereafter until 285min post-indentation (Fig. 2). The experimental $r_d(t)$ data were calculated by defining a circle within which the mean intensity of the grayscale fluorescent image I (0-255) was twice that of unloaded regions of the BAMs (Fig. 2). The $r_d(t)$ data were substituted in eq. (1) to determine the lowest strain levels that caused cell death $E^c(t)$ at each time point t (Fig. 3). Finally, a closed-form function was fitted to the experimental $E^c(t)$ data points in order to describe the strain-time threshold in a manner suitable for incorporation in analytical or numerical models aimed at extrapolating from local strain magnitudes to cell viability. A Boltzmann-type decreasing step sigmoid function was shown to fit the experimental $E^c(t)$ data from the BAMs with nonlinear correlation ratios that were consistently above 0.95 (Fig. 3):

$$E^c(t) = \frac{K}{1+e^{\alpha(t-t_0)}} + C \qquad (2)$$

where K, t_0 [min], α [min^{-1}] and C are empirically-determined constants, which are obtained from curve fitting to experimental $E^c(t)$ for each individual trial. The asymptotic strain at short times approximated from eq. 2 is $K+C$, and the asymptotic strain at long times is C.

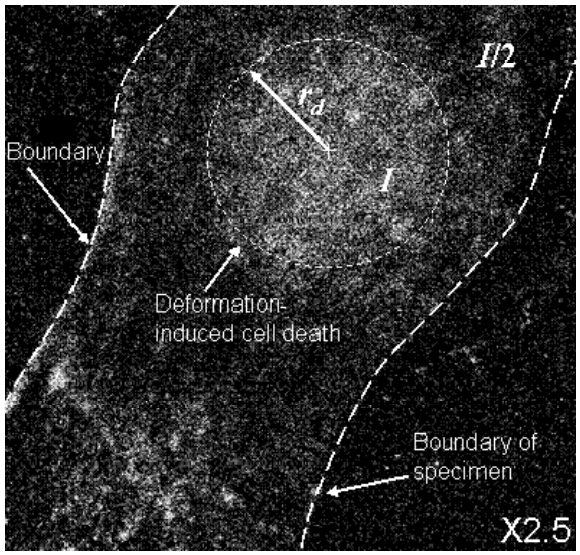

Figure 2. Fluorescent image of propidium-iodide-stained necrotic region in a tissue-engineered muscle specimen 165min post-indentation. *I* = mean image intensity (0-255).

RESULTS

The method described in this paper was successfully applied to determine the strain-time threshold of 15 BAMs. Examples for experimental $r_d(t)$ data, experimental $E^c(t)$ data and the fitting of eq. 3 are provided in Fig. 3. The $r_d(t)$ always increased with time, and the experimental $E^c(t)$ data of the BAMs consistently fitted a decreasing step sigmoid function, with mean parameter values of K=0.134, t_0=126min, α=0.0353min^{-1} and C=0.4158. The coefficients of variation in the parameters of eq. 2 were 53%, 33%, 29% and 21% for the K, t_0, α and C parameters, respectively. The strain endurance of the C2C12 cells at short times (order of few min) $K+C$ was hence 0.55 (engineering strain 73%), and the asymptotic strain at long times (over four hours) C was 0.42 (engineering strain 52%).

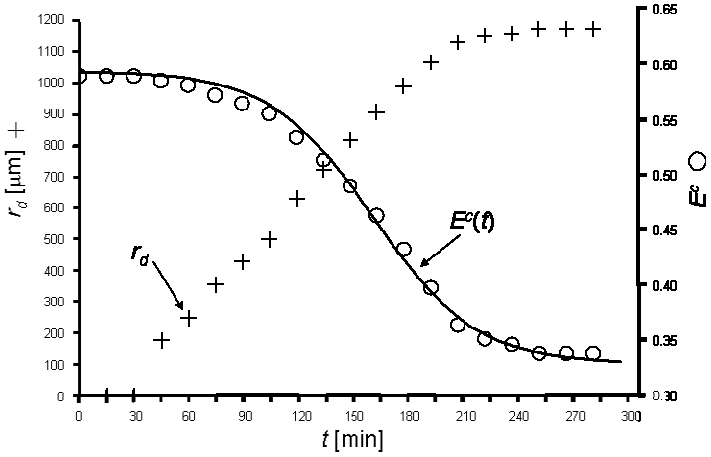

Figure 3. Example of experimental data of damage radius r_d (marked +) versus time from one test specimen. The corresponding strain-time threshold $E^c(t)$ (marked ○) and the closed-form sigmoid fit (solid line) are also shown

DISCUSSION

A method to determine strain-time thresholds of cells in planar tissue-engineered constructs was developed, and tested with 3D-cultured C2C12 skeletal muscle cells in BAMs. This new method indicated that engineering strains exceeding ~70% caused C2C12 cell death immediately, whereas lower strains that still exceeded ~50% caused cell death after approximately 3-4 hours. It is very likely that the high strain levels mechanically destroyed cell structures such as the membranes and cytoskeletons, and thus caused instantaneous death [3]. The death of cells subjected to the lower strains at the 3-5 hours time interval can be explained by severe disruption of homeostasis in those compressed cells, e.g. due to hindered diffusion of nutrients from the medium, local accumulation of lactate and other waste products, or apoptotic signaling from cells which died previously.

REFERENCES

1. Ning, X., Lovell, M., and Slaughter, W.S. 2006, "Asymptotic solutions for axisymmetric contact of a thin, transversally isotropic elastic layer," Wear, 260, pp. 693-8.
2. Gawlitta, D., Li, W., Oomens, C.W., Baaijens, F.P., Bader, D.L., Bouten, C.V. 2007, "The relative contributions of compression and hypoxia to development of muscle tissue damage: an in vitro study," Ann. Biomed. Eng., 35, pp. 273-84.
3. Peeters, E.A., Oomens, C.W., Bouten, C.V., Bader, D.L., Baaijens, F.P. 2005, "Mechanical and failure properties of single attached cells under compression," J. Biomech. 38, pp. 1685-93.