Quantification of cell viability in engineered tissues

Quantification of cell viability in engineered tissues

A. Mol, R.G.M. Breuls, R. Petterson, C.V.C. Bouten
Eindhoven University of Technology, Department of Biomedical Engineering

Introduction

Cell viability assessment is a key issue in monitoring the fate of engineered tissues. Ideally, a viability assay is quantitative, real-time, non-destructive, and three-dimensional. Current methods only incorporate some of these aspects [1]. We developed a novel approach, which fulfills all requirements.

Material and methods

Material

The tissue is loaded with a dual fluorescent staining: CellTracker Green (CTG) and Propidium Iodide (PI). CTG is used for staining living cells, whereas PI stains nuclei of dead cells. Images are acquired using a Confocal Laser Scanning Microscope (CLSM), see *Fig. 1*. Scanning with the CLSM allows three-dimensional, real-time, and non-destructive imaging of the tissue sample.

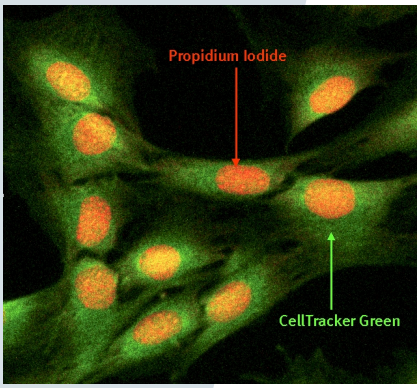

Figure 1 *Muscle cells stained with CTG and PI. The cells are currently dying, thus showing both expression of CTG and PI.*

Methods

To obtain quantitative measurements, first we proved that the CTG and PI signal intensities are linear proportional to the number of living and dead cells, respectively (*Fig. 2*).

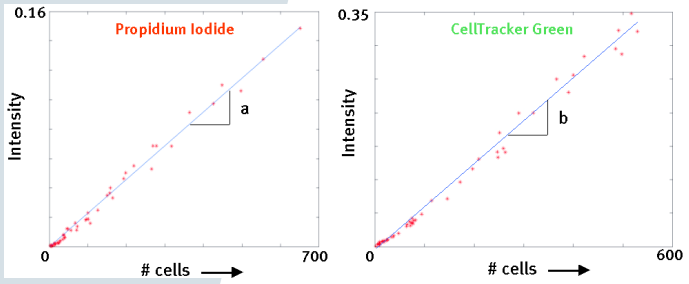

Figure 2 *Linearity of the CTG and PI signal intensities.*

Undesired influences of background staining and signal fading are compensated with mathematical algorhitms. The corrected intensities were used to determine viability, defined as:

$$Viability = \frac{CTG(t_0) - (PI(t) \cdot \frac{b}{a})}{CTG(t_0)} \cdot 100\%$$

The terms a and b refer to the slope of the linearity curves of PI and CTG, respectively, as shown in *Fig. 2* and $CTG(t_0)$ refers to the initial CTG intensity. This approach was applied to track damage evolution in an engineered muscle tissue construct (see *Fig. 3*). Damage was induced by decreasing the temperature. The vability was monitored for 18 hours.

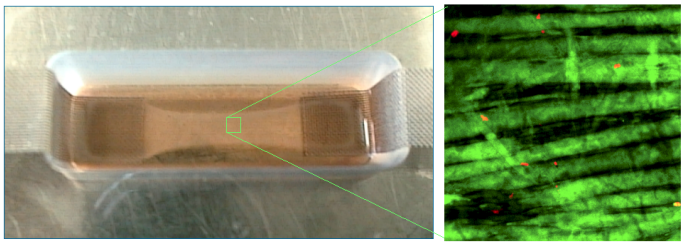

Figure 3 *The tissue construct used for damage evolution.*

Results

The intensitity courses of CTG and PI, and the resulting viability, are shown in *Fig. 4*. Statistical analysis has shown that the viability can be determined with an accuracy of 5-10%.

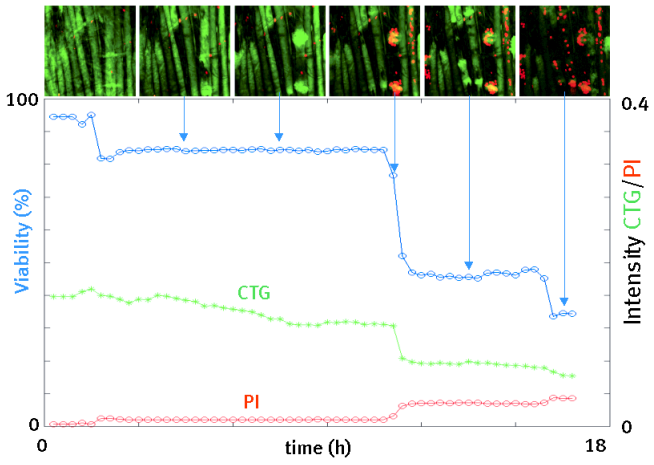

Figure 4 *Damage evolution in engineered muscle tissue.*

Conclusions

An accurate, quantitative, non-destructive, real-time, and three-dimensional viability assay has been developed for monitoring engineered tissues.

References:

[1] Park, J., Hwang, Y., Suh H.: *Viability evaluation of engineered tissues* (Yonsei Medical Journal, 2000, vol. 41(6), pp: 836-844)

PO Box 513, 5600 MB Eindhoven, the Netherlands