Reversal of Apoptosis by Cyclic Stretch

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Introduction: Apoptosis is a process of programmed cell death critical for tissue morphogenesis and homeostasis which is triggered by various stimuli including biochemical signals, oxidative stress, low ECM mechanical resistance, and restricted cell spreading. This tightly controlled process is marked by specific cell events such as cell blebbing and chromatin condensation. Once initiated, the process generally proceeds to cell death. However, if still in the early stages and the stimulus is removed, e.g., jasplakinolide [1] or p53 [2] in cancer cells, or external stimuli are added, e.g., neuron growth factor in neurons [3], apoptosis has been shown to be reversible. In the current study, we test the hypothesis that cyclic mechanical stretch can reverse apoptosis induced by culture on soft (low elastic modulus) substrates which limit generation of traction forces and cell spreading.

Materials and Methods: Stretchable Elastosil silicone substrates (CellScale) were coated with 0.6 kPa polyacrylamide gels per [4]. Also, the PA gels were coated with collagen. Porcine valvular interstitial cells (VICs, passages 8-9) were seeded (5000 cells/$1.96x10^{-1}$ mm^2 well). VICs were chosen as a cell type that experiences highly dynamic mechanical stimuli *in vivo*. The cells were cultured overnight on the soft substrates which have been shown to induce apoptosis at a significantly higher rate than on stiff substrates [5]. Two treated groups were stretched 10% uniaxially at 1 Hz (approximately the average heart rate) with an MCFX motorized system (CellScale) for nine hours; a control group was cultured statically in parallel. Cleaved caspase 3/7 and propidium iodide (PI) were used to quantify early-stage apoptosis and cell death, respectively. The return rate (% saved from apoptosis) was calculated as the number of the cells which were caspase-positive and PI-negative at the beginning of the experiment and negative for both markers at the end.

Results and Discussion: After 9 hours of loading, the proportion of apoptotic cells decreased substantially. In all groups, after overnight culture, 8.4% of the cells were caspase+ (n=537), then after 9 hours 3.2% were caspase+ in the static control group (n=186 cells) and 2.3% were caspase+ in the dynamically stretched groups (n=351). The return rate for the control group was 27% (n=11 cells) and 57% (n=14) and 71% (n=7) for stretched groups (see Figure 1). On static soft substrates, apoptosis may be induced by the inability of the cells to generate sufficiently high traction forces and/or due to the inability of the cells to spread out. These data suggest that external mechanical loading can reverse the pro-apoptotic stimulus from the soft substrate, possibly by increasing traction forces and/or cell spreading, both of which we have observed for cells cultured with dynamic stretch on soft substrates previously [4].

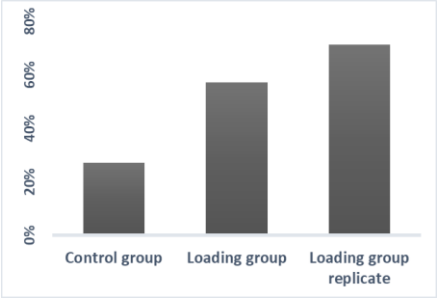

Figure 1. The return rate of VICs (proportion of cells undergoing apoptosis which revert to healthy status after culture in static and cyclic stretch environments for nine hours).

Conclusions: When exposed to a dynamic environment similar to the native valve, valvular interstitial cells are able to reverse the apoptotic pathway. Future experiments are needed to make statistical comparisons and to determine the extent to which cyclic stretch can reverse apoptosis at other stages and induced by other stimuli.

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References:

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