Fibroblast response to mechanical stress: role of the adhesion substrate

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Objective of the research:
The role of mechanical stimuli in tissue functioning and in cellular responses is still poorly understood because of the enormous complexity of the issue (1). The aim of the present study was to check metabolic changes in fibroblasts determined by parameters of the applied mechanical stress (amplitude, frequency, nature of the adhesion substrate etc.)

Novelty of the work:
We used the CELLDRUM® Technology developed in our laboratory to study the consequences of cyclic mechanical stress on 3T3 fibroblasts (2). Prior to the stimulation, the cells were immobilized on a silicone membrane using different adhesion substrates, natural (fibronectin) and artificial (CellTech®). Changes in proteome, in gene expression, as well as in cell morphology were studied in parallel for different surface coatings.

Methods and techniques:
Mechanical stimulation: The used device was a drum-like construction (well) consisting of cylindrical body to which a silicone membrane had been glued. The membrane (1µm thick and 16mm in diameter) was pre-coated with different substrates. NIH 3T3 mouse fibroblasts were seeded on the membrane (1.5×10^5 cells per well) and allowed to grow for 48h in DMEM medium. After that, six such wells were placed in a holder and were subjected to a cyclic sinusoidal strain at 0.25 Hz for 2.5 h (or 48h for the fluorescence microscopy observation). The membrane exerted the applied strain (maximal indentation 1mm (0.25%)) to the attached fibroblasts monolayer.
Observation: Cytoskeleton structural rearrangements were observed by confocal fluorescence microscopy using phalloidin-AlexaFluor 488 staining. Gene expression was investigated on RNA and protein levels by real-time PCR and two-dimensional SDS PAGE. For evaluating in a Real Time PCR, one housekeeping gene (GAPDH) and two target genes (ITGβ5 and p53) were selected. Primers for the selected genes were designed using the Vector NTI program (version 5.0 demo; Invitrogen) and than were synthesized by Karl ROTH Company and MWG Genomic Company. AurumTM Total RNA mini kit (Biorad cat. No 732-6820), Perfect RNATM Eukaryotic Mini Kit (Eppendorf cat. no. 00322006.108) and RNeasy Mini Kit (Qiagen cat. no. 74124) were used for RNA isolation. RNA was converted to cDNA, quantitatively analyzed and specifically multiplied using Thermal Cycler (Bio-Rad Co. USA).

Main results:
Mechanical stimulation of the cells resulted in evident changes in the cell morphology, protein composition and gene expression. Microscopically, additional formation of stress fibers accompanied by cell re-arrangements in a monolayer was observed. Also, significant activation of p53 gene was revealed as compared to control. Interestingly, the use of CellTech membrane coating induced cell death after mechanical stress had been applied. Such an effect was not detected when fibronectin had been used as an adhesion substrate.

Conclusions:
The combined "morphology-genome-proteome-based" approach appears to be a helpful tool in general for examining the cell status at various mechanical conditions. The cell-substrate adhesion molecules and interactions for CellTech coatings seem to differ significantly from the ones for fibronectin coatings. The role of mechanical stimulation in p53 induction needs to be further discussed.

References:
[1] Loscalzo J. Proteomics in cardiovascular biology and medicine. *Circulation.* 2003; 108: 380–383.
[2] Trzewik, J., M Ates, GM Artmann. A novel method to quantify mechanical tension in cell monolayers. *Biomed Tech (Berl)*, 2002; 47 Suppl 1 Pt 1: 379-81.