FOCAL ADHESIONS OF FIBROBLASTS ON DINAMIC SURFACES: AN ADDITIONAL APPROACH FOR TISSUE ENGINERING

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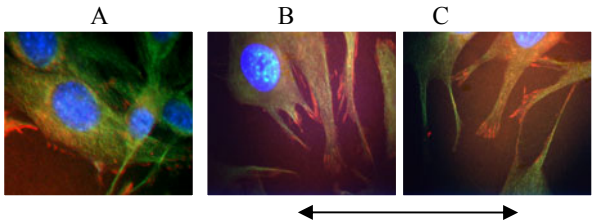
Statement of purpose:
Mechanical forces play an important role in the organization, growth and function of tissues (1). The ability of cells to transduce mechanical signals is governed by focal adhesions, that link cells to other cells and to extracellular matrix. The mechanism of signal through the cell is of particular interest, expecially the transmission of mechanical forces followed by the transduction into biological signals (2). One hypothesis is that mechanical forces produce changes in the assembly or disassembly of integrin-associated linker proteins, such as vinculin, that form the cytoskeletal backbone of the focal adhesion (3). We applied cyclic and static mechanical strain and monitored cell orientation together with morphometric changes in vinculin expression.

Methods:
Silicone sheets 0.010" (SMI Inc**.** Saginaw, MI, USA) 1 cm^2 have been used as deformable substrate for cell seeding and after coating with fibronectin 10µg/ml (Sigma, Italy) for 1h at room temperature. Human fibroblasts MRC5 (ATCC CRL 171) have received mechanical stress by a Instron 5564 (Instron Corporation, Canton MA). Different deformations (1-8%) and different frequencies (0.25-3 Hz) have been tested for 3 hours and compared to not stressed cells and cells treated with static 2% deformation. Cellular orientation has been measured using a grid with different oriented angle (0-30°; 30-60° and 60-90°). To evaluate vinculin expression cells have been labelled with anti-vinculin antibody (Oncogene, Italy) TRITC conjugated (Santa Cruz, Italy). Anti-tubulin antibody (Sigma, Italy) FITC conjugated for cytoplasmatic microtubular localization and DAPI staining for nuclei have been used. By fluorescence microscopy (Leica, DM 2500) the focal contacts have been quantified also for area and length using Q-Win software (Leica).

Results/discussion:
Preliminary experiments evidenced a cellular orientation perpendicular (angle 60-90°) to the deformation starting from 1% substrate deformation reaching statistically higher orientation (> 50%) at 2% substrate deformation). These values remain unmodified at 5% substrate deformation while 8% substrate deformation induced cellular death. It was also shown that percentage of cells oriented perpendicularly to the deformation was not influenced by increased frequency of cyclical deformations (0.25-3 Hz). No differences were seen between cells cyclically deformed (B) respect to the cells cultured on 2% static (not cyclic) deformation (C). in both cases >50% cells oriented perpendicular to mechanical forces respect to control (A). Immunicytochemical and morphometrical analysis evidenced a higher focal adhesion positivity on stressed cells; focal contacts have the same area (4.4µm^2) and resulted longer (7.6±1.3 respect to control 3.7±1.3 µm) and localized at the terminal ends of tubulin filaments.



Conclusions
Recently it became evident that focal adhesions are not stable but move to enable cell migration and ECM formation. Different cellular orientation, the cytoskeletal organization as well as the number and size of focal adhesions depend on the substrate deformation, and is not determinant if the stress applied is cyclic or not. Cellular organization resulted influenced by variation in the surface mechanical properties and this finding may have a great impact for the fabrication of biomimetic surfaces used in tissue engineering.

Refernces:
[1] Lele TP, J. Cell. Biochem. 97: 1175-1183, 2006.
[2] Goffin JMJCB 172: 259-268, 2006.
[3] Lele TP. J. Cell. Physiol. 207 :187-194, 2006.