Relazione Bari 10/11 Febbraio 2006

Unità Operativa: Prof. Alfonso Barbarisi, NAPOLI -WP1-

Biomateriali: PET con superfici florurate nanostrutturate via plasma: Unità Operativa di Bari

Adhesion and proliferation of fibroblasts on plasma-deposited nanostructured fluorocarbon coatings: evidence of FAK activation.

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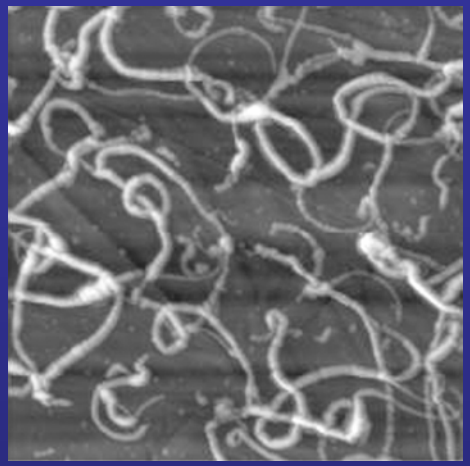
a



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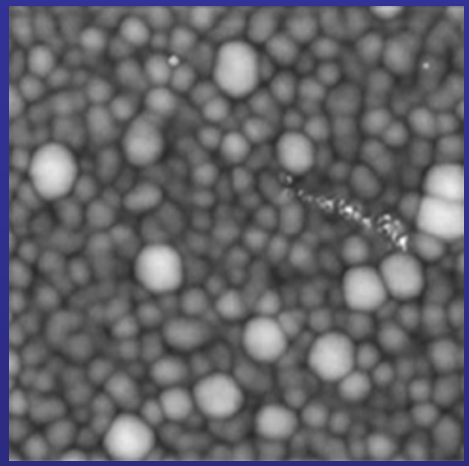


Figure 1. AFM images of fluorocarbon coating deposited in from C2F4 (flow rate 6 sccm; power 100W; pressure 200 mTorr) in MD conditions at different duty cycle values:
a) 20 mm x 20 mm scan of a 120±25 nm rough ribbon-like nano-structured coating deposited at 5 % duty cycle (time on = 16 ms; time off = 304 ms);
b) 5 mm x 5 mm scan of a 10±3 nm rough globular nano-structured coating deposited at 50 % duty cycle (time on = 160 ms; time off = 160 ms).

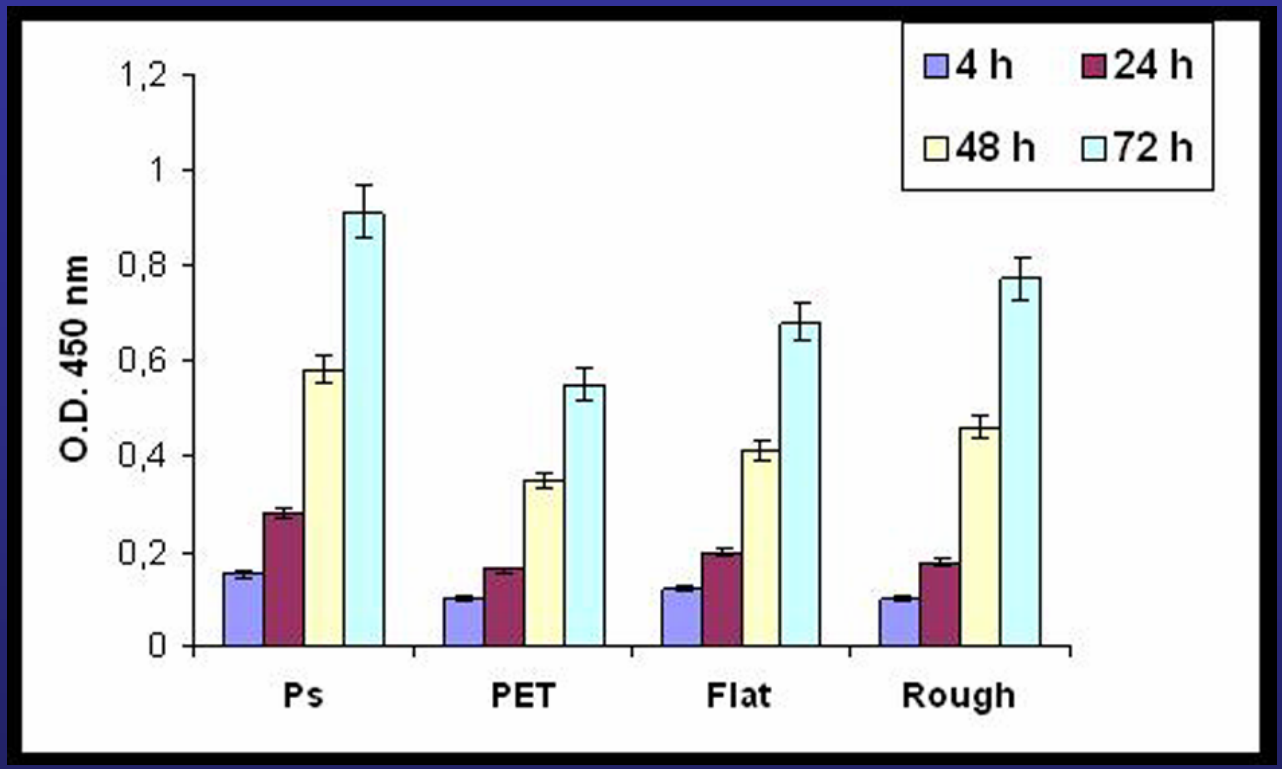


Figure 3. Proliferation of 3T3 Swiss Albino Mouse Fibroblasts on tested biomaterials. Ps, polystyrene flasks; PET, non treated polyethylene terephthalate (PET); Flat, polyethylene terephthalate conformally fluoroarbon coated with flat surface topography ; Rough, polyethylene terephthalate conformally fluoroarbon coated with nanostructured surface topography.

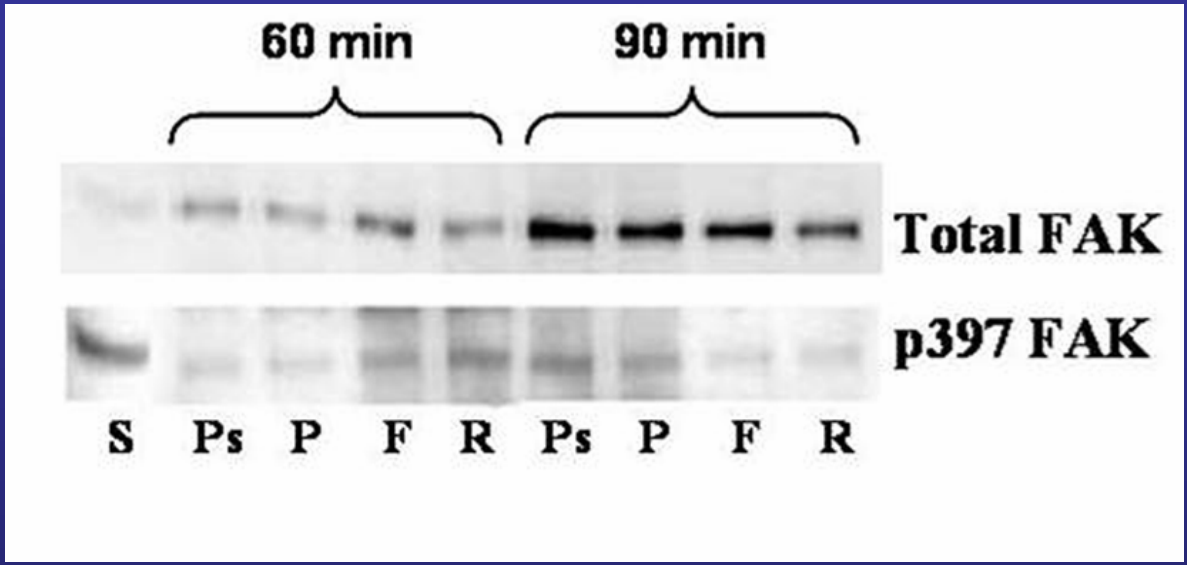


Figure 4. Effect of surface chemistry and topography on FAK tyrosine 397 phosphorylation. Quiescent cultures of 3T3 Swiss albino Mouse Fibroblasts were seeded on surfaces for 60 min and 90 min. Cells lysates were immunoprecipitaded using anti-FAK mAb and immunoblotted with anti-phospho (tyr 397) FAK and anti-FAK mAb. The autoradiograms shown are represantative of at least three indipendent experiments. S, experimental phosphoFAK (tyr 397) control; Ps, polystyrene flasks; PET, non treated polyethylene terephthalate (PET); Flat, polyethylene terephthalate conformally fluoroarbon coated with flat surface topography ; Rough, polyethylene terephthalate conformally fluoroarbon coated with nanostructured surface topography.

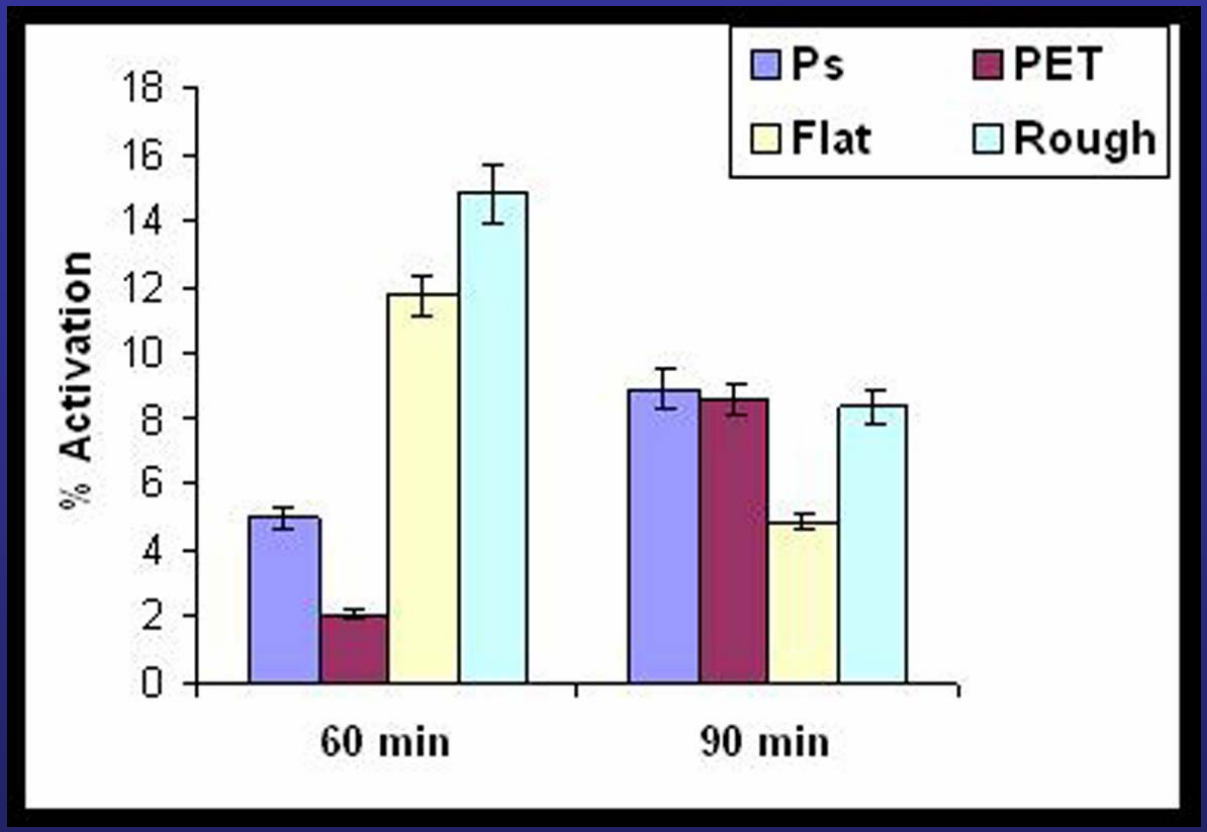


Figure 5. FAK activation. The bars illustrate the results of densitometric analysis, as X ± SE of the ratio of phosphorylated (tyr 397)) to total FAK (n=5). Ps, polystyrene flasks; PET, non treated polyethylene terephthalate (PET); Flat, polyethylene terephthalate conformally fluoroarbon coated with flat surface topography; Rough, polyethylene terephthalate conformally fluoroarbon coated with nanostructured surface topography.

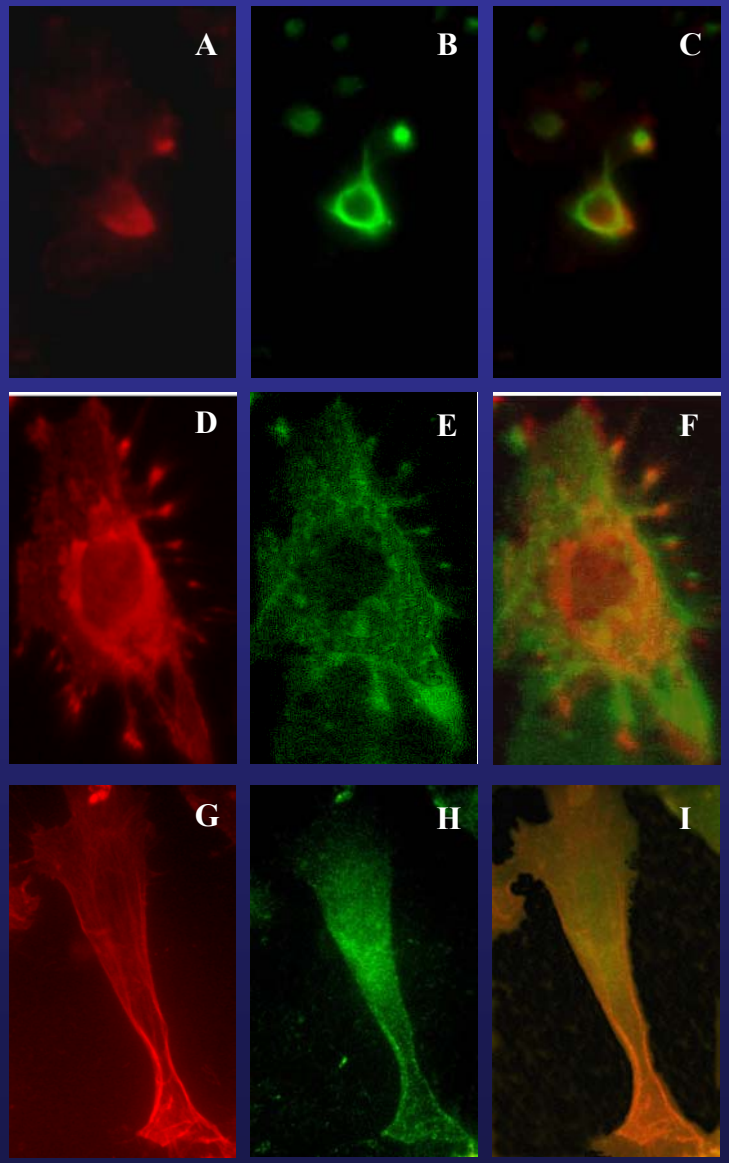


Figure 6. Effect of of surface chemistry and topography on phosphoFAK (tyr 397) distribution and cytoskeletal organization. Quiescent cultures of 3T3 Swiss albino Mouse Fibroblasts were seeded on surfaces. After 60 min cells were washed, fixed, and permeabilized with Triton X-100. Immunofluorescence was performed with Texas Red-phalloidin (0.1 mg/ml) to stain actin and with anti-phosphoFAK (tyr 397) to stain phosphoFAK . The experiment was repeated three times with similar results. Panels A, D, G cytoskeletal organization of cells seeded on PET, FLAT and ROUGH surfaces respectively. Panels B, E, H phosphoFAK distribution in cells seeded on PET, FLAT and ROUGH surfaces respectively. Panels C, F, I overlaied pictures. Magnification X 100.

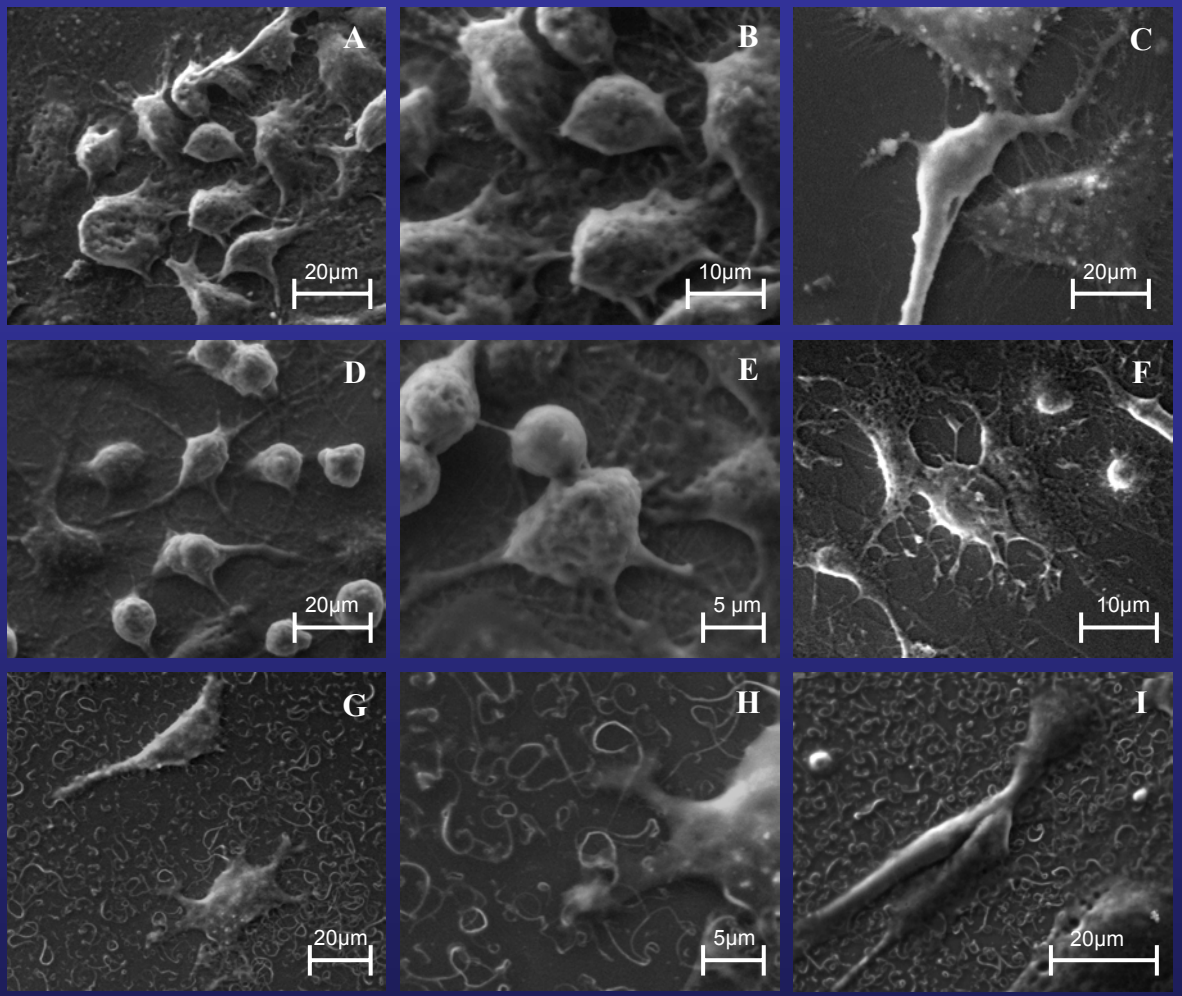


Figure 7. Environmental Scanning Electron Microscopy (ESEM). Micrographs showing 3T3 Swiss Albino Mouse Fibroblasts morphologies. Panels A, B; cells after 60 min of seeding on PET surfaces. Panels D, E cells after 60 min of seeding on FLAT surfaces. Panels G, H cells after 60 min of seeding on ROUGH surfaces. Panels C, F, I cells after 24 h of seeding on PET, FLAT and ROUGH surfaces respectively.

Biomateriali : PET nanostrutturato con Acido Ialuronico,
Unità Operativa di Siena, Prof. R. Barbucci

Esperimenti Effettuati: Valutazione della Proliferazione cellulare;
Organizzazione del citoscheletro cellulare.

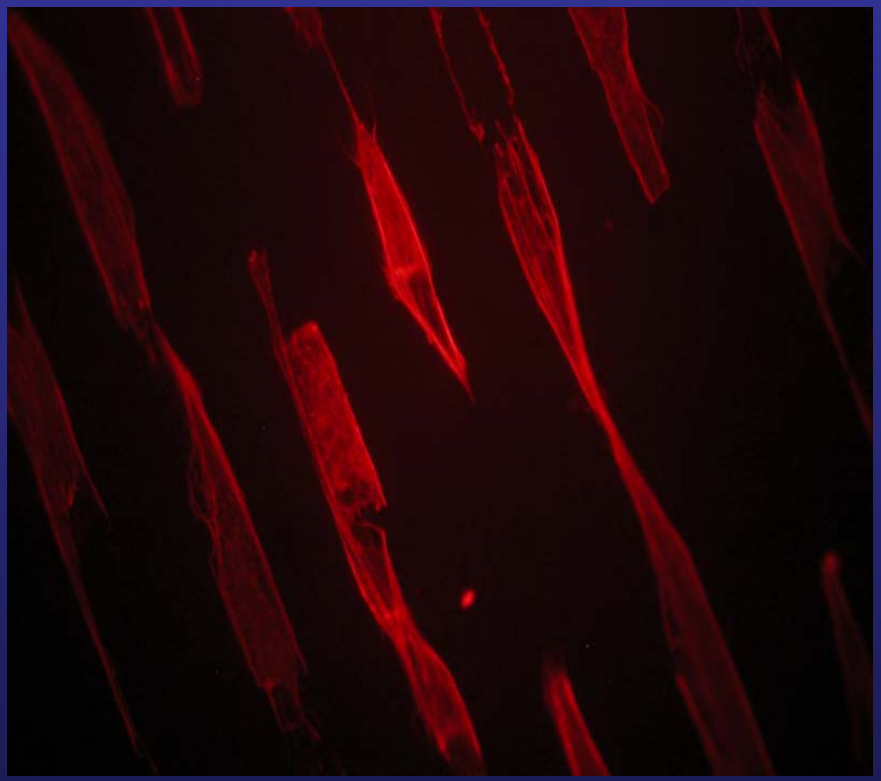
Dopo 24 ore di incubazione la proliferazione dei fibroblasti era del 35% rispetto al controllo;

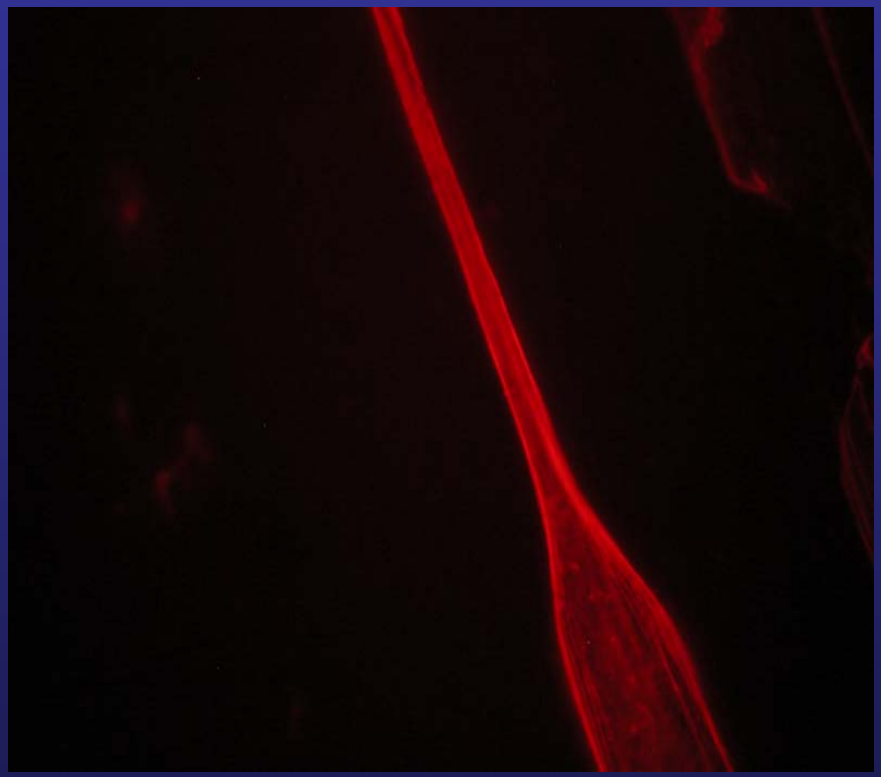
Dopo 48 ore di incubazione la proliferazione dei fibroblasti era del 30% rispetto al controllo.

Mancanza di una campionatura adeguata per il prosieguo

ORGANIZZAZIONE DEL CITOSCHELETRO CELLULARE

L'organizzazione del citoscheletro cellulare è stata studiata mediante microscopia ottica a fluorescenza (Phalloidin-Texas Red)





A (Ingrandimento 1000X), B (Ingrandimento 400X). Fibroblasti su PET nanostrutturato (Siena). Citoscheletro ben organizzato, che ricalca la disposizione cellulare tra due strisce adiacenti di acido Ialuronico.

Studio dell'adesione di fibroblasti 3T3 su PET trattato con fasci ionici (Realizzato dall'Unità Operativa di CATANIA)

Materiali testati

- PET non trattato (P0)
- PET irradiato (P1)
- PET non trattato + RGD (P0R)
- PET irradiato + RGD (P1R)
- PET non trattato + FBS (P0F)
- PET irradiato + FBS (P1F)

- valutazione della proliferazione cellulare (numero significativo di campioni +SD) ;
- Analisi biochimica della p397 fosfoFAK e FAK totale (solo su P0 e P1)

RISULTATI OTTENUTI

Il PET deposto su wafer di silicio e trattato con i fasci ionici non favorisce la proliferazione dei fibroblasti,

Qualche miglioramento della proliferazione è stato ottenuto sui materiali rivestiti con RGD e FBS.

Siamo in attesa di ricevere una campionatura adeguata dei seguenti materiali:

PET non trattato + RGD (P0R)
PET irradiato + RGD (P1R)
PET non trattato + FBS (P0F)
PET irradiato + FBS (P1F)