Effect of nanoscale surface roughness on the adhesion and proliferation of normal skin fibroblasts and HT1080 fibrosarcoma cells

Kanioura Anastasia[a], Bourkoula Athanasia[a], Tsougeni Katerina[b], Petrou Panagiota[a], Kletsas Dimitris[c], Tserepi Aggeliki[b], Gogolides Evangelos[b], Kakabakos Sotirios[a]

[a] Institute of Nuclear and Radiological Sciences & Technology, Energy & Safety, NCSR "Demokritos", Aghia Paraskevi, 15310 Athens, Greece
[b] Institute of Nanoscience & Nanotechnology, NCSR "Demokritos", Aghia Paraskevi, 15310 Athens, Greece
[c] Institute of Biosciences and Applications, NCSR "Demokritos", Aghia Paraskevi, 15310 Athens, Greece
e-mail:nkanioura@ipta.demokritos.gr

Separation and enrichment of cancer cells from a mixture with normal cells are important steps for cancer diagnosis. The methods used for the separation of cancer from normal cells are based on observation of morphological features, labeling of the cells with specific markers or differences in physical properties between cancer and normal cells (e.g. cell size, density, adhesion, dielectric properties) [1]. Surface nanotopography, as it has been reported in the literature, affects cell adhesion, proliferation and viability [2]. However, there are few reports about the potential of such nanostructured surfaces for separation/enrichment of cancer cells from mixtures with normal ones [3]. Here, we investigated the effect of surface nanotexturing on the adhesion, viability, and proliferation of normal fibroblasts and fibrosarcoma HT1080 cancer cells on thin PMMA films nanotextured through oxygen plasma etching in comparison to flat untreated surfaces.

Randomly nanotextured PMMA film surfaces were prepared following a published procedure [4]. Briefly, a 25% (w/w) PMMA solution was spin coated on Si wafers at 1500 rpm followed by baking for 1.5 h at 150 ^{o}C. The films were treated with O_2 plasma. The etching conditions were: bias voltage: -100 Volts; electrode temperature: 15 ^{o}C; etching time: 3 min; source power: 1900 W; pressure: 0.75 Pa and oxygen flow: 100 sccm in a Helicon Plasma reactor (MET system, Adixen). The plasma treated surfaces (Fig. 1) along with untreated ones were then used as substrates to culture 10000 cells/ml normal fibroblasts or HT1080 cells for periods of 1 and 3 days. The adhered cells were fixed and stained with phalloidin-Atto 488 (F-actin) and DAPI (nucleus) for cell counting and observation using an epifluorescence microscope, as described previously [5].

It was found that on the untreated surfaces after 1-day culture the number of adhered cells per surface area was approx. 1200 and 1000 cells/cm^2 for the HT1080 and the normal fibroblasts, respectively. After 3 day culture the HT1080 cell population increased 4 times on these surfaces and the normal fibroblasts 1.5 times. On the other hand, concerning O_2 plasma treated surfaces after 1-day culture approx. 2500 HT1080 cells and 800 normal fibroblasts had been adhered per sq. cm. A significant finding was that after 3-day culture the HT1080 cells population per surface area was increased almost 4 times (9800 cells/cm^2) whereas, the number of normal fibroblasts was decreased by 20% (640 cells/cm^2) compared to 1-day culture. In addition, as it is depicted in Fig. 2A and B the morphology of normal fibroblasts on the nanotextured surfaces was considerably affected after 3-day culture, as witnessed by the excessive distortion of cytoskeleton, compared to the untreated surfaces. In contrast, surface nanotexturing did not influence the morphology of HT1080 cells (Fig. 3A and B). The reduced cell population of normal fibroblasts on the rough surfaces after 3-day culture was not due to apoptosis as it was proved through staurosporine assay. Therefore to explain this finding we performed focal points staining using fluorescently labeled anti-vinculin antibody. It was found that the number of focal points per normal fibroblast cell was reduced by 40% whereas, that of HT1080 was increased by 30% on the nanotextured surfaces compared to the untreated ones. Therefore the decreased number and the distortion of the cytoskeleton of normal fibroblasts on the nanotextured surfaces could be ascribed to the considerable decrease of the focal points formation, which affects cell adhesion and viability. In conclusion, taking into account that adhesion and proliferation of normal skin fibroblasts is inhibited on O_2 plasma nanotextured PMMA surfaces in contrast to HT1080 cells, these surfaces could be useful for the enrichment and isolation of fibrosarcoma cells derived from tissues suspected for neoplasias and help to improve cancer diagnosis.

References

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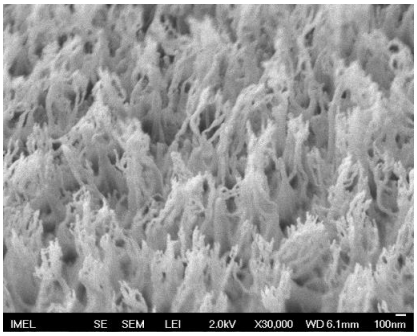

Figure 1. SEM images of O_2 plasma treated PMMA surfaces at bias voltage 100V for 3 min.

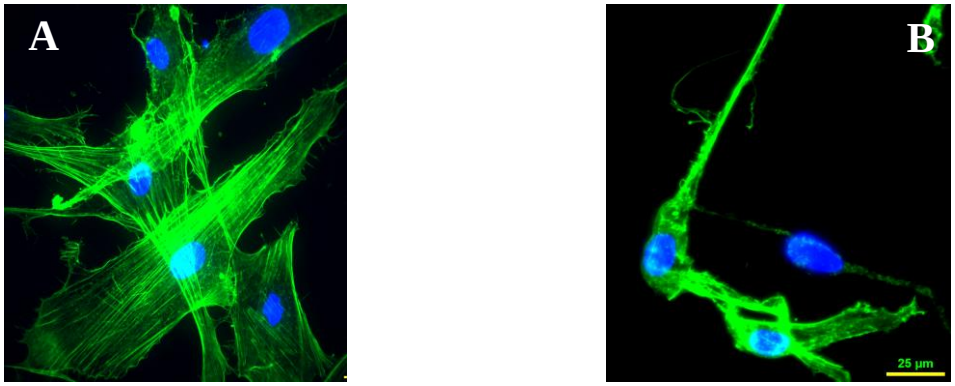

Figure 2. Fluorescence microscope images of normal skin fibroblasts cultured for 3 days on untreated flat PMMA surfaces (A) or plasma treated surfaces (100 V, 3 min) (B). Cytoskeleton (F-actin) was stained with phalloidin-Atto488 and cells nuclei with DAPI.

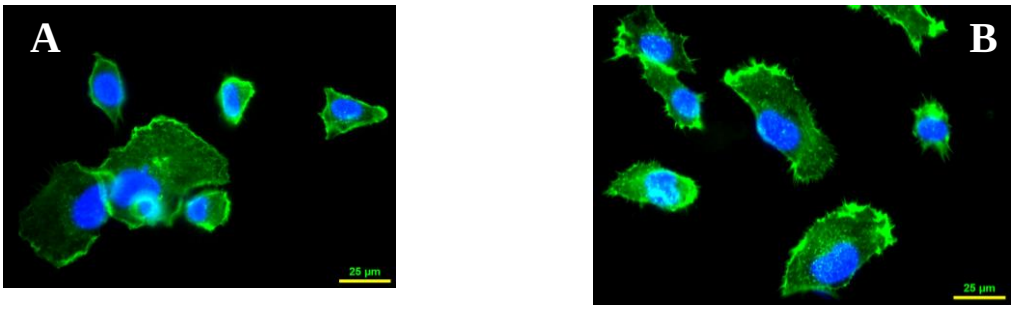

Figure 3. Fluorescence microscope images of HT1080 fibrosarcoma cells cultured for 3 days on untreated flat PMMA surfaces (A) or plasma treated surfaces (100 V, 3 min) (B). Cytoskeleton and cells nuclei were stained as described in Fig. 2.