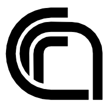
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Relazione Scientifica

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Study of B16 mouse melanoma cell dynamics on micro-structured surfaces

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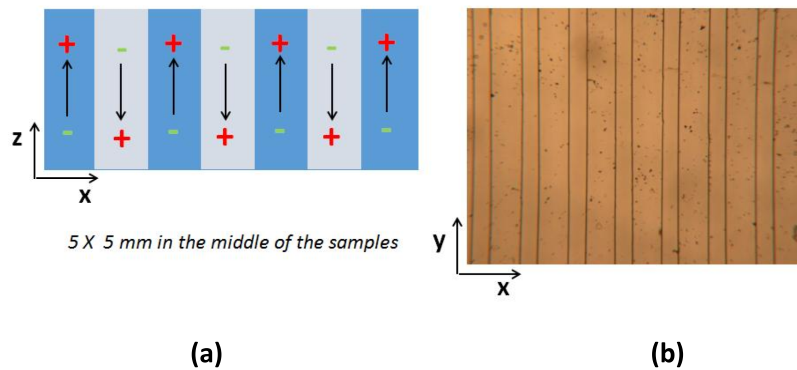
Abstract

Recently lithium niobate-based lab-on-chip have been applied to the biological field spacing from biosensing application to cell dynamics and morphology studies. In this work B16 mouse melanoma cell dynamics was studied on periodically-poled-lithium niobate surfaces. Cell morphology and dynamics was evaluated via optical microscopy and liposome-based cell uptake was also evaluated.

Results and Discussion

In Figure 1 a schematic representation and an optical microscope image of the periodically poled lithium niobate domains are shown.



(a) **(b)**

Figure 1. Schematic representation (a) and optical microscope image (b) of PPLN

1. **Healing effect**

Below the procedure adopted for the healing effect evaluation is reported.

- B16 melanoma cells were cultured on PPLN surfaces for 24 hours and the cell culture was performed in parallel on standard plates and cover slips as reference.
- After cell culture, a scratch was performed on the cell surface by using a 20 µl micropipette tip
- With an optical microscope, the images of the scratch healing effect were taken with an interval of 1 hour between each point
- The healing effect was evaluated for all the surfaces tested both in the centre and in the edge of the samples

In the next figure, the healing effect is clearly visible from optical microscope images.

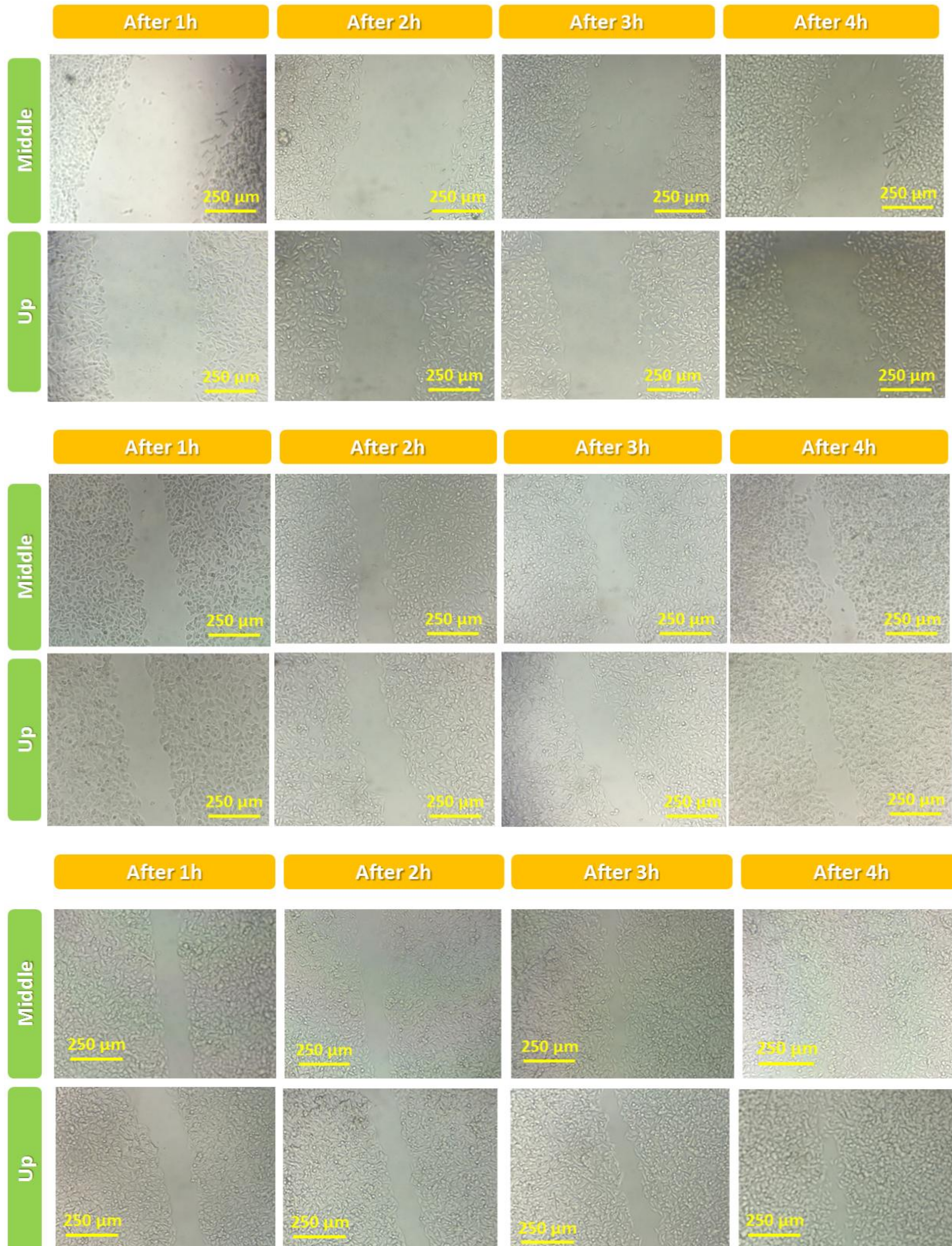


Figure 2. The healing effect on the three different surfaces teted: the standard plates, the cover slips and the PPLN surfaces

From the characterization shown above the cell healing effect was higher on the PPLN surfaces compared to the other surfaces tested. Indeed a scratch full coverage was obtained after 4 hours on the PPLN surfaces while on the other surface types the healing effect resulted slower.
This consideration is resumed in Figure 3.

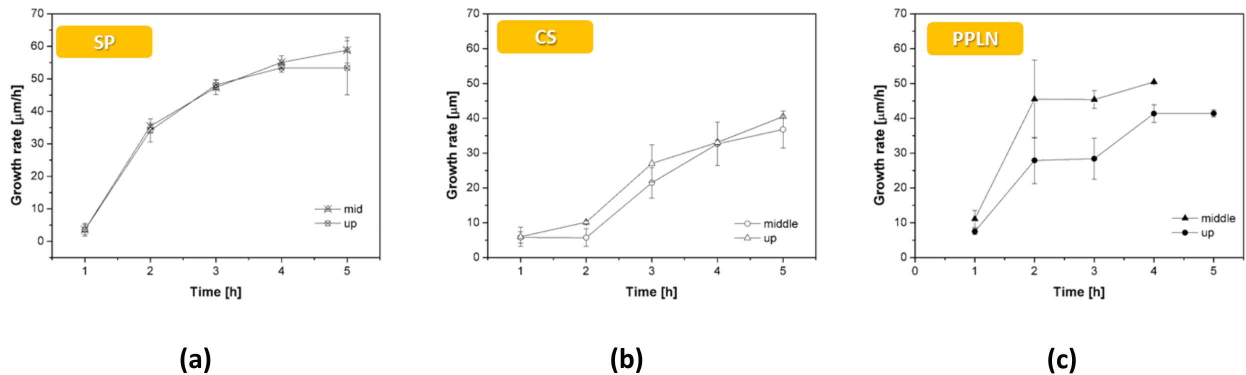


Figure 3. The healing effect on the standard plates (a), on the cover slips (b) and on the PPLN surfaces (c).

2. Cell toxicity and uptake

2.1. Physicochemical characterization of liposomes

Particle size of vesicle dispersions was measured (at a lipid concentration of 0.4 mg/ml) by dynamic light scattering (DLS) (Malvern Nano-Zs, Malvern Instrument, UK) at 25 0C (173o angle, to avoid backscattering).

Table 1. *Liposome mean diameter, Polydispersity Index (PI) and MAb attachment yield of both LIPs constructed (control [PEG LIPs] and targeted [OX-26 LIPs]). Values are means (±SD values), from 3 different samples.*

Samples	Size distribution	Polydispersity Index	MAb attachment yield (%)
PEG LIPs	103.3±3.606	0.290±0.006	-
OX-26 LIPs	110.3±0.3536	0.225±0.012	68.5±10.18

2.2. Proliferation studies

The biocompatibility of PPLN surfaces was tested quantitatively by MTT assay. Cells were incubated on the different substrates tested for 24 and 48 h periods. The cells were seeded at a density of 1.5x106 cells/well for 24h incubation and 0.75x106 cells/well for 48h incubation on the three substrates (PPLN, LN and Cover Slip [CS]) and the same number of cells on a 6-well plate was used as a control for each time point. After

incubation, surfaces were transferred to new 6-well plates and 1.5ml of fresh medium containing 0.5mg/ml 3-(4,5 Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added. Cells were then further incubated for 2h at 37oC and acidic isopropanol (0.33%) was added (at 37oC for 30 min) to dissolve the formazan crystals that formed. Alive cells were calculated according to the OD of cells at the 0 time point for 24 h and for 48 h.

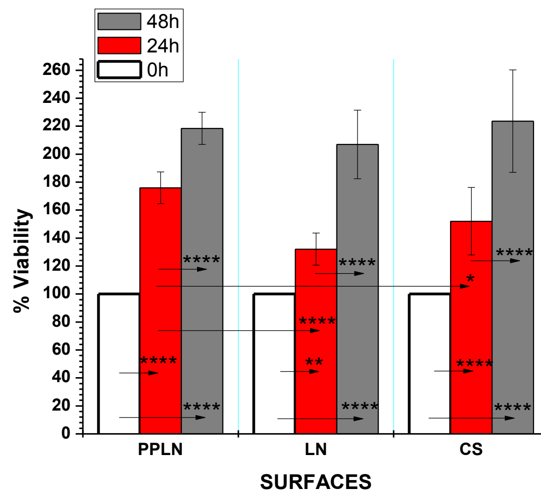


Figure 4. Cell growth (Viability (%)) on the three different substrates. Asterisks' denote significant differences between the corresponding values (* p<0.05, ** p<0.01, ***p<0.001).

As seen from results (Figure 4), the 24 h proliferation rate is higher on PPLN surfaces (compared to CS and LN surfaces), while there is no significant differences after 48 h for all substrates. In fact, the cell number increases by ~70% on PPLN surfaces and by ~40-50% on LN and CS, after 24 h, while after 48 h the population increases by ~120% for all surfaces. These results demonstrate that the surface of PPLN can be considered biocompatible.

2.3. Uptake study

In order to test if the cells attached on the various surfaces retain the potential to overexpress specific receptors which could be used for the uptake of targeted nanocarriers (as liposomes) by receptor-mediated endocytosis, the uptake of targeted liposomes (functionalized with a monoclonal antibody against the transferrin receptor, which is known from the literature to be overexpressed on the membrane of B16 cells) by B16 cells was investigated. As control liposomes, pegylated liposomes without any targeting ligand on the surface were used.

For LIP uptake by cells (PEG LIPs and targeted OX-26 LIPs), FITC-dextran-labeled vesicles were initially prepared and incubated with B16 cells (200 nmoles liposomal lipid/0.75x106 cells) in medium (containing 10% (v/v) FCS) at 37°C, for 3 h, then washed in ice-cold PBS (x3), detached from plates, re-suspended in PBS and assayed by FI

(after cell lysis in 2% Triton X-100). Cell auto fluorescence was always subtracted. Protein also was measured for each sample by the Bradford assay.

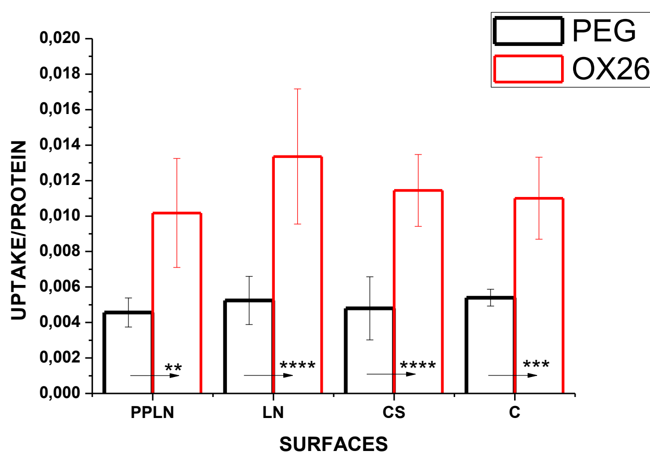


Figure 5. Uptake/protein (% Uptake /ug of protein) of targeted (OX-26 LIPs) and control LIPs (PEG LIPs) by B16 cells, after 3h of incubation of 200 nmoles of LIP-lipid /750.000 cells. Each result is the mean of 6 repetitions and the corresponding SD of the means are added as bars. Asterisks' denote significant differences between the corresponding samples (* p<0.05, ** p<0.01, ***p<0.001).

As seen from the results (Figure 5) the uptake values of OX-26 LIPs by B16 cells were substantially higher, compared to control LIPs (PEG LIPs) for all the substrates evaluated. More analytically, uptake of targeted LIPs was from 2.04 -2.55 times higher than that of control LIPs for each surface; [2.23 for PPLN, 2.55 for LN, 2.39 for CS and 2.04 for C]. For each independent surface there is a significant difference between control and targeted LIP uptake, but there is no significant difference between the different surfaces/substrates on the uptake of LIPs for both LIP-types tested (PEG or OX-26 sample), indicating that the targeted LIPs attached to both PPLN and also LN surfaces retain their capability to overexpress the transferring receptor in a way that enables uptake of nanocarriers by receptor-mediated mechanisms. This is, to the best of our knowledge the first time that such studies have been carried out and is of particular importance when the potential of using PPLN/LN surfaces in microfluidic devices related with drug delivery applications is considered.

Conclusions

In this work we studied the B16 melanoma cell dynamics on periodically-poled lithium niobate surfaces demonstrating the biocompatibility of the material adopted at first and then the possibility of apply it to mote complex bio-chip aimed at cell uptake tests.