Rat hepatoma (FAO) and human hepatoma (Huh7) cells were used. FAO cells were cultured in a mixture of 50 % HAM F12 medium (Sigma Aldrich) and 50 % NCTC 135 medium (Eurobio) supplemented with 5 % foetal bovine serum, 2 mM L-glutamine and 2 mM gentamicine (Sigma). Huh7 cells were cultured in a mixture of 25 % 199-2020 medium and 75 % 2021 medium (Eurobio) supplemented with 5 % foetal bovine serum, 2 mM L-glutamine, 5 µg/ml insulin (Sigma), 0.5 µM hydrocortisone hemisuccinate and 2 mM gentamicine. Cells were cultured in 7 cm2 flask at 37 °C in an humidified atmosphere of 5 % CO2 in air. Cells were passaged every week (1/2 dilution for FAO cells, and 1/6 dilution for Huh7 cells) with trypsin/EDTA (Sigma), the medium was renewed every 2 or 3 days. FAO cell culture on nanoneedles. After trypsinisation, FAO cells were centrifuged for 5 min at 1500 rpm and resuspended in the culture medium, 3 ml of FAO suspension was put on several nanoneedle chips (heights and low nanoneedles density) in 60 mm Petri dish, with a common cell concentration of 1.105 cells/ml on a 700 mm2 surface corresponding to 400 Cells/mm2. Chips were kept at 37 °C for culture and cell viability evaluation. Viability was monitored by cell proliferation after several culture days. Cell culture was stopped on the different chips after respectively 1, 3 and 7 days. FAO cells were fixed with formaldehyde 3.7 % in PBS and deshydrated with increasing ethanol concentration (60 % to 100 %). Visualisation was made with a scanning electronic microscope (C.M.B.A., Rennes University, France).

Huh7 cell culture on nanoneedles. After trypsinisation, Huh7 cells were centrifuged for 5 min at 1500 rpm and resuspended in the culture medium, 5 ml of Huh7 suspension was put on several nanoneedle chips (heights and low nanoneedles density) in 60 mm Petri dish, with a common cell concentration of 4.105 Cells/ml (2.105 Cells/mL, a 2827 mm2 surface corresponding to 700 Cells/mm2). Chips were kept at 37 °C. Cell's membrane poration was monitored by visualization of a fluorescent labeled F-actin (Texas Red-X phallolid, Molecular Probes), which is one of the cytoskeleton's protein and present at high concentration at the membrane vicinity for cytoskeleton anchoring purpose. Cell culture was stopped on the different chips after respectively 1 and 3 days. Huh7 cells were fixed with formaldehyde 3.7 % in PBS and labeled with Texas red-X phalloidin (1/100 of phalloidin in PBS) and 50 % NCTC 135 medium (Eurobio) supplemented with 5 % foetal bovine serum, 2 mM L-glutamine. Nanoneedles labeled Huh7 cells were then mounted on a glass coverslip and visualized with a confocal microscope (Rennes University, France). Confocal's observation was made between the first layer of the nanoneedle's support and the middle of the cell, corresponding to a height of 6.94 µm. For each observation, six planes were made, pictures are space out 1.15 µm.

Conclusion

Through long-term cultures, we have demonstrated the biocompatibility of new types of devices made of fields of sharp nanoneedles.

Those nanoneedle fields are interesting because of the tight interaction (observed through SEM and confocal imaging) they allow with the cells membrane and inside. Those nanoneedles fields opens the door to massively parallel applications of nanoneedles as extends of existing techniques thanks to the sharp nanoneedles. Such a nanoneedled chip, allowing specific interaction, opens the door to a large number of exciting and valuable applications such as nanorobotics for translocation or internal cell potential recording.

Nanoneedle fields devices

- Si and SiO2 materials : biocompatible, - 7-10 µm high sharp needles, - few hundreds nanometers tip diameter, - 4 to 40 nanoneedle per 100 µm2, - field density controlled during the process,

Cells viability

- evaluated through proliferation dynamic,
- cultures fixed after 1, 3 and 7 days,
- few hundreds nanometers tip diameter,
- field density controlled during the process,
- 4 to 40 nanoneedle per 100 µm².

Cell/nanoneedle interaction

- through Scanning Electron Microscopy (SEM).

Culture and preparation protocols

Cell culture: Rat hepatoma (FAO) and human hepatoma (Huh7) cells were used. Huh7 cell culture on nanoneedles. After trypsinisation, Huh7 cells were centrifuged for 5 min at 1500 rpm and resuspended in the culture medium, 5 ml of Huh7 suspension was put on several nanoneedle chips (heights and low nanoneedles density) in 60 mm Petri dish, with a common cell concentration of 1.105 cells/ml on a 700 mm2 surface corresponding to 400 Cells/mm2. Chips were kept at 37 °C for culture and cell viability evaluation. Viability was monitored by cell proliferation after several culture days. Cell culture was stopped on the different chips after respectively 1, 3 and 7 days. FAO cell culture on nanoneedles. After trypsinisation, FAO cells were centrifuged for 5 min at 1500 rpm and resuspended in the culture medium, 3 ml of FAO suspension was put on several nanoneedle chips (heights and low nanoneedles density) in 60 mm Petri dish, with a common cell concentration of 1.105 cells/ml (2.105 Cells/mL, a 2827 mm2 surface corresponding to 700 Cells/mm2). Chips were kept at 37 °C. Cell's membrane poration was monitored by visualization of a fluorescent labeled F-actin (Texas Red-X phallolid, Molecular Probes), which is one of the cytoskeleton's protein and present at high concentration at the membrane vicinity for cytoskeleton anchoring purpose. Cell culture was stopped on the different chips after respectively 1 and 3 days. Huh7 cells were fixed with formaldehyde 3.7 % in PBS and labeled with Texas red-X phalloidin (1/100 of phalloidin in PBS) and 50 % NCTC 135 medium (Eurobio) supplemented with 5 % foetal bovine serum, 2 mM L-glutamine and 50 % NCTC 135 medium (Eurobio) supplemented with 5 % foetal bovine serum, 2 mM L-glutamine. Nanoneedles labeled Huh7 cells were then mounted on a glass coverslip and visualized with a confocal microscope (Rennes University, France). Confocal's observation was made between the first layer of the nanoneedle's support and the middle of the cell, corresponding to a height of 6.94 µm. For each observation, six planes were made, pictures are space out 1.15 µm.

SEM Images of nanoneedle fields. High density field in the left column and low density field in the right column.