PIEZOELECTRIC NANOPARTICLES' BIOCOMPATIBILITY ON NEURON-LIKE CELL LINE

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Aim

Nanoparticles are compounds with small size and unique physicochemical properties and are currently used in many fields such as biomedicine, to develop new system for drugs delivery or cancer treatment, but also in the production of everyday consumer products like foods, cosmetics etc. The aim of this study was to test the in vitro biocompatibility of two piezoelectric nanoparticles: barium titanate and lithium niobate, currently tested to develop new self-powered cochlear implant, more performing device and with a better patient's quality of life. Both nanoparticles were tested on differentiated PC12 cell line analyzing cell viability, morphological changes, Cytochrome c distribution, ROS production and complexity of the neuritic network.

Methods and results

Cell viability

The MTS Assay was performed to evaluate the nanoparticles' toxicity on PC12 cells.

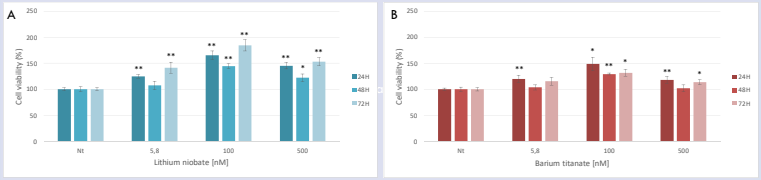

Fig.1 PC12 cells treated with different concentrations of barium titanate and lithium niobate for 24h, 48h and 72h. Cell viability was expressed as mean value percent ± standard error (SE) *vs.* control cells (Nt). Asterisks indicate significant differences in comparison to Nt. * = <0.05; ** = p<0.01.

The results showed in fig.1 that the treatment with lithium niobate (A) and barium titanate (B) not only didn't affect the cell viability of differentiated PC12 cell line, but also, significantly enhanced the vitality at all time and doses tested.

Morphological analysis

The morphological analysis was performed to evaluate the capacity of nanoparticles to induce morphological alterations on PC12 cells.

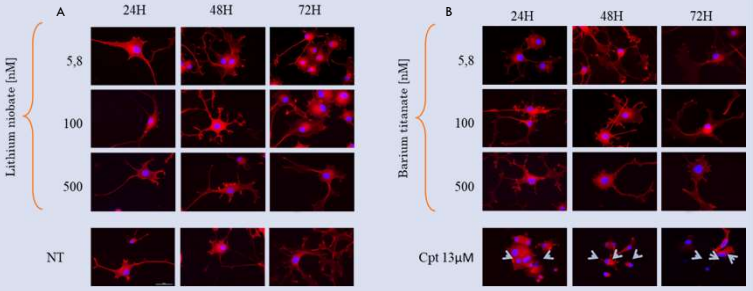

Fig.2 The PC12 cells were differentiated and treated with different concentrations of nanoparticles and with Cisplatin (Cpt) 13 μM for 24h, 48h and 72h. The nuclei were stained in blue by DAPI, the cytoskeleton was stained in red by phalloidin-TRIC in red. Arrows indicate apoptotic cells. Merged images. Magnification 40X

The morphological features of the cytoskeleton and the nuclei of PC12 cells didn't change after the treatment with lithium niobate (A) and barium titanate (B) at all incubation times and concentrations tested; in contrast the treatment with Cisplatin induced neurotoxic effect.

Apoptosis

We analysed the Cytochrome c distribution on PC12 cells to evaluate whether nanoparticles promote apoptosis.

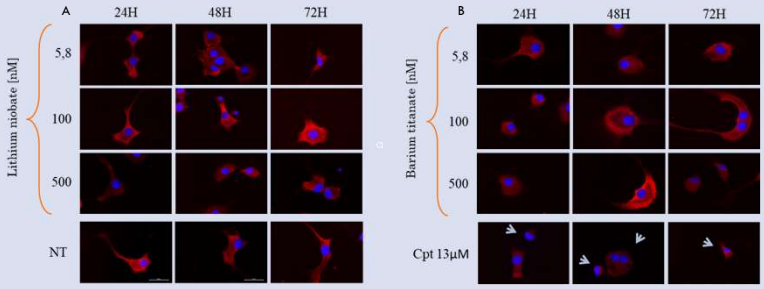

Fig.3 The PC12 cells were differentiated and treated with different concentrations of nanoparticles and with Cisplatin (Cpt) 13 μM for 24h, 48h and 72h. Cytochrome c staining in red and DAPI in blue, arrows indicate apoptotic cells. Merged images. Magnification 40X..

The Cytochrome c distribution in PC12 cells didn't change after the treatment with lithium niobate (A) and barium titanate (B) all incubation times and concentrations tested; in contrast the treatment with Cisplatin induced neurotoxic effect.

ROS production

The DCFDA assay (2′,7′-Dichlorodihydrofluorescein diacetate) was performed to evaluate the ROS (Reactive Oxygen Species) production in PC12 cells treated with nanoparticles.

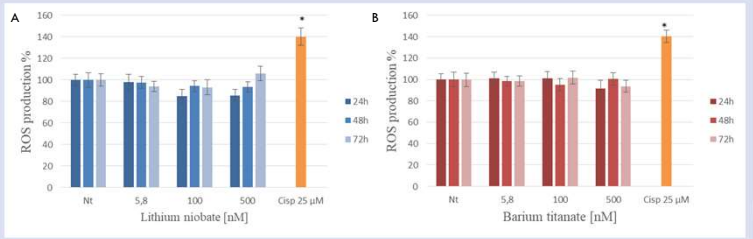

Fig.4 The PC12 cells were treated with different concentrations of nanoparticles for 24h, 48h and 72h; cells treated with Cisplatin at concentration of 25 μM for 24 h were used as positive control. ROS production was expressed as mean value percent ± standard error (SE) *vs.* control cells (Nt). Asterisks indicate significant differences in comparison to Nt. * = <0.05; ** = p<0.01.

The results showed that the amount of ROS produced by cells treated with lithium niobate (A) and barium titanate (B), at all time and doses tested, did not significantly change in comparison with control cells; in contrast, the treatment with Cisplatin increased the reactive oxygen species produced by 40 % vs Nt.

Neurotoxicity

The neurotoxicity induced by nanoparticles on PC12 cell line, was evaluated comparing the complexity of neuritic branches produced by control cells to those treated with piezoelectric compounds and Cisplatin.

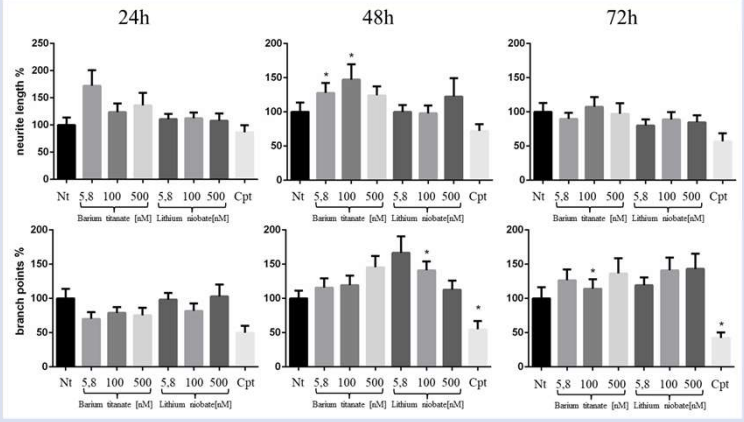

Fig.5. The PC12 cells were treated with different concentrations of barium titanate and lithium niobate for 24h, 48h, and 72h. Cells treated with Cisplatin 13 μM were used as positive control (Cpt). After incubation times thirty fields per treatment were acquired with and analysed measuring the length and the number of branch points of neurites using the ImageJ software (https://imagej.nih.gov/ij/). The data were expressed as mean value percent ± SE *vs.* control cells (Nt). Asterisks indicate significant differences in comparison to Nt. * = <0.05.

The results showed that the treatment with lithium niobate and barium titanate, did not negatively affect the complexity of neuritic network produced by PC12 cells. Barium titanate at concentrations of 5,8 and 100 nM significantly increased the neuritic length after 48h. Similarly, Barium titanate at concentration of 100 nM after 48h and lithium niobate at 100 nM after 72h increased the number of branch points produced by the cells. In contrast, the treatment with Cisplatin at concentration 13 μM significantly reduced the number of branch points after 48h and 72h.

Conclusions

Barium titanate and lithium niobate nanoparticles showed an high biocompatibility on PC12 cell line at all times and doses tested, therefore these piezoelectric nanoparticles could be used to produce new devices that can stimulate the spiral ganglion neurons without inducing side effects.

This study was performed as part of the Italian Health Ministry project "New self-powered devices for cochlear stimulation based on piezoelectric nanomaterials" (RF-2011-02350464) and the project "4NanoEARDRM" (EuroNanoMed 3).

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