# NANOSTRACTURED SILICON NANOWIRES AND ELECTROSPUN NANOFIBRE POLYMER INTERFACES MODULATE ASTROCYTES PHYSIOLOGY AND BIOPHYSICAL PROPERTIES *IN VITRO*


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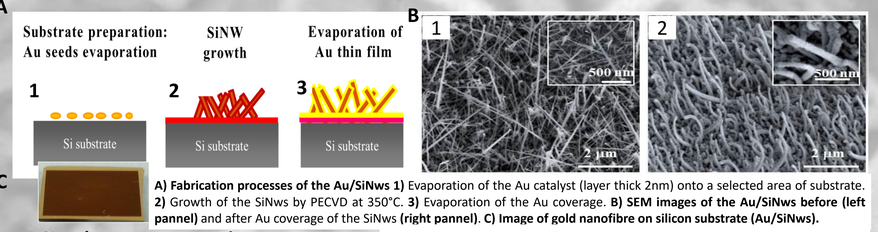
## Abstract

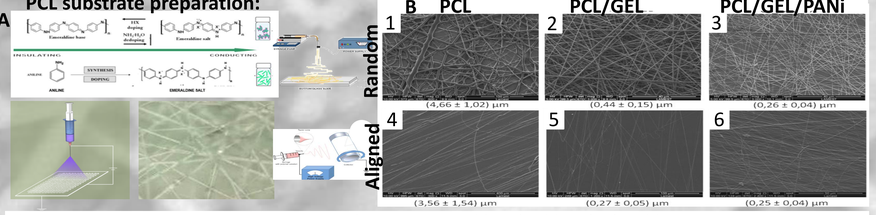

**Introduction.** Astrocytes ion channels and aquaporins play important roles in brain physiological function and dysfunction. There is a need of models to study, modulate and understand astrocytes physiology and pathology. Astrocytes grown in culture lose the peculiar morphological, molecular and functional phenotype that they display *in vivo*. **Aim.** Our goal is to define and characterize *in vitro* models where astrocytes resemble their features *in vivo* and to identify molecular, biophysical and chemical factors that drive their polarization/differentiation process. In this view, we sought to validate nanostructured biomaterials, such as gold coated silicon-nanowires (Au/SiNw) and polycaprolactone (PCL) electrospun nanofibers as substrates to grow and differentiate astrocytes in vitro. **Methods and Results.** Cell viability assays indicate that Au/SiNw and PCL nanofibers enable strong astrocytes adhesion and growth without need of additional coating. Fluorescent imaging, SEM and AFM reveal that astrocytes respond to the substrate topography by morphological differentiation with elongation sprouting from the cell body. GFAP analyses showed that the cells differentiation was not due to gliotic reaction, confirming the differentiation capability of astrocytes in the proposed models. Confocal imaging evidentiated that astrocytes align their process along with PCL nanofibre with F-actin fibre alignment and vinculin polarization. Immunofluorescence on ion channels and AQP4 revealed their localization in endfeet of astrocytes elongation grown on Au/SiNw. The electrophysiological properties of differentiated astrocytes have been characterized by patch clamp. **Conclusions.** Our results validate PCL nanofibre and Au/SiNw as novel glial interface that enables the growth, differentiation and polarization of astrocytes *in vitro*. Also a device made with Au/SiNw has been fabricated to perform extracellular recording of astrocytes current properties, in order to define a model suitable to predict and define effectiveness and toxicology of nanomedicine.


## 1) Nanostructured silicon nanowires and electrospun nanofibre polymer interfaces preparation and topographic characterization



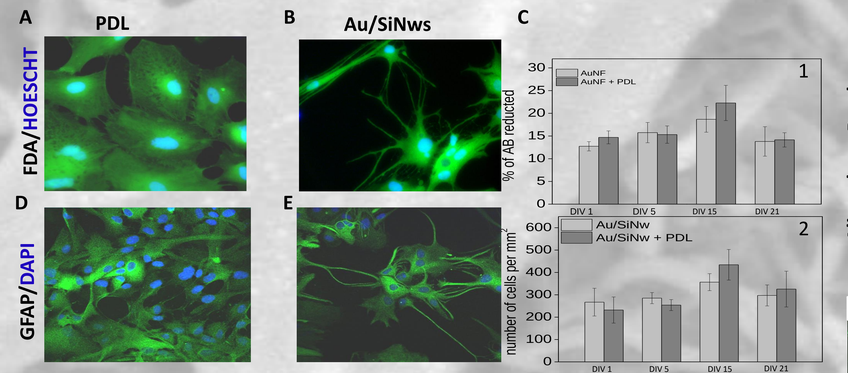

**A) Fabrication processes of the Au/SiNws 1)** Evaporation of the Au catalyst (layer thick 2nm) onto a selected area of substrate. **2)** Growth of the SiNws by PECVD at 350°C. **3)** Evaporation of the Au coverage. **B) SEM images of the Au/SiNws before (left pannel)** and after Au coverage of the SiNws **(right pannel)**. **C) Image of gold nanofibre on silicon substrate (Au/SiNws).**

**PCL substrate preparation:**

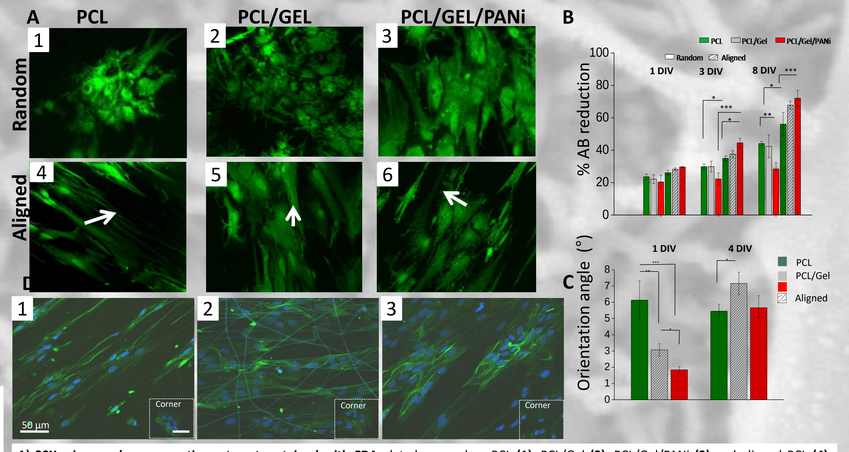



**A) Fabrication processes of the PCL electrospun nanofibers B) SEM images** of PCL **(1)**, PCL/Gel **(2)**, PCL/Gel/PANi **(3)** random electrospun fibers and PCL **(4)**, PCL/Gel **(5)**, PCL/Gel/PANi **(6)** aligned electrospun fibers.

## 2) Astrocytes viability and morphological differentiation on nanostructured silicon nanowires an on electrospun nanofibre polymer interfaces

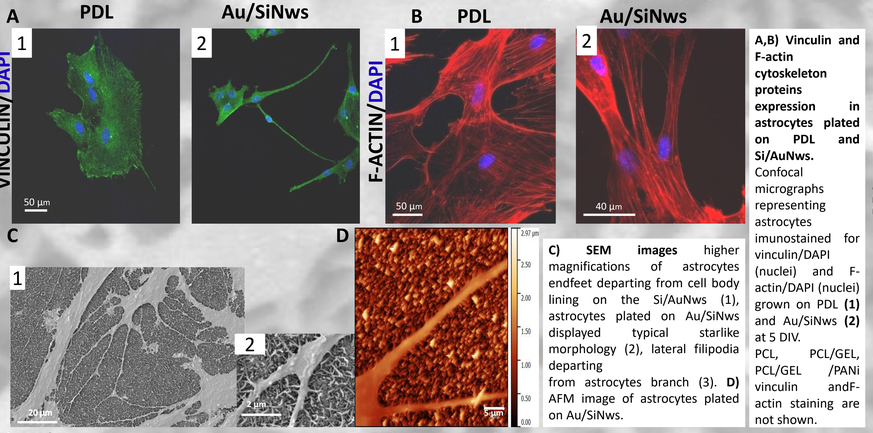



**Morphology and viability of astrocytes on PDL and Au/SiNws films. (A, B)** Fluorescent microscopy image of astrocytes stained with FDA/HOESCHT 33342 (nuclei) plated on PDL **(A)** and Au/SiNws **(B)** captured after 5 DIV. **C)** Histogram plot depicting time course of astrocyte viability on Si/AuNw and Si/AuNw + PDL, investigated by AB assay at different time points. Data are plotted as the averaged percentages of reduced AB ± Standard Error (SE) at 1, 5, 15 and 21 DIV **(1)**. Histogram plot reporting the averages of astrocyte nuclei number, counted on images of cells grown on Au/SiNw and on Au/SiNw +PDL. ($p > 0.05$, ANOVA test) **(2)**. **D,E)** Confocal imaging of **GFAP protein expression** in astrocytes plated on PDL **(D)** and Au/SiNws **(E)**.




**A) 20X micrographs representing astrocytes stained with FDA** plated on random PCL **(1)**, PCL/Gel **(2)**, PCL/Gel/PANi **(3)** and aligned PCL **(4)**, PCL/Gel **(5)** and PCL/Gel/PANi **(6)** substrates, captured after 4 DIV. **B)** Time course of astrocytes viability, carried out using AB assay. The percentages of AB reducted are expressed as means ±SE (n= 8. *: P<0,05; **: P<0,01; ***: P<0,001). **C)** Histogram plot reporting the averages of orientation angles measured between astrocytes and fibers (1 DIV, 4 DIV) plated on PCL (green bar), PCL/Gel (gray bar) and PCL/Gel/PANi (red bar) aligned substrates. **D)** Single plane 40 X confocal images of GFAP expression in astrocytes seeded on aligned PCL **(1)**, PCL/Gel **(2)**, PCL/Gel/PANi **(3)**, captured after 3 DIV. Elongated GFAP-positive intermediate filaments extending from astrocytes the portion of the coverslip devoid of aligned fiber is reported as control on insets.

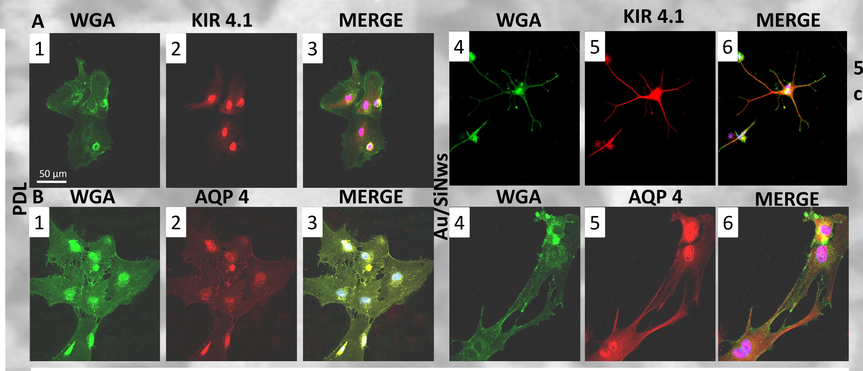
## 3) Effects on nanostructured silicon nanowires and on electrospun nanofibre polymer interfaces



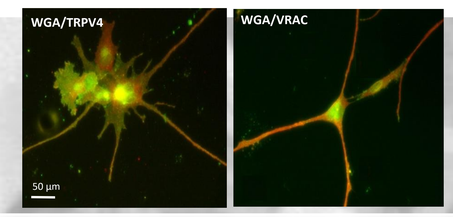

**A,B) Vinculin and F-actin cytoskeleton proteins expression in astrocytes plated on PDL and Si/AuNws.** Confocal micrographs representing astrocytes imunostained for vinculin/DAPI (nuclei) and F-actin/DAPI (nuclei) grown on PDL **(1)** and Au/SiNws **(2)** at 5 DIV. PCL, PCL/GEL, PCL/GEL /PANi vinculin andF-actin staining are not shown.

**C) SEM images** higher magnifications of astrocytes endfeet departing from cell body lining on the Si/AuNws (1), astrocytes plated on Au/SiNws displayed typical starlike morphology (2), lateral filipodia departing from astrocytes branch (3). **D)** AFM image of astrocytes plated on Au/SiNws.

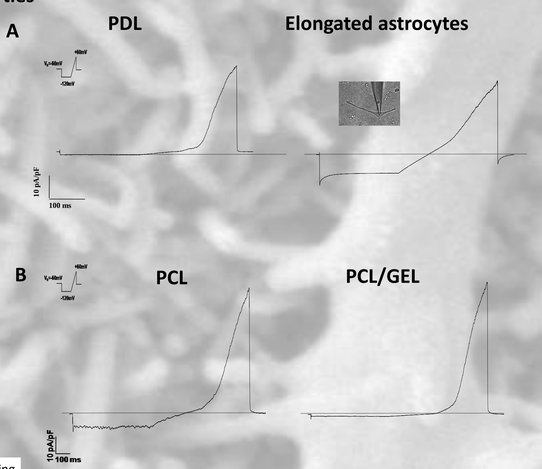
## 4) Membrane properties of astrocytes grown on nanostructured silicon nanowires




**A,B) Kir 4.1 and AQP4 protein expression in astrocytes plated on PDL and Au/Sinws**. a) Confocal imaging of astrocytes grown on PDL and Au/Sinws stained for WGA **(1,4)**, KIR 4.1 **(2,5)** and merge **(3,6)**; **B)** Confocal imaging of astrocytes grown on PDL and Au/Sinws and stained for WGA **(1,4)**, AQP 4 **(2,5)** and merge **(3,6)**, **C)** Fluorescent microscopy of astrocytes grown on AuSinws and stained for WGA and TRPV4 and VRAC.

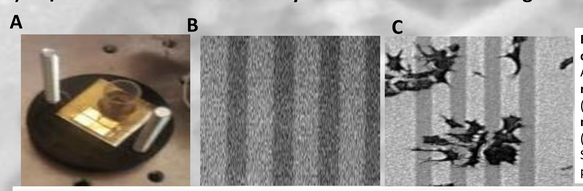



## 5) AuSiNw and electrospun nanofibre polymer interface–astrocytes interaction modulate cells electrophysiological properties




**A, B)** Current traces recorded stimulating astrocytes with a voltage ramp protocol from Vh of -60 mV, fro-120 to +60 mV (100 ms) on Au/Sinws and PCL, PCL/GEL.

## 6) Au/SiNw device enable astrocytes extracellular recording




**Extracellular recording astrocytes current plated on Au/Sinws electrode. A)** Image rapesenting Au/Sinws device. **B) Representative trace of recording performed on** Au/Sinws electrode (inset) without cells C) **Representative trace of recording performed on** Au/Sinws electrode (inset) with astrocytes plated on the device (inset). Spikes can be clearly resolved when recording was performed with cells.

**The results reported here are: 1) Au/SiNws and PCL are valuable material interface for adhesion, growth and differentiation of astroglial cells in vitro without the need of any chemical coating; 2) Astrocytes morphology and ion channels as well as AQP4 on Au/SiNws interfaces are resembling the one of astrocytes observed in vivo 3) The differentiation on Au/Si Nw is not due to astrocytes gliosis; 4) Electrospun nanofibres of PCL allow the alignment and the direction of astrocytes elongation; 4) Collectively results indicate that astrocytes membrane protein expression and function is responsive and can be manipulated by to nanostructured surface as well as by material chemistry;**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