Project n°: STRP 032652

BIODOT

Sensing BIOsystems and their Dynamics

in fluids with Organic Transistors

EXECUTIVE PUBLISHABLE SUMMARY

Start date of project: October 1, 2006

COORDINATOR	RESPONSIBLE	PERIOD COVERED
CNR	Fabio Biscarini	1 October 2006 - 30 September 2009

Project overview

The detection of biological and chemical species is central to many areas of health care and the life sciences, ranging from diagnosing diseases, to the discovery and screening of new drug molecules. Neuroscience, as well as diagnostics and therapies of neurological diseases, demand for development of new devices with a highly sensitive mechanism of transduction of the biological and chemical signals.

Devices based on organic semiconductors emerge as a powerful and versatile class of ultra-sensitive electrical transducers for label-free detection of biological species. These devices can be fabricated and integrated with micro- and nanofluidics devices by the use of sustainable nanofabrication techniques; they can be downscaled and endowed with specific recognition functionality by materials design. BIODOT has addressed and demonstrated a hybrid bio-organic technology for label-free transduction of biomolecules' slow dynamics and cell signals in-vitro.

The technology able to treat organic materials and devices, membranes and biomolecules, cells on same footings has been developed. This is a non trivial outcome, and has requested a considerable effort in designing collaborative experiments with integration of competences at the same node, devising of new protocols of operations and interactions, and coordinate an intense networking. Patterning and device fabrication have been aligned to the sterility requirements of cell cultures, a result which is not trivial to obtain since sterilisation of organic devices cannot be made by traditional laboratory methods.

Two device layouts based on organic thin film transistors integrated with microfluidics have been developed within the project: i) a single gate ultra-thin film transistor; ii) a dual gate ultra-thin film transistor, with a reference electrode. These devices respond to changes of the electrostatic charge at the interface between the biosystems in the solution and the organic semiconductor within the Debye-Helmholtz layer. The former device by a modification in the density of charge carriers similar to doping of the organic semiconductor; the latter, by a change of the electrostatic surface potential.

BIODOT has brought the knowledge and the technology of the biological transducer based on organic semiconductors at the state-of-the-art. Sensor operations in aqueous media, the possibility to interface the active layer to systems of increasing complexity such as biomolecules (DNA, antibodies, beta amyloid peptides), recognition layers to neural cells and networks, and finally the transduction of signals correlated to the viability of cells have been demonstrated. The project has also achieved a substantial control on reproducibility, stability and sensitivity level of the devices, as well as on the spatial control of the biological system integrated in the device, which has lead us to assess the technology developed.

Specific results achieved concern:

i) the understanding of the mechanism of amyloide 1-40 peptide aggregation in mesoscopic channels, with experiments carried out in microfluidics setups;
ii) the specific recognition of anibody-antigene interactions on organic semiconductor surfaces;
iii) the simulation of amyloide peptide aggregation at organic surfaces and upon application of electric fields;
iv) the integration of murine stem cells on a variety of organic semiconductors, and their differentiation into neural networks;
v) the control of adhesion, proliferation and spatial positioning of human neurons and astrocytes from secondary lines on ultra-thin films of organic semiconductors;
vi) the fabrication of dual gate FETs with buried electrodes using polymeric mutilayers or thin organic films as second gate dielectric for stable device operations under water.
vii) the understanding of transduction of single and dual gate FETs;

viii) the transduction of noise from differentiated stem cell cultures after two weeks of operations in an incubator.

An unforeseen outcome of BIODOT has been the founding of a new spin off company, Nano4bio Srl, in Bologna Italy. Its mission is to develop patterning technology for cell cultures and tissues. Another outcome is that the partner Scriba Nanotecnologie Srl is now designing and manufacturing microfluidics setups on demand for the research market.

More than 22 papers on high impact international journals have been published and other 9 have been published after the end of the project. A patent application has been deposited.

BIODOT has effectively demostrated the concept of converging technologies by merging two enabling technological platforms, viz. bio- and nanotechnology, in which EU has invested substantial resources. It has opened broad market segments to multifunctional materials and organic electronics, beyond consumer electronics and displays. The outcome of BIODOT and its long-term objectives are consistent with the concepts of ERA and the development of a knowledge-based economy as stated in the Lisbon conference

S &T Objectives

BIODOT aimed to develop a hybrid technology and organic electronic devices that allow monitoring dynamical phenomena of biosystems at in-vitro conditions. The objectives of BIODOT were:

i) the development of a fluidics/bio-organic technology;
ii) the design and fabrication of the new device;
iii) the electrical transduction of dynamical phenomena of biomolecules;
iv) the scalability of the response down to the level of finite numbers of (bio)molecules;
v) the quantification of the response of the device;
vi) the integration of functional membranes and neural cells in the device;
vii) the assessment of the technology towards the development of new tools for research and therapy of neurological diseases;
viii) the evaluation of the societal issues in the context of neurological diseases.

These objectives have been largely met. Partial success concerns limitations at point iv) owing to the lack of standardised single gate transducers, and to the lesser sensitivity of the standardised dual gate transducers. The achieved transduction level corresponds to an equivalent coverage of several percent by charged molecules. Overall, BIODOT has proven the feasibility of the approach, and identified technological bottlenecks, which can be surpassed by different routes (e.g. integration of hybrid organic-inorganic materials, standardisation of organic materials, AC vs DC operations).

Partnership:

The consortium brings together several EU centres of excellence in nanoscience, processing and nanofabrication, organic electronics, biophysics, neuromedicine and neuroscience. Partnership includes a major electronics industry research centre at the forefront of innovation, and a spin-off company developing products based on unconventional nanofabrication. The complementary expertise is carefully balanced according to the objectives.

Role	Participant name	Country
Coordinator (CO)	***CNR*** - Consiglio Nazionale delle Ricerche-ISMN Bologna	I
CR	***CSIC*** - Consejo Superior de Investigacíones Cientificas - IMM Madrid	E
CR	***IEM-HAS*** - Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest	HU

CR	***LMU*** - Ludwig Maximilians Universitaet, Munich	D
CR	***Philips*** - Philips Research Laboratories, Eindhoven	NL
Contractor (CR)	***RuG*** - University of Groningen	NL
CR	***Scriba*** - SCRIBA Nanotecnologie Srl, Bologna	I
CR	***UNIBO*** - Università di Bologna	I

Project objectives and major achievements in the first year (1/10/2006-30/09/2009)

Objectives of BIODOT in the first year were:

- Deposition of relevant biomolecules from water-based solutions on organic semiconductors (WP1)
- Choice of materials and fabrication approaches for dual gate devices (WP2)
- Initial assessment of stability of organic semiconductors and devices in water or exposed to humidity and physiological buffers (WP3)
- Elementary technological steps for the deposition of peptide molecules and bilayer membranes onto organic semiconductors (WP4)
- Design of a first generation microfluidics layout for integration in FETs; Choice of prototyping technique for microfluidics (WP4)
- Assessment of the compatibility of both materials and processing with biological systems; alignment of biological protocols with the requirements for organic semiconductors; Evaluation of the growth and viability of neural cells on organic semiconductors (WP6)

Partners have agreed on and signed the Consortium Agreement text by Summer 2007. A good level of networking among groups has been established, by overcoming jargon barriers level and achieving an effectiveof cross-disciplinary understanding; open internal discussions and frequent exchange of samples and information have been achieved; dissemination has started with several talks at international conferences and papers.

More specifically, the achievements of the work-packages (WP) are:

WP1: i) First high resolution AFM images of adsorbed single IgG molecules; visualisation of antibody-antigen interaction by force microscopy (D2 achieved);
ii) imaging and control of early stage aggregation of beta-amyloid peptides on organic semiconductors and bilayer membranes patterned on pentacene;
iii) deposition of a bilayer membrane across a large area of a pentacene film.

WP2: iv) first generation test patterns with single gate transistors has been delivered by Philips to CNR and RuG for processing organic semiconductors, etc. (D1 achieved);
v) evaluation of high-k dielectrics as second gate dielectric for dual gate devices;
vi) first sensor response to benzoic acid achieved with a dual gate transducer operated as ISFET;
vii) structural and morphological stability of pentacene ultra-thin films in water positively assessed.

WP3: viii) control of size distribution of aggregates of beta amyloid peptides in mesoscopic channels by MIMICS achieved;
ix) patterning synthetic bilayer membranes onto pentacene 2 ML thick films;
x) micrometer scale patterns of fibrin monolayers on pentacene and sexithiophene. These three results contribute to the achievement of D3.

WP4: xi) design of a self- aligned microfluidics/device layout.
xii) Fluid cell for XRD reflectivity experiments in water fabricated.

WP5: xiii) first device models of dual gate device worked out;
xiv) binding energy and electrostatic screening of water dipoles interacting with holes in organic semiconductor have been predicted.

WP6: xv) test pattern and substrate cleaning, high vacuum sublimation of organic semiconductor thin films, patterning adhesion proteins are all processes which do not introduce bacterial contamination; samples can be safely and routinely delivered to partners for cell growth;
xvi) glia cells (astrocytes) were grown on bare pentacene thin films and pentacene transistors;
xvii) number of viable glia cells can be modulated by pentacene thickness/ roughness in the range 1-10 ML; there is an optimum thickness for layered pentacene thin films.
xviii) Neural stem cells adhere on pentacene films;
xix) Neural stem cells on pentacene can be differentiated into neurons forming dense networks of viable cells, growing onto aggregates of glial cells; no serum is needed. Glia cells and neurons remain viable on pentacene for many days.

Project objectives and major achievements in the 2nd year (1/10/2007-30/09/2008)

During the second year, the research activities were aimed to assess and optimize the layout of the bio-OFETs and to test the integration of the living cells on organic devices. This effort has produced a deep integration of the competences of the partners and the work has been carried out in an efficient framework. Joint experiments have been envisioned and performed with positive results.
The breakthrough was to demonstrate organic semiconductor ultra thin film transistors operating in aqueous solutions, including a physiological medium with living cells for several days.
Major achievements both in the fundamental knowledge and the technology of the hybrid bio-organic systems are:
i) the demonstration of operation of organic field effect transistors in water environment;
ii) the integration of an additional organic capping layer on the organic semiconductor to protect the semiconductor from water;
iii) the integration of microfabricated fluidic cell with 800 nm wide 180 nm high channels on standard size organic transistor and the control of the concentration;
iv) the simulation of β-amyloid peptide conformation and nucleation in water, close to surface and under electric field;
v) the modelling of signal formation and scaling by a continuum approach and theoretical analysis of the sensitivity as a function of the basic sensor parameters;
vi) the fabrication by Local Oxidation Nanolithography of periodic nanostructures on pentacene films with a lateral periodicity below 1 μm for anchoring antibodies;
vii) the understanding of the non-selective attachment of neural progenitors to non-toxic surfaces;
viii) space-oriented cell attachment and neurite growth on surfaces patterned by gradients of different adhesive molecules;
ix) the application of a synthetic adhesive for the attachment and differentiation of neural stem/progenitor cells;
x) the determination of the critical nucleus size of peptide aggregation in solutions confined in mesocopic channels, and the transduction of peptide aggregation.

The results i-vi show that the BIODOT approach is viable, and there are no major fundamental technological barriers for OFETs used as biosensors. The results VII-IX shows that living cells can be integrated in the OFET and that the mechanism of attachment and differentiation has been approached. The results X show that the electronic transduction of peptide aggregation works. The activities has been disseminated by 16 publications and more than 10 presentations at international conferences. During the second reporting period there have been active exchanges of researchers for training in different disciplines from those belonged. Overall, the project is developing, with a very high level of cooperation among academic partners and industries, with a substantial networking and open flow of information. The flow of information and experiment planning has been carried out by all the partners in a constructive way. The hired young researchers has brought in the research staffs their different competences increasing the multidisciplinarity of the consortium.

The knowledge has been protected with a patent and the transfer of technology has been implemented through the foundation of a spin-off company “Nano4bio srl”, established in Italy to develop hybrid technology and “smart” substrates for in vitro cell cultures. The company stems from a majority group of women scientists and professionals, with different backgrounds.

Project objectives and major achievements in the 3rd year (1/10/2008-30/09/2009)

During the third year, the research activities have been focused to optimise the layout of the OFET transducers and to characterize the integration and signalling of biomolecules and living cells on organic devices. Major achievements have been:

- The demonstration of single gate transducer based on pentacene ultra-thin (5 ML thick) films grown layer-by-layer, or conjugated polymers (PTAA), also in differential operations, interfaced with microfluidics setup.
- The demonstration of a different dual gate field-effect transistor based on pentacene, fabricated on TOPAS as a flexible polymer dielectrics, and with a 50 nm thin film as second gate dielectric.
- The optimisation of the dual-gate field-effect transistor using polytriarylamine (PTAA) as active layer, with an insulating stack applied on the top, made of a first polyisobutylmethacrylate (PIBMA) layer and then a second layer of a Teflon derivative (AF-1600). The electrodes are manufactured on silicon wafers acting as a common bottom gate, and integrated with a microfluidic set-up and channel, to supply and confine the electrolyte to the sensing area. A reference electrode is used in both the dual gate configurations.
- A demonstration of specific sensing of antibody-antigene interaction using Si nanowire transistors fabricated by a combination of local anodic oxidation and etching steps. This device consisting of ultra-thin Si nanowires scalable down to 4 nm width were not foreseen originally in the proposal, and have been explored as a proof of concept.
- Multiple feed-through *microfluidics* was developed in order to perform simultaneous measurement with different solutions in direct contact with the sensor area. In particular two parallel microchannels were used with analyte+solvent (*i.e.* amyloid+water; or cells+medium) and solvent (*i.e.* water; or medium) solutions, to control the effect of concentration of the analyte and estimate a range of sensitivity in the device response.
- Pentacene FETs capable to operate in water solutions for many days (up to 2 weeks) have been demonstrated; a complete system to systematically characterize the behaviour of the OFETs working as bio-sensors in time and changing the concentration of bio-molecules has been demonstrated.
- New experimental set-up for monitoring signals from a neural cell culture in vitro was designed and home-built by modifying a cell incubator in order to include the proper connection to electrical measurement instruments, allow the control of the temperature, humidity and CO_2 pressure during cell growth.
- Functionalization, by soft lithographical techniques, of the surface of organic field-effect transistors and of Si nanowire transistors was achieved, in order to electrically recognize specific protein-protein interactions in real time.
- Functionalization with the extracellular matrix protein laminin of the surface of devices was performed, in order to control and promote the adhesion of neural cells on the sensing region of the transducer, by a new soft lithographic approach. These findings evidenced that laminin pattern is an excellent template for inducing highly preferential, if not selective, cell adhesion (i.e. human neuroblastoma cells).
- Suitable protocols for growing cells onto different substrates, different adhesive gradients and cell inducing protocols have been established, depending on the adhesive features of the investigated cells (NE-4C neural stem cells, radial glia-like neural progenitors and freshly isolated neurons). Gradient adhesive surfaces have been established by controlled perfusion of PDMS microfluidic channels with different adhesive substrates from the opposite side-reservoirs against the central well. The selective adhesion preferences have been used up to orient the growth of non-induced progenitors, the migration of developing neuronal precursors and the elongation of neurites.

Overview of the WP objectives and achievements

WP. N.	Objective Description	State-of-the-art	Innovation by BIODOT
WP1 WP3	Control of the biological/ organic semiconductor interface	Bio-organic semiconductor interfaces largely unexplored; Biomolecules at functionalised silicon-oxide surfaces or Au extensively studied; Single molecule experiments by SPM, optical tweezers, FRET with artificial constraints.	➢ **Specific recognition on bio-organic semiconductor interfaces**: measurement of single molecule recognition processes (antibody-antigen) on pentacene surfaces has been demonstrated by TREC-AFM mode. Patterns (stripes and dots) by AFM local oxidation on pentacene were fabricated as first step of constructive lithography of a recognition layer. Periodicity below 1 μm has been achieved. The patterned samples have been used as templates to direct the deposition of IgG antibodies. The scheme has been upscaled to a larger area using parallel local oxidation by soft Au-coated stamps, or by direct lithographically controlled wetting deposition of IgG antiobodies. Local Oxidation Nanolithography has been also been applied to fabricate silicon nanowire transistors, a few tens nm wide down to 4 nm. These devices have been integrated with 10 micron wide microfluidics channels, and the detection of antigen-antibody interactions was demonstrated. The ultimate goal would be to detect neuronal activity. ➢ **Amyloid peptide dynamics on organic semiconductor surfaces**: Simulation of β-amyloid peptide fragments (17-42) on semiconductor interfaces and upon application of electric field has lead to propose pathways of conformational changes and aggregation. These simulations have been extended to the investigation of β-amyloid oligomers interacting with prion protein on semiconductor interfaces, in an effort to design new specific sensors. Here, coarsegrained multiscale simulations (diffusive particle dynamics) which is state-of-the-art, have been used to describe the time evolution of the aggregated oligomers at the device surfaces. The simulation work has complemented a systematic experimental investigation of the kinetics of aggregation of the 1-40 beta amyloid peptide vs concentration and time. The experiments have been carried out in microfluidics environment, with the aim to finely control concentration throughout the 24 hour duration of the experiment. The microfluidics mimick the conditions of cellular crowding. The experimental populations of oligomers, aggregates, protofibrils were fit with a nucleation-and-growth model accompanied by a molecular dynamics simulation, to yield a critical nucleus size equal 3. This result hints that tetramer is the most stable oligomer of AB 1-40, in good agreement with the simulations. ➢ **Functional membranes** Functional membranes and organic capping layers were formed on pentacene surfaces. Their structure, dynamics, dielectric properties and the interactions with beta-amyloid peptides were studied by a combination of GIXD, XRR, fluorescence microscopy, AFM.

WP 2	Design and fabrication of a new transducing device.	- Optical transduction widely used; - Electrical transduction limited to MEAs. - Tailoring/biocompatibility difficult; expensive downscaling. - OFET largely unexplored as bio-transducers.	➢ **Single Gate FET:** Pentacene FETs with no capping are operated in aqueous environment in linear regime below -1 V drain bias. Pentacene FETs (5 nm thick) capable to operate in water solutions for many days (up to 2 weeks) have been demonstrated; a complete system to systematically characterize the behaviour of the OFETs working as bio-sensors in time and changing the concentration of bio-molecules has been built and demonstrated. ➢ **Dual Gate FET as Transducers operating in Aqueous Environment:** Theoretical model that simulates the DGFET and charges on the interfaces with the gate dielectic was developed. The sensitivity is demonstrated to depend on the ratio of the capacitance of the two dielectric layers. Dual Gate FETs were manufactured. The photo-lithography process for DGFET was designed and tested. Final outcome are substrates with buried electrodes manufactured and up-scaled to 150 mm wafers.The technology platform has been defined upon a screening of different semiconducting polymers and a variety of dielectrics (inorganic, polymer multiplayers). Optimum choice is PTAA as standardised active layer and a polymer multilayer PIBMA/Teflon AF1600 as second gate dielectric to protect the device from aqueous environment. Another choice which has worked effectively has been pentacene FET on a flexible substrate, TOPAS, and with second gate dielectric made of a long chain alkane thin film (50 nm thick). Operations in water demonstrated that DGFET is sensitive to gating by electrolyte, provided the solution potential is fixed by a reference electrode. Improved modelling of the transistors lead to fully understand the response of the bio-transducer. Stability has increased by reduction of the hysteresis and minimization of the leakage currents. This allows applying much higher drain biases, resulting in higher currents and thus more reliable measurements. Sensitivity (around 60 mV) is limited by thickness of the second dielectric layer, but is suitable for substantial enhancement. Both devices are integrated with microfluidics setups for static and dynamic monitoring.
WP 4	Development of a scalable fluidics/ bio-organic technology	- Microfluidics has been integrated into organic electronics devices for molding components; - unconventional patterning of biomolecules and membranes largely unexplored; - experimental gap between single molecules and large ensembles.	➢ **Microfluidics for sensing devices:** Two processes for integration pursued: 1) single step techniques (glass structures); 2) alignment of PDMS devices on top of existing FET. Effort in testing the seal as the solution is infilled with an external actuator. Successful integration of micro-fabricated fluidic cell on FET devices with channel length 1.5-40 μm. ➢ All the transducers can be operated with a microfluidics circuit integrated on it. In some layout (e.g. SiNW FETs) the microfluidics screens water completely out of the electrodes. ➢ **Microfluidics for biomolecules and cells:** Specific microfluidics devices for the investigation of peptide aggregation in microconfined environment were designed and fabricated for long-term static incubation at controlled concentration. These devices also allow us to pattern peptide nanostructures down to 50 nm lateral feature size, and with single molecule control in thickness. Patterns of biomolecules /organic interface with rms roughness better than 1 nm achieved. Simple microfluidics with thermostated pools and inlet/outlet flow of medium and or stimuli were designed and fabricated. Conditions for long-term (up to 14 days) neuronal cultures

			under PDMS channels/confinements of different width (20 – 200 μm) were assessed, where neural cells remain viable and electrical signal monitoring can be performed.
WP 5	Electrical transduction of static and dynamical phenomena in biomolecules	Dynamic effects are usually not accounted in sensing Transduction of biomolecular events at surfaces by optical methods is not quantitative.	➢ **Proof-of-concept with single gate FET and biomolecules:** Electrical characteristics on dried DNA/pentacene FETs were studied as a function of DNA concentration in the solution. Label free transducers suitable to operate in regimes of high molecular entanglement demonstrated. Devices exhibit a sensitivity of about $7 \cdot 10^5$ bp per μm^2, suitable for a 600 time improvement as FET is standardised (threshold voltage dispersion below 50 mV). ➢ **Assessment of the sensitivity and scaling of the transduced signal:** the modelling of signal formation and scaling was done by a continuum approach; analysis of the sensitivity as a function of the basic sensor parameters; electrical tests were performed under water using dual gate field-effect transistor. Device testing under water and under ionic solution was successful. This demonstrated that the detection of ionic species is essentially limited by the ratio of the capacitance of the dielectric layers. ➢ **Transduction of amyloid peptide aggregation:** The study of peptide aggregation in a confined environment has been translated to a microfluidics interfaced with a DGFET. The detection scheme is based on a differential circuit, one with the reference solution containing just the medium and the other with the analyte. As the monomer disappears from the solution due to aggregation, the signal detected (apparently the shift in pinch-off voltage) decreases. The onset of protofibril formation from aggregate correspond to a minimum followed by an increase of the signal vs concentration. ➢ **Transduction of antibody-antigene specific interactions:** Patterns of laminin have been fabricated by soft lithography of the highly hydrophobicTeflon surface of the DGFET transducers. The functionality of patterned laminin has been demonstrated by a specific immunofluorescence assay. These patterns have been then used to integrate cells on the DGFET transducer. The aim was to detect the formation of focal contacts on the device at the early stages. We also demonstrated recognition of proteinBSA with SiNW FETs with Anti-BSA deposited with a microfluidics in the channel. ➢ **Transduction of noise from cell cultures:** pentacene ultra-thin film FET and DGFET were interfaced to stem cell cultures (see WP6), then differentiated into neural networks after 10-14 days. The experiment probed the noise level at 140 Hz from the cell culture, by means of a lock-in amplifier. Cells are stimulated by microvolt ms-long pulses at the drain voltage, and a correlation between number of viable cells and noise intensity is observed. This experiment proves the feasibility of long-duration operations of OFET transducers in cell culture conditions, and the possibility to detect a noise, although direct correlation to cell signal has yet to be demonstrated.
WP 6	Integration of neural cells in the	Growth of neural cells on a few types of	➢ **Growth of neural cells on organic semiconductors and functional primers**: The adhesion, growth, and neural induction of mouse neural cells NE4C on pentacene ultra-

	technology	substrates. Microarrays with neural networks show often poor contacts and noise.	thin films was achieved. Immuno-cytochemical identification of the cell types developing on the various surfaces. It was demonstrated that i) pentacene is structurally and morphologically stable to the prolonged contact with water, physiological buffer and cell culture medium; ii) neural stem cells adhere to pentacene and remain viable on it for at least up to 15 days; iii) densely interconnected neural networks and glial cells develop on the pentacene surface after several days. This technology can be transferred to interface neural networks to devices. Neural cells were grown also on semiconductor and dielectric surfaces functionalized with adhesive peptides (poly-L-lysine, laminin, fibronectin and RGD peptide-mimetics), with and without serum supplement. ➢ **Rationalisation of the non-selective attachment of neural progenitors to non-toxic surfaces**. Demonstration and quantification of a large-scale production of extracellular matrix molecules by neural stem/progenitor cells. • Application of a synthetic adhesive peptide-conjugate for serum-free attachment and differentiation of neural stem/progenitor cells; • Isolation, characterization and cloning of "native" radial glia-like neural progenitors; timed neuron-production by in vitro induction of radial glia cells. • Demonstration of bioelectrical activity of neuronal networks grown on functionalized surfaces • Growth factor supported orientation and guided elongation of axons on surfaces functionalized with gradients of adhesive proteins in microfluidic channels • Description of conditions (minimum fluid compartment, maximum stimulus intensity and duration) for transistor assays on living cell responses. ➢ **Cell Patterning:** Patterned arrangements of neural stem/progenitor cells obtained on adhesive peptide-stamped surfaces. Space-oriented cell attachment and neurite growth on artificial surfaces patterned by gradients of different adhesive molecules. Growth of neural stem cells on artificial lipid membranes, also functionalized with adhesive peptide. Patterned growth of neurons on pentacene and insulator surfaces functionalized with adhesive peptides. ➢ **Integration of neural cells into a device structure** This is achieved by microfluidics containing cell onto test devices bare or pre-patterned with laminin (as for DGFET). The characterization of electrical transducers of neuronal activity implies that the device is measuring while a cellular culture is developing on it. The ideal conditions for cellular culture are orthogonal to the standard requirements for the electrical characterization of OFET. Excitation of device and the response of the cells does not depend on either the cell population or the intensity of the stimulus, the power density of the sidebands will be an indication of the cells response. Preliminary results clearly show difference between apoptotic and healthy cell populations.

WP7	Assessment of the technology towards development of new tools for research on and therapy of neurological diseases	Local therapies slow down and even make neurological diseases to regress; Loco-regional therapies are based on clinical observation.	**Social impact of the technology and implications in diagnosis and therapy of neural deseases** A hybrid bio-organic devices flexible, biocompatible and integrable with biomolecules and neuronal cells has been explored, developed and assessed. The demonstration of new methodological approaches in vitro can help to better understand the evolution of the phenomena of peptide aggregation which are involved in the ethiogenesis of Alzheimer's disease, in a context that simulates more closely cell crowding and the synaptic contacts. The patterning and microfluidics technology developed are important basic steps towards the realization of new assays for markers on solid surfaces and integrated into optical or electronic devices. The organic transducer is an important first step for the development of low-cost single-shot multiparameter sensors, fabricated in large numbers, which can be used for massive screening and diagnostics of AD. Current state of the art envisions six biomarkers for AD, although the diagnosis of the pathology is based still on late clinical evidence. The development of a technology to interface neural networks and devices, together with the demonstration of the viability of the cells on such devices for long time, opens the road to exploratory work towards flexible devices which can be interfaced to neural cultures and brain tissue. The knowledge created in BIODOT has lead to found a new spin off company, Nano4bio Srl based in Bologna, which nowadays is developing patterned Petri dishes for cell and tissue cultures, and has started collaboration with cell factories. Microfluidics has become a product offered by Scriba Nanotecnologie Srl on demand to customers. The BIODOT technology has the potential to enable early detection at nanoscale of biomarkers for neurodegenerative diseases, and to improve diagnosis and anticipate the treatment of diseases (small size sensing and local therapy). Other by-products of the technology developed apply to the controlled growth of neural networks from stem cells, which is important for regeneration of brain connections and nerves, and more widely can be a paradigm for regenerative medicine.

Conclusion

Based on many original and breakthrough results obtained, BIODOT is a successful project. None of these existed or was consolidated or simply demonstrated in crash test before BIODOT started. These results have demonstrated the feasibility of a new technology based on hybrid bio/organic systems, which can be promising and effective for interfacing sensors to living tissues and possibly extended in a future to animal models. The project has shown the possibility to probe signals, although many issues remain open, such as the sensitivity, the selectivity, and reproducibility of the

signal upon repeated measurements. All these issues must be faced with an activity of standardisation which has to be beyond the scope of BIODOT.

The amount of the technical-scientific results has been very high, considering the diversification of problems and competences. This supports the validity of the converging technology approach, despite a first impression of serendipity. The possibility to interact among different disciplines has also revealed to be a very effective driver for the collaborations and the motivations of young researchers involved in the project. The level of exchange of information, secondments, samples has been exceptionally intense. It has also been very useful to test the compatibility among groups which have not interacted before and have always operated in fields rather distant. This has been one of the greatest achievements of BIODOT, as jargon and disciplinary barriers were a problem for the first few months, but have been effectively overcome later. Another interesting aspect that emerged from BIODOT is the fact that even with the most open collaboration, it is difficult to perform breakthrough experiments just by circulating samples. There is a clear need for a common laboratory/facility where experiments involving the different competences and tools can be carried out. In the context of BIODOT, the CNR node played this role of common laboratory for all the experiments involving devices and cells. Finally, the IP produced by BIODOT is substantial, and beyond producing patents, has lead to the foundation of a new spin-off company and to diversify the product offer by Scriba Nanotecnologie Srl.

As an outlook, there are many different avenues of research that could follow up starting from the knowledge acquired in BIODOT: from transducers to (multi-parameter) sensors; standardisation and demonstration of analogue circuits; high throughput manufacturing of biosensors based on flexible devices; measurement response of neural networks to external stimuli by non invasive sensors which capture signal from the whole network and not from single cells; coupled micro-fluidic circuits and devices for cell actuation, stimulation, fingerprinting, and sorting; rational design of neural networks on soft matter; micro-fluidics as a tool for aggregation studies for a variety of conformational diseases.