nanotechnology based cochlear implants

SECOND PUBLISHABLE SUMMARY

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Functional hearing can be restored in the majority of deaf patients using a neuroprosthesis called cochlear implant (CI). However, some limitations remain, which are mainly caused by the anatomical gap between auditory neurons and the electrode array in the cochlea.

NANOCI aims at improving CI performance by creating a gapless human-machine interface in the inner ear. Methods of biomedical engineering, regenerative medicine and nanotechnology are used in concert to achieve this ambitious goal.

Eliminating the gap between neurons and electrode array could in theory improve efficiency of auditory nerve stimulation. Physically moving the device closer to the neurons remains impractical and potentially traumatic to cochlear tissues. An alternative option is to attract the peripheral processes of the auditory nerve towards the electrode array.

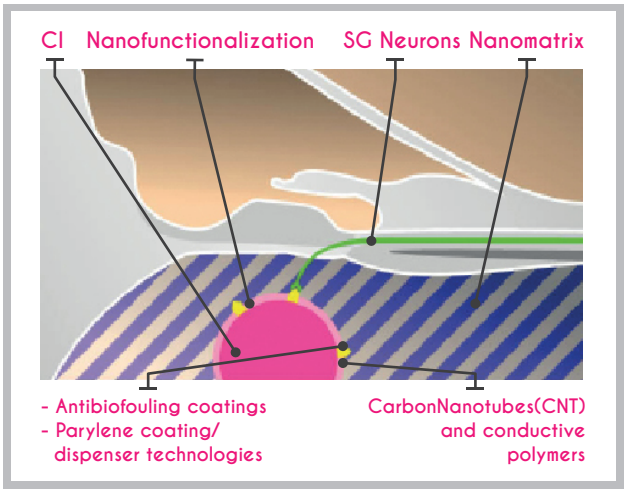


Figure 1. Schematic of the NANOCI electrode: Spiral Ganglion (SG) neurons in green, CI in pink. A bio-functionalized injectable nanomatrix (violet stripes) will be used to fill Scala Tympani and guide SG neurons to the modified electrode array including: Surface coating (nanoparticles with antibiofilm activity and Parylene coating for drug delivery) and Electrode Coating (conductive polymers and carbon-nanotubes).

The NANOCI project aimed at bringing about evidence for the development of a novel CI design, which permanently eliminates the gap between the electrode array and the auditory neurons in the cochlea (Figure 1) through bio- and nano-technological modifications of the current CI.

A number of developments and achievement have been obtained through this fruitful collaborative and multidisciplinary approach in the second project period and will be discussed in detail in the next paragraph.

Briefly, novel bioactive compounds have been developed and tested for their capacity to stimulate neurite outgrowth. A functionalized nanomatrix, containing laminin epitopes has been developed and validated. Novel nanoparticles have been developed with antibacterial activity, for electrode coatings. Nanomaterials have been tested as functional modifications to optimize electrode conductivity, reduce impedance and improve neuron-electrode coupling in the scenario of a gapless interface. Dispenser technologies have been investigated in order to obtain growth-factor/neurotrophin release from the CI electrode surface. All these components have been tested *in vitro* as well as *in vivo* for their efficacy. Bioassays on multielectrode arrays (MEAs) have been developed to analyze in detail the response profile of auditory neurons to gapless stimulation modalities and *in silico* models to define optimal stimulation strategies have been created. A novel pressure sensor technology has been developed. Finally, novel imaging approaches have been tested to visualize neurotrophin release *in vivo* through CI.

An animal-grade, multichannel, cochlear implant prototype has been developed by the NANOCI consortium, including multiple functionalization.

Overall, the proof of concept to bridge the gap between the cochlear neurons and the implant array was successfully achieved *in vivo* as well as the proof of concept for a potential decrease in energy consumption of the NANOCI device in case of a gapless interface between auditory neurons and the electrodes.

Achievements in the second period

1. Development of nanomaterials and bioactive compounds

We have investigated a new 3D nanomatrix, functionalized with extracellular protein (ECM) epitopes to allow for growth and guidance of nerve fibers. The leader in this part of the project was EMC microcollections GmbH (**EMC**).

A commercially available peptide-based gel was identified (PuraMatrix®) as most compliant with the physico-chemical specifications required for the application in inner ear surgery.

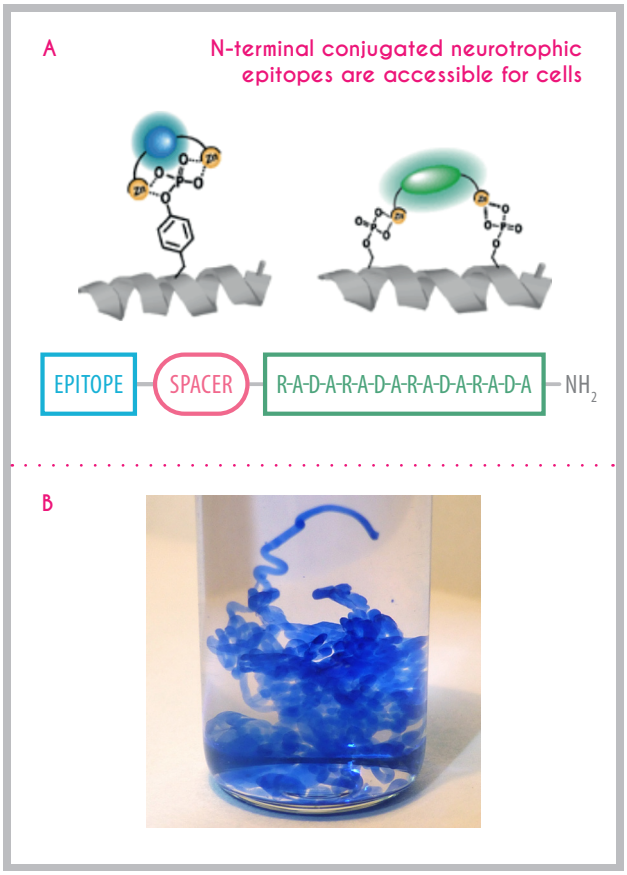


Figure 2. (A) The peptide IKVAV-GGG-$(RADA_4)$-NH_2 was synthesized, terminally carrying a laminin epitope (IKVAV) for neurotrophic functionalisation. Self-assembly through stacking of the linear peptides results in the formation of fibers with functional ECM domains presented on the surface. (B) Gel formation after injection of peptide solution into artificial perilymph.

The NANOCI Consortium

Universität Bern - UNIBE Switzerland

Universität Tübingen - UT Germany

Uppsala Universitet - UU Sweden

Tampereen yliopisto - UTA Finland

Haute Ecole Spécialisée de Suisse Occidentale - HES-SO Switzerland

Bar-Ilan University - BIU Israel

Project Coordinator

PD Dr. med. PASCAL SENN
Inselspital
Universität Bern

www.nanoci.org
info@nanoci.org

EMC microcollections GmbH - EMC Germany

MED-EL Elektromedizinische Geräte GmbH - MED-EL Austria

SCIPROM Sàrl - SCIPROM Switzerland

Gelation is based on fiber formation as a result of changes in ionic strength and pH of the aqueous peptide solution, and could be triggered by interaction with the inner ear fluid perilymph after injection. The laminin epitope IKVAV was selected as most interesting candidate based on literature for neuron-extracellular matrix interaction.

The final gel forming peptide IKVAV-GGG-$(RADA_4)$-NH_2 was synthesized (Figure 2), characterized by different analytical methods and tested for gelation properties and kinetics *in vitro*.

This nanomatrix and optimized protocols for gel formation were delivered to multiple partners for *in vitro* and *in vivo* testing and further characterisation. *In vitro* experiments with murine spiral ganglion auditory neurons at the University of Bern (**UNIBE**) and the University of Tübingen (**UT**) and human vestibular neurons at the University of Uppsala (**UU**) confirmed the suitability of this gel for auditory neuron sprouting. *In vivo* experiments in guinea pigs models (**UT**) further corroborated these findings.

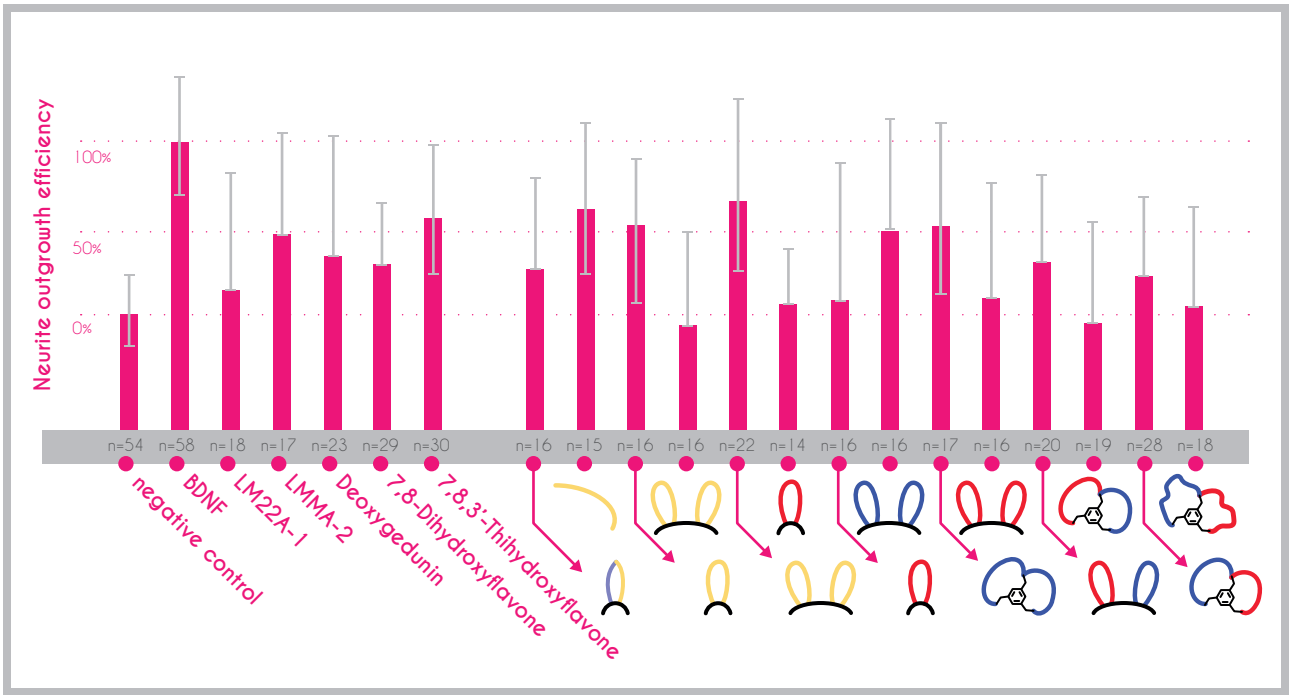


Figure 3. Neurite outgrowth efficiency using murine spiral ganglion explant culture in vitro is assessed through a neurite length index established by image analysis after incubation of the explants with different BDNF mimetics.

EMC was also leading the development of BDNF mimetics for stimulation of neuronal sprouting and survival. Based on the crystal structure of the BDNF molecule and putative interaction sites with the BDNF receptor TrkB, different loop mimetics were designed and synthesized. A schematic is given in Figure 3.

Linear and cyclic biomimetic peptides (homodimers and heterodimers) were synthesized and tested *in vitro* by UT for neuronal sprouting.

The most promising structures, 7,8,3'-trihydroxyflavone (THF) and a loop mimetic L2, were investigated in *in vitro* and *in vivo* experiments at **UT** and distributed to the consortium partners engaged in bioassay development/testing. L2 structure is now under further optimization to improve stability and activity.

2. Nanofunctionalization of the surface and Electrode

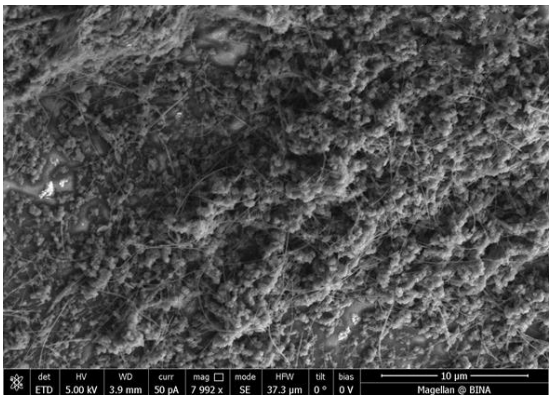
A conductive hybrid carbon nanotube (CNT)/polythiophene (polyTh) coating was developed and used to functionalize platinum (P)t electrodes for *in vitro* and *in vivo* testing.

Bar-Ilan University (**BIU**) further optimized previously developed methods for grafting thiolated carbon nanotubes onto the Pt surface of the electrode array contacts in combination with a novel developed conductive polymer. Single and multi-walled CNTs (SWCNTs and MWCNTs respectively) were chemically modified through oxidation, in order to further bind neurotrophic factors and eventually functionalized by cysteamine for attachment onto the Pt electrode pads. The conductive polymer poly($EDOT_3$:$EDOT\text{-}COOH_1$) was synthesised and grafted onto Pt electrodes in combination with ox-MWCNT, resulting in a new conductive nanocomposite (NC-A5).

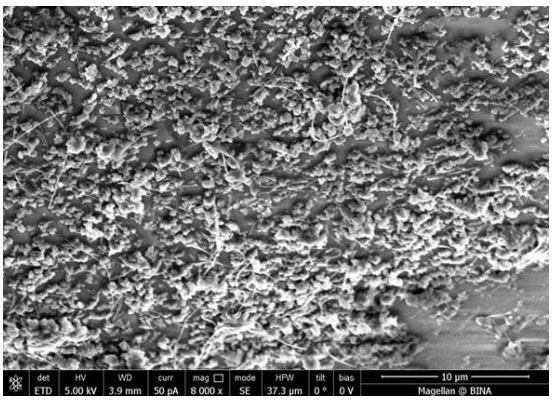
Pt electrodes modified with the newly synthesized nanocomposite displayed better conductivity and impedance profiles moreover were tested on ex vivo culture of SG neurons using multielectrode arrays (**UNIBE**), with promising results as well as *in vivo*, on the final CI prototype.

These modifications greatly improved the conductivity of the electrodes and resulted in a uniform coating of the electrode surface (Figure 4), however displayed limited resistance to

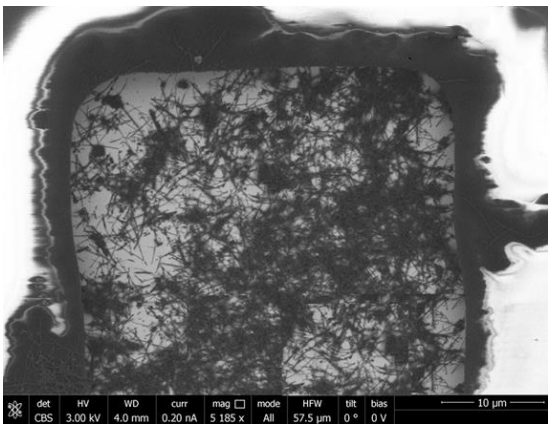
A Pt grey surface (NC-A_5)



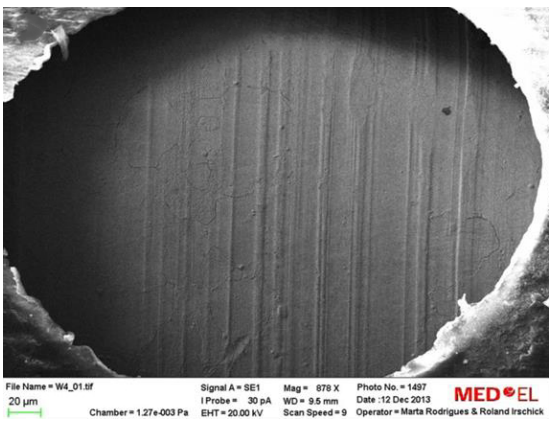
B Pt surface (NC-A_5)



C Pt grey surface (MWCNTcyst)



D Neat Pt electrode



mechanical stress, possible limiting their *in vivo* application.

Additional surface modifications applied to the silicone area surrounding the Pt pads, consisted of incorporation of nanoparticles with antibacterial properties (**BIU**) and coating with Parylene-C for the development of novel dispenser technologies by the University of Applied Sciences and Arts Western Switzerland (**HES-SO**).

BIU optimized the deposition of mixed ZnO-MgF_2 antibacterial agents on the surface of the parylene/silicone substrate. The most uniform distribution of the both kinds of nanoparticles was achieved when the co-deposition was performed by layers. The biological test at **BIU** demonstrated that the optimized co-deposition of both ZnO and MgF_2 NPs displayed a synergistic activity against *Streptococcus Pneumoniae*, as the most typical for the CI infections. At the same time, the CuZnO nanocomposite demonstrated good antibiofilm properties against *Streptococcus Pneumoniae*, *Staphylococcus Aureus* and *Escherichia Coli*. The toxicity towards mammalian cells, including macrophages and SG neurons of ZnO-MgF_2 and CuZnO coating was controlled at **UU** and the University of Tampere, Finnland (**UTA**), and the decrease in toxicity for the low concentrated samples was demonstrated.

Concerning the development of novel dispenser technologies, **HES-SO** focused on dry-dispenser approaches that could provide suitable methods for long-term storage of bioactive compounds compatible with industrial CI production. Parylene-encapsulation of the bioactive compound (L2 mimetic generated by **EMC**) or direct incorporation into medical grade silicone were tested. *In vitro* release was validated by spectroscopy, fluorescence microscopy

Figure 4. HR-SEM images of chemically modified Pt electrode (MEAs UNIBE). (A): Pt black surface coated with poly($EDOT_3$:$EDOT\text{-}COOH_1$)cyst/$MWCNT_{cyst}$ (NC-A5). (B) Pt standard surface coated with poly($EDOT_3$:$EDOT\text{-}COOH_1$)cyst/$MWCNT_{cyst}$ (NC-A5). (C) Pt grey surface coated with sole $MWCNT_{cyst}$. (D) Neat Pt surface (MED-EL electrode).

and HPLC-MS. Incorporation into silicone using drop dispensing was found to be the best suited way for incorporation into the electrode silicone surface. L2 mimetic was found to be released from the silicone, possibly though swelling and water uptake, dissolving the dry L2 peptide and releasing it into perilymph.

3. Modeling, coding and information transfer

UNIBE developed a robust *in vitro* bioassay to analyze the response profiles of auditory neurons on multielectrode arrays (Figure 5). The platform allowed for the analysis of new electrode induced stimulation pulses, able to induce SG responses using the low current amplitude and therefore low energy levels.

Long biphasic pulses as well as pulses containing an interphase gap were found to be efficient in inducing neuronal activity ex vivo into regrown SG neuron peripheral processes.

These results should allow for the design of novel stimulation patterns in the frame of a gapless interface between neurons and CI electrodes. These data, obtained with murine culture were further confirmed using human fetal spiral ganglion neuron.

Based on these finding and literature on the expression of ion channels expressed in SG neurons a model for neuronal activation was developed by MED-EL Elektromedizinische Geräte GmbH (**MED-EL**) to predict response profiles as a function of distance and pulse shape/amplitude. This should allow for the optimization of coding strategies for the novel NANOCI device (Figure 6).

Feedback sensors

HES-SO developed a new optical feedback sensor prototype based on an optical fibre, first to exploit the bending-loss in the fibre for the implant curvature determination during the

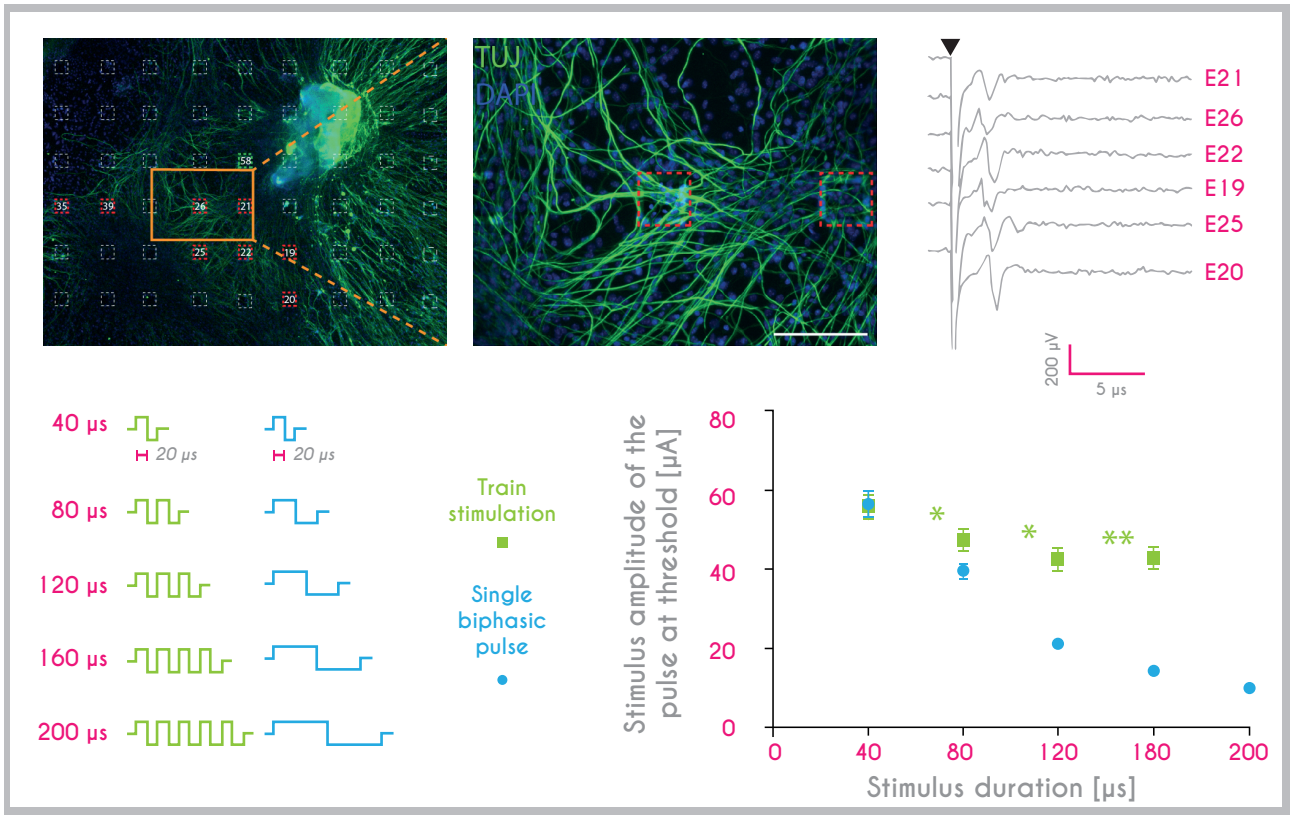
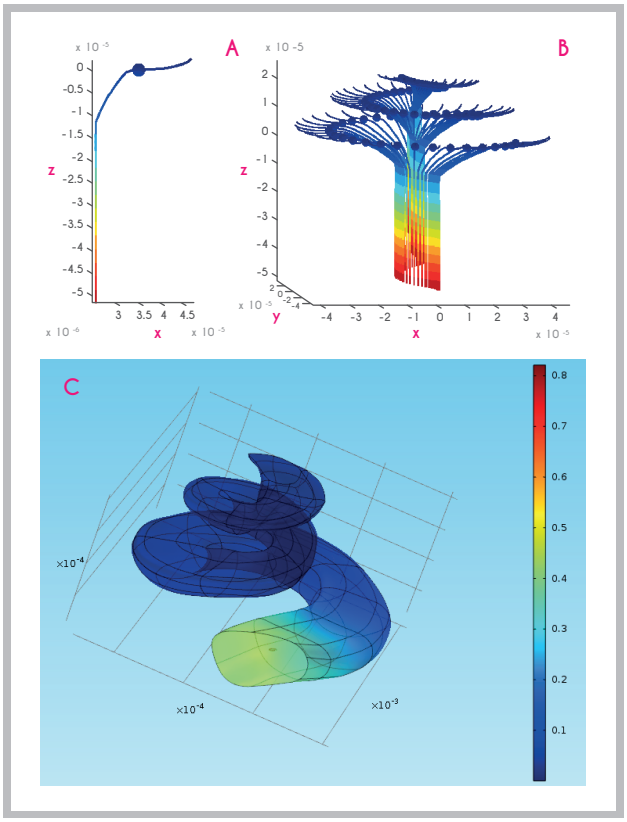


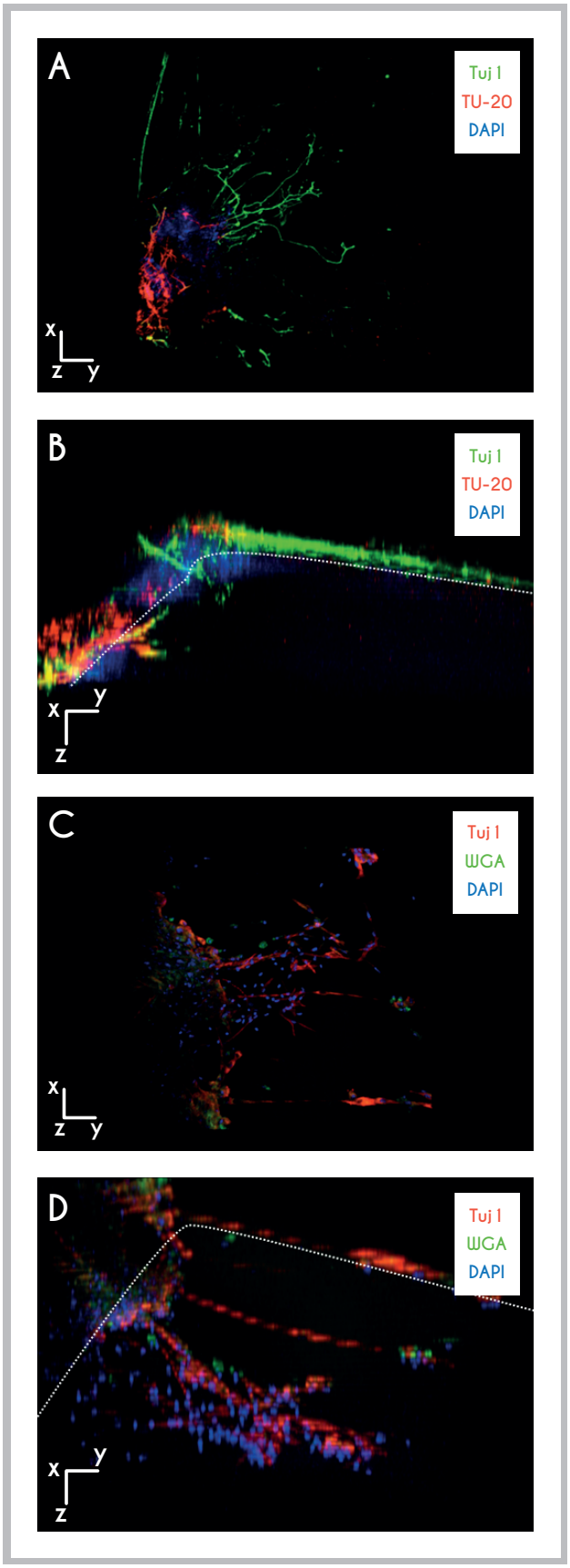
Figure 5. SG neurons (immunostained for the neuronal marker TUJ) on multielectrode array (68 electrodes 40x40 µm²) and representative traces of responding electrodes after electrical-induced response (upper panels). An example of the pulse shapes tested (left) and corresponding threshold values to elicit response (right graph).

Figure 6. ▼ Morphology of one example SGN. The sample neuron has been divided into 25 compartments, depicted by the different colors. (B) 80 instances of the neuron shown in (A), rotated and shifted in space according to the structure of the human cochlea and forming the beginning of the auditory nerve. (C) Volume conductor model of a human scala tympani, containing one stimulating electrode at the base (disk). Colors depict the voltage across the tympanic space, ranging from 1 V (dark red) to 0 V (dark blue).



surgery and second to probe the extremity of the cochlea by an optical sensor incorporated on the top of the tip. The fabrication of the sensitive optical part was based on the patented SOLID technology and the deformation of a liquid drop encapsulated by parylene. The deposition of a gold mirror on the parylene was performed in order to monitor the drop deformation by the feedback laser light measurement, and thus be able to detect the extremity of the cochlea.

Figure 7. ► 3-D confocal imaging of human and murine cultures in modified PuraMatrix® with IKVAV motif. (A) A murine spiral ganglion cell culture, (top view, XY) with Tuj1 positive (green) processes located peripherally whilst TU-20 (red) is visible in the central region. (B) Tilted side view (YZ) of the same area as shown in A. Dotted line indicates outline of gel. (C) A human vestibular ganglion culture (top view, XY) showing Tuj1 positive cells on the gel. (D) Tilted side view of (C) (YZ) shows both nuclei (blue) and Tuj1-positive cells (red) residing inside the matrix unlike. Dotted line indicates outline of gel.



4. Bioassay

Through the design of complementary and alternative approaches, spiral ganglion neuron sprouting in response to BDNF mimetics (**UT**) provided as soluble factors or incorporated into dispenser technology (**UNIBE** and **HES-SO**) or into nanomatrix (**UT**) was assessed and validated *in vitro*. L2 mimetic was found to be the most promising candidate, together with a previously reported TrkB agonist THF. Additionally, the IKVAV functionalized PuraMatrix® gel was demonstrated to be suitable for neuronal sprouting-penetration using murine SG neurons (**UNIBE**) as well as human adult vestibular neurons (**UU**) as shown in Figure 7.

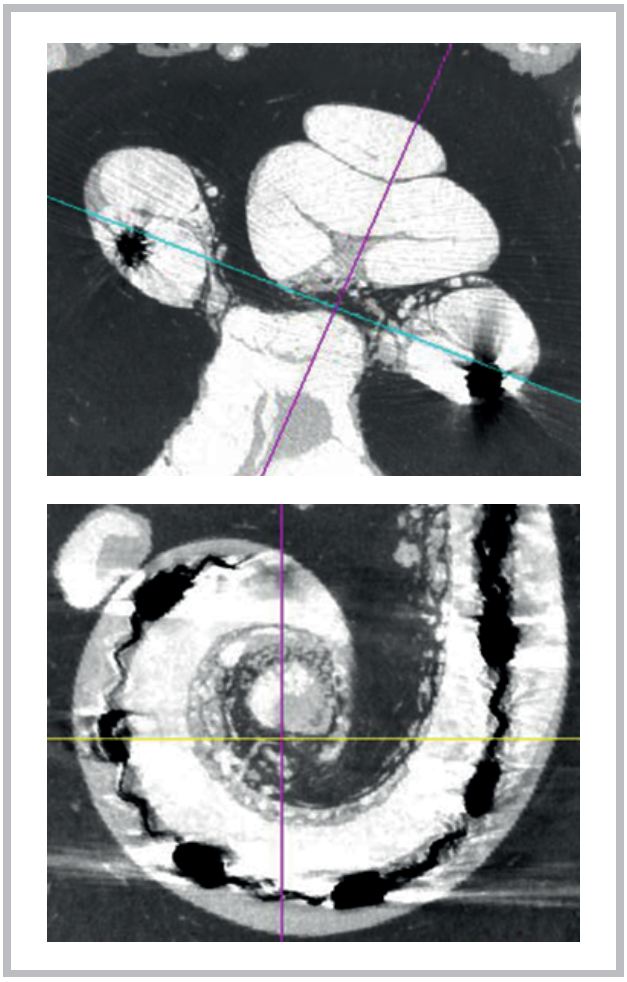


Figure 8. CI electrode in the human cochlea. Top (CBCT image): Location of the CI electrode in the scala tympani below the basilar membrane away from the modiolus. Bottom (µCT image): CI electrode array with visible wires and contacts inserted into the cochlea.

5. Imaging, biocompatibility and release dynamics of the functional CI electrode

Methods to visualize and to determine neurotrophin release dynamics from a nanomatrix and from a multifunctional cochlear implant (CI) electrode were developed by the group at **UTA**. *In vitro* systems to study the diffusion of BDNF mimetics through perilymph or nanomatrix were developed.

Ultra-small cerium cation ($Ce^{3/4+}$)-doped maghemite (γ-Fe_2O_3) NPs (developed by **BIU**) were used for coupling to L2 mimetic and visualization using MRI. Alternatively, a novel cone-beam computed tomography (CBCT) was developed to visualize cochlear implant (CI) electrode arrays in the human inner ear. Imaging parameters using CBCT were optimized regarding frame rate, filter-system, delivery voltage and radiation levels on cadaver temporal bones and in human dummy heads (Figure 8).

6. Pilot animal grade NANOCI multicomponent validation

Different prototype electrodes were produced by **MED-EL**. These included: animal grade with single or multiple (n=4) contacts for *in vivo* experiments (in guinea pigs) and ex vivo tests (NP release, impedance, drug delivery). Additionally, human grade electrode arrays featuring up to 36 contacts were developed as a proof of concept of the possibility to increase the number of electrodes in the scenario of a gapless interface *in vivo*.

Stability of the nanoparticle coating (MgF_2, ZnO and CuZnO) on Pt pads was confirmed. Increase in conductive properties of the electrode (increased double layer capacitance and reduction in the resistive component of impedance) modified with ox-MWCNT or the novel nanocomposite (NC-A5) were reported.

Finally, multi-functionalized electrode arrays were used for *in vivo* experiments using a Guinea pig model at **UT**. Guinea pig electrodes containing 4 Pt electrode contacts, modified

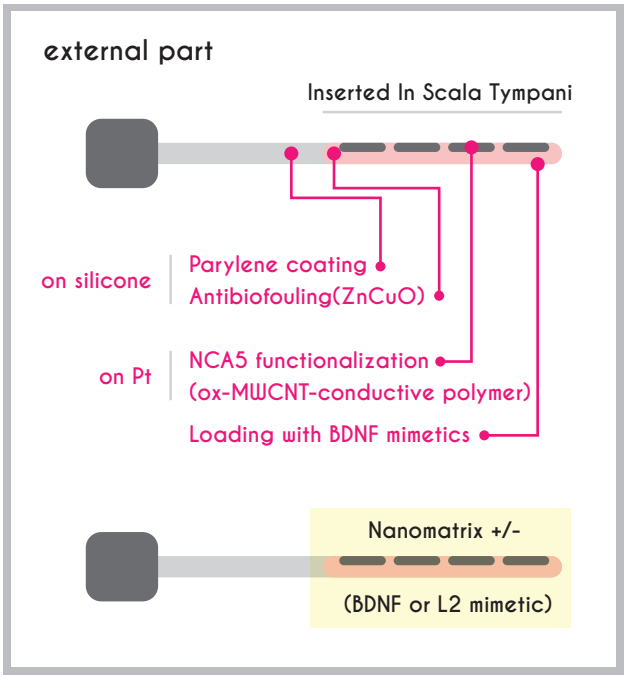
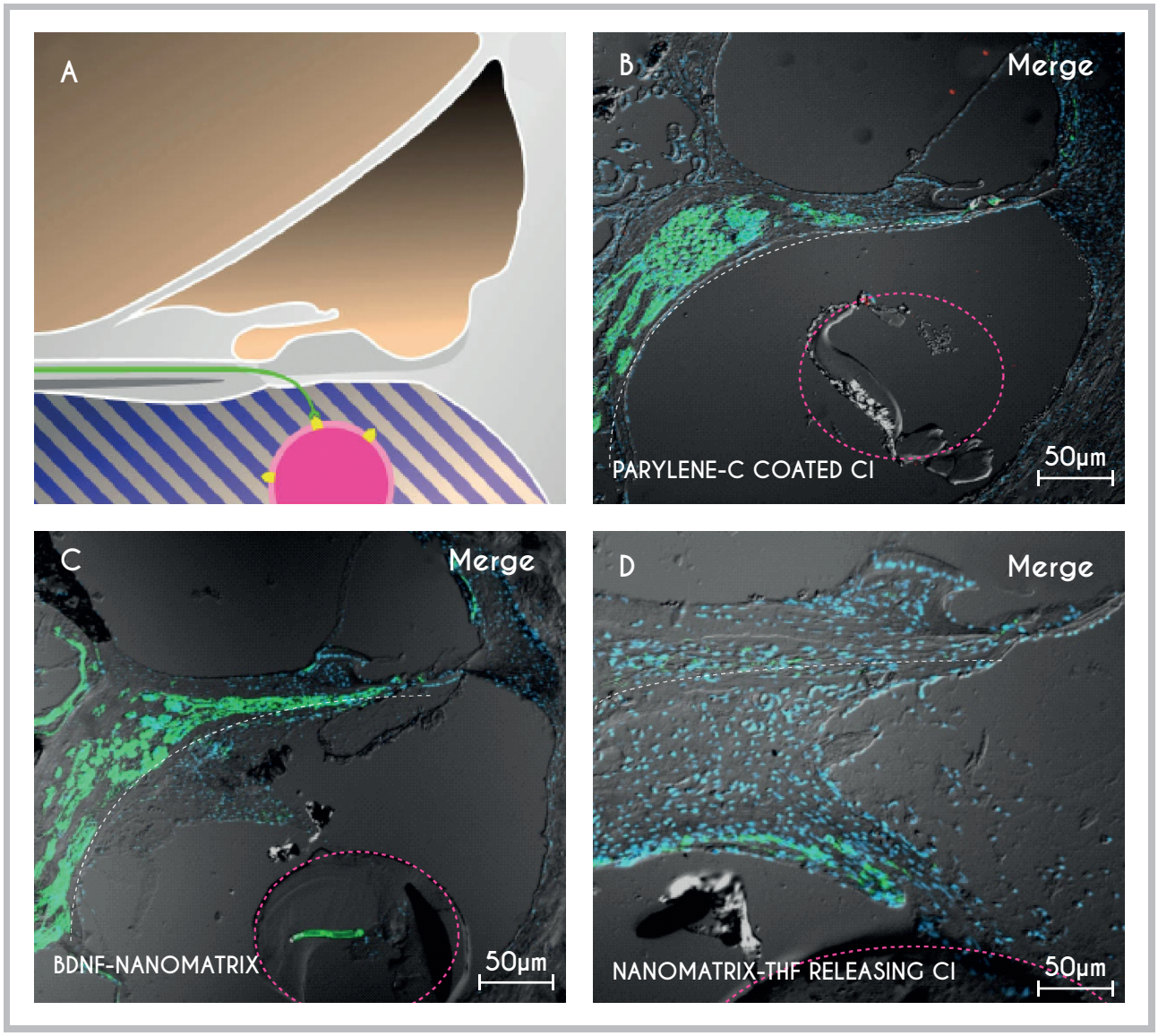


Figure 9. ◄ Summary of the different modifications implemented onto the guinea pig prototype electrode.

Figure 10. ▼ (A) Schematic of the NANOCI electrode recruiting SG neurons (green) onto the electrode array (pink) modified by nanotechnology (yellow) through bio-functionalized 3D nanomatrix (violet stripes). (B) Histological analysis of Guinea Pig cochlea at day 42 after CI implantation. Electrodes are removed for histology. Possible location is indicated with pink dotted lines based on artifacts. SG neurons are stained for TUJ (green), cell nuclei for DAPI (blue). No SG neurons (green) are observed in Scala Tympani (border along white dashed line) in unmodified electrodes (parylene coated). (C and D) Representative examples of SGN invasion into Scala Tympani in presence of biofunctionalized nanomatrix (C: BDNF loaded; D: THF releasing electrode).



using different approaches described above (parylene coating, antibiofouling nanoparticles, nanocomposites, BDNF-analogue reservoir) were inserted either in hearing animals, to test adverse effect of these modifications, or in deafened animals to assess the functionality of the new devices. The effect of the novel design 3D nanomatrix loaded with BDNF or the newly synthesized L2 mimetic were assessed alone of in combination with the CI. A schematic is given in Figure 9.

Physiological measurements using CAP (Compound Action Potential evoked by acoustic stimulation), ABR (Auditory Brainstem Response evoked by acoustic stimulation) and eCAP (Compound Action Potential evoked by electrical stimulation) and eABR (Auditory Brainstem Response evoked by electrical stimulation) were performed and histological assessment was used to evaluate the outgrowth of neurites to the Cochlear Implant 42 days after implantation.

No detrimental effects were observed in hearing animals after implantation of parylene-coated or antibiofouling-coated electrodes, neither on hearing function nor at the histological level. Some small improvements were observed in hearing thresholds and amplitudes after application of the nanomatrix in combination with CI.

Outgrowth of spiral ganglion neurons into scala tympani was observed in the presence of the 3D nanomatrix, either loaded with BDNF or THF and outgrowth towards the CI was identified in different treatment groups as shown in Figure 10.

These results give strong evidence for the possibility of recruiting neurons into the CI using the NANOCI design.

7. Potential impact of the NANOCI project

Combining all developments of the NANOCI project, the proof of concept for the gapless interface between auditory neurons and the cochlear implant electrodes has been obtained *in vivo* (Figure 10). The *in vitro* setup confirmed the hypothesis that a significant reduction in energy used to stimulate the auditory neurons can be achieved, notably a five-fold reduction for the gapless position and a four-fold reduction, if stimulus parameters are optimized for the new interface (Figure 5). Together, these key findings lay the foundation to develop cochlear implant systems in the future with more specific and more energy-efficient stimulation of auditory neurons. Whereas the higher specificity of stimulation may result in better auditory resolution and improved hearing performance in music or background noise, the reduced energy consumption may be the key to develop smaller and more cost-efficient cochlear implants. If only a part of the energy-reduction obtained in the *in vitro* setup can be carried over to a clinical-grade cochlear implant system in the future, this may help to develop fully implantable devices, which would make hearing loss invisible, thereby increasing the acceptance by hearing impaired patients.

The other achievements of the project offer additional opportunities for researchers and industry in Europe. Of particular interest are the lead structures for BDNF mimetics and antibiotic nanoparticles, which alone may be worth the whole project's investment, in case they can be brought to the world-wide market.

Overall, NANOCI was to our own judgment a highly successful project yielding new evidence on many different topics and bringing together scientific groups and industry from a variety of backgrounds. We would like to express our gratitude to the Seventh Framework Programme of the EU for the support of this exciting endeavor.