# FINAL PUBLISHABLE SUMMARY

1.09.2012

31.08.2015

nanoCI
nanotechnology based cochlear implants

Co-funded by the European Union

**The NANOCI project may help to improve auditory resolution at decreased energy consumption in future cochlear implants, world-wide used prostheses to restore hearing in the deaf. Proof of concept was obtained for guided growth of auditory neurons towards the stimulating electrodes *in vivo* and for an up to five-fold reduction of energy needed for stimulation *in vitro*, thereby offering a solution to improve the man:machine interface in the inner ear.**

Functional hearing can be restored in the majority of deaf patients today using a neuroprosthesis called cochlear implant (CI, Figure 1). Over 300,000 of these devices are currently in use worldwide and allow the majority of deaf-born children and deafened adults to use the sense of hearing to a degree that spoken language can be understood, thereby enabling oral communication.

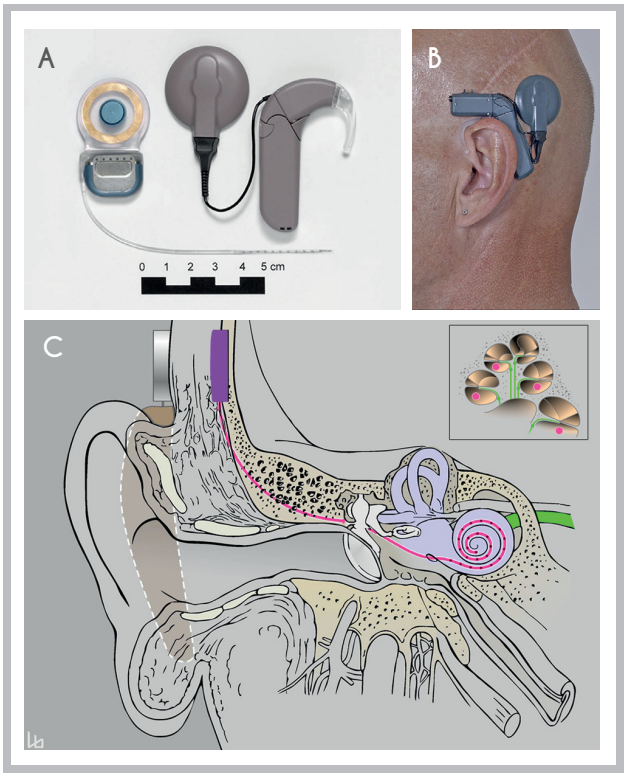
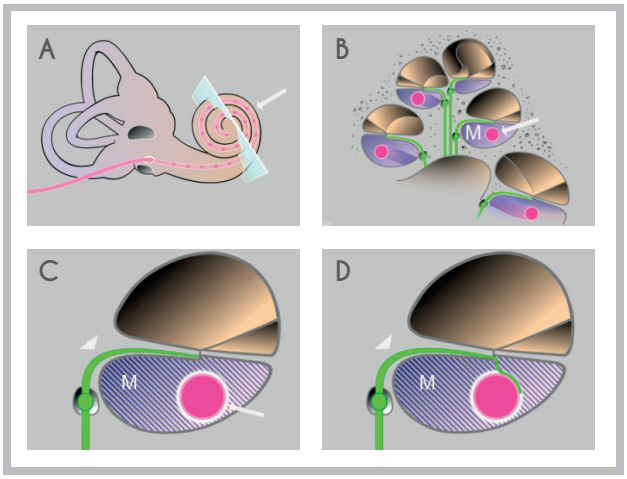



Figure 1. ▲ MED-EL cochlear implant system with implantable stimulator/receiver unit and externally worn speech processor (A). Clinical CI system with behind-the-ear speech processor (B). Schematic view with CI electrode array (pink) inserted into the cochlea of the inner ear for direct stimulation of the auditory neurons (green, C).

Despite the success, some limitations remain, which are mainly caused by the anatomical gap between auditory neurons and the electrode array in the cochlea. NANOCI aimed at improving CI performance by creating a gapless man:machine interface in the inner ear (Figure 2). Methods of biomedical engineering, regenerative medicine and nanotechnology have been used in concert to achieve the ambitious goal defined at the beginning of the project.




## Proof of concept for guided growth of auditory neurons *in vivo*

Brain-derived neurotrophic factor (BDNF), tetrahydroflavone (THF) or a small protein mimetic of BDNF (L2) were chronically released over 4 weeks in the cochlea of deafened guinea pigs from reservoirs on the animal-grade *nanoCI* electrode and from the *nanoCI* gel-matrix in the scala tympani. In response to this treatment, auditory neurons in BDNF and THF treated deaf animals were guided through the nanomatrix-filled scala tympani towards the neurotrophin source on the electrode array (Figure 3). In most of the cases, the auditory neurons took a sharp turn immediately before the organ of Corti region (Figure 3A) growing downward and

Figure 2. ▲ The main aim of the NANOCI project is to eliminate the anatomical gap between the intracochlear electrode array (arrow in Figs. A-D) and the peripheral processes of the auditory neurons (arrowheads in Figs. C, D). The nerves will be attracted through surface release of growth factors from the array (arrow in Fig. C), supported and guided through the scala tympani using a neurotrophic nanomatrix (M) and permanently locked on the array through nanostructuration of the surface (D).

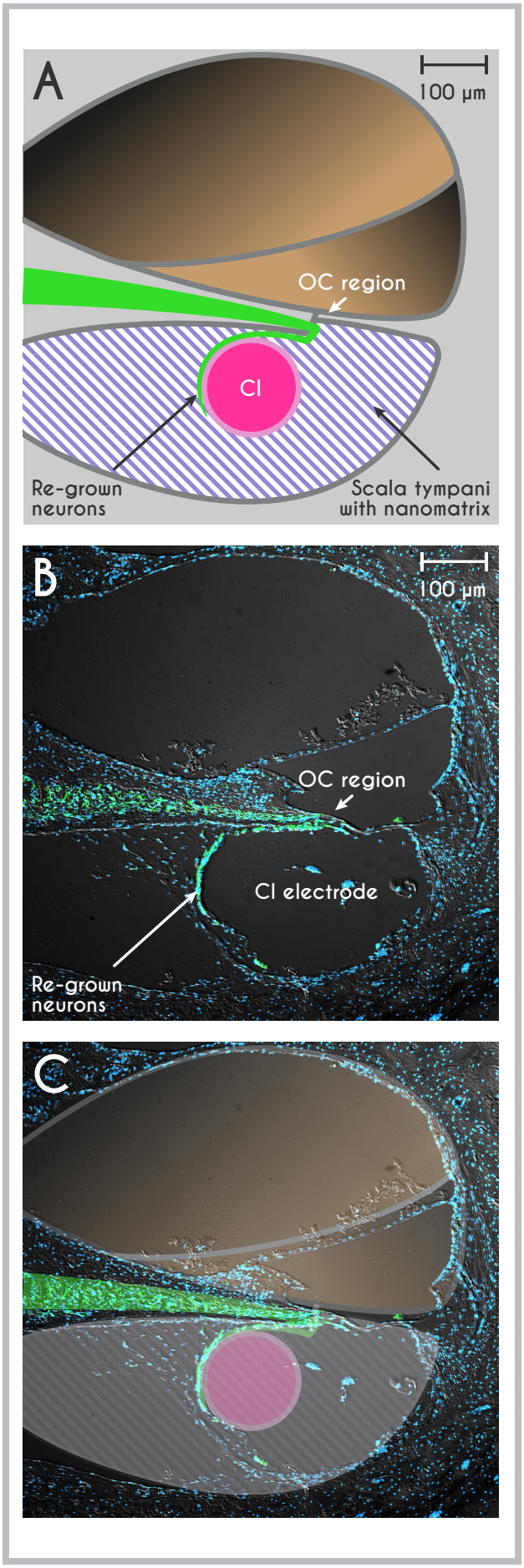



Figure 3. Guided growth of auditory neuronal fibers (green) from the organ of Corti (OC) region towards the drug-releasing nanomatrix (blue-shaded area) and cochlear implant electrode array (CI, pink) to form a gapless neuron:electrode interface in concept (A), in vivo (B) and merged (A+B = C). The electrode materials (silicone and platinum) are difficult to section and visualize, however the space occupied by the CI electrode is well delineated (B). The CI electrode implanted was larger compared to the original concept (difference of CI electrode area in C).

along the border of the *nanoCI* electrode array to form a gapless neuron:electrode interface (Figure 3B), very close as foreseen in the original concept of the project (Figures 3C).

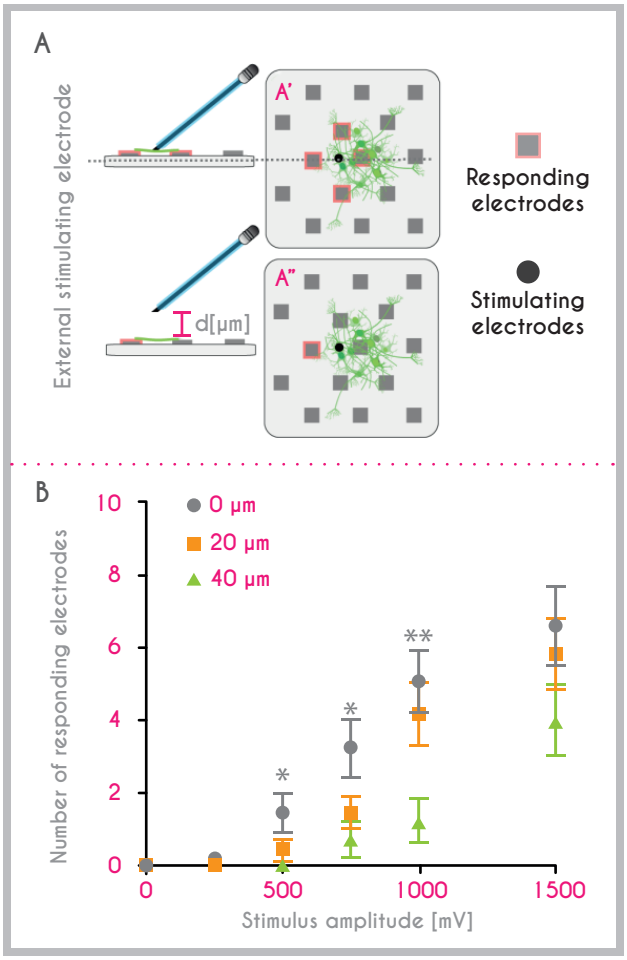
## The gapless neuron:electrode interface opens the door for energy-efficient stimulation

Objective hearing tests showed some small improvements in hearing thresholds and amplitudes in deafened animals implanted with *nanoCI* gel-matrix and a combination of gel-matrix and *nanoCI* electrode array (as shown in Figure 3) compared to deafened control animals *in vivo*. However, these differences did not reach statistical significance, mostly due to the small numbers of animals tested in each group. The clear trend to improved functional results was corroborated instead by highly significant advantages of the gapless neuron:electrode interface in the *in vitro* setup, where response profiles of murine and human auditory neurons were obtained on multi-electrode arrays (Figure 4).

By modifying stimulus parameters in the gapless position on the multi-electrode arrays, an up to four-fold reduction of energy needed to elicit a response was observed (Figure 5).

## Other significant achievements

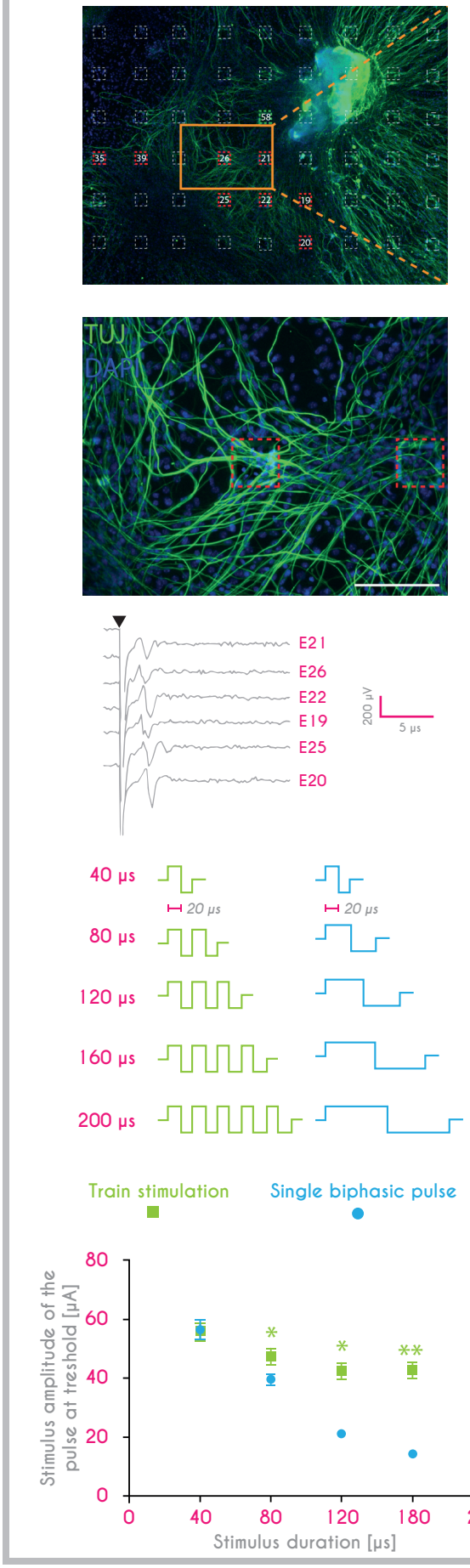
Novel bioactive compounds have been developed and tested for their capacity to stimulate neurite outgrowth. Most notably, a lead structure of a small-peptide mimetic of BDNF has been identified, which is ready for patenting. The functionalized *nano*CI gel-matrix (Figure 2 "M" and Figure 3) containing laminin epitopes was successfully applied *in vivo*. New nanoparticles have been developed with antibacterial activity at reduced toxicity for coatings of the

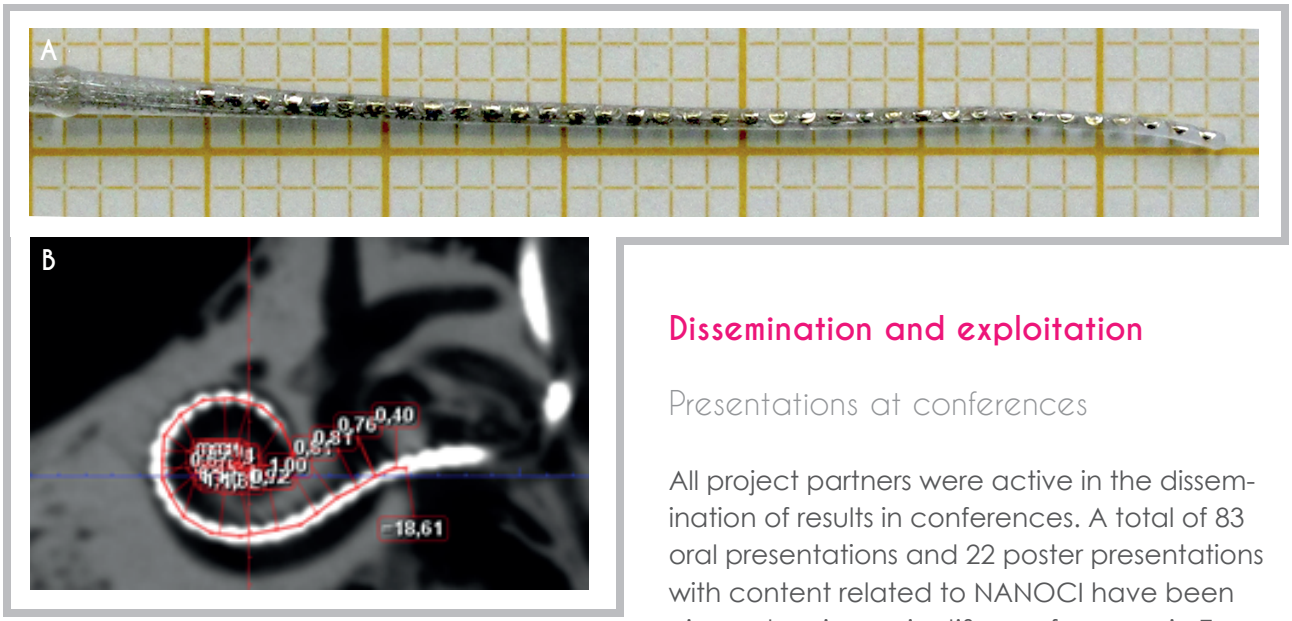



electrode array. Other nanomaterials have been produced for functional modifications to optimize electrode conductivity, reduce impedance and improve neuron-electrode coupling in the scenario of the gapless interface. It is important to mention that all nanomaterials produced during the project have been tested for toxicity in appropriate and validated bioassays. Dispenser technologies have been investigated in order to obtain growth-factor/ neurotrophin release from the CI electrode surface. Methods to visualize this neurotrophin-re-

Figure 4. ◮ Murine auditory neurons were cultured on multi-electrode arrays (MEAs) and stimulated from an externally mounted cochlear implant electrode array at various distances, ranging from 40 µm to 0 µm (A). In the gapless position (0 µm), more electrodes responded, enabling the reduction the stimulus amplitude and thereby also the minimal energy needed to elicit a response (B). We calculated a five-fold energy reduction when stimulating in the gapless position compared to a distance of 40 µm (1.5 nJ vs. 7.5 nJ).

Figure 5. ► Auditory neurons (immunostained for the neuronal marker TUJ) on MEAs (68 electrodes 40x40 µm²) and representative traces of responding electrodes after electrical-induced response (upper panels). An example of the pulse shapes tested (lower middle graph) and corresponding threshold values to elicit response (bottom graph).






lease and also to analyze the electrode location in animal and human inner ears have been optimized. Fusing all these developments, the animal-grade *nanoCI* electrode was produced for the successful multicomponent testing *in vivo*, shown in Figure 3.

In order to exploit the theoretical advantages of the gapless interface in future human-grade cochlear implant systems, a theoretical, morphological and functional model of the auditory neuron and the auditory nerve was created. This model, implementing response profiles obtained in experiments as shown in Figure 5, lays the foundation to design optimum stimulation patterns suited to the gapless interface. Similarly, a patent-ready optical feedback sensor has been developed and tested in human temporal bones for monitoring insertion forces of the electrode array to minimize insertion trauma.

Finally, a human-grade prototype of cochlear implant electrode array featuring 36 electrode contacts and channels was built to assess the limits of todays manufacturing processes, thereby tripling the number of contacts and channels currently available from this manufacturer.

Figure 6. ▲ Human-grade, experimental, *nanoCI* prototype featuring 36 contacts and 36 channels produced to assess the limits of current manufacturing methods (A). Despite the three-fold increase in contacts and wires, stiffenning and enlarging the diameter of the array, it could be subtotally inserted into a human cadaveric temporal bone, as shown on a conebeam computed tomography (CBCT) image (B).

## Dissemination and exploitation

### Presentations at conferences

All project partners were active in the dissemination of results in conferences. A total of 83 oral presentations and 22 poster presentations with content related to NANOCI have been given at various scientific conferences in Europe, USA and Asia. In general, the interest for the project has been found to be very high. Final presentations on the project were given in Europe and the US, respectively.

### Publications

Overall, 19 articles have been published in peer-reviewed journals, three articles have been submitted and several others are in preparation. Seven articles have appeared in professional magazines and trade journals. All published or accepted publications are cited in the references at the end of this article.

### Dissemination to the general public in print and over the project website

The dissemination of the results to the wider general public was of importance to the consortium and 6 articles were published in newspapers, journals of hearing impaired patients or professionals working in the field (see reference list).

In addition the project hosts a website www.nanoci.org, where over 5,000 visits with over 15,000 page views have been counted during the project duration. Most visitors were from European Countries, but the US, Russia, China, Japan, Australia and many others are also well represented. NANOCI has actively promoted the site on various other websites. The website

will remain active for another 5 years beyond the end of the official project duration.

### Exploitation

A total of 4 patents to protect intellectual property related to NANOCI have been filed or are in preparation at the end of the project. In addition, over a dozen individual exploitation tracks have been identified, which will be pursued beyond the NANOCI project. These exploitation tracks include further developments of neurotrophin biomimetic molecules, nano-matrix gels, conductive and antibiotic nanoparticles, optic sensors, mathematical neuron models, *in vitro* and *in vivo* bioassays (as shown in Figures 3, 4 and 5), controlled drug-release and medical imaging technologies (as shown in Figure 6), among others.

## 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 3). 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 (Figures 4 and 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 SMEs in Europe. Of particular interest are the lead structures for BDNF – biomimetics and antibiotic nanoparticles, which may be worth the whole project's investment, in case they can be carried over into the world-wide market.

Overall, NANOCI was to our own judgment a successful project yielding new evidence on many different topics and bringing together scientific groups and SMEs 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.


### The NANOCI Consortium

**Universität Bern**
Bern, Switzerland

**Universität Tübingen**
Tübingen, Germany

**Uppsala Universitet**
Uppsala, Sweden

**Tampereen yliopisto**
Tampere, Finland

**Haute Ecole Spécialisée de Suisse Occidentale**
La Chaux-de-Fonds, Switzerland

**Bar-Ilan University**
Ramat Gan, Israel

**EMC microcollections GmbH**
Tübingen, Germany

**MED-EL Elektromedizinische Geräte GmbH**
Innsbruck, Austria

**SCIPROM Sàrl**
St-Sulpice, Switzerland

Project Coordinator

**PD Dr. med. PASCAL SENN**
Inselspital Universität Bern

**www.nanoci.org info@nanoci.org**

## Reference list

### Peer-reviewed publications in scientific journals


Response Profiles of Murine Spiral Ganglion Neurons On Multi-Electrode Arrays. **Stefan Hahnewald, Anne Tscherter, Emanuele Marconi, Jürg Streit, Hans Rudolf Widmer, Carolyn Garnham, Heval Benav, Marcus Mueller, Hubert Löwenheim, Marta Roccio, and Pascal Senn.** Journal of Neural Engineering. Accepted for publication.

Targeted Delivery of Contrast Agents to the Tympanic Medial Wall at Minimum Amount and the Efficient Uptake in the Inner Ear through Oval and Round Windows. **Jing Zou, Stella Ostrovsky, Liron L Israel, Jean-Paul Lellouche, and Ilmari Pyykkö.** Austin J Radiol. 2015; 2(6): 1034.

Calcium Metabolism Profile in Rat Inner Ear Indicated by MRI after Tympanic Medial Wall Administration of Manganese Chloride. **Jing Zou, Ilmari Pyykkö. Ann Otol Rhinol Laryngol**. Posted online before print on July 29, 2015. doi:10.1177/0003489415597916

Methyl methacrylate embedding to study the morphology and immunohistochemistry of adult guinea pig and mouse cochleae. **Peter Bako, Mohamed Bassiouni, Andreas Eckhard, Imre Gerlinger, Claudia Frick, Hubert Löwenheim, Marcus Müller.** Cell Tissue Res. 254, 86–93, 2015. doi:10.1016/j.jneumeth.2015.07.017

Molecular organization and fine structure of the human tectorial membrane: is it replenished? **Hisamitsu Hayashi, Annelies Schrott-Fischer, Rudolf Glueckert, Wei Liu, Willi Salvenmoser , Peter Santi , Helge Rask-Andersen.** Cell Tissue Res. Posted online on Jun 18, 2015. doi:10.1007/s00441-015-2225-5

Auditory Nerve Preservation and Regeneration in Man - Relevance for Cochlear Implantation. **Helge Rask-Andersen and Wei Liu.** Neural Regeneration Research, 10(5), 710-712–65, 2015. doi:10.4103/1673-5374.156963

Fine control of drug delivery for cochlear implant applications. **Alexandra Homsy, Edith Laux, Julien Brossard, Harry J. Whitlow, Marta Roccio, Stefan Hahnewald, Pascal Senn, Pavel Mistrík, Roland Hessler, Teresa Melchionna, Claudia Frick, Hubert Löwenheim, Marcus Müller, Ute Wank, Karl-Heinz Wiesmüller, and Herbert Keppner.** Hearing, Balance and Communication. Vol. 13, Issue 4, 2015, 153-159. doi:10.3109/21695717.2015.1048082

X-ray microtomographic confirmation of the reliability of CBCT in identifying the scalar location of cochlear implant electrode after round window insertion. **Jing Zoua, Markus Hannul, Kalle Lehto, Hao Feng, Jaakko Lähelmä, Antti S. Aula, Jari Hyttinen, Ilmari Pyykkö.** Hearing Research, 326, 59–65, 2015. doi:10.1016/j.heares.2015.04.005

Solid on liquid deposition, a review of technological solutions. **Alexandra Homsy, Edith Laux, Laure Jeandupeux, Jerome Charmet, Roland Bitterli, Chiara Botta, Yves Rebetez, Oksana Banakh, H. Keppner.** Microelectronic Engineering, 141, 267–79, 2015. doi:10.1016/j.mee.2015.03.068

Imaging cochlear implantation with round window insertion in human temporal bones and cochlear morphological variation using high-resolution cone beam CT. **Jing Zou, Jaakko Lähelmä, Juha Koivisto, Anandhan Dhanasingh, Claude Jolly, Antti Aarnisalo, Jan Wolf and Ilmari Pyykkö.** Acta Otolaryngol. 135(5), 466-472, 2015. doi:10.3109/00016489.2014.993090

Macromolecular organization and fine structure of the human basilar membrane - RELEVANCE for cochlear implantation. **Wei Liu, Francesca Atturo, Robair Aldaya, Peter Santi, Sebahattin Cureoglu, Sabrina Obwegeser, Rudolf Glueckert, Kristian Pfaller, Annelies Schrott-Fischer and Helge Rask-Andersen.** Cell Tissue Res. 360(2), 245–262, 2015. doi:10.1007/s00441-014-2098-z

Imaging Optimization of Temporal Bones With Cochlear Implant Using a High-resolution Cone Beam CT and the Corresponding Effective Dose. **Jing Zou, Juha Koivisto, Jaakko Lähelmä, Antti Aarnisalo, Jan Wolff, and Ilmari Pyykkö.** Ann Otol Rhinol Laryngol 124(6) 466-473, 2015. doi:10.1177/0003489414565004

MeV ion beam lithography of biocompatible halogenated Parylenes using aperture masks. **Harry J. Whitlow, Rattanaporn Norarat, Marta Roccio, Patrick Jeanneret, Edouard Guibert, Maxime Bergamin, Gianni Fiorucci, Alexandra Homsy, Edith Laux, Herbert Keppner, Pascal Senn.** Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms. 354, 34–36, 2015. doi:10.1016/j.nimb.2014.10.024

The pre- and post-somatic segments of the human type I spiral ganglion neurons – Structural and functional considerations related to cochlear implantation. **Wei Liu, Fredrik Edin, Francesca Atturo, Gunde Rieger, Hubert Löwenheim, Pascal Senn, Michael Blumer, Annelies Schrott-Fischer, Helge Rask-Andersen, Rudolf Glueckert.** Neuroscience, 284, 470-482, 2015. doi:10.1016/j.neuroscience.2014.09.059

3-D gel culture and time-lapse video microscopy of the human vestibular nerve. **Fredrik Edin, Wei Liu, Hao Li, Francesca Atturo, Peetra U. Magnusson, Helge Rask-Andersen**. Acta Oto-Laryngologica, 134(12), 1211-1218, 2014. doi:10.3109/00016489.2014.946536

Differentiation of human neural progenitor cell-derived spiral ganglion-like neurons: a time-lapse video study. **Fredrik Edin, Wei Liu, Marja Boström, Peetra U. Magnusson, Helge Rask-Andersen.** Acta Oto-Laryngologica, 134(5), 441-447, 2014. doi:10.3109/00016489.2013.875220

Immunohistological analysis of neurturin and its receptors in human cochlea. **Wei Liu, Helge Rask-Andersen.** Auris Nasus Larynx, 41(2), 172-178, 2014. doi:10.1016/j.anl.2013.07.016

Possible role of gap junction intercellular channels and connexin 43 in satellite glial cells (SGCs) for preservation of human spiral ganglion neurons. **Wei Liu, Rudolf Glueckert, Fred H. Linthicum, Gunde Rieger, Michael Blumer, Mario Bitsche, Elisabeth Pechriggl, Helge Rask-Andersen, Annelies Schrott-Fischer.** Cell and Tissue Research, 355(2), 267-278, 2014. doi:10.1007/s00441-013-1735-2

Nanoparticle based inner ear therapy. **Ilmari Pyykkö.** World Journal of Otorhinolaryngology, 3(4), 114-133, 2013. doi:10.5319/wjo.v3.i4.114

Distribution of pejvakin in human spiral ganglion: An immunohistochemical study. **Wei Liu, Anders Kinnefors, Marja Boström, Fredrik Edin, Helge Rask-Andersen.** Cochlear Implants International, 14(4), 225-231, 2013. doi:10.1179/1754762812Y.0000000027

## Professional magazines and trade journals

The clarity of sound. Pan European Networks: Science and Technology 13, Dec 2014. link

EU-Projekt NANOCI. Mehr Hörqualität für CI-Träger. Audio Infos. 162, 1-5, October 2014.

Vom Glück der Hörnervenzellen. Fachzeitschrift Schnecke 84(6), 42, 2014. link

Eine faszinierende Gehörforschung. Hörakustik, 5, 96-97, 2014.

Bald bessere Ohrimplantate durch Nano-ci-Forschungsprojekt? dezibel 2, 11-12, 2014. link

Bessere Ohrenimplantate für Gehörlose. Unilink, die Nachrichten der Universität Bern, 12, 16-17, 2013. link

«Natürliches» Hören ist das Ziel.» Mit Stammzellen Cochlea-Implantate verbessern - Medical Tribune, 5(1), 6, 2013.