PROJECT FINAL REPORT

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3.1 Final publishable summary report

4.1.1 Executive summary

The BIOHYBRID consortium consists of three SMEs and seven academic partners with long-standing substantial expertise relevant for the successful execution of the project. Main goal of BIOHYBRID was the development of an artificial nerve device to improve the outcome after reconstruction of injured nerves. Although peripheral nerves display regenerative capacities in contrast to the central nervous system, regeneration after substantial nerve loss is very poor. When regenerated nerves in the arm, for example, reach distal muscles these are atrophied in the meantime and might not regain function. Peripheral nerve injuries represent a major cause for morbidity and disability in the affected patients. Although persons involved are often young males after motorcycle accidents, for example, severe nerve lesions might occur at any age and result from many other different types of traumas. The incidence of peripheral nerve injuries lies at about 300,000 cases per year in Europe. In addition, also in the context of traumatic limb amputations, peripheral nerve lesions are clinically relevant with an incidence of 2/100,000 persons per year recorded for hand amputations, for example. Therefore, there is a considerable need for innovative therapies in the area of repair, reconstruction and regeneration of peripheral nerve injuries.

Basically, in the first 18 months chitosan-based materials were used in *in vitro* and *in vivo* studies to evaluate the most efficient formulation in the context of nerve regeneration and to select the most promising types for the more complex approaches in the second 18 months. We demonstrated that fine-tuned chitosan conduits with a degree of acetylation of ~5%, allow functional and structural regeneration across a 10-mm sciatic nerve gap in healthy and diabetic rats to a similar extent as autologous nerve grafts. These chitosan nerve conduits (Reaxon® Nerve Guide) also allow regeneration across 15-mm gaps in 57% of the animals, while no regeneration occurred through classic silicone guides.

During the second 18 months the selected hollow chitosan tubes were modified with luminal fillers, biomatrices, and/or (genetically modified) cells and analysed in *in vitro* and *in vivo* studies, in order to then further support functional recovery especially in advanced animal models, across long gaps or even after delayed repair (45 days after nerve transection injury), respectively. According to the Work Packages the main modifications of hollow chitosan nerve conduits were: (A) Different genetically modified Schwann cells (SCs) over-expressing neurotrophic factors were seeded into a hydrogel, NVR-Gel, and introduced into the lumen of the conduits. (B) Chitosan films (non-perforated or perforated) were longitudinally introduced into the centre of the lumen of the conduits as regenerative guidance structure. Reconstruction of 15-mm rat sciatic nerve gaps was performed as either acute intervention (in (A) + (B)) or delayed reconstruction (in (B)). Functional recovery and histomorphometrical parameters after chitosan conduit repair were compared to repair with autologous nerve transplants. Several fundamental data were selected, for example, regarding the immediate reconstruction scenario, the results from (B) demonstrated significantly increased regeneration outcome in comparison to hollow conduits (~ 40% of animals), with reinnervation of the tibialis anterior muscle 58% (non-perforated film) and 86% (perforated film) of the operated animals. In the last 12 months promising devices were finally analysed in a preclinical setting using the advanced animal models, like diabetic Goto-Kakizaki (GK) rats and delayed nerve repair, respectively.

Regarding the translational work Reaxon® Nerve Guide obtained the CE mark in January 2014, market entry of Reaxon® Nerve Guide was performed in June 2014. The product has already been implanted in patients with defects in digital nerves in several German clinics. Moreover, FDA submission has been initiated. In addition, finalising the translational work for clinical application of the hollow tube (ethics applications) and continuation of translational work for complex nerve conduits are ongoing activities. The preparation of a multicentre clinical trial on Reaxon® Nerve Guide in median and ulnar nerves is already completed.

4.1.2 Summary description of the project

The BIOHYBRID consortium consists of three SMEs and seven academic partners with long-standing substantial expertise relevant for successful execution of the project. The main goal of BIOHYBRID is the development of an artificial nerve device to increase the reconstruction of injured nerves. Although peripheral nerves display regenerative capacities in contrast to the central nervous system, regeneration after substantial nerve loss is very poor. When regenerated nerves of the arm, for example, reach distal muscles these are atrophied in the meantime and might not regain function. Peripheral nerve injuries represent a major cause for morbidity and disability in the affected patients. Although persons involved are mainly young males after motorcycle accidents, severe nerve lesions might result from many other different types of traumas. The incidence of peripheral nerve injuries lies at about 300,000 cases per year in Europe. In addition also in the context of traumatic limb amputations, peripheral nerve lesions are clinically relevant with an incidence of 2/100,000 persons per year recorded for hand amputations, for example. Therefore, there is a considerable need for innovative therapies in the area of repair and regeneration of peripheral nerve injuries.

We are looking for the production of a bio-inspired and bio-active nerve graft device, e.g. an artificial organ which not only passively substitutes the lost nerve tissue but also activates the biological processes inside the receiving tissue (bio-activation). We aim to promote the regeneration capacities through various strategies: Creating a construct – with optimal porosity and layers degrading at a programmed rate – which activates the biological repair process by its 3D-structure formed by a hydrogel or nanofibres; manipulating the main properties of the construct (e.g. length, diameter, rigidity, permeability, degradability, interior surface morphology, luminal constitution, etc.) in order to meet the clinical requirements. In particular, the components which are most suitable to promote nerve regeneration are cells (especially glial cells) and bio-active molecules (such as neurotrophic factors) that are main players in the degeneration/regeneration scenario after nerve injury; the artificial nerve guide device can thus be enhanced with the enrichment of supportive cells and exogenous neurotrophic factors into the lumen. A neurotrophic factor delivery system and/or preformed instructive neurotrophic factor gradients should be part of the biohybrid artificial nerve in order to provide temporally balanced neurotrophic factor supply. Transplantation of supportive cells further creates the potential to induce the synthesis of various factors which are important for the activation of biological regeneration processes through gene transfer.

In addition to the experimental processes, continuous supervision of the facilitation of product transfer into future clinical trials of different lesioned nerves will accompany the project. This work includes scaling-up ISO/GMP production, preparation of a design for clinical trials, arranging regulatory work, and performance of risk analyses.

4.1.3 The main scientific and technological achievement specified according to the Work Packages

4.1.3.1 *Work Package 1: In vitro and in vivo testing of hollow tubular devices for nerve regeneration*

The tasks of WP1 cover largely basic requirements for the whole project. Therefore, the experiments started within the first six months and were mainly accomplished before the end of the second year. Only the manufacturing (Task 1.4) was continuing during the entire runtime of the project.

Task 1.1: In vitro: hollow tube - porosity, biocompatibility, biodegradability

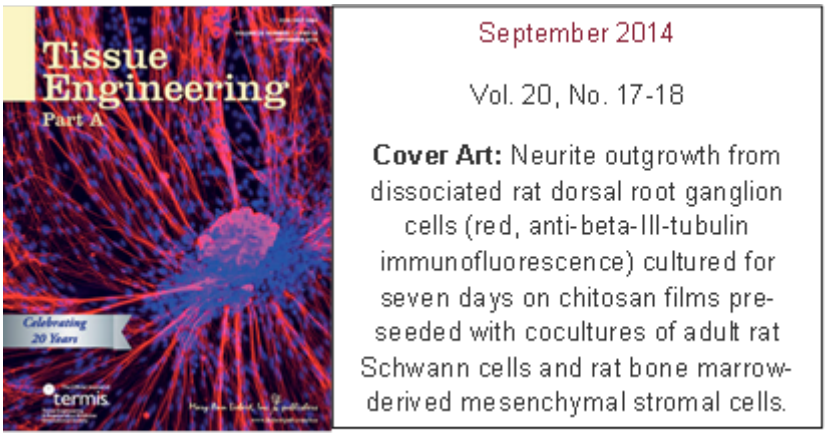
Direct contact assays of rat bone marrow mesenchymal stem cells (rBMSCs) and Schwann cells cultured on the luminal surface of chitosan tubes (half-shells). rBMSCs displayed a poor adhesion on these structures. Cells could be found after two days in culture, but almost no cells were observed after 7 days. Schwann cells (rat neonatal, rat adult) did not adhere to the inner but only to the outer surface of the original half shells. Also immortalised rat Schwann cells, although adhering to the inner surface, demonstrated better adhesion on the outer surface. As a second material chitosan membranes were tested for their adhesive properties, because they were easier to handle than chitosan half shells. In order to remove possible cell adversive manufacturing residues, the washing procedure was extended resulting in better cell adhesion on the membranes. After changes in the manufacturing process of the half-shells it was decided that chitosan tubes-Variant 1 will be used for all future in vitro and in vivo experiments. Direct contact assays of rBMSCs, rat adult Schwann cells (raSCs) and their co-cultures on chitosan membranes from different DA were performed by an exchange student between MHH and UMINHO. Results revealed that rBMSCs and raSCs adhere to the surface of the membranes and there were interactions between both cell types when in co-culture.

Morphological evaluation (light microscopy, SEM, and time lapse analysis): Adult primary Schwann cells and RT4-D6P2T (Schwann cell line) adhered adequately to the chitosan membrane only after a washing pre-treatment or with a coating of NVR gel.

Biomolecular analysis of changes in a Schwann cell-line (RT4-D6P2T) cultured on chitosan membranes of different DA: this analysis was carried out by RT- PCR with the goal of determining the best DA for chitosan conduits. The analysis was carried out looking for different gene categories: apoptosis genes; autophagy; oxidative stress; mitochondrial TCA cycle; stress response; ErbB family. The data demonstrate that the transcription of several apoptosis-associated genes (both anti- and pro-apoptotic) is increased in cells seeded on chitosan membranes with high DA. Accordingly, the expression of genes associated with oxidative stress, autophagy, mitochondrial TCA cycle and oxidative stress response increases in cells seeded on highly acetylated membranes.
Taken together, these results indicate that the high DA is less compatible with cell viability, while low and medium acetylated membranes are suitable substrates for cell adhesion and growth. These results were also supported by the in vivo studies (see Task 1.3).

In conclusion, data from these technical and basic studies were part of several comprehensive publications (e.g. Haastert-Talini et al., Biomaterials 2013).

In deviation from the original DOW, an additional objective was addressed - the biocompatibility of chitosan films. The chitosan films can be used as luminal fillers in hollow tubes (see WP2).
These films proved to provide possible luminal enrichment to hollow chitosan tubes. Schwann cells (SCs, immortalised and neonatal) and bone marrow derived mesenchymal stromal cells (BMSCs) could be cultured on these films which also provided a cell culture surfaces with superior properties for sensory dorsal root ganglion (DRG) neurite outgrowth over classical cell culture surface.



Wrobel et al. **Tissue Eng Part A** 2014

Task 1.2: Standardisation of the methodology for in vivo studies

Three training courses regarding the different aspects of the in vivo experiments were conducted:
19th – 21st March 2012 in Malmö/ULUND, BIOHYBRID course on surgical procedures
25th – 27th September 2012 in Torino/UNITO, BIOHYBRID course on histological techniques
28th – 30th January 2013 in Barcelona/UAB, BIOHYBRID course on electrophysiology and functional tests

On the internal part of the BIOHYBRID website in vivo standard protocols and the developed Handbook for in vivo experiments Version 1 from May 2012 were filed:
- Standard Protocols
- In vivo standard protocols
- Factsheet in vivo, Factsheet in vivo Rev 01, Pre-SOP 004, Handbook in vivo

After the first 3 months in vivo experiment at the MHH, S. Geuna (UNITO) attended to ensure that the standard procedures could immediately be adjusted among the partners MHH and UNITO during explantation and fixation procedures if needed.

Task 1.3: In vivo: hollow tube – selected conditions

The work related to this task was performed by several partners and several papers were published (Haastert-Talini K., Geuna S., Dahlin L.B., Meyer C., Stenberg L., Freier T., Heimann C., Barwig C., Pinto L.F., Raimondo S., Gambarotta G., Samy S.R., Sousa N., Salgado A.J., Ratzka A., Wrobel S., Grothe C. Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. **Biomaterials**. 2013 Dec;34(38):9886-904. doi: 10.1016/j.biomaterials.2013.08.074. Epub 2013 Sep 17;

Shapira Y., Tolmasov M., Nissan M., Reider E., Koren A., Biron T., Bitan Y., Livnat M., Ronchi G., Geuna S., Rochkind S. Comparison of results between chitosan hollow tube and autologous nerve graft in reconstruction of peripheral nerve defect: An experimental study. **Microsurgery**. 2015 Apr 22. doi: 10.1002/micr.22418. Epub ahead of print).

In this task a comprehensive and ground-breaking paper concerning the chitosan hollow tubes of different acetylation, including the key technical in vitro data (see Task 1.1), the in vivo (10 mm gap) short-term data (5 and 18; 21 days post OP), the complete data of the 3-month in vivo study, and the material data of manufacturing and chemical composition after explantation was published in Biomaterials (Haastert-Talini et al., Biomaterials 2013). In this fundamental study hollow chitosan conduits of different acetylation were evaluated with regard to the capacity to support nerve regeneration, with regard to immunological reactions and the behavior of the connective tissue at the implantation site and the regenerated nerve. Following key results concerning the important issue of the different acetylation grades of the chitosan tubes were achieved:
DAIII chitosan tubes (DA: 20%) revealed a too fast degradation accompanied with considerable related tissue reaction, like immunological reactions, increased connective tissue formation, significant higher number of cells stained for cleaved caspase 3 (i. e. apoptosis) in the regenerative matrix, all parameters which can affect the regeneration process.
DAI chitosan tubes (DA: 2%) revealed an unintended sprouting effect; enhanced sprouting could eventually become a limiting factor for the outcome of functional recovery and thus argues against the use of DAI chitosan tubes for future trials.
DAII chitosan tubes (DA: 5%) revealed as the most supporting ones for peripheral nerve regeneration and as the first choice for ongoing applications in nerve reconstruction and the further studies in BIOHYBRID. On the basis of this seminal study the translational work and the market introduction of the REAXON® nerve guide was accomplished.

Task 1.4 Pilot manufacturing

During the entire project duration chitosan material was continuously produced as demanded by the partners. Moreover, several technical parameters were evaluated and optimized during the time.
At the beginning of the project scaling-up of chitosan tube production process with the medical grade chitosan from ALTAKITIN was performed. MEDOVENT was able to produce chitosan material with different degrees of acetylation. Production was performed under controlled ISO 13485 conditions. In addition to chitosan tubes, half shells, microfibers, and films were manufactured out of chitosan (different degrees of acetylation). Moreover, MEDOVENT conducted within in the first 18 months aging studies and biocompatibility and toxicology tests (in completion to those under Task 1.1). Moreover, samples sent by MEDOVENT were analysed by GPC for evaluation of MW variation after sterilisation steps and samples aging. These tests were ongoing.

Significant results for WP1

A comprehensive in vivo study evaluating the chitosan hollow tubes of different acetylations was published in a high impact factor journal. This study, together with the results of the other in vitro and in vivo studies of WP1, was an important basis for the translational work (see WP5) and the market introduction of the REAXON® Nerve Guide.

4.1.3.2 Work Package 2: In vitro and in vivo testing of functionalised tubular devices

The main goal of WP2 was to functionalise the hollow nerve conduits with luminal fillers and/or biomatrices and to deliver neurotrophic factors within the conduits. It is divided into six tasks all of which were planned to be started along the first 18 months. During the second 18 months most of the in vitro studies were finished and the results published in peer-reviewed international journals. In the third period on-going studies were finished and manuscripts submitted; however, there are still some studies which will be finished after the end of Biohybrid (because of the long postoperative intervals). – Since most of the in vivo experiments with functionalized tubular devices were already performed in the advanced rat model using 15 mm gap (because of the promising results using 15 mm gap and hollow tubes), the experiments/data/papers are described under WP4.

Task 2.1: In vitro: functionalisation of luminal fillers with trophic factors

Tubular chitosan devices were about to be functionalised by luminal fillers and/or biomatrices and neurotrophic factors (NTFs) conjugated to iron-oxide nanoparticles (np-NTFs). At MHH several in vitro tests have been performed in close collaboration between a MHH PhD student (Sandra Wrobel) and a visiting PhD student from UNITO (Michela Morano). Additional work has been performed at UNITO after the end of the student exchange (see below). The prolonged bioactivity of np-NTFs in comparison to free NTFs has successfully been demonstrated in neurite outgrowth/formation assays utilising sensory dorsal root ganglia (DRG) neurons or PC-12 cells, respectively. In vitro assays at MHH were performed in 0.5% NVR-Gel (mainly composed of hyaluronic acid and laminin) as substrate and potential luminal hydrogel filler for hollow chitosan tubes.
Moreover, NVR performed a series of in vitro studies using organotypic cultures of spinal ganglia to compare the effects of different free neurotrophic factors with iron nano-particles conjugated neurotrophic factors.

In conclusion, these systematically performed in vitro experiments revealed biological data on the different combinations of chitosan membranes with NVR gel and neurotrophic factors which were published in a number of papers in peer-reviewed international journals

(e.g. Ziv-Polat O., Shahar A., Levy I., Skaat H., Neuman S., Fregnan F., Geuna S., Grothe C., Haastert-Talini K., Margel S. The role of neurotrophic factors conjugated to iron oxide nanoparticles in peripheral nerve regeneration - in vitro studies. **Biomed Res Int. 2014**;2014:267808. doi: 10.1155/2014/267808. Epub 2014 Jul 16;

Morano M.*, Wrobel S.*, Fregnan F., Ziv-Polat O., Shahar A., Geuna S., Grothe C., Haastert-Talini K. Nanotechnology versus stem cell engineering - In vitro-comparison of neurite inductive potentials. **Int J Nanomedicine. 2014** Nov 14;9:5289-306. doi: 10.2147/IJN.S71951. eCollection 2014).

Furthermore, thanks to the in vitro research, UNITO was able to demonstrate the greater stability of NRG1β1 conjugated with iron nano-particles and to apply for a national patent entitled: "Enhancing effect of NRG1-beta 1 on the peripheral nerve regeneration through covalent conjugation to iron nanoparticles".

Task 2.2: In vivo: functionalisation of luminal fillers with trophic factors

The objective of this task was to evaluate nerve regeneration after repair with chitosan conduits filled with NVR-Gel functionalised with neurotrophic factors. Taking into account the good outcome of the in vivo tests carried out in the course of WP1, after some initial experiments several in vivo tests of WP2 were conducted by applying the functionalised device to repair a longer gap in the rat sciatic nerve (15 mm), thus pre-drawing WP4 (these experiments are described in WP4).

However, one study was conducted using rats with 12 mm gap, - low acetylation tube (DAI) filled with fibrin clot, - low acetylation tube (DAI) filled with NVR gel, - medium acetylation tube (DAII) filled with NVR gel, - high acetylation tube (DAIII) filled with NVR gel, autograft was used as control. Regeneration outcome was comprehensively evaluated with regard to behavior, electrophysiological function, and morphometry (Rochkind S, Mandelbaum-Livnat M, Morano M, Raimondo S, Viano N, Nissan M, Koren A, Biran T1, Yifat B, Reider E , Ziv-Polat O, Shahar A, Geuna S. Peripheral Nerve Reconstruction using Different Acetylation Chitosan Conduits Filled with NVR-Gel and Magnetic Fibrin Hydrogel. *In preparation*).

Important conclusion from these in vivo studies is the fact that filling of conduits interfers with nerve fiber regeneration, although we found very promising effect on survival and neurite outgrowth in the combination of NVR gel and neurotrophic factors in vitro. As general take message it can be concluded that in vivo experiments are necessary to evaluated devices/molecules in the animal context.

Task 2.3: In vitro: functionalisation of biomatrices with trophic factors

UMINHO and NVR analysed the impact of the three different matrices (GG-FN; NVR Gel, and Collagen) using MSCs derived from adipose tissue and DRG explants. It was shown that the hydrogels that were mostly used during the project have adequate properties for neurite and MSCs growth, although there are some variations among them.

R. Assunção-Silva, C. Costa-Oliveira, O. Ziv-Polat, E.D. Gomes, A. Shahar, N. Sousa, N.A. Silva, A.J. Salgado. “Induction of Neurite Outgrowth in 3D Hydrogel-Based Environments. **Biomedical Materials**, 2015 Oct 20; 10(5):051001. doi: 10.1088/1748-6041/10/5/051001.

In conclusion, these in vitro studies revealed that Gellan Gum (GG) hydrogels in their tested different formulations are not the suitable substrate for nerve regeneration. The GG hydrogel has to be and can be combined with extracellular matrix proteins. This problem was overcome by grafting the peptide used for GG-FN (GRGDS) simultaneously to those peptides obtained from laminin (Silva N.A., Moreira J., Ribeiro-Samy S., Gomes E.D., Tam R.Y., Shoichet M.S., Reis R.L., Sousa N., Salgado A.J. Modulation of bone marrow mesenchymal stem cell secretome by ECM-like hydrogels. **Biochimie**. 2013 Dec;95(12):2314-9. doi: 10.1016/j.biochi.2013.08.016. Epub 2013 Aug 30).

Moreover, since the in vitro experiments with GG were not promising following experiments were performed in deviation from the original DOW. UAB evaluated collagen-based matrices enriched with full-length laminin or fibronectin. DRG explants and spinal cord organotypic slices were cultured within 3D matrices and neurite outgrowth was measured. It can be concluded that collagen enriched matrices are a permissive substrate for the elongation of motor and sensory neurites. When enriched with laminin or fibronectin, neurite growth was enhanced

with interesting differences for motor and sensory neurons (Gonzalez-Perez F., Alé A., Santos D., Barwig C., Freier T., Navarro X., Udina E. Substratum preferences of motor and sensory axons in postnatal and adult rats. **Eur J Neurosci** 2015 Aug 31. doi: 10.1111/ejn.13057). A review article focusing on the use of such extracellular matrix components in peripheral nerve regeneration was published in a peer-reviewed journal (Gonzalez-Perez F., Udina E., Navarro X. Extracellular matrix components in peripheral nerve regeneration. **Int Rev Neurobiol**. 2013;108:257-75. doi: 10.1016/B978-0-12-410499-0.00010-1).

Task 2.4: In vivo: functionalisation of biomatrices with trophic factors

UAB performed functionalization of tubular devices with biomatrices. Chitosan DAII conduits have been filled with biomatrices based on collagen-type I, enriched with fibronectin or laminin and incorporated either as horizontally polymerized gels or tethered aligned ones. Electrophysiological tests showed that enrichment of collagen gels with fibronectin favoured muscle reinnervation above the other matrices. Furthermore, tethered aligned gels increased muscle reinnervation compared to their respective horizontally polymerized gels. The histological and morphometrical analysis of the regenerated nerves confirmed the functional findings (a manuscript with these results has been *submitted for publication*: Gonzalez-Perez F, Cobianchi C, Heimann C, Phillips JB, Udina E, Navarro X. Stabilization, rolling and addition of other ECM proteins to collagen hydrogels improves regeneration in chitosan guides for long peripheral nerve gaps in rats).

Task 2.5: Combination

Since the results using NVR-Gel and Gellan Gum, respectively, alone were not very promising the consortium had already decided during the second 18 months to prescind from further experiments applying different combinations of these materials.

Task 2.6: Pilot manufacturing

In addition to the information given in Task 1.4 materials were manufactured and delivered to the partners by MEDOVENT, ALTAKITIN, NVR, and UMINHO.
ALTAKITIN performed “Best use before” methodology for chitosan assessment: The methodology for the “Best use before” testing protocol for 3 year validation of KiToMed® - Medical grade chitosan. Moreover, since the hydrogels revealed not to be suitable in in vivo experiments modifications of the materials were performed. In coordination with NVR, to perform in vivo and in vitro studies, Altakitin produced several materials based on purified chitosan, hylauronic acid, and NVR-gel, both supplied by NVR. These productions were done in two main production batches, which allowed an improvement of production methods and a broadening of the materials’ scope. These studies are still ongoing.

Significant results for WP2

Significant results were achieved in in vitro experiments with regard to the very promising use of iron oxide nanoparticle-conjugated neurotrophic factors (np-NTFs). By conjugation the factors obtained a prolonged stability and therefore possess a long-lasting efficiency. The np-NTFs display an innovative tool which will be applied in further enhanced animal models for peripheral nerve regeneration. A request for patenting is under way.
The second important result was already achieved during the second period and supported by additional/modified experiments in the third period regarding the failure of hydrogel fillings like

NVR gel and Gellan Gum to increase nerve regeneration efficiency potential of tubes. However, functionalization with collagen gel +/- laminin or fibronectin, respectively, substantially enhanced the regeneration outcome. Study in the advanced sciatic nerve model is still running.

4.1.3.3 Work Package 3: In vitro and in vivo testing of cell-enriched tubular devices

The tasks of WP3 cover the use of Schwann cells and Mesenchymal stem cells (rBMSCs), their standardised application in culture and their genetic modification to induce over-expression of neurotrophic factors. The evaluation of the cells in functionalised tubular devices in vitro was performed with a continuous feedback to WP2. In close exchange with WP2, Schwann cells and rBMSCs were cultured on several chitosan-based substrates, first to evaluate the handling of the different devices and their suitability to serve as substrate without the presence of neurotrophic factors and cytokines. In addition to Schwann cell cultures a co-culture was performed with DRG neurons. In this context we were able to establish a novel method to obtain early onset of myelin in organotypic DRG cultures in the presence of NP-neurotrophic factors (GDNF, NGF, FGF-2).

Task 3.1: In vitro: Schwann cells

Standardised culture conditions were introduced in all partner laboratories. In this context, a training course on in vitro procedures was performed at MHH (16^{th}-19^{th} January 2012).
On the internal part of the BIOHYBRID website in vitro standard protocols and the developed Handbook for in vitro experiments Version 1 from May 2012 have been filed:

- Handbook in vitro Version 2 (Feb 2012),
- Factsheet in vitro F002 Rev00 (Feb 2012), Pre-SOP 002 Rev 01 (July 2012).

Furthermore, techniques for cell seeding into the different scaffolds were established and two related milestones (MS14 and MS15) achieved.
Although most of the experiments were finished in the first and second 18 months, there were promising studies ongoing in the last period, regarding the analysis of different biomatrices; publication is in preparation. UAB selected cell-seeded scaffolds for peripheral nerve regeneration. Chitosan DAII tubular devices have been filled with tethered aligned biomatrices based on collagen-type I, enriched with fibronectin or laminin combined with Schwann cells (SC) or Mesenchymal Stem cells (MSC). Analysis was performed with regard to survival and proliferation of the cells.
Data from these in vitro studies were combined with the in vivo studies and comprehensively published (see below).

Task 3.2: In vitro: Mesenchymal Stem cells

To evaluate the bioactivity of the engineered rBMSCs a comparable set-up as used for the engineered SCs was established. Together with the experiments undertaken in the presence of recombinant and NP-neurotrophic factors in WP2 results are combined and the results were included in at least two manuscripts (Morano M.*, Wrobel S.*, Fregnan F., Ziv-Polat O., Shahar A., Geuna S., Grothe C., Haastert-Talini K. Nanotechnology versus stem cell engineering - In vitro-comparison of neurite inductive potentials. **Int J Nanomedicine**. 2014 Nov 14;9:5289-306. doi: 10.2147/IJN.S71951. eCollection 2014; Wrobel S., Serra S.C., Ribeiro-Samy S., Sousa N., Heimann C., Barwig C., Grothe C., Salgado A.J., Haastert-Talini K. In vitro evaluation of cell-

seeded chitosan film for peripheral nerve tissue engineering. **Tissue Eng Part A**. 2014 Sep;20(17-18):2339-49. doi: 10.1089/ten.TEA.2013.0621. Epub 2014 Apr 22).

Although most of the experiments were finished in the first and second 18 months, there were promising studies ongoing in the last period, regarding the analysis of secretome of the MSCs; publication is in preparation.

Task 3.3: Engineered cells in scaffolds

Schwann cells and rBMSCs cells were successfully transfected non-virally to over-express NTFs. Expression plasmids (pCAGGS-EGFP-FLAG) induce expression of EGFP which carries a FLAG-Tag in order to facilitate the identification of the cells within the scaffolds (GG/NVR) and the composite nerve grafts. Bioassays after seeding of the transfected cells in NVR-gel were performed with regard to survival and support of neurite outgrowth and compared with the efficiency of np-NTFs in NVR-gel (Morano M.*, Wrobel S.*, Fregnan F., Ziv-Polat O., Shahar A., Geuna S., Grothe C., Haastert-Talini K. Nanotechnology versus stem cell engineering - In vitro-comparison of neurite inductive potentials. **Int J Nanomedicine**. 2014 Nov 14;9:5289-306. doi: 10.2147/IJN.S71951. eCollection 2014).

On the basis of the in vitro studies Schwann cells combined with NVR-gel were selected for the animal experiments, whereas BMSCs were excluded from future in vivo tests, since they displayed a less reliable support of neurite outgrowth. The in vitro data were published in combination with the animal study data (see Task 3.4 and WP4; Meyer C., Wrobel S., Raimondo S., Rochkind S., Heimann C., Shahar A., Ziv-Polat O., Geuna S., Grothe C., Haastert-Talini K. Peripheral nerve regeneration through hydrogel enriched chitosan conduits containing engineered Schwann cells for drug delivery. **Cell Transplant**. 2015 Apr 14. [Epub ahead of print]).

Task 3.4: In vivo: Different cell types

UAB performed preliminary experiments using virally transfected SCs over-expressing NTFs. UAB previously proved that addition of FGF-2 to spinal cord and dorsal root ganglia (DRG) explants cultured in a 3D collagen matrix caused a potent increase of motor neuron axonal growth. To further explore the potential capability of FGF-2 to promote axon regeneration, UAB and collaborators produced a lentiviral vector (LV) to overexpress FGF-2 (LV-FGF2) in the injured rat peripheral nerve. Cultured Schwann cells transduced with FGF-2 and added to collagen matrix embedding spinal cord or DRG explants significantly increased motor but not sensory neurite outgrowth. LV-FGF2 was as effective as direct addition of the trophic factor to promote motor axon growth *in vitro* (Allodi I., Casals-Díaz L., Santos-Nogueira E., Gonzalez-Perez F., Navarro X., Udina E. FGF-2 low molecular weight selectively promotes neuritogenesis of motor neurons. **Mol Neurobiol**. 2013 Apr;47(2):770-81. doi: 10.1007/s12035-012-8389-z. Epub 2012 Dec 30).

To investigate the *in vivo* effect of FGF-2 overexpression on axonal regeneration after nerve injury, Schwann cells transduced with LV-FGF2 were grafted in a silicone tube used to repair the resected rat sciatic nerve. These studies have provided essential proof of concept that selected NTFs are able to enhance axonal growth in a relatively selective manner for neuronal populations (Allodi I., Mecollari V., González-Pérez F., Eggers R., Hoyng S., Verhaagen J., Navarro X., Udina E. Schwann cells transduced with a lentiviral vector encoding Fgf-2 promote

motor neuron regeneration following sciatic nerve injury. **Glia**. 2014 Oct;62(10):1736-46. doi: 10.1002/glia.22712. Epub 2014 Jul 2).

UAB performed in addition to the in vitro studies (Task 3.1) also in vivo studies with regard to analysing a selection of cell-seeded scaffolds for peripheral nerve regeneration: Chitosan DAII tubular devices have been filled with tethered aligned biomatrices based on collagen-type I, enriched with fibronectin or laminin combined with Schwann cells (SC) or Mesenchymal Stem cells (MSC).

Chitosan DA II devices were used to bridge a 15 mm nerve defect. Since we used this long gap, these experiments are also part of WP4; these experiments are still ongoing.
Since the tasks of WP3 concern the development of a tubular device filled with luminal fillers and/or biomatrices enriched with engineered cells that can support regeneration, the experiments were performed in parallel to WP2 activities. Moreover, taking into account the good outcome of the *in vivo* tests carried out in the course of WP1, after some starting experiments all further *in vivo* tests of WP3 were conducted by applying the cellularised device to repair a greater gap length (rat 15 mm) thus anticipating WP4. Two comprehensive in vivo studies were conducted (and published) using tubular devices filled with luminal fillers and/or biomatrices enriched with engineered cells that can support regeneration (see Task 4.1).

Task 3.5 Pilot manufacturing

Pilot manufacturing for WP3 is performed in parallel to the manufacturing for WP1 and WP2.
In addition, tubes with inserted chitosan films were developed. Chitosan tubes and films were first separately produced at Medovent. Chitosan films with a thickness of 0.06–0.1 mm were inserted into the DAII (~5%) tubes. After that, the tubes and films were cut into the right size. The films were cut into pieces of different lengths (4 mm shorter than the tube length) and a width of 0.5cm. Moreover, production of chitosan films with holes for introduction into the tubes proceeded and was performed in cooperation with MHH and Medovent. These devices were applied in two in vivo studies (see Task 4.1).

Significant results for WP3

Significant results were achieved both in *in vitro* and *in vivo* studies. We demonstrated that genetically modified Schwann cells over-expressing neurotrophic factors within chitosan nerve guides support peripheral nerve regeneration. They especially are able to compensate the obstacle originating from the presence of NVR-Gel in vivo (see WP2).
In vitro investigations of naïve and genetically modified mesenchymal stem cells provided important insights on the effects of their secretome on neurite outgrowth and their potential as supportive cellular component in biohybrid nerve guides.

4.1.3.4 Work Package 4: Final preclinical in vivo testing

Task 4.1: 15 mm gap rat

Because of the promising results in WP1 the consortium decided at its 2nd General Assembly to anticipate the control experiments within the 15mm gap repair rat model using hollow tubes of DAI and DAII, although experiments for WP4 should start on month 28. UAB performed the

first evaluation of chitosan conduits for the effective repair of a limiting nerve gap (15 mm) in the rat sciatic nerve. Two types of chitosan tubes (differing in the degree of acetylation, DAI, 2%; DAII, 5%), provided by Medovent, were tested, in comparison with the gold standard autologous nerve graft repair and the classical silicone tube repair. Both groups with chitosan tubes showed similar degree of functional recovery, evaluated by electrophysiology and algesimetry tests, and similar number of myelinated nerve fibres at mid tube (7.5mm distal to the original proximal nerve end) after 4 months of implantation. The results with chitosan tubes were significantly better compared to silicone tubes. Although hollow chitosan tubes are still inferior to the autologous nerve graft repair, they seem promising conduits to construct artificial nerve grafts, combined with intratubular tropic and trophic support, for the repair of long gap nerve lesions (Gonzalez-Perez F., Cobianchi S., Geuna S., Barwig C., Freier T., Udina E., Navarro X. Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat. **Microsurgery** 2015 May;35(4):300-8. doi: 10.1002/micr.22362. Epub 2014 Dec 4).
Because of these promising results all further experiments in the second and the last period (also belonging to WP2 i.e. Tasks 2.2 and 2.4 and WP3 i.e. Task 3.4) were performed in the 15 mm long gap sciatic nerve model.
A comprehensive analysis of fine-tuned chitosan nerve guides (CNGs) enhanced by introduction of a longitudinal chitosan film to reconstruct critical length 15 mm sciatic nerve defects in adult healthy Wistar or diabetic Goto-Kakizaki rats was performed by MHH, ULUND, UAB, and UNITO. Short and long term investigations demonstrated that the CNGs enhanced by the guiding structure of the introduced chitosan film significantly improved functional and morphological results of nerve regeneration in comparison to simple hollow CNGs. Importantly, this was detectable both in healthy and in diabetic rats (short term) and the regeneration outcome almost reached the outcome after autologous nerve grafting (long term). Hollow CNGs provide properties likely leading to a wider clinical acceptance than other artificial nerve guides and their performance can be increased by simple introduction of a chitosan film with the same advantageous properties. Therefore, the chitosan film enhanced CNGs represent a new generation of medical device for peripheral nerve reconstruction. Since already long gaps and diabetic rats, respectively, were used, this study is also part of WP4 (Meyer C, Stenberg L, Gonzalez-Perez F, Wrobel S, Ronchi G, Udina E, Suganuma S, Geuna S, Navarro X, Dahlin LB, Grothe C, Haastert-Talini K. Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral Nerves. **Biomaterials** 76, 33-51, 2016 - <u>One comment of a reviewer:</u> These enhanced nerve guides, all their components can be produced under ISO standards, are likely to receive a wider clinical acceptance than hollow chitosan nerve guides!)
Other papers were already mentioned, although long nerve gaps were used as well; e.g. Meyer C, Wrobel S, Raimondo S, Rochkind S, Heimann C, Shahar A, Ziv-Polat O, Geuna S, Grothe C, Haastert-Talini K. Peripheral nerve regeneration through hydrogel enriched chitosan conduits containing engineered Schwann cells for drug delivery. **Cell Transplant**. 2015 Apr 14. [Epub ahead of print]

Task 4.2: 15 mm gap rat - delayed repair

ULUND has continued the experiments to analyse the nerve regeneration after a delayed nerve repair in healthy and diabetic rats (i.e. 45 days delay), where we initially had problems with autotomy in the rats. However, the surgical technique was rearranged and healthy and diabetic GK rats have been operated, where a 15 mm gap in the sciatic nerve was bridged by DAII-film conduits or empty DAII-conduit and evaluated after additional 8 weeks. The specimens were analysed with the same technique and methods applied in the previous studies (e.g. Haastert-Talini K., Geuna S., Dahlin L.B., Meyer C., Stenberg L., Freier T., Heimann C., Barwig C., Pinto L.F., Raimondo S., Gambarotta G., Samy S.R., Sousa N., Salgado A.J., Ratzka A., Wrobel

S., Grothe C. Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. **Biomaterials**. 2013 Dec;34(38):9886-904. doi: 10.1016/j.biomaterials.2013.08.074. Epub 2013 Sep 17; Meyer C, Stenberg L, Gonzalez-Perez F, Wrobel S, Ronchi G, Udina E, Suganuma S, Geuna S, Navarro X, Dahlin LB, Grothe C, Haastert-Talini K. Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral Nerves. **Biomaterials** 76, 33-51, 2016; Stenberg L, Kodama A, Lindwall-Blom C, Dahlin LB. Nerve regeneration in chitosan conduits and in autologous nerve grafts in healthy and in type 2 diabetic Goto-Kakizaki rats. **Eur J Neurosci**. 2015 Sep 10. doi: 10.1111/ejn.13068. [Epub ahead of print]). MHH is contributing to this task with long-term studies. Based on the study results obtained so far within the BIOHYBRID project, hollow chitosan tubes are compared to chitosan tubes enriched with a central chitosan film (n=10 animals/group).
Summary of preliminary results:

1. Both conduits supported tissue regrowth and functional recovery following delayed repair: empty chitosan conduit enabled earlier regeneration, chitosan tubes enriched with chitosan membranes slightly improved final regeneration outcome.
2. Both conduits induced initial recovery of muscle weight.
3. Both conduits resulted in partial recovery of compound muscle action potentials (CMAPs) and nerve conduction velocity
4. Both conduits resulted in partial recovery of the mechanical pain threshold.

Together with the short term observations (ULUND) a manuscript is currently drafted for publication.

Task 4.3: 15 mm gap - diabetic (GK) rat

The first study with diabetic (GK) rats was performed in short-term nerve regeneration studies after reconstruction of a 10 mm sciatic nerve defect with either a hollow chitosan conduit or an autologous comparing healthy Wistar with diabetic Goto-Kakizaki (GK) rats. After 21 days, axonal outgrowth, presence of activated and apoptotic Schwann cells, as well as thickness of the formed matrix in the conduits were measured. Axonal regeneration was better in autologous nerve grafts compared to hollow chitosan conduits and was enhanced in diabetic GK rats compared to healthy rats after reconstruction. This study provides insights into the nerve regeneration process in a clinically relevant diabetic animal model; published in Stenberg L, Kodama A, Lindwall-Blom C, Dahlin LB. Nerve regeneration in chitosan conduits and in autologous nerve grafts in healthy and in type 2 diabetic Goto-Kakizaki rats. **Eur J Neurosci**. 2015 Sep 10. doi: 10.1111/ejn.13068. [Epub ahead of print]. Interestingly, longer lengths of axonal outgrowth were seen in diabetic rats than in the healthy rats, which is in contrast to the preceding work performed in this WP, where healthy rats show longer axonal outgrowth lengths after sciatic injury and nerve repair without the use of chitosan tubes (direct suture; end-to-end; Stenberg L and Dahlin LB. Gender differences in nerve regeneration after sciatic nerve injury and repair in healthy and in type 2 diabetic Goto-Kakizaki rats. **BMC Neurosci** 2014). In the latter study gender aspects were also considered with an improved regeneration in male rats compared to female rats.

Task 4.4: 4 cm gap rabbit

At UNITO (with K. Haastert-Talini, MHH), the procedures to analyse peripheral nerve regeneration in the rabbit median nerve model where established. In this model it is possible to use longer conduits (up to 3cm) than in rat sciatic nerve model. In deviation from the original DOW, the rabbit sciatic nerve model was not established in respect of animal care issues,

because in the median nerve model automutilation was not expected to occur as frequently as described for the sciatic nerve model.
A pilot study was conducted to repair a gap of 26mm length with 30mm-long medium acetylation (ChDAII) tube (diameter of 2.1mm).
The experiment was on -3 adult female New Zealand White rabbits SPF: a more flexible chitosan conduit (DAII) has been used to repair the gap. -3 animals were operated with autologous nerve graft. -All experimental groups were sacrificed 2 months after the surgery.
For the enhanced rabbit model, technical difficulties were found. For this reason, two pilot studies were necessary to standardize the method (anesthesia, type of tube, landmark for median nerve transection, sterile conditions degree, post -surgery care). **A *SOP for BIOHYBRID rabbit in vivo studies* was generated.** After deciding the best procedures, a new set of animals was operated in March 2015.

Study 1
Gap length: 26mm; Tube: 30mm-long medium acetylation (ChDAII) soft tube (diameter of 2.1mm); Autograft (length of 26 mm); - 6 adult female New Zealand White rabbits SPF a median nerve defect consisting of 26mm gap length, where 20 mm of the proximal median nerve stump was removed prior to transplantation of the tube was bridged using a 30mm-long medium acetylation (ChDAII) tube (diameter of 2.1mm); - 6 adult female New Zealand White rabbits SPF was used as control, operated with autologous nerve graft .
Rabbits from this study have been sacrificed in July 2015 at UNITO. During withdrawal, the chitosan graft was found disconnected in all six animals tested despite all the precautions taken during surgery and in the post-operative period, thus proving that the rabbit model is not easily applicable to our study of peripheral nerve regeneration.

Task 4.5: Production of final biohybrid construct

At the end of the project term the final BIOHYBRID construct immediately applicable in the clinical setting is the chitosan conduit filled with chitosan film with holes. This construct was produced and successfully evaluated in the advanced animal models with extended nerve defects and in diabetic GK rats. However, beyond the project term after finishing the ongoing studies, we expect some further developments regarding the combination of the film-containing conduits functionalized for example with collagen +/- laminin or fibronection, respectively, with trophic factors conjugated to iron oxide nanoparticles adsorbed to this functionalized surface, and seeded with genetically modified Schwann cells overexpressing for example FGF-2^{18kD}.

Significant results for Work package 4

1. The DAII chitosan tubes support peripheral nerve regeneration across long gaps (15 mm in rats) significantly better than the classic silicone tubes. They provide properties likely leading to a wider clinical acceptance than other artificial nerve guides and their performance can be increased by simple introduction of a chitosan film with the same advantageous properties.
2. The chitosan nerve guides containing chitosan films with holes represent a new generation medical device for peripheral nerve reconstruction.
3. Characterisation of differences between peripheral nerve regeneration in healthy and diabetic rats as well as between male and female subjects is of high importance for the ongoing studies. Using chitosan films-containing chitosan nerve conduits, longer lengths of axonal outgrowth were seen in diabetic rats than in the healthy rats, which is in

contrast to the preceding work performed in this WP, where healthy rats show longer axonal outgrowth lengths after sciatic injury and instant nerve repair.

Deviation from plan for Work package 4

The work of this WP4 has been pre-drawn in deviation from the original DOW so that the work with enhanced rat models started already before months 28. This is related to the promising results we achieved already with the hollow DAII tubes in WP1.

In deviation of the original DOW the rabbit model has been shifted from the sciatic nerve model to the median nerve model which still provides the possibility to study regeneration across longer peripheral nerve gaps than in rats but takes into account an optimised animal welfare during the experiments. However, it turned out that the rabbit median nerve model is not easily applicable to our study of peripheral nerve regeneration

4.1.3.5 Work Package 5: Translational work for clinical application

Work in WP5 started already in month 1 with the design and documentation of the experiments with regard to the ISO/GMP production. In addition, development of documentation models for future clinical trials and the regulatory work were started.

Task 5.1: Scaling-up ISO/ GMP production

Within the BIOHYBRID project Medovent used its long-standing experience in processing and modifying chitosan. For the in vitro and in vivo experiments performed within the BIOHYBRID project Medovent delivers chitosan hollow tubes, thin fibres, and chitosan films. All prototypes for BIOHYBRID are produced under controlled ISO 13485 conditions in Medovent's clean room facilities using Medovent's own extrusion technology.

<u>Preparation of GMP processes and application for GMP manufacturing authorisation</u>
One of Medovents main tasks in BIOHYBRID WP5 was the implementation of GMP manufacturing processes with regard to future entry into international markets. In order to adjust Medovent's ISO 13485 quality system to the GMP requirements a third party mock audit followed by a gap analysis has been performed at Medovent's facilities. In addition Medovent joined a 21CFR820 training. Based on the gap analysis and the training inputs Medovent was working on the implementation of GMP standards and adjustment of the product documentation. Formal adjustments in the QM documents according to 21CFR820/GMP were performed. The product documentation was adjusted according to the different understandings of Design History File, Device Master Record and Device History Records. The international marking system for medical devices (UDI = Unique Device Identifier) was implemented and an international vigilance system was prepared.
With scientific support by the project partner MHH Medovent prepared the FDA 510(k) submission documents for Reaxon® Nerve Guide and applied for 510(k) approval.

Task 5.2: Development of new documentation models for future clinical trials

Clinical trials

Medovent is coordinating the preparation of a multicentre clinical trial on Reaxon® Nerve Guide in median and ulnar nerves. After the discussion of the rough clinical study design within the consortium at the General Assembly in Torino in January 2014, Medovent prepared a first study protocol (NG-002) according to ISO 14155 in close corporation with the clinical expert BIOHYBRID partners ULUND and SMC. Medovent, ULUND and UNITO worked together to prepare and submit the first ethic applications. The clinical study is already approved in Sweden and Germany. The approval process in Italy is running.

We have also thoroughly judged the documentation models available to assess outcome after nerve injury and repair/reconstruction, particularly after median and ulnar nerve injuries in the forearm; a suitable condition to study e.g. a new product on the market. The existent internationally accepted and validated documentation model (*Rosén and Lundborg, J Hand Surg Am. 2000 May;25(3):535-43*) was found to be suitable also for examining the Reaxon® nerve guide after median and ulnar nerve injuries. The model was recently used to examine patients with a median and/or ulnar nerve injury with a median follow up of 30 years (*Chemnitz et al., J Bone Joint Surg Am. 2013 Feb 20;95(4):329-37. doi: 10.2106/JBJS.L.00074*). Thus, a clinical study has been designed to assess the effectiveness of Reaxon® nerve guide to repair and reconstruct median and ulnar nerve injuries.

Summary of the clinical investigation plan NG-002:
A Controlled, Randomised, Comparison, Blind Evaluation of Repair of Median and Ulnar Nerve Lesions in Man Using an Implanted Reaxon® Nerve Guide

The clinical investigation NG-002 is planned as a multicentre, parallel, controlled, randomized, blind evaluation of the repair of median and ulnar nerve lesions (distal portion of the upper extremity).

The aim of this clinical investigation is to collect post-market clinical follow-up (PMCF) data to confirm the medium- and long-term safety and performance of the chitosan-based Reaxon® Nerve Guide. So far very poor clinical data for median and ulnar nerve lesions are available as most clinical studies focus on digital nerves. Beside the comparison of treatment of gaps from 6 to 26 mm with either autograft or Reaxon® Nerve Guide another important aspect of the clinical study is to compare treatment of short gaps up to 5 mm with either the standard end-to-end-suture or the tensionless alternative Reaxon® Nerve Guide.

Five centres, located in Sweden, Germany, Spain, Italy and Israel, will participate in this clinical investigation which is split into two substudies, each investigating 76 subjects with traumatic median or ulnar nerve injuries. In substudy 1, subjects with very short gaps of median and ulnar nerves (< 5 mm) in alternative to direct end-to-end suture are eligible for inclusion. In substudy 2 subjects with median and ulnar nerve injuries (> 5 < 26 mm gap) in whom surgical repair may not allow end-to-end direct suture of the nerve ends, and in whom the nerve tissue gap would indicate the use of an autograft of equal or less than 26 mm are eligible for inclusion.

The anticipated clinical benefits of this clinical evaluation are besides the expected potential of the Reaxon® Nerve Guide to accelerate and enhance the regeneration of the injured nerve, in substudy 2, the Reaxon® Nerve Guide will spare the patient an additional surgery to harvest the donor material and the associated loss of function of the donor nerve. The removal of the autograft can result in a number of post-surgery complications like delayed wound healing or neuroma. Regarding the end-to-end suture performed in substudy 1, Reaxon® Nerve Guide

provides a particularly gentle treatment of the nerve lesion. As it is known that even slight tension can negatively affect the nerve regeneration, treatment of very short gaps up to 5 mm by implantation of Reaxon® Nerve Guide instead of conventional end-to-end suture is a complete tensionless alternative.

The primary objective of substudy 1 is to demonstrate that the Rosén Score (functional recovery after nerve repair) 24 months after surgery will be superior in the Reaxon® Nerve Guide test group compared to the control group receiving an end-to-end suture. The primary objective of substudy 2 is to demonstrate that the Rosén Score (functional recovery after nerve repair) 24 months after surgery will be not inferior in the Reaxon® Nerve Guide test group compared to the control group receiving an autologous nerve graft.

In both substudies the subjects will be evaluated for sensory and motor function in the injured and non-injured hand and the components attributing to cold sensitivity and hyperesthesia/allodynia are also monitored according to the Rosén Score protocol which was established at the Department of Hand Surgery, Rehabilitation Unit, Skåne University hospital and which is based on the guidelines developed by Rosén [1] and published by Rosén and Lundborg [2, 3].

The secondary outcome of the follow-up examinations is to ascertain regeneration of nerve fibres through the nerve guide conduit and reinnervation of the target organs (sensory receptors in skin and muscle fibres). In addition, there will be evaluations of common post-operative complications associated with peripheral nerve repair surgery, i.e. painful neuroma formation, assessed by pain on percussion, allodynia, and dysaesthesia (pain and/or numbness) in both the Reaxon® Nerve Guide and control groups. Time to remove the autograft and cutting-and-suture time will also be evaluated. Furthermore, the injured nerve will be examined electrophysiologically.

[1] B. Rosén, Recovery of sensory and motor function after nerve repair. A rationale for evaluation. J Hand Ther 1996; 9(4):315-27
[2] B. Rosén and G. Lundborg, The long term recovery curve in adults after median or ulnar nerve repair: a reference interval. J Hand Surg 2001; 26(3):196-200
[3] B. Rosén and G. Lundborg, A Model Instrument for the Documentation of Outcome After Nerve Repair. J Hand Surg 2000; 25(3):535-43

Task 5.3: Regulatory work

Quality management and product approval

Based on the promising outcomes of the first short gap animal studies Medovent decided, with the full support of the BIOHYBRID Board, to bring an empty chitosan tube for short gap nerve repair (Reaxon® Nerve Guide) to regulatory approval already at an early stage of the BIOHYBRID project. The following studies had to be performed:

- Production validation
- Sterilisation validation
- Biocompatibility and toxicology studies according to ISO 10993
- Aging studies
- Transport tests

The technical documentation has been prepared including complete risk analysis, preclinical and clinical evaluations, usability file and documentation of the fulfilment of the essential

requirements according to MDD 93/42/EEC annex I.CE mark of Reaxon® Nerve Guide was approved in January 2014.

Preparation of FDA submission

With regard to BIOHYBRID task D5.2 and future US market entry Medovent started to prepare an FDA submission process. In order to adjust Medovent's quality system to the requirements of the FDA a third party mock audit followed by a gap analysis was performed at Medovent's facilities in June 2014.

Translational work and clinical application

Reaxon® Nerve Guide received the CE mark in January 2014. The product was introduced first into the German market and has already been implanted in patients with peripheral nerve defects at several clinics across the country. A multicentre clinical trial on Reaxon® Nerve Guide in median and ulnar nerves is in preparation.

Training

Training presentations on QM requirements and the market approval processes in the EU and the US as well as training on the preparation of clinical trials were given by Medovent in the Grand Assemblies in Malmö in April 2013, in Torino in January 2014, and in Hannover in November 2014.

Task 5.5: Preparation of biocompatibility and toxicological tests

CE-Certification

Medovent's main tasks in the BIOHYBRID project included the production of chitosan-based hollow tubes, half-shells, films and fibres for in vitro and in vivo studies. The prototypes of an artificial hollow tube device for the BIOHYBRID project were produced under controlled ISO 13485 conditions in Medovent's clean room facilities with the purified chitosan from Altakitin.
Preclinical work was finished in cooperation with the BIOHYBRID partners to optimize the chitosan hollow tube production at Medovent.

The CE certification process of a chitosan hollow tube, named Reaxon® Nerve Guide, was completed in January 2014. Reaxon® Nerve Guide is on the German market since July 2014.

REAXON® NERVE GUIDE

The Reaxon® Nerve Guide is made of the naturally occurring biopolymer chitosan and is used to bridge short peripheral nerve defects. It is a flexible, long-term stable nerve guide that actively guides the regrowing nerve fibers and inhibits the ingrowth of fibroblastic tissue and scar formation.

The transparent hydrogel wall supports the transport of nutrients and oxygen to the regenerating nerve. Reaxon® Nerve Guide provides an optimal environment for the vitality and the growth of Schwann cells, because it protects the growing nerve against undesirable external influences and facilitates the transport of nutrients and oxygen due to its hydrogel properties.
Extensive animal testing has confirmed that Reaxon® Nerve Guide is as effective as the autologous nerve graft, the current gold standard (Haastert-Talini et al., Biomaterials 34 (2013) 9886-9904).

Reaxon® Nerve Guide and the autologous nerve graft are comparable in the Functional Sciatic Index, Somato-Sensory-Evoked Potential and Compound Muscle Action Potential. It supports axonal regrowth to a similar extent and a similar thickness of the myelin layer at the proximal and distal stumps of the sciatic nerve compared to the autologous nerve graft.
The Reaxon® Nerve Guide is a 30 mm long chitosan hollow tube (Fig. 16), available with five different inner diameters (between 2.1 mm and 6 mm). It can be used to reconstruct injured peripheral nerves up to a maximum defect length of 26 mm.

Figure shows: Reaxon® Nerve Guide with different inner-diameters.

The tube-shaped Reaxon® Nerve Guide is packaged in double blisters (Fig. 17+18) and is intended exclusively for single use. The inner diameter of each guide is indicated on the packaging.

Figure shows primary packaging (double-blister) of Reaxon® Nerve Guide.

Figure shows secondary packaging of Reaxon® Nerve Guide.

Significant results for Work package 5

The clinical application of Reaxon® Nerve Guide in several clinics in Germany and Italy is a significant result and great success.
Medovent, ULUND, and UNITO worked together to prepare and submit the first ethic applications. The clinical study is already approved in Sweden and Germany. The approval process in Italy is still running. The FDA submission process has been initiated.

Publications of WP5: Hallgren et al., BMC Surg 2014; IF 1.240; Chemnitz et al., BMC Musculoskel Dis 2013; IF 1.898; Dahlin, Int Rev Neurobiol 2014; IF 2.457

Deviation from plan for Work package 5

Although not included into the original DOW, a new medical device, the Reaxon® nerve guide was already introduced into the market. The Chitosan Nerve Guide is to be classified as a Medical device according to National regulations (Germany: Medical Devices Act, "Medizinproduktegesetz", MPG). The FDA submission process is advanced.

4.1.4 Potential impact of the project

4.1.4.1 Socio-economic impact

Nervous system diseases greatly compromise the quality of life for patients and have an enormous socio-economic impact. About 3% of all trauma patients are afflicted by injuries of peripheral nerves which in many cases result in life-long disabilities. In Europe alone, at least 100,000 surgeries are performed annually to repair or reconstruct injured nerves leading to significant costs for the health system. In the majority of cases, patients who suffer from traumatic injuries are at working age so that treatment aims at the full restoration of nerve functionality to avoid unemployment and long-term burden to the social systems.

The current gold standard is the use of a donor nerve (autograft) to bridge the severed nerve ends, a procedure which is associated with several disadvantages. These include the need for an additional surgery to harvest the donor material and the associated loss of function at the donor site. Moreover, graft harvesting may result in donor site morbidity such as scarring or neuroma formation. Success rates are estimated at 30%-70%.

Synthetic nerve guides (nerve conduits) were introduced as an alternative to autografts. Currently used conduits show similar results in short-gap nerve repair compared to autografts. However, materials used are bioinert and cannot stimulate nerve growth over long distances. Another frequent problem is the rapid loss of mechanical strength due to fast degradation, associated with the collapse of the conduit before completion of the healing process, and resulting in nerve compression.

The BIOHYBRID consortium of renowned academic institutions, hospitals and SMEs has aimed at the development of novel, improved devices for peripheral nerve repair and reconstruction, thereby contributing to the main impacts expected for projects in the Call "HEALTH.2011.1.4-2: Tools, technologies and devices for application in regenerative medicine":

- Projects should lead to new tools, technologies or devices which will assist the establishment of regenerative therapies in the clinic
- Projects must involve the European biotechnology industry, especially the SME sector

A major outcome based on the successful results of the Biohybrid project was the development of a novel nerve conduit made from chitosan. The Reaxon® Nerve Guide which was CE approved and commercialized by one of the SME partners in the consortium, Medovent, in 2014 provides guidance and protection for regenerating axons whilst preventing the undesired interference from fibrotic tissue ingrowth. The anticipated clinical benefits include the potential to accelerate and enhance the regeneration of the injured nerve. Moreover, Reaxon® Nerve Guide

will spare the patient an additional surgery to harvest the donor material and the associated loss of function of the donor nerve. It thereby offers an alternative to the current gold standard autograft.
The most beneficial features of a chitosan-based nerve guide include:

- Bioactivity – supports nerve regeneration
- Anti-adhesive – inhibits scar and neuroma formation
- Hydrogel – facilitates the transport of essential nutrients and oxygen
- Transparent – easy and comfortable suturing

as well as very important supplementary properties, such as:

- Long-term stability – enables the maturation of the regenerating nerve
- Biocompatibility – prevents irritation or inflammation
- Non-toxicity – degradation products are natural constituents of the body
- Antibacterial – limits or prevents infections

Reaxon® Nerve Guide has the potential to overcome significant limitations of the currently used materials, thereby providing much benefit to the trauma patient. This, in turn, provides treatment options to the health system which are more cost-efficient and associated with reduced times for functional recovery and post-operative rehabilitation, thus leading to a higher percentage of patients getting back to work and less unemployment resulting from traumatic injuries. Moreover, Reaxon® Nerve Guide is also applicable in patients with diabetes.

In addition, Biohybrid has generated new approaches for long-gap nerve repair which, after commercialization, can be expected to rapidly obtain a broad acceptance by the medical community. Such technological advantage creates a leading market position for a newly developed product in this application, which can be expected to become the material of choice for large as well as short nerve defects.

A successful market commercialization of the novel nerve guide by the Biohybrid partner Medovent has therefore significant socio-economic impacts to the European community. Increasing sales will create jobs particularly in production and sales, but also in R&D, quality management and others, which requires training of new employees and building-up of new and extended infrastructure. Based on estimations of the current and future market potential of peripheral nerve guides sales in the two-digit million Euro range are to be expected within the first five years in the market alone.

After market entry, new research can focus on the transfer of the technology to other medical fields. Furthermore a global market entry is planned after the European approval. All these activities lead to a significant impact on the manufacturer's employees, suppliers, distributors, retailers, and, most importantly, customers which is the surgeon and his/her patient. At the same time, technology development and transfer from academic institutions will be fundamental, affecting employment, infrastructure, communication and scientific strategies in the research community.

A most important impact anticipated is the improvement of the quality of life for patients suffering from peripheral nerve injury. Feedback from surgeons and patients received so far confirms the efficacy of the novel nerve guide. Reaxon® Nerve Guide has been first introduced in Germany and is already the standard of use in major trauma clinics across the country. Two prospective multicenter clinical trials are ongoing to provide additional evidence of the efficacy of the product. Another multicenter study is being planned as a large European trial. Moreover,

surgeons are suggesting extensions to the current product design and application, adding to the long-term sustainability of socio-economic impacts resulting from the successful outcome of the Biohybrid project.

4.1.4.2 Wider societal implications of the project so far

The above mentioned outputs and the attainment of the project goals to provide solutions to concrete health impairments were the core of this collaborative project. In addition, the project's activities also contributed to ensuring the sustainability and acceptability of scientific research within the EU, thus strengthening the positive reputation of both scientific research and interdisciplinary collaborations beyond the EU.

Ethics
All partners were bound by high ethical standards when carrying out their work. Apart from the personal interest in ethically sound work, ethical standards were enforced through regulating bodies at the institutional level that implemented and ensured strict guidelines to comply with EU and national legislation. Furthermore, in the face of increased public awareness it is paramount to ensure the compliance with ethical standards in a transparent and accountable manner to safeguard continued support and thus sustainability for clinical research.

Gender
All involved parties had a strong basis of female researchers who were active as the coordinator, PhD students, post-doctoral researchers, laboratory technicians, quality control and scientific management. Even though an active gender mainstreaming plan was not implemented it is self-evident that researchers and support staff are primarily selected on the basis of their merit, qualifications and suitability for the research tasks at hand.

Public outreach and dissemination
In line with the above two points, all parties were active in the dissemination of the project's activities and results not only within the scientific community but also to the general public. Attendance of international conferences, presenting the current research and publishing in internationally renowned journals was undertaken meticulously as the record of publications in table Template A1 shows. Furthermore, as can be seen in Template A2, numerous events such as open days, poster presentations, radio broadcasts and press releases contributed to the visibility and accessibility of the project. In the event of feedback following such events the researchers made sure to respond to queries posed (also by Email) by members of the public.

Outreach to policy makers
The coordinator also actively reached out the policy makers within the EU, thus the project officer was continually informed and invited to workshops and symposia which he also reciprocated. The *1st International Workshop on Intrinsic and Extrinsic Mechanisms of Axonal Regeneration* was held on May 27th, 2014 in Brussels with the express aim of including policy makers in Brussels.

Interdisciplinarity and private sector involvement
The project as such had a wide scope, starting from sourcing suitable materials, to the production and distribution of nerve conduits and their fillers and conducting studies to fulfil the project's aims. The involvement of the private sector, in the form of SMEs, was an integral part in

securing the expertise, infrastructure and future collaboration of companies in the disciplinary fields of biomaterials science and bioengineering. In addition, several experts from different medical disciplines such as neuroanatomy, reconstructive surgery and veterinary science collaborated closely over 4 years to generate and test knowledge and products. The involvement of young researchers, as PhDs or in post-doctoral positions provided ideal conditions for training and qualifications. As such 6 PhD students were involved and obtained/will obtain their PhD degrees as part of their work in the project.

4.1.4.3 Main dissemination activities and exploitation of results

Website

In line with the project kick-off in October 2011, several activities were undertaken to announce BIOHYBRID: A dedicated public website with a project overview, partner profiles, news, event announcements, publications etc. was set up. In addition this website also hosts an internal part that allows all partners to access the workflow tables, SOPs (*In vitro standard protocol, Factsheet in vivo, Pre-SOP 002 in vitro, Pre-SOP 002 Rev 01; Factsheet in vivo, Factsheet in vivo Rev 01 , Pre-SOP 004)* and handbooks (*Handbook in vitro; Handbook in vivo)*, gantt charts, milestone and deliverable tables and reports, short (3-months) internal reports, training opportunities and minutes of coordination board meetings and general assemblies. On occasion it has also been used to share large files with raw data.

Project main website: http://kongress.mh-hannover.de/biohybrid/

Horizon Health: http://www.horizonhealth.eu/project/biohybrid-templates-peripheral-nerve-regeneration/238

CORDIS: http://cordis.europa.eu/project/rcn/101289_en.html

Embedded in the Website of the coordinator and partners:
http://intranet.mh-hannover.de/biohybrid.html?&MP=175-8345

Apart from the dedicated website, the ***2nd and 3rd International Symposia on Peripheral Nerve Regeneration*** in January 2014 and September 2015 had separate website set up to keep the scientific community up to date.

Media and press

Right from the beginning, the consortium has been active in issuing press releases to mark occasions such as the kick- off, with articles featuring in the regional newspaper “Hannoversche Allgemeine Zeitung on 06.12.2011” and online media such as http://www.medport.de/nw_read.php/165975.

As the project progressed press releases were issued on the occasion of the publication of the paper ***Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects*** in Biomaterials 34 (2013) which again resulted in newspaper and online articles in the “Hannoversche Allgemeine Zeitung” on 17.10.2013 and “Labor Journal” the German edition of Lab Times issue 11/2013. Following this a radio interview with Deutschland Radio Kultur was conducted with the coordinator Prof. Claudia Grothe and Prof. Kirsten Haastert-Talini on 18.11.2013: http://www.deutschlandradiokultur.de/nervenreparatur-implantate-aus-krabbenpanzer.1067.de. html? dram:article_id=269061.

More press releases and announcements were issued on the occasion of the ***2nd International Symposium on Peripheral Nerve Regeneration,*** in January 2014 in Torino, Italy by the

consortium partner UNITO and taken up by several media outlets such as Torino Repubblica.it on 23.01.2014.

Dissemination to scientific community

Apart from the regular publications of articles, book reviews and abstracts, a letter to the editor was published in the Journal of the Peripheral Nervous System: "BIOHYBRID-Biohybrid templates for peripheral nerve regeneration", Grothe et al. , Journal of the Peripheral Nervous System 17:220–222 (2012).
For more detail on publications and conferences please refer to table A2 below.

Furthermore, open presentations about the coordination of EU-projects were given at university-wide events such as the MHH EU-experience exchange meetings on 12.06.2012 and 29.06.2015 by the coordinator ("EU-Projekte: Erfahrungsbericht einer Koordinatorin").
Biohybrid related talks were also presented during Graduate School Day of the Hannover Graduate School for Veterinary Pathobiology, Neuroinfectiology, and Translational Medicine (HGNI), open to all universities at Hannover, Germany (23rd/24th Nov 2012)

Open days

On several occasions the consortium partners were active on open days of their respective institutions: UNITO: NICO open doors; Turin, Italy (15th October, 2014 and 16th May, 2015) which were also covered by local press and was a good occasion also for the young researchers to illustrate the impact of their studies.
MHH: November der Wissenschaft in 2012 and 2014 in Hannover, Germany. The Hannover-wide event is an important platform for all tertiary education and research institutions in Hannover with an attendance of over 10,000 members of the public spread all over the city.

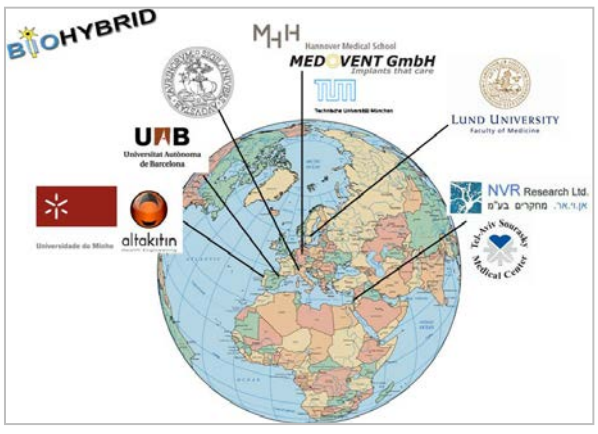
Exploitation of results

Issues of intellectual property rights were negotiated and defined within the Consortium Agreement which was implemented in time before the start of the project.
Medovent, as the lead partner provided deliverable reports on market surveys and follow-up exploitation plans on a regular basis.

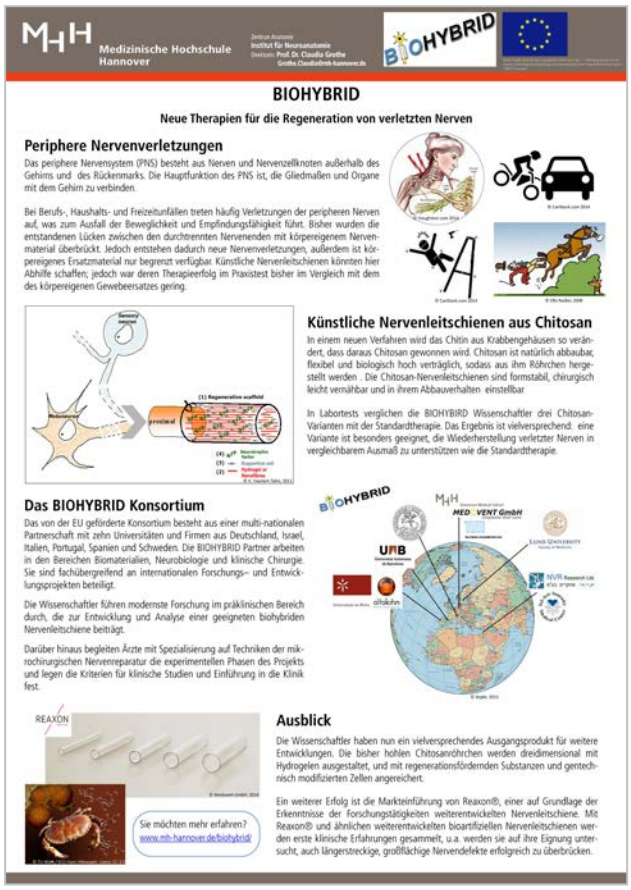
One significant outcome from the project was the patent on **Heregulin beta1 -conjugated iron oxide nanoparticles** by the partners NVR and UNITO, illustrating the fruitful collaboration between university research and the private sector.

Some examples of outreach material:

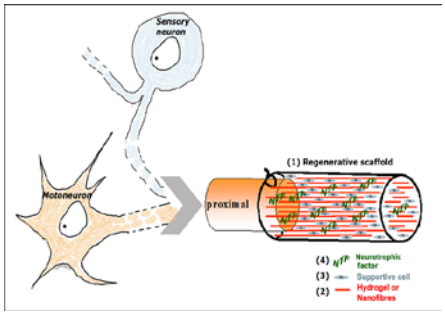
BIOHYBRID Logo



Global partnership



Poster presented at the "Nacht der Wissensschaft" in Hannover, Nov. 2014



Biohybrid Concept

3.2 Use and dissemination of foreground

- Section A

This section should describe the dissemination measures, including any scientific publications relating to foreground. **Its content will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

- Section B

This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES*

No.	Title	Main *(First)* author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers† (if available)	Is/ Will open access‡ provided to this publication?
1	Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects	Haastert-Talini K.	Biomaterials IF 8.312	34(38), Dec 2013	Elsevier		2013	9886-904	doi: 10.1016/j.biomaterials.2013.08.074. Epub 2013 Sep 17	yes
2	Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat	Gonzalez-Perez F.	Microsurgery IF 2.421	35(4), May 2015	Wiley		2014/2015	300-8	doi: 10.1002/micr.22362. Epub 2014 Dec 4	yes - repository

* **The first 8 papers are ordered by importance, the following then are listed chronologically.**

† A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

‡ Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

3	Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves	Meyer C.*, Stenberg L.*, Gonzalez-Perez F.* (*contributed equally)	Biomaterials IF 8.557	76, 21 Oct 2015	Elsevier		2015	33-51	doi: 10.1016/j.biomaterials.2015.10.040. [Epub ahead of print]	yes
4	In vitro evaluation of cell-seeded chitosan film for peripheral nerve tissue engineering	Wrobel S.	Tissue Eng Part A IF 4.254	20(17-18), Sep 2014	Mary Ann Liebert		2014	2339-49	doi: 10.1089/ten.TEA.2013.0621. Epub 2014 Apr 22	yes
5	Nanotechnology versus stem cell engineering - In vitro-comparison of neurite inductive potentials	Morano M.*, Wrobel S.* (* contributed equally)	Int J Nanomedicine IF 4.195	9, 14 Nov 2014	DovePress		2014	5289-306	doi: 10.2147/IJN.S71951. eCollection 2014.	yes
6	Induction of Neurite Outgrowth in 3D Hydrogel-Based Environments.	Assunção-Silva R.	Biomed Mater. IF 3.697	10(5), 20 Oct 2015	IOP Science		2015	051001	doi: 10.1088/1748-6041/10/5/051001	no
7	Peripheral nerve regeneration through hydrogel enriched chitosan conduits containing engineered Schwann cells for drug delivery	Meyer C*, Wrobel S* (*contributed equally)	Cell Transplant IF 3.127	14 Apr 2015	Cognizant		2015		2015 Apr 14. [Epub ahead of print]	yes
8	Specificity of peripheral nerve regeneration: interactions at the axon level	Allodi I.	Prog Neurobiol IF 9.035	98(1), Jul 2012	Elsevier		2012	16-37	doi: 10.1016/j.pneurobio.2012.05.005. Epub 2012 May 15	yes
9	Differential effects of activity dependent treatments on axonal regeneration and neuropathic pain after peripheral nerve injury	Cobianchi S.	Exp Neurol IF 4.617	240, Feb 2013	Elsevier		2012/2013	157-67	doi: 10.1016/j.expneurol.2012.11.023. Epub 2012 Nov 30	no
10	The role of timing in nerve reconstruction.	Dahlin L.B.	Int Rev Neurobiol IF 2.457	109, 2013	Academic Press Elsevier		2013	151-64	doi: 10.1016/B978-0-12-420045-6.00007-9.	yes - repository

11	BIOHYBRID - Biohybrid templates for peripheral nerve regeneration.	Grothe C.	J Peripher Nerv Syst IF 2.57	17(2), Jun 2012	Wiley		2012	220-2	doi: 10.1111/j.1529-8027.2012.00399.x	no
12	FGF-2 low molecular weight selectively promotes neuritogenesis of motor neurons	Allodi I.	Mol Neurobiol. IF 5.286	47(2), Apr 2013	Springer		2012/2013	770-81	doi: 10.1007/s12035-012-8389-z. Epub 2012 Dec 30	no
13	Schwann cells transduced with a lentiviral vector encoding Fgf-2 promote motor neuron regeneration following sciatic nerve injury	Allodi I.	Glia IF 6.031	62(10), Oct 2014	Wiley		2014	1736-46	doi: 10.1002/glia.22712. Epub 2014 Jul 2	no
14	Activity dependent therapies modulate the spinal changes that motoneurons suffer after a peripheral nerve injury	Arbat-Plana A.	Exp Neurol IF 4.696	263, Jan 2015	Elsevier		2014/2015	293-305	doi: 10.1016/j.expneurol.2014.10.009. Epub 2014 Oct 23	no
15	Consequences and adaptation in daily life - patients' experiences three decades after a nerve injury sustained in adolescence	Chemnitz A.	BMC Musculoskelet Disord IF 1.898	14, 22 Aug 2013	BioMed Central		2013	252	doi: 10.1186/1471-2474-14-252	yes
16	Assessment of sensory thresholds and nociceptive fiber growth after sciatic nerve injury reveal the differential contribution of collateral reinnervation and nerve regeneration to neuropathic pain	Cobianchi S.	Exp Neurol IF 4.696	255, May 2014	Elsevier		2014	1-11	doi:10.1016/j.expneurol.2014.02.008	no
17	Neuregulin 1 isoforms could be an effective therapeutic candidate to promote peripheral nerve regeneration	Gambarotta G.	Neural Regen Res IF 0.22	9(12), 15 Jun 2014	Wolters Kluwer		2014	1183-5	doi: 10.4103/1673-5374.135324	yes
18	Identification and validation of suitable housekeeping genes for normalizing quantitative real-time PCR assays in injured peripheral	Gambarotta G.	PLoS One IF 3.234	9(8), 21 Aug 2014	PLoS One		2014	e105601	doi: 10.1371/journal.pone.0105601. eCollection 2014.	yes

	nerves.									
19	The Use of Chitosan-Based Scaffolds to Enhance Regeneration in the Nervous System.	Gnavi S.	Int Rev Neurobiol IF 2.457	109, 2013	Academic Press Elsevier		2013	1-62	doi: 10.1016/B978-0-12-420045-6.00001-8	no
20	Extracellular matrix components in peripheral nerve regeneration	Gonzalez-Perez F.	Int Rev Neurobiol IF 2.457	108, 2013	Academic Press Elsevier		2013	257-75	doi: 10.1016/B978-0-12-410499-0.00010-1	no
21	Subjective outcome related to donor site morbidity after sural nerve graft harvesting: a survey in 41 patients	Hallgren A.	BMC Surg IF 1.24	13, 24 Sep 2013	BioMed Central		2013	39	doi: 10.1186/1471-2482-13-39	yes
22	Trends in Bioabsorbable Osteosynthesis Devices: Introduction to a Novel Production Process of Chitosan-Based Implants	Oliveira N.G.	J Chitin Chitosan Sci IF not available	1(3), Dez 2013	American Scientific Publishers		2013	1-11	doi: 10.1166/jcc.2013.1030	no
23	In vitro assessment of three dimensional dense chitosan-based structures to be used as bioabsorbable implants.	Oliveira N.G.	J Mech Behav Biomed Mater IF 3.417	40, Dec 2014	Elsevier		2014	413-25	doi: 10.1016/j.jmbbm.2014.09.014. Epub 2014 Sep 28.	no
24	Modulation of bone marrow mesenchymal stem cell secretome by ECM-like hydrogels	Silva N.A.	Biochimie IF 3.123	95(12), Dec 2013	Elsevier		2013	2314-9	doi: 10.1016/j.biochi.2013.08.016. Epub 2013 Aug 30	no
25	Gender differences in nerve regeneration after sciatic nerve injury and repair in healthy and in type 2 diabetic Goto-Kakizaki rats	Stenberg L.	BMC Neurosci IF 2.665	15, 13 Sep 2014	BioMed Central		2014	107	doi: 10.1186/1471-2202-15-107	yes
26	The role of neurotrophic factors conjugated to iron oxide nanoparticles in peripheral nerve regeneration - in vitro studies	Ziv-Polat O.	Biomed Res Int IF 3.169	2014, 2014	Hindawi		2014	267808	doi: 10.1155/2014/267808. Epub 2014 Jul 16	yes
27	The Mouse Median Nerve Experimental Model in Regenerative Research	Buskbjerg Jager S.	Biomed Res Int	July-August 2014	Hindawi		2014	1-6	10.1155/2014/701682	yes

			IF 1.579							
28	Competitiveness of chitosan-based implants	Oliveira N.G.	Ciência & Tecnologia dos Materiais IF 0.00	26, July-December 2014	Elsevier		2014/2015	77-88	doi:10.1016/j.ctmat.2015.03.001	no
29	Processing and characterization of 3D dense chitosan pieces, for orthopedic applications, by adding plasticizers	Figueiredo L.	Procedia Engineering IF 0.00	110, 2015. 4th International Conference on Tissue Engineering, ICTE2015, An ECCOMAS Thematic Conference	Elesevier		2015	175-182	doi:10.1016/j.proeng.2015.06.182	yes
30	Activity dependent therapies modulate the spinal changes that motoneurons suffer after a peripheral nerve injury	Arbat-Plana A.	Exp Neurol IF 4.696	263, Jan 2015	Elsevier		2014/2015	293-305	doi: 10.1016/j.expneurol.2014.10.009. Epub 2014 Oct 23	no
31	Substratum preferences of motor and sensory axons in postnatal and adult rats	Gonzalez-Perez F.	Eur J Neurosci IF 3.181	31 Aug 2015	Wiley		2015		doi: 10.1111/ejn.13057. [Epub ahead of print]	no
32	Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models. A critical overview	Navarro X.	Eur J Neurosci IF 3.181	30 Jul 2015	Wiley		2015		doi: 10.1111/ejn.13033. [Epub ahead of print]	no
33	Early increasing-intensity treadmill exercise reduces neuropathic pain by preventing nociceptor collateral sprouting and disruption of chloride cotransporters homeostasis after peripheral nerve injury	López-Álvarez V.M.	Pain IF 5.213	156(9), Sep 2015	Wolters Kluwer		2015	1812-25	doi: 10.1097/j.pain.0000000000000268	no
34	Comparison of results between chitosan hollow tube and autologous nerve graft in reconstruction of peripheral nerve	Shapira Y.	Microsurgery IF 2.421	22 Apr 2015	Wiley		2015		doi: 10.1002/micr.22418. [Epub ahead of print]	no

	defect: An experimental study									
35	In vitro models for peripheral nerve regeneration	Geuna S.	Eur J Neurosci IF 3.181	26 Aug 2015	Wiley		2015		doi: 10.1111/ejn.13054. [Epub ahead of print]	no
36	New insights on the standardization of peripheral nerve regeneration quantitative analysis	Ronchi G.	Neural Regen Res IF 0.22	10(5), May 2015	Wolters Kluwer		2015	707-9	doi: 10.4103/1673-5374.156962	yes
37	The Effect of Electrospun Gelatin Fibers Alignment on Schwann Cell and Axon Behavior and Organization in the Perspective of Artificial Nerve Design	Gnavi S.	Int J Mol Sci IF 2.862	16(6), 8 Jun 2015	Multidisciplinary Digital Publishing Institute (MDPI)		2015	12925-42	doi: 10.3390/ijms160612925	yes
38	The Neuregulin1/ErbB system is selectively regulated during peripheral nerve degeneration and regeneration	Ronchi G.	Eur J Neurosci IF 3.181	8 Jun 2015	Wiley		2015	?	doi: 10.1111/ejn.12974	no
39	Local delivery of the Neuregulin1 receptor ecto-domain (ecto-ErbB4) has a positive effect on regenerated nerve fiber maturation	Gambarotta G.	Gene Ther IF 3.104	4 May 2015	Nature Publishing Group		2015		doi: 10.1038/gt.2015.46	yes
40	Update on stereology for light microscopy	Geuna S.	Cell Tissue Res IF 3.656	360(1), Apr 2015	Springer		2015	5-12	doi: 10.1007/s00441-015-2143-6. Epub 2015 Mar 6	no
41	The sciatic nerve injury model in pre-clinical research	Geuna S.	J Neurosci Methods IF 2.025	243, 30 Mar 2015	Elsevier		2015	39-46	doi: 10.1016/j.jneumeth.2015.01.021. Epub 2015 Jan 25	no
42	Nerve regeneration in chitosan conduits and in autologous nerve grafts in healthy and in type 2 diabetic Goto-Kakizaki rats	Stenberg L.	Eur J Neurosci IF 3.181	10 Sep 2015 [Epub ahead of print]	Wiley		2015		doi: 10.1111/ejn.13068. [Epub ahead of print]	no
43	Application of iron oxide nanoparticles in neuronal tissue engineering	Ziv-Polat O.	Neural Regen Res IF 0.22	10(2), Feb 2015	Wolters Kluwer		2015	189-91	doi: 10.4103/1673-5374.152364	yes

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES[§]

[§] **Some smaller events are summarised**

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES**								
No.	Type of activities††	Main leader	Title	Date/Period	Place	Type of audience‡‡	Size of audience	Countries addressed
1	Press releases	MHH	Kick-off of BIOYHBRID Project	October 2011	Hannover, Germany	General public and scientific community		Global
2	Conference	MHH with UNITO	2nd International Symposium on Peripheral Nerve Regeneration	23.-24.01.2014	Turin, Italy	Scientific Community	162	Global
3	Press releases	UNITO	Announcement for 2nd International Symposium on Peripheral Nerve Regeneration 23.01.-24.01.2014	3-6 months leading up to 23.01.2014	Italy	General public and scientific community		Global
4	Workshop	MHH and whole Consortium	1st International Workshop on Intrinsic and Extrinsic Mechanisms of Axonal Regeneration	27.05.2014	Brussels, Belgium	Scientific Community, esp. young researchers	56	Global
5	Conference	MHH	3rd International Symposium on Peripheral Nerve Regeneration	24.-25.09.2015	Hannover, Germany	Scientific Community	173	Global
6	Open days	MHH, UNITO, ALTAKITIN	"November der Wissenschaft" and various	Throughout the project lifetime	Hannover, Germany, Turin, Italy, Portugal	General public		National/ regional
7	Online media	MHH	Project website and HorizonHealth	October 2011 and November 2013 and throughout the project lifetime	www. http://kongress.mh-hannover.de/biohybrid/6349.html	General public and scientific community		Global/ EU wide
8	Press releases, newspaper articles and radio Interview	MHH	Following the publication of *Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects* in Biomaterials 34 (2013). Interview on_ Deutschlandradio Kultur	October/ November2013	Hannover, Germany	General public and scientific community		National

** **Some smaller events are summarised**

†† A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

‡‡ A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

Section B (Confidential[§§] or public: confidential information to be marked clearly)
Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[***]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	YES	n/a	EP12163992.6	Manufacturing an N-acylchitosan article with a memorized shape	MEDOVENT
Patent	YES	n/a	US13/850,568	Processing of acylchitosan hydrogels	MEDOVENT
Trademark	NO	n/a	012294492	Reaxon	MEDOVENT
Patent	NO	n/a	102015000071499	Coniugato della neuregulina 1 per il trattamento delle lesioni dei nervi periferici . (Enhancing effect of Neuregulin1 on the peripheral nerve regeneration through covalent conjugation to iron nanoparticles)	UNITO and NVR

[§§] Not to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[***] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Type of Exploitable Foreground[†††]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[‡‡‡]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial exploitation of R&D results	Processing of chitosan tubes	NO	n/a	Nerve conduit	C32.5 - Manufacture of medical and dental instruments and supplies	Nerve conduit (Reaxon® Nerve Guide) in commercial application since 2014	US Patents 8,414,925, 9,034,379; CA Patent 2,622,342; EP applied	MEDOVENT

Exploitable foreground includes technology for the processing of chitosan tubes which can be further processed into nerve guidance channels. Several **international patents** have been issued to Medovent to protect this unique technology. **Medovent has started the commercialization of a novel chitosan-based nerve conduit (Reaxon® Nerve Guide) in 2014**. Related technology is protected in pending patent applications (see Part B1). **Further research includes novel designs** to allow the application of nerve conduits for large gaps. The socio-economic impact is enormous, considering the **high number of patients** who suffer from nerve injuries (more than 100,000 annually in Europe alone). **Reaxon® Nerve Guide has the potential to overcome significant limitations of the currently used materials**, thereby providing much benefit to the trauma patient and health economic system (see Section 4.1.4.1).

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[‡‡‡] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

3.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	EU-FP7-278612
Title of Project:	BIOHYBRID: Biohybrid templates for peripheral nerve
Name and Title of Coordinator:	Prof. Dr. rer. nat. Claudia GROTHE

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
• Did the project involve Human biological samples?	No
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	Yes
• Were those animals transgenic small laboratory animals?	No
• Were those animals transgenic farm animals?	No
• Were those animals cloned farm animals?	No
• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	No
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	No
DUAL USE	

• Research having direct military use	No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	0
Work package leaders	1	7
Experienced researchers (i.e. PhD holders)	20	13
PhD Students	7	5
Other	11	13

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	11
Of which, indicate the number of men:	7

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

○ Yes
✓ No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

✓ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

✓ Yes- please specify: Open days such as November der Wissenschaft in Hannover, Germany, Talks at graduate schools etc.

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

✓ Yes- please specify: In vitro standard protocols; Handbook in vitro; In vivo standard protocols; Handbook in vivo

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

✓ Main discipline[12]: 3

✓ Associated discipline: 1.5

✓ Associated discipline: 2.3

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

○ Yes
✓ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

✓ No

[12] Insert number from list below (Frascati Manual).

- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- ○ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ Yes ✓ No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ✓ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ✓ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		***Public Health***	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ✓ National level
- ✓ European level
- ✓ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**42**
To how many of these is open access[13] provided?	**19**
How many of these are published in open access journals?	**6**
How many of these are published in open repositories?	**2**
To how many of these is open access not provided?	**23**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[14]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**1**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**1**
	Registered design	
	Other	
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	✓	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
✓	Difficult to estimate / not possible to quantify		

[13] Open Access is defined as free of charge access for anyone via Internet.
[14] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
	?
Difficult to estimate / not possible to quantify	✓

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ✓ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes ✓ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

✓	Press Release	❑	Coverage in specialist press
✓	Media briefing	✓	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
✓	Radio coverage / report	❑	Coverage in international press
✓	Brochures /posters / flyers	✓	Website for the general public / internet
❑	DVD /Film /Multimedia	✓	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

✓	Language of the coordinator	✓	English
✓	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

5. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1. MHH	977,600.00
2. UAB	472,400.00
3. ULUND	400,400.00
4. UMINHO	400,400.00
5. SMC	400,400.00
6. UNITO	508,400.00
7. NVR	1,002,400.00
8. MEDOVENT	1,250,400.00
9. ALTA	335,600.00
10. MED-TUM	7,200
Total	**5,755,200.00**