1. Executive Summary

Peripheral nerves are basic communication structures guiding motor and sensitive information from CNS to effector or receptor units. Severe nerve injuries include axon bundles section and Schawnn cells destruction, which results in loss of motion control and sensorial perception. After the lesion, cells present in damaged nerves activate spontaneously self-regeneration programs that might facilitate further treatment. Nerve autograft is the “gold standard” surgical intervention that demands autologous tissue extraction and corresponding function loss. The goal of the project is the validation of biomaterials structural plasticity and those compatible manufacturing technologies that will enable the generation of a tubular structure containing an intraluminal microstructure based on an array of aligned channels or fibers. The regenerative properties of this prototype will be also validated *in vivo* in a sciatic nerve section animal model. This project proposal will take advantage of partners’ experience in the design of medical devices composed of natural and synthetic biomaterials and in scaled-up production mechanization technologies for the generation of the most effective peripheral nerve implant.

2. Summary description of the project context and main objectives

2.1 Concept of the Project

Post-traumatic peripheral nerve repair is one of the major challenges in restorative medicine and microsurgery. Primary causes of damage are traumatic accidents, tumour resection, iatrogenic side effects of surgery or repetitive compression (tunnel syndromes). **At present, peripheral nerve injuries are cause of medical consultation in more than 1,000,000 patients per year in the United States and Europe, with more than 100,000 cases undergoing surgery**. Severe nerve injury has a devastating impact on patients' quality of life. Typical symptoms are sensory and motor function defects that could result in complete paralysis of an affected limb or development of intractable neuropathic pain. Despite the progress in understanding the pathophysiology of peripheral nervous system injury and regeneration, as well as advancements in microsurgical techniques, peripheral nerve injuries are still a major challenge for reconstructive surgeons.

The surgical treatment for the complete severing of a nerve with small gap length (≤5mm) and no loss of tissue is direct suturing of opposite nerve stumps. In this particular case, nerve co-aptation with fascicle alignment and tension-free suturing is feasible because peripheral nerves are phenotypically driven to regenerate spontaneously following injury. However, when direct suturing is not possible because it would cause tissue tension affecting nerve regeneration or when there is a discontinuity (> 1 cm) between both distal and proximal stumps, tissue engineering approaches are required. During nerve regeneration, axons grow randomly forming a nerve fibre mass called bands of Büngner. Unless surgically intervened, these regenerative sprouts will result in complete axonal degeneration affecting motor control and sensory perception. Nerve autografting is still the gold standard technique for nerve gap repair. Autografts are primarily taken from purely sensory nerves, since this allows the obtaining of longer grafts with lower donor-site morbidity than from motor or mix nerves, as the primary complication is often temporary localized numbness rather than a motor deficit. The most commonly donor source is the sural nerve, which allows for the harvest of up to 50 mm of nerve graft (up to 30 mm nerve gaps), with quite well-tolerated adverse effects ranging from sensory deficit around the lateral foot (9,1 - 41% of patients), to neuroma formation and unbearable pain (6,1 - 8,1% of cases). Autograft has several disadvantages such as limited sources of donor nerve, the need for a second surgery to obtain the donor nerve, loss of nerve function in transplantation, and a lack of correspondence between the repaired nerve and the graft for the cross sectional area. **According to these drawbacks, the success rate in patients treated with sural nerves is limited to 50%.**

Thanks to progress in the field of tissue engineering, it appears increasingly possible to use artificial conduits for reconstruction of nerve gaps. **Implantable nerve guidance conduits (INGCs)** offer a promising alternative to conventional treatments, supporting and guiding the axons during their growth, while avoiding scar tissue infiltration in the gap. Fundamental requirements for effective nerve tissue regeneration demands a tubular scaffold that should be biocompatible, have sufficient mechanical stability during nerve regeneration, be flexible (with mechanical properties close to that of nerve tissues to prevent compression of the regenerating nerve), be porous to ensure supply of nutrients, and degrade in a proper time (after nerve regeneration) into nontoxic products to prevent long-term irritation.

Biodegradable materials, of either synthetic or natural origin, have been studied for INGCs production because they offer several advantages. The most interesting one is that biodegradation acts on biomaterial as a time-controlled elimination system as well as avoiding nerve compression and fibrosis formation. Between them, synthetic polymers show highly tunable possibilities, as variations in their

chemical or engineering properties may change biocompatibility, degradation behaviour, flexibility, porosity, and mechanical strength. On the other hand, natural-derived materials show a good biocompatibility, although most lack adequate mechanical strength and water stability and thus need cross linking.

To date, several single tubes developed using synthetic or natural polymers have shown to be capable of physically guiding the linear growth of regenerated axons to some extent. Some of them are now available in the market as will be described later. A major problem of these hollow INGC in the market comes from **axon misdirection**: Oriented structures still differ substantially from the guiding basal lamina microchannels in nerve autografts, and hence lead to very limited positive outcomes in the patient's recovery, too short degradation time and inappropriately high Young´s modulus (linked to the limited range of biomaterials used for hollow tube production). Thus, for these commercial tubes, there is a mismatch in mechanical properties between the device and native nerve tissue (Ultimate tensile stress of ≈11.7 MPa for rabbit tibial nerve). Another drawback of tubulization with empty hollow tubes is the maximum nerve gap allowable for successful recovery: 3cm nerve defects. Due to these limitations, **commercially available hollow conduits fail to match the regenerative levels of autograft.** For pronounced discontinuities (> 3cm), 3D scaffold based on processed nerve allografts are used for bridging the nerve gap. Disadvantages of this nerve allografting include the risk of rejection and complications related to immunosuppression. An improved alternative to hollow tubes, similar to nerve allografts and currently in the market, are decellularized mammalian implants that contain the structure of a mature nerve. However, these implants cost thousands of Euros each unit and are exposed to anatomical variability.

Since the basal lamina microchannels in nerves are known to play a significant guiding role in the linear growth of regenerating axons, INGCs with architecture and dimensions resembling the basal lamina microchannels sutured to nerve stumps are expected to provide a promising alternative for bridging nerve gaps. Thus, recently, several designs have been proposed to construct **biomimetic tubular devices** that incorporate an internal structure in the lumen of the tubes aiming to increase the regenerative surface, providing an array of aligned conduits to direct nerve regeneration through the tube and minimizing axon disorientation. *In vivo* tests (rat, dog and mouse) have shown that nerve recovery is significantly greater in these biomimetic devices than in a hollow conduit. However, the designs proposed to date **lack the mechanical properties required to mimic those of the native nerve tissue**. Regenerative properties of these devices strongly depend on the intraluminal channel (or fiber) density and, again, there is **no**t **a general consensus about the number (and dimension) of intraluminal channels needed to bridge a nerve gap higher than 3 cm**. Thus, these drawbacks lead to a decrease in nerve recovery ability of these biomimetic tubular devices compared to nerve autograft. Additionally, proper **scale – up** of the manufacturing technologies used for obtaining biomimetic tubular devices at lab scale is required to ensure the final industrialization of the product/device, covering different range of gaps in a cost – effective manner.

In this scenario, **NEURIMP project** aims to produce a novel biomimetic nerve prostheses paying special attention to **device structure, biomaterials and their combination with high throughput manufacturing methods, which play a vital role in the industrialization process.** In a first stage, **NEURIMP** will study those biomaterials previously tested in pre-clinical *in vitro* and *in vivo* experiments and clinical trials, having shown a positive outcome for nerve tissue regeneration due to axon growth facilitation, myelinating cell proliferation, reactive gliosis inhibition, anti-inflammatory activity and revascularization. These materials will be evaluated in terms of manufacturability, analysing their ability to be processed into predefined microstructures to address the challenges described above. In a second stage, new formulations of biomaterials, based on the prior study, and

combinations of different materials (copolymers and blends) will be developed to overcome the limitations observed in the first stage. The main objective here is exploring which of the most promising biomaterials (natural and synthetic), or combinations thereof, are compatible with the recently established technologies, potentially scalable to generate valid microstructures containing nerve devices. **The final goal is to scale up the biomaterial production and manufacturing technologies in order to generate a next generation of peripheral nerve devices that will overcome the limitations of state of the art INGCs in terms of regenerative capacity, biodegradability, physical properties and manufacturability.**

The INGC proposed in this project consists of a combination of stiffer and softer materials where the biological and physicochemical properties of each biomaterial will define the role that it will play into the scaffold and therefore, its location in it. Stiffer materials will be required to create the external suturable tube and the internal skeleton core of the scaffold that will confer structural stability to the device during the regeneration period. Softer hydrogels, will be incorporated into the lumen of the channels created in the axonal regenerative core. The device will contain a set of intraluminal structures (channels or aligned fibers) especially designed to optimize axon contact surface, improve guided axon growth and bridge nerve gaps larger than 3 cm. The idea behind the **NEURIMP** concept is **to develop a 3D scaffold mimicking the nerve tissue leading to positive outcomes close to 100% for short (<20 mm) and large gaps (>30 mm and up to 50 mm)**. These figures for positive outcomes would generate **an increase in the market value respect to that associated to hollow tube conduits and allograft implant**. Moreover, **NEURIMP** is intended to reduce the cost for peripheral nerve implant as well as to avoid dependence on potential donors or complicated and expensive removal of cellular components as needed for allograft implants. A successful **NEURIMP implant in large limiting nerve gaps will be considered to promote an earlier and more effective regeneration in case of being implanted in smaller nerve gaps (<20 mm), overcoming the limitations of single tube conduits linked to axon misdirection.**

NEURIMP will select candidate biomaterials according to their biocompatibility, mechanical properties, biotoxicity and biodegradability, evaluating their adequacy as a function of these parameters and nerve cell proliferation, axon growth facilitation and myelination in a **2D and 3D *in vitro* system**. Optimization of biomaterial composition and configurations (blends) will be developed according to these in vitro tests. **NEURIMP** will implement state-of-the art technologies to generate the desired absorbable implants, restricting the selection and development of **novel biodegradable materials to those that will enable production of INGCs for peripheral nerves with manufacturing technologies that permit the spatial resolution needed for the construction of the internal microchannels array.** Dimensions to mimic the nerve, referring to the size, shape and distribution of intraluminal structures, as well as, the dimensions of the external tube will be determined from final resolution of each fabrication technology, in vitro degradability analysis and validated in ***in-vivo* experimentation in rats**. **NEURIMP** will define the production protocols on the basis of optimal biological properties, technical feasibility of manufacturing and economic costs and will scale-up the production of limited pilot prototypes according to the combination of biomaterials and manufacturing technology. These prototypes will be tested for biodegradability in a 3D *in vitro* system, as well as biodegradability and biocompatibility in an *in vivo* model in rats, analyzing growth facilitation and myelination in both systems. Finally, INGCs produced in **NEURIMP** will be validated *in vivo* by evaluating functional recovery and nerve regeneration into the device implanted in a rat sciatic nerve defect model.

NEURIMP will consider all regulatory affairs as an integral part of the project: Medical Device Directive (MDD) 93/42/EEC, standardization (ISO standards), manufacturing (GMP), developing an IMPD at the end of the project. NEURIMP medical device will be fabricated according to the relevant provisions of the Medical Device Directive (MDD) 93/42/EEC and to assure that the medical device is safe for use and functional as intended. It will ensure that all biomaterial synthesis, protocols for manufacturing, scale-up of the combination biomaterials-technology for production and *in-vitro* and *in-vivo* experiments are accorded to regulation. Therefore, if successful, the component could be ready for introduction in clinical trials in humans at the end of the project.

2.2 Objectives of the project

Objective 1: Develop advanced synthetic-natural biohybrid materials with improved biocompatibility and biodegradability (18 – 24 months), regenerative capacity (nerve gaps > 3cm) and mechanical properties (≈ 11.7 MPa) suitable for the generation of 3D micropatterned structures comprising with selective porosity and controlled degradation. Determine the optimal physical parameters required by biomimetical endoneural tubes to pave for an efficient regeneration of both sensory and motor axons. Scale – up biomaterial production to industrial levels.

Objective 2: Develop advanced manufacturing technologies for the generation of biomimetic endoneural tubes with precise morphologies and sizes (intraluminal microchannels or fibers with high aspect ratio). Scale – up manufacturing technologies to industrial levels.

Objective 3: Understand the interplay between scaffolds and the endothelial cells, the Schwann cells and neurons (via in vitro assays) to promote the generation of Bands of Büngner and revascularization inside the INGCs, and provide the trophic and tropic conditions for an optimal axonal regeneration and remyelination.

Objective 4: Design, fabricate and optimize a new generation of Neural Guides composed of two clearly differentiated parts: i) An outer wall with selective porosity for nutrient exchange and a slow-degrading degradation ratetax to reduce fibrosis, to protect the newly formed nerve cord, and to provide physical stability that avoids the INGC collapse while regeneration progresses; ii) An inner endoneural-like microstructure to provide a topographical axonal regeneration. This part will be composed of a biomaterial with a regulated degradation tax according to the repaired gap length, being replaced once the Bands of Büngner and the axons have regenerated across the INGC. Afterwards, the system would behave as a natural interstump reconnecting nerve cord, and thus avoid secondary compressive damage of regenerated axons.

Objective 5: Characterize, in a clinically relevant animal model of sciatic nerve injury, the performance of the produced INGCs for key parameters such as the maximum gap length that can be repaired, their ability to promote the regeneration of both motor and sensory axons, and their ability to pave for precise target reinnervation with as resulting in improved functional recovery. Comparison to regenerative capacity of autografts.

Objective 6: Scaled up production of the new generation of INGCs taking into account standards, regulatory affair and economical issues. Exploitation plan.

3. Description of the main S&T results/Foreground

WP1 Development, synthesis/production and characterization of natural and synthetic polymers to be used in the manufacture of the nerve guidance conduits.

The objective of this work package was the production and characterisation of both natural and synthetic polymers with predefined physicochemical properties pertinent for the development of novel nerve conduit. Three main polymers that have been investigated within this work package include highly hydrophilic, hyaluronic acid (natural), a family of natural polyesters, Polyhydroxyalkanoates and synthetic polymers, Polylactide and Polycaprolactone.

Task 1.1. Production and characterisation of natural polymers (Polyhydroxyalkanoates and Hyaluronic acid)

The primary objective of this task was to carry out microbial production of Polyhydroxyalkanoates (PHAs) and hyaluronic acid (HA) with the range of physicochemical, mechanical and biological properties suitable for their use in the fabrication of nerve conduits. UoW focused on the production of a range of novel PHAs via bacterial fermentation. Extensive screening experiments were carried out using different bacterial strains, carbon sources and operating conditions. Materials were characterized chemically using FTIR, GC-MS and NMR. Molecular weight determination was carried out using GPC, mechanical properties were analysed using tensile testing, whereas thermal characterisation was carried out using DSC. Four different PHAs such as P(3HB), P(3HO), P(3HO-3HD) and P(3HO-3HD-3HDD) were successfully produced. By Month 12, biosynthesis of these novel PHAs were completed and corresponding results were reported in *Deliverable 1.1 "Production of a range of scl-PHAs and mcl-PHAs, their purification and characterization"*. These biomaterials were found to have a wide range of properties, in compliance with the requirements of NEURIMP DoW. In addition to the large scale production of the chosen PHAs, UoW also synthesized oligomeric PHA derivatives via acidic depolymerisation for both scl- and mcl-PHAs. These oligomer derivatives, characterised by higher acidic numbers, were further functionalised in Task 1.3. In parallel, CONTIPRO focused on the microbial production of HA. Furthermore, they focused on the derivatisation of HA for the synthesis of cross-linkable, biocompatible and biodegradable materials. The covalent bonding of several commercially available photo-crosslinkable linkers (α,β-unsaturated carboxylic acids) was studied. The synthesis was carried out reproducibly and scaled up successfully. The outcomes of this task was reported in detail in *Deliverable D1.2 "Production and characterisation of a range of purified HAs and crosslinked HAs"*.

Task 1.2. Production and characterisation of synthetic polymers (Polylactic acid (PLA), polyglycolic acid (PGA) and poly-ε-caprolactone (PCL) and their copolymers)

The main aim of this task was to develop synthetic polyesters to be used for nerve conduit fabrication as material 1 and material 2. PLA and PCL were successfully synthesized in $scCO_2$ and *Deliverable 1.3 "Production of Polylactic acid (PLA), Polyglycolic acid (PGA), Poly-ε-caprolactone (PCL) and their copolymers and their characterization"* was successfully completed by Month 12. After extensive testing, it was established that neat PLLA was too stiff to be considered as the material of choice. Additionally, PCL was deemed unsuitable with poor performance in early material trials. To overcome this, copolymers of lactic acid and caprolactone were successfully synthesised in $sc\text{-}CO_2$ using ring opening copolymerisation. Copolymers of wide compositional range have been synthesised. The caprolactone content was varied between 10 and 40 mol%. Strength and stiffness of copolymers decreased with the increase in caprolactone content. The most ductile copolymer with caprolactone content was synthesised at 25 g scale and resulted in copolymer of high purity. With dedicated time and effort, Vornia were able to synthesize of 60 grams in a time period of 40 hours. Additionally,

photocurable precursors based on PCL and PLA were also developed within Task 1.2. As a result, *Deliverable 1.4 "Production of photocurable forms of Polylactic acid (PLA), polyglycolic acid (PGA), poly-ε-caprolactone (PCL)"* was completed successfully. Derivatisation was validated with a combination of FT-IR, ^{1}H NMR.

Task 1.3. Production of unique innovative novel materials including synthetic and natural polymers

The primary objective of this task was to develop novel blends of medium chain length Polyhydroxyalkanoates (mcl-PHAs) with Poly(3-hydroxybutyrate) (P(3HB), and the synthetic polyesters. Based on the physicochemical properties and processability, natural and synthetic polymers were chosen for the development of these blends. The rationale behind developing these novel blends was to achieve a variation of mechanical properties in order to narrow the gap between a group of stiff, brittle materials and a group of soft, ductile materials, resulting in PHA-based blends with different mechanical and biological properties, biodegradability profiles and processability suitable for the development of nerve conduits. From the R&D work, it was understood that the mechanical properties of mcl-PHA/P(3HB) blends were dependent on the compatibility of the blend components. Mcl-PHA-enriched blends were found to be highly ductile with mechanical properties suitable for the fabrication of nerve conduits. Blends of natural mcl-PHAs and synthetic polyesters poly-(L-lactic acid) and polycaprolactore (PCL) were obtained. These blends were immiscible which resulted in significant decrease of ductility even for the blends with low content of PLLA. However, small PLLA additives significantly improved the Young's modulus which was even higher than that induced by P(3HB). These novel biodegradable blends were reported in the successfully completed *Deliverable 1.5 "Production of novel materials as blends of PHAs/PLA/PGA and PCL.*

Additionally, novel Hyaluronic acid (HA) based conjugates were developed to enhance the processability of the biomaterials developed within this task. Bioconjugates of HA-P(3HB), HA-PCL, HA-PLA and HA-PLGA were considered as alternative hybrid materials, which expected to meet the biological, physical and manufacturability properties desired for nerve conduits. For the development of P(3HB)-HA conjugates, P(3HB) was hydrolysed in an acidic media. NMR analysis validated the acidic hydrolysis. Thermal characterization was carried out to check batch variability. This was carried out at the University of Westminster. Based on the cytotoxicity results, the HA-P(3HB) conjugates were focused on and conjuagates of acceptable purity was synthesized in two separate reactions. The first reaction was carried out in the presence of trichlorobenzoyl chloride (TCBC) and benzoyl chloride (BC) whereas the second reaction was carried out in the presence of N,N′ -Carbonyldiimidazole (CDI). Both the reactions were successfully used to graft P(3HB) to HA. The success of HA grafting was analysed using 1D and 2D NMR. Ash determination validated the reproducibility between batches after chemical modification.

Following this, other PHAs such as Poly(3-hydroxyoctanoate),P(3HO), was also covalently grafted to HA. Hydrolysed P(3HO) was successfully grafted to HA and characterized using Diffusion Ordered NMR Spectroscopy (DOSY). From the R&D work, it was understood that the polarity of the solvent was crucial for the formation of linkages. *In vitro* biocompatibility studies using NIH-3T3 cells on the P(3HB)-HA conjugates confirmed their cytocompatible nature. In addition to PHAs, PLGA was successfully grafted to HA using a new reaction pathway (amidation). Outcome of these studies have been reported in deliverable *D1.6"Production of novel hydrogels with HA in combination with PHA/PLA/PGA(or PLGA)/PCL"*.

Optimization of the production of both natural and synthetic polymers

In months 18-24, the production of the chosen natural and synthetic materials were optimised. From the synthetic polymers PLCL6040 was identified as the most promising material candidate of Vornia's synthetic polymer range. The co-polymer showed excellent physico-chemical properties, particularly in terms of flexibility and degradation rate. Considerable effort was put into optimising the production protocol and the characterization, in view of large-scale commercial production. At the University of Westminster (UoW), production of P(3HB) and mcl-PHAs was initiated in tandem. Extensive optimization studies were conducted by varying process parameters such as air flow rate, pH, stirrer speed, media composition and the mode of fermentation. The overall objective was to augment biomass as well as PHA yields. As a result of these optimisation studies, there was a threefold increase in the polymer yield and a 7-fold increase in the amount of biomass produced. This was considered significant since a wild type bacterial strain was used for all the fermentation processes.

Key results:

- Successful production of four novel different PHAs including scl and mcl-PHAs such as (P(3HB), P(3HO), P(3HO-3HD), P(3HO-3HD-3HDD). Synthesis of these novel PHAs was completed by Month 12. These polymers were found to have a wide range of values, in compliance with the requirements of the NEURIMP DoW. Hence, a proper combination of these PHAs leads to the formulation of natural materials that meet the requirements established for the final implant. These novel PHAs have now been patented (UK Application no. 1714816.4).

- Synthesis of copolymers of PLA and PCL with improved elasticity and lower glass transition temperature (Vornia). Inclusion of both ethylene glycol and caprolactone monomers to form novel lactic acid copolymers was selected as a strategy for the improvement of material properties. Mechanical properties of this copolymer closely matched the requirements for the material for nerve conduit production. Blends of natural mcl-PHAs and synthetic polyesters (poly-(L-lactic acid)) showed significantly improved Young's modulus values.

- Novel blends of medium chain length Polyhydroxyalkanoates (mcl-PHAs) with Poly(3-hydroxybutyrate) (P(3HB), and synthetic polyesters with properties suitable for nerve conduits were developed successfully. These blends supported neuronal cell attachment, spreading, proliferation and neurite extension *in vitro*.

- Two photocurable derivatives of HA were synthesized and used in WP2 for the processing into aligned tubes. PCL and PLLA were successfully methacrylated and consequently rendered photocurable (US). These synthetic materials can be handled to manufacture internal and external tubes via microstereolithography and casting technologies.

- Bioconjugates of HA-P(3HB), HA-P(3HO) and HA-PLGA were synthesized for the first time. The novel HA-PHB conjugates were used for incorporation in a hydrogel and in a nanofiber mat as part of WP2 activities.

Work Package 2. Design and fabrication of microcomponents for peripheral nerve regeneration

WP2 was comprised of four objectives, which were structured according to four work package tasks:

Objective 1 + Task 2.1 - Define precise physical dimensions of sciatic nerve for the *in vivo* model to be used (USFD and HNP). *Duration – 3 months (1-3).*

Objective 2 + Task 2.2 - Manufacture nerve guides from materials produced in WP1 as a tube by microSL (TKN and USFD). *Duration – 21 months (3-24).*

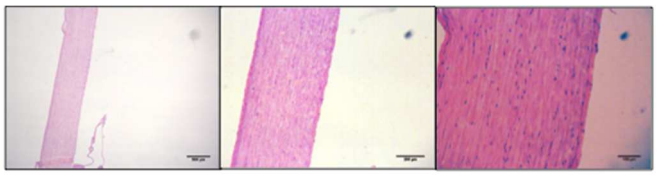
Objective 3 + Task 2.3 - Manufacture nerve guides from materials produced in WP1 as a tube containing microchannels (Tekniker and USFD). *Duration 21 months (3-24).*

Objective 4 + Task 2.4 - Manufacture aligned electrospun filaments from materials produced in WP1 (nano to micrometer range) and combine with nerve guide tubes made by microSL (USFD and Contipro). *Duration 18 months (6-24).*

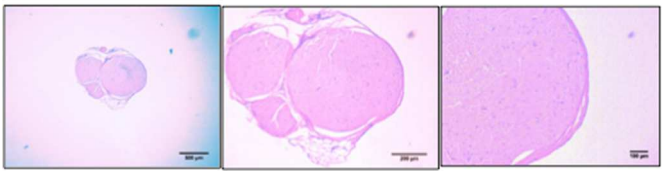
Objective 1 + Task 2.1 - Define precise physical dimensions of sciatic nerve for the in vivo model to be used (USFD and HNP). *Duration – 3 months (1-3).*

Introduction - for the precise manufacture of external nerve guide tubes in advance of evaluation in the rat sciatic *in vivo* nerve model of partner HNP-SESCAM, it was necessary to be supplied with rat nerve samples from there model for anatomical and histological analysis. We took approximate dimensions from the literature, and compared them with actual nerve dimensions to ensure that the micro-fabricated devices produced fitted the rat sciatic *in vivo* model of partner HNP-SESCAM. HNP supplied 4x fixed and 4x blocked rat sciatic nerves (left and right from 3x animals) to partner US who undertook transverse and longitudinal histological sections. Blocks were be stained by haematoxylin and eosin (H&E) to ascertain the average: i) epineurial diameter; ii) the average number of fascicles per nerve and average diameter and iii) the number of average myelinated axons and average diameter per nerve. This information was used to inform the inner diameter of the outer tube structure produced by microSL (with a typical increase of 50% for the inner diameter of the original nerve to allow for swelling induced by the transection injury of the *in vivo* model.) Information on the number of fascicles per nerve was also deduced to inform the exact number of channels per device.

Key results



H&E of rat sciatic nerve in longitudinal (top panel) and transverse (bottom panel) section, showing low (left), intermediate (middle) and high (right) magnification images.



From this, quantification of nerve diameter and fascicles was established. Transverse sections were used to provide data (N.B. longitudinal measurements were also deduced, but not considered reliable due to point of section uncertainty).

Epineurial diameter

Minimum **= 900 μm ± 23 μm** (n=4)

Maximum **= 1183 μm ± 85 μm** (n=4)

Transverse section measurements - Maximum diameter (plus epineurium)

a) Tibial branch **= 692 μm ± 30 μm** (n=4)

b) Peroneal branch **= 404 μm ± 44 μm** (n=4)

c) Sural branch **= 252 μm ± 41 μm** (n=4)

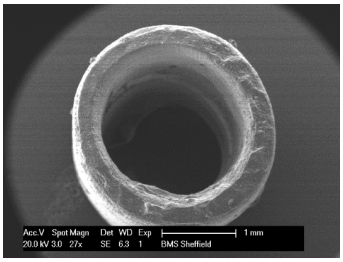
d) Cutaneous branch **= 90 μm ± 25 μm** (n=4)

The above data was compared with and was comparable to data published in *Schmalbruch, H. (1986) Fiber composition of the rat sciatic nerve. Anat Rec; 215 (1): 71-81.* A 50% addition was made to the identified size for device fabrication to take account of nerve swelling from injury.

Objective 2 + Task 2.2 - Manufacture nerve guides from materials produced in WP1 as a tube by microSL (TKN and USFD). *Duration – 21 months (3-24).*

Introduction - A major aim of NEURIMP was to micro-fabricate a range of biocompatible and biodegradable nerve guides from natural and synthetic materials. We considered a range of manufacturing technologies (microSL, microinjection molding and microextrusion) to broaden the number of polymer versions to include HA, PCL, PHAs (including P3HB) and PGA copolymers. Acrylated pre-polymer forms of polymers manufactured in WP1 were used as substrates to fabricate materials into nerve guides by microSL, by adding methacrylate end groups. Photocurable resin mixtures were used to construct 3D external guide structures by microSL. Resins were placed into a receptacle and platform lowered at a constant velocity into the resin and hence the in situ construction of prototype devices. The internal diameter of sciatic nerve defined by the H&E in task 2.1 was used initially to make a range of PCL tube sizes for testing in the HNP model (internal diameter - 0.9mm, 1.1mm, 1.3mm, 1.5mm, 1.7mm). High-throughput manufacturing technologies (microextrusion and microinjection molding) were also evaluated for fabricating external tubes. Material modifications were evaluated to obtain a polymer material suitable for injection molding and / or extrusion. In the case of microinjection molding and extrusion, a micro-mold was designed and manufactured to replicate the geometry of the external tube, based on work in task 2.1 and the microSL outcome of task 2.2. Different mold designs for replication of the internal component (as channels) considered demolding forces (versus friction).

Key results

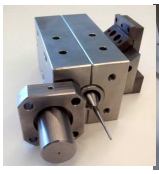


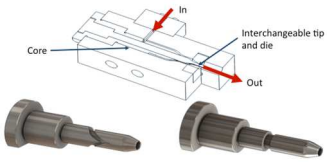
PCL tubes with 250 μm wall thickness and 0.9, 1.1, 1.3, 1.5 and 1.7 mm internal diameter and 8mm length were sent to HNP for compatibility with the *in vivo* model.

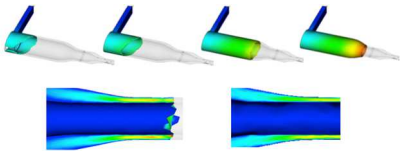
Inner diameter = 1.5 mm

Wall thickness = 250 μm. Length = 8 mm

Production of photocurable PCL was straightforward, and led to the production of devices above. Photocurable PHA synthesis proved challenging. Two approaches were taken: i) Transesterification, in which the bulk polymer size was reduced in order for the polymer to reach a more optimal size for methacrylation and ii) using a hydrolysed form of PHA. PHBv was used as a starting material due to availability compared with other PHAs. While both approaches yielded potential methacrylated products, variations in tube manufacture arose run to run, with meniscus effects, varying amounts of materials required, and irregular shapes, with neither generated material suitable for structuring NGCs. Methacryalted HA was discarded as not being suitable when cured, as HA structures did not have



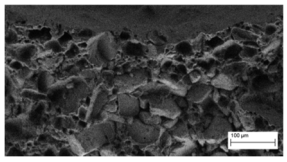
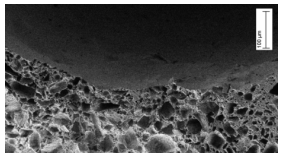
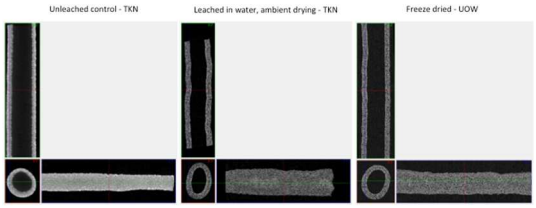


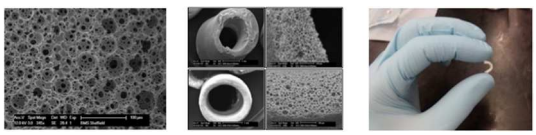


sufficient structural integrity to form layers greater than nanometer length scale. In contrast, microextrusion was successfully employed to extrude single lumen tubes.

At this point in the programme a new task was included – the introduction of micropores in to the external fabricated tube. The rationale was that such structures would allow the exchange of nutrient and waste product exchange, and in so doing encourage the wound bed repair process and facilitate axonal regeneration and potential reinnervation. Porogen incorporation was considered using NaCl or glucose crystals, and was successfully employed in the extrusion of single lumen tubes using a combination of polymer / porogen materials summarized in the table below. In conjunction, an extrusion die head and extrusion system was designed and constructed for the fabrication of tubes. The filling of the die was analyzed by finite element and core designs compared for polymer velocity and density at the die outlet. Spiral path solution and eccentric designs showed similar polymer behaviour, so an eccentric design was been selected based on it being more economical, easier to use and modify.

Extrusion tests were conducted combining porogens and two leaching methods: i) ambient drying and ii) freeze drying, with samples analysed by SEM below showing before (left) and after (right) leaching, and sent to USFD for microCT analysis.



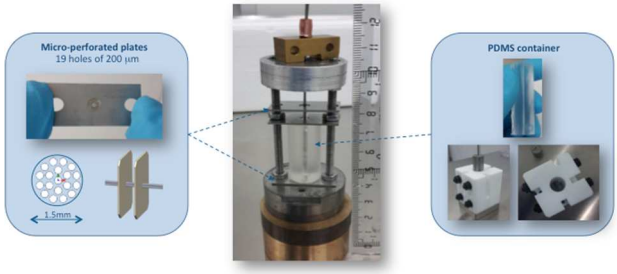
An additional method for porogen introduction was also studied in the form of polymer high internal phase emulsions (pHIPEs). These tubes while being restricted to PCLm, and could be fabricated by microSL and microextrusion with inclusion of UV as a catalyst.

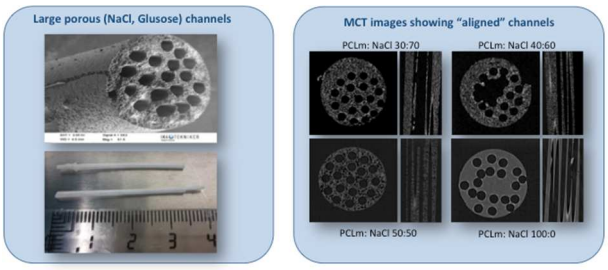
Thus, tubes could be extruded with ID = 1.6 mm or 2 mm with connected porosity (across a range of 1-10 μm) and tube lengths of 20-30 cm, and in turn cut to a desired length as required for the *in vivo* model (10-15 mm).

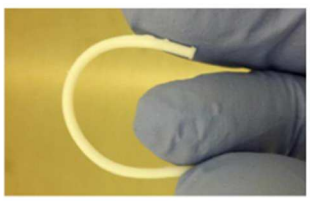
Objective 3 + Task 2.3 - Manufacture nerve guides from materials produced in WP1 as a tube containing microchannels (Tekniker and USFD). *Duration 21 months (3-24).*

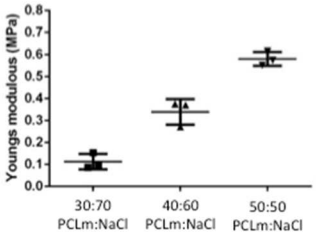
Different manufacturing processes were studied to analyze the channel fabrication suitability as function of the material and processability characteristics: i) photopatterning via microSL; ii) photopatterning-based 3D micromoulding; iii) soft-lithography-based 3D micromoulding; iv) injection and cast micromoulding and v) microextrusion. Photopatterning and soft-lithography based micromoulding was developed but was deemed unsuitable due to the large number of holes and length (cm) required for state-of-the art nerve guide alignment. Photopatterning via microsterelithography was also investigated but a maximal theoretical lateral resolution of 10 μm and a practical working resolution of 50 μm (using PCLm), produced difficulties in maintaining the regularity of channels throughout the NGC, most likely due to polymer viscosity. Thus, micromoulding technologies were optimized and used with UV-methacrylated materials, which included the design of micro perforated plates containing 19 holes of 200 μm diameter. Mechanical testing was developed in final prototypes to validate the mechanical properties, and found to mirror those measured for a rat sciatic nerve. En route, thermal and solvent casting was evaluated, but optimal processing was obtained using UV casting. Thus, UV cast microchanneled porous PCLm was produced, which recovered its shape after manual handling and was flexible when leached.

Material : Porogen	Porogen size and concentration
PLCL6040 : NaCl	<63um 70%wt
P3HOcoHD/P3HB 95/5 : Glucose	>25um <50um 70%wt
PLCL6040 : NaCl	>25um <50um 70%wt









Borschel et al. (2003) = rat sciatic nerve Young's Modulus of 0.58 ± 0.015 MPa.

PCLm/NaCl 50:50 = Young's modulus of 0.58 ± 0.016 MPa

Objective 4 + Task 2.4 - Manufacture aligned electrospun filaments from materials produced in WP1 (nano to micrometer range) and combine with nerve guide tubes made by microSL (USFD and Contipro). *Duration 18 months (6-24).*

HA, PHA, P3HB and PGA copolymers were produced by aligned fibre electrospinning, with sheets spanning a range of uniform diameters (100 nm to 5 µm). Polymers were dissolved suitable solvents (DCM / chloroform) or water/alcohol solution and spun using a high mandrel speed rig (2000 rpm), with processing conditions devised for each polymer (flow rate, voltage, needle/emitor to collector distance, needle size, collector speed and spin duration time, in conjunction with polymer molecular weight and concentration. Fibre physical dimensions were determined by SEM, and the best performing fibres determined by *in vitro* cell culture using neuronal and Schwann cells in WP3. Selected candidate polymer materials were taken forwards to WP3 for *in vitro* evaluation:

1) PCL (5µm and 8µm fibres);

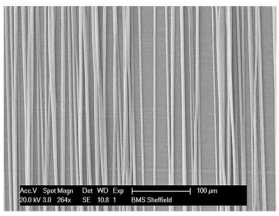
2) P(3HO-co-3HD):PLLA (50:50) (5µm and 8µm fibres);

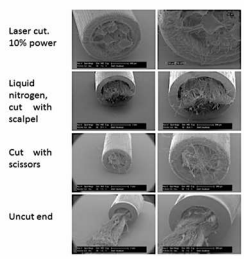
3) P(3HB):P(3HO) (50:50) (5µm and 8µm fibres);

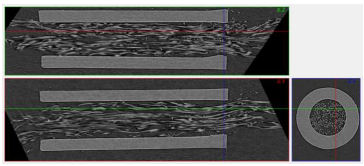
4) P(3HO-co-3HD):P(3HB) (50:50) (4µm fibres fabricated at Contipro)

The best performing outer tube material was identified in WP5 and fibre scale up was conducted by Contipro (4SPIN technology) for optimally performing fibres from WP3. A summary of the different polymer blends is shown in the below table together with the range of diameters, and an example of P3HB fibres of 4.2µm fibres by SEM. Fibres of >20cm were then threaded in to tubes (10 or 15 mm) in length and cut to approximate size. A precise end-section cutting method was then determined using liquid nitrogen and a scalpel to obtain a high fibre surface area without fibre annealing.

Material/Blend	Aligned electrospun fibres fabricated?	Range of fibre diameters fabricated (µm)
PCL	Yes	1-5
P(3HB)	Yes	2.3-8.07
P(3HO)	No	N/A
PGA	Yes	1.55-4.32
P(3HO) P(3HB) (25:75)	Yes	1.77-10.59
P(3HO) P(3HB) (50:50)	Yes	1.99-9.11
P(3HO) P(3HB) (75:25)	Yes	3.73-6.61
P(3HO-co-3HD) P(3HB) (95:5)	Yes	2.68-6.46
P(3HO-co-3HD) PLLA (95:5)	Yes	8.97
P(3HO-co-3HD) PLLA (50:50)	Yes	3.04-8.07
P(3HO-co-3HD) PCL (95:5)	No	N/A







Thus, at the end of WP2 the following candidate polymer materials, manufacturing methods and implant parts were confirmed:

	PCL	PLCL	PCLm	P(3HO-3HD): PCL	P(3HO-3HD): PLLA	P(3O-3HD): P3HB	P(3HO): P(3HB)
External		(60:40) Extrusion with porogen	µSL + HIPE	(75:25) Extrusion with porogen**		(X:100-X), X<95 Extrusion with porogen	(75:25) Extrusion with porogen
Internal fibres	Electro-spinning*				(X:100-X), X<95 Electrospinning	(X:100-X), X<95 Electrospinning**	(50:50) Electrospinning
Internal channels			UV-moulding	(75:25) Thermal moulding**	(X:100-X), X<95 Thermal - moulding	(X:100-X), X<95 Thermal-moulding	(75:25) Thermal - moulding

Work Package 3 Product validation with pre-clinical data (In vitro assays)

In this work package the goal is to pre-clinically validate *in vitro* the materials selected for use in production of the final nerve conduits prototypes for their biocompatibility, biodegradability and potential to support nerve regeneration.

Task 3.1.: Analysis of Biocompatibility and biodegradability biomaterials

In this task Histocell has leaded the biological validation of the materials used for the final product coming from WP1 and WP2.

For biocompatibility determination, in vitro cytotoxicity assays according to guidelines described in ISO 10993-5 has been performed. The specific guidelines for cytotoxicity assays were communicated to the partners. This in order that the materials produced in WP1 and manufactured in WP2 are provided according to these guidelines and proper cytotoxicity testing can be performed.

The specifications of packaging and sterilization methods were defined in this WP. It has been decided that the sterilization method used in the Neurimp project will be gamma irradiation, due to its penetration into the material, innocuous and lack of chemical residues.

In the first 18 months, Histocell has tested the cytotoxic properties of 10 synthetic materials produced in WP1 by UoW, Vornia, US and Contipro and manufactured in WP2 by Tekniker and US. Cytotoxicity of materials has been tested using the MTT assay, which was performed according to the guidelines described in ISO 10993-5. These experiments were performed with a minimum n=3 samples per material. Repeating a Cytotoxicity assay was necessary after changing a manufacturing step.

The specific guidelines for biodegradation assay according to guidelines described in ISO 10993-13 has been defined and communicated to the partners in order that the materials produced in WP1 and manufactured in WP2 are provided according to these guidelines and the biodegradation assay can be performed properly. Degradation experiments for photocurable PCL (M14USFD003) will start in month 18. Furthermore, samples will be sent in the next month (M19) by the University of Westminster for degradation evaluation. Eventually all definitive materials will be tested by this degradation protocol in next 6 months.

From month 19 to24, final candidate materials and blends of these were tested for cytotoxic properties, using *in vitro* cytotoxicity assays according to guidelines described in ISO 10993-5. These experiments were performed with a minimum of n=6 samples per material or blend. From these experiments, non-cytotoxic materals and blends were identified and these were used to continue with degradation experiments.

Furthermore, cytotoxic evaluation for use of glucose and salt as porogens for final IGNC's was evaluated in Histocell. For this leached and non-leached PCLm cylynders were evaluated using the same *in vitro* cytotoxicity assays previously used with mouse fibroblast cell line L929 and human neuroblastoma cell line SH-SY5Y cells. From these experiments it became clear, that on a cellular level, without taking into account properties such a melting point of glucose and salt, glucose would be the most suitable porogen for future use.

In Histocell, 5 final candidates were evaluated for their degradation properties using oxidative degradation protocol described in ISO 10993-13. Furthermore, as a control, in parallel a hydrolytic degradation was performed as well. From these experiments, PCLm and PLCL 60/40 were identified as materials that would fit the predefined and desirable degradation properties.

For detailed information please refer to **deliverable 3.1: "Evaluation of biocompatibility and biodegradability properties in accordance with ISO 10993 of produced biomaterials and blends of these"**

Task 3.2.: *In vitro* high throughput screening of biomaterials adequacy for neuroregeneration

In this task, adequacy of materials for the regeneration of the peripheral nerves and compatibility with cell lines derived from nervous tissue was assessed in vitro. Nerve cell viability and generation of neurites are the two fundamental aspects that were analyzed due to their relevance in the reconnection needed in the NGCs.

Viability of NG108-15 neuronal cells seeded on substrates composed of P(3HO)/P(3HB) and PCL was assessed by UoW. Generation of neurites formed by NG108-15 cells cultured on 6 different synthetic materials developed in the University of Westminster and University of Sheffield were assessed. Confocal microscopy images of NG108-15 neuronal cells inmunocytochemically-labelled for beta-III tubulin were acquired.

In Histocell, 5 candidate materials identified in task 3.1 were evaluated for their potential to support neuronal cell attachment and precursos proliferation as well as neuroregeneration. This was done by culturing human neuroblastoma SH-SY5Y cells directly upon these materials. In these experiments it was shown that all 4 candidates evaluated in Histocell were suitable for use in a final device and support the properties stated above. P(3HO-co-3HD):P(3HB) (95:5) was observed to be the most suitable candidate since it showed highest average neurite length and maximum neurite length measured and SH-SY5Y cells cultured on this materials showed the highest proliferation rate.

For detailed information please refer to **deliverable 3.2: *"Description of neuroregenerative capacity of previously selected biocompatible-biodegradable biomaterials, based on their neuroregeneration capacity"***

Task 3.3: In vitro 3D culture model of pheripheral nerve regeneration

In task 3.3, electrospun fibres fabricated in WP2 were evaluated using 2D and 3D in vitro culture models. Due to two separate deliverables, task 3.3 was split into two sections: The in vitro testing of candidate nerve guides and fibres using an ex vivo DRG model, and the in vitro evaluation of candidate parallel-aligned filaments using neuronal and Schwann cell culture.

In the in vitro evaluation of candidate parallel-aligned filaments, using neuronal and Schwann cell culture, NG108-15 neuronal cells were seeded onto fibres, and after 6 days stained for live/dead analysis and β III tubulin expression (neurite marker). Rat primary Schwann cells were isolated from sciatic nerves, and were cultured onto fibre samples. After 6 days, Schwann cells were stained for live/dead analysis, and S100 (a Schwann cell marker) to determine cell viability as well as cell phenotype. Co-cultures were also performed. Measurements of average and maximum neurite lengths, number of neurites expressed per neuron and the average Schwann cell length were taken, as well as cell viability assays performed, to select the optimum fibre diameter, and most efficient material and/or blend for to aid peripheral nerve repair.

Using an ex vivo DRG culture model, set up in the Haycock laboratory at The University of Sheffield, Dorsal Root Ganglion bodies were isolated from male Wistar rats (1-2 months) and vertically placed onto conduits containing 5 and 8µm fibres of the candidate material blends. After 21 days, samples were fixed and the fibres removed for analysis. Fibres were stained for β III tubulin (for axon filament

elongation) and S100 (for Schwann cell identification and migration). Measurements of average and maximum neurite lengths, number of neurites expressed per neuron and distance of migrating Schwann cells were taken, from the ex vivo DRG study, to determine the most efficient material blend and diameter of fibre for nerve regeneration. Deliverable 3.3 also assessed the development of a 3D system for ex vivo nerve regeneration, the optimisation of proccessing methods for 3D samples as well as the DRG regeneration in candidate NGC devices.

For detailed information please refer to **deliverable 3.3**: "**In vitro evaluation of candidate test nerve guides using 3D DRG culture"** and **deliverable 3.4**. **"In vitro evaluation of candidate parallel-aligned filaments and microchannels using neuronal and Schwann cell culture."**

Task 3.4: *Ex vivo* proof-of-concept of peripheral nerve regeneration

In task 3.4 (deliverable 3.5), two *ex vivo* models were proposed to bridge a gap of 6 mm between two nerve explants, or a nerve explant and a DRG. Even though some regenerating axons and infiltrated glial cells were observed inside the tubes, the results obtained were inconsistent and not very reproducible. Due to technical limitations and the difficulty to develop a standard protocol for histological processing, it was decided to test nerve regeneration in vivo in a short gap of 6 mm in the rat sciatic nerve. This contingency was eventually preview in the project

For detailed information please refer to **deliverable 3.5: *"Determination in a ex vivo model the best biomaterial and internal microestructure to promote the maximum length in cell migration and interstump reconection"*.**

Key results:

Regarding **Task 3.1,** 5 materials were identified that were suitable to do further experimentation in task 3.2. P(3HO-co-3HD)/P(3HB) 95/5, P(3HO-co-3HD)/PLLA 95/5, P(3HO-co-3HD)/PCL 95/5, PCLm and PLCL 60/40 were proven to be non cytotoxic, according to in vitro cytotoxicity testing assays. Furthermore, the latter 2 were observed to possess the most suitable (in vitro) degradation properties for the final device.

The detailed results of **Task 3.1** are compiled in the document **D3.1**: "***Evaluation of biocompatibility and biodegradability of produced biomaterials and blends of these***".

In **Task 3.2**, P(3HO-co-3HD), P(3HO-co-3HD-co-3HDD), P(3HO-co-3HD):PLLA (95:5), P(3HO-co-3HD):PCL (95:5), P(3HO-co-3HD):P(3HB) (95:5) and PLCL 60/40 were all proven to support neuronal cell attachement, proliferation and neuroregeneration.

The detailed results of **Task 3.2** are compiled in the document **D3.2**: "***Description of neuroregenerative capacity of previously selected biocompatible-biodegradable biomaterials. based on their neuroregeneration capacity***".

In Task 3.3, Based on equivalent viability and subtle increases in regeneration the following electrospun materials were selected as most suitable.

- P(3HO-co-3HD):PLLA (50:50) (5µm and 8µm fibres)
- PCL (5µm and 8µm fibres)

- P(3HB):P(3HO) (50:50) (5µm and 8µm fibres)
- P(3HO-co-3HD):P(3HB) (50:50) (4.26µm fibres fabricated at Contipro)

A novel 3D culture system suitable for ex vivo testing was developed to yield maximal DRG attachment into which electrospun fibres were thread into external NGC conduits

A novel technique termed ENACT (Enhanced antibody computed tomography) was developed to overcome the destructive shortcomings and limitations of conventional histological processing and enable molecular specific labelling of neuronal populations non-invasively within NGCs.

5µm and 8µm P(3HO-co-3HD):PLLA (50:50) fibres could not be threaded reproducibly therefore were discarded as candidate internal electrospun fibres

Despite maximal attachment there was some variability in observable regeneration, not related to material composition and as in the in vitro tests no single material was significantly better than any other. Some fibres may bear significance with increased reproducibility of our ex vivo assay. Although the potential biological variability of regeneration from different DRGs was not adequately tested.

All materials caused regeneration from 3D ex vivo cultured DRGs however no single intra luminal was significantly better than any other.

Therefore 5µm P(3HO):P(3HB)(50:50) (USFD), 8µm P(3HO):P(3HB)(50:50), 4µm P(3HB):P(3HO-co-3HD) and 8 µm P(3HO):P(3HB)(50:50) were all considered as suitable internal filler materials

A key parameter not fully considered prior to commencement of this work package was the reproducibility in threading aligned fibres into external NGCs and whilst biological properties as based on in vitro parameters identified all materials to be suitable, not all materials could be thread accurately due to the different handling properties of electrospun fibres and acted as a selection mechanism for final candidates.

The detailed results of **Task 3.3** are compiled in the documents ***D3.3: "In vitro evaluation of candidate test nerve guides using 3D DRG culture"*** and ***D3.4. "In vitro evaluation of candidate parallel-aligned filaments and microchannels using neuronal and Schwann cell culture."***

In task 3.5, PLCL 60/40 was identified as a great candidate material to use in a future INGC.

Work Package 4: Up-scaling of targeted microcomponents and biomaterials and high throughput manufacturing technologies

The objective of the work package was to select the most suitable polymers (or blends) in terms of scalability and suitability for peripheral nerve repair. A second objective was to manufacture a small batch of prototypes to be used for implantation in animal model.

Task 4.1. *Upscale of natural material production under GMP conditions*

The Production and characterization of poly(-3-hydroxybutyrate) (P3HB), poly(3-hydroxyoctanoate-co-3-hydroxydecanoate) P(3HO-3HD) in semi production scale was performed by University of Westminster. All the fermentation processes were carried out via fed batch mode in 10L Applikon bioreactors. For P(3HB) production, nitrogen limitation increased significantly both biomass (7 g/L) as well as yield (1.8 g/L). Similarly, for P(3HO-3HD), there was a drastic increase in both biomass (8 g/L) as well as yield (1.6 g/L). The specification of the materials was prepared according to the following criteria: Appearance (visual), Identification (GCMS, NMR), Monomer composition, mol%, Melting temperature (TGA), Glass transition, Molecular Weight (GC) and Polydispersity. The characterization of three independent batches was included. The synthesis was carried out in laboratory scale. After that, Fermentation conditions that resulted in highest PHA yield were used as baseline conditions for further optimisation at Centre for Process Innovation (CPI, UK). P(3HB) and P(3HO) were produced using 15L, 20L and 72L Electrolab fermenters. Optimised fermentation conditions will be used for the final scale up of both P(3HB) and P(3HO-3HD) using 750 L bioreactors.

Task 4.2. Upscale of synthetic biodegradable material production under GMP productions

Poly L-lactide-co- ε-caprolactone (PLCL) with prepared. The specification was written: structure, appearance, determination of residual monomers, analysis of heavy Metals (not incl. Sn), residual solvents, molecular weight and PDI and Viscosity. The last polymer was Poly L-lactide co ε-caprolactone (PLCL-6040). Moreover, Vornia designed and manufactured two larger reactor vessels to up-scale the production of synthetic polymers. In addition, a workstation was designed and fabricated to house these new vessels. This work represents an increase in batch size per reaction from 60 grams to simultaneous batches of 140 and 500 grams. Vornia supplied PCL, PLLA, PLCL in laboratory quality. The scale up and quality of the polymers was improved. The synthesis of PCL, PLLA, PLCL are directed to the medical-grade synthetic polymers, processed, and packaged in a controlled cleanroom environment, following the (ISO Class 7 (FED equiv. = Class 10,000)) Norm. GMP production of up-scaled batches of PLCL 6040 and PCL were carried out in a cleanroom to achieve consistent results. DSC results showed signs of crystallization in the PLCL 6040 after storage at room temperature for 2 months. Therefore, the material was removed from the final composition. GPC and NMR analysis were conducted on 6 sets of tube samples after extrusion and storage in various conditions. GPC results clearly demonstrated material degradation during storage.

Vornia staff continued to gather data relating to materials upscaled in task 4.2 and 4.3, namely PLCL 6040 and PCL. Long-term degradation studies were conducted to better the understanding of the materials.

Task 4.3. Upscale of biopolymer blends and copolymers

Two different blend compositions were prepared:

(1) Poly (3-hydroxyoctanoate-co-3-hydroxydecanoate) identified in short as P(3HO-3HD) and mixed with Polycaprolactone (PCL) 75:25

(2) P(3HO-3HD) and Poly(3-hydroxybutyrate) or P(3HB) 85:15

The above-mentioned blends were chosen for testing in processing into nerve guidance conduits due to their optimum mechanical properties as described in WP1 (Task 1.3). Chemical, Thermal, and Mechanical tests were carried out after 7 weeks of storage to ensure ageing of the polymer did not affect the quality of the final polymer.

PLCL6040 was also produced by Vornia and reported in up-scaled conditions. This work represents an increase in batch size per reaction from 60 grams to simultaneous batches of 140 and 500 grams. Three batches of PCL were produced with a molecular weight of 120 kDa.

Two blends were prepared for the manufacture and the characterisation of melt-extruded tubes: P(3HO-3HD)/P(3HB) containing glucose particles (85:15) and P(3HO-3HD)/PCL+ glucose particles (75:25), both on 35 g/batch.

Based on the data obtained from the in vivo experiments, P(3HO-3HD)/PCL 75/25 demonstrated higher nerve regeneration capacity and therefore, higher amount (105g) of P(3HO-3HD)/PCL 75:25 was prepared and supplied for further *in vivo* experiments.

Task 4.4. Up-scaling of manufacturing technologies for production of small series of INGC

4.4.1. Up-scaling of electrospinning process

A series of microfibers were produced in CONTIPRO to find out the feasibility of the process in high scale as described previously by University of Sheffield).

For the up-scale the composition P(3HB): P(3HO) (50:50) was selected to produce microfibers. Experimental parameters were controlled: Temperature of the chamber (22-23°C), humidity (17%), air filtration, voltage (12.5 V) and speed of the collector (2000 rpm) were maintained constant during the process. A flow rate = (50µl/min) was implemented.

Two different materials were used for the fibers mats collection (aluminium foil and conductive fiber - cloth). The last one was found to improve the productivity of the process.

In parallel, blends of P(3HO)/P(3HB) was used to obtain electrospun fibres at the University of Sheffield. These fibres were successfully threaded through the hollow tube to obtain internal channels. For the final in vivo experiments, UoW have continued to collaborate with the University of Sheffield and Contipro to supply P(3HB) and P(3HO) for developing electrospun blend fibres.

4.4.2. Up-scaling of micro-SL process

For the outer tube, regarding the up-scaling of the microSL process, and the upscaling of PCL-methacrylated (PCLm), at USFD, should not go forward as there was difficulties achieving this.

4.4.3. Up-scaling of microinjection moulding and microextrusion

Microextrusion of hollow tubes from P(3HO-3HD)/PCL 7525 was performed following an upscaled protocol. Some of the tubes were used for threading of fibers (US) and subsequent implantation in rats and others were sent to HNP to be implanted in the pending medium and large gap trials in HNP.

4.4.4. Scale-up of soft lithography and photo-patterning-based 3D micromoulding

Furthermore, fabrication of microchanneled PCLm following an upscaled UV-moulding protocol was developed. The porosity was created by both glucose porogen introduction and High Internal Phase Emulsion (HIPE). The inner tubes threaded into the mentioned hollow tubes were carried out to be implanted in medium and large gap trials in animal model.

Task 4.5. Production of small series of products for clinical trials under GMP conditions

Hollow tubes of PLCL (6040) with salt as pore generator and of P(3HO-3HD)/PCL 75/25 with glucose as porogen were extruded. Samples of tubes were sorted and prepared to be sent to HNP for the implant in animal models.

The tubes made of PLCL (6040) were sent leached, and in case of P(3HO-3HD)/PCL tubes, the dispatched samples were both in leached and non-leached condition. The tubes shown wall thicknesses of 250 µm and 500 µm for P(3HO-3HD)/PCL tubes, while 500 µm was determined for PLCL tubes.

The tubes, once in HNP, were subsequently implanted in rats for hollow tube medium gap in-vivo experiments (gap of 10mm). Additionally, biodegradability tests were carried out by HISTOCELL. P(3HO-3HD)/PCL75:25+70% glucose (not leached) tubes were stiff and brittle as characterised by their high Young's Modulus and very low % elongation at break values. On the contrary, P(3HO-3HD)/PCL75:25+70% glucose tubes upon leaching were significantly elastomeric as characterised by their % elongation at break values. There was a drastic decrease in the stiffness upon leaching as expected. PLCL6040 tubes upon leaching were significantly elastomeric, as characterised by their high % elongation at break values.

Selected samples of PLCL60/40 and P(3HO-3HD)/PCL 75/25 extruded tubes, were stored in freezer and in desiccator at RT; periodically, samples were tested with DSC to study the ageing of the materials and compare their evolution in freezer and in desiccator at RT.

Pure glucose was sieved in 25-50µm particle size range to be sent to UoW, for the extrusion of new hollow tubes using P(3HO-3HD)/PCL 75/25 for in vivo implantation in medium and large gap performed in HNP.

Microextrusion of hollow tubes from a new batch of P(3HO-3HD)/PCL 7525 was performed, some of them were sent to USFD for threading of fibers and subsequent implantation in rats and others were sent to HNP to be implanted in the pending medium and large gap trials in HNP.

Key results:

Using the expertise developed in WP4, a series of tubes (a-d) were produced and implanted in vivo. The knowledge generated during the project allowed the production of tubes for 10 mm and 15 mm gap with the characteristic enlisted below:

a) First a series of hollow tubes made of the blend P(3HO-3HD)/PCL 75/25, which was non-leached and is characterized by 500 µm wall thickness.

b) Also, a series of tubes made of a blend P(3HO-3HD)/PCL (external)+ PCLm (internal) with porogen.

c) Also, a model consists on a P(3HO-3HD)/PCL hollow tube and PCLm (internal) with porogen created by High Internal Phase Emulsion (HIPE).

d) Finally, two series of tubes with the composition of P(3HO-3HD)/PCL as a hollow tube and P(3HO):P(3HB) 50:50 fibers of 5 and 8 µm for the inner structure.

The fabrication of all prototypes were developed following different upscaled manufacturing processes such as microextrusion, UV-moulding and fibers electrospinning and posterior threading into the hollow tubes.

Work Package 5 Product validation with pre-clinical data (In vivo assays)

The objective of the work package is the validation *in vivo* of the final devices/prototypes to be used for peripheral nerve repair. This work package has been leaded by HNP.

Task 5.1. *In vivo* assessment of prototypes in a non-critical gap.

Experiments in Task 5.1 were divided in trials performed at 6 mm and 10 mm gap.

First, we implanted the following devices in a 6 mm gap to choose the potential external prototypes to be used in longer gaps. Here we contemplated three different variables: the biomaterial, the thickness of the wall and the pre-treatment before implantation, in order to define the best prototype of hollow tube regarding *in vivo* biocompatibility, capacity to support regeneration and structural stability of the NGC after 4 weeks of implantation.

Table 1: List of animals implanted in the 6 mm gap

Group	N
SILICONE. 1,5 mm i.d.	2
PEG	3
PLCL 60:40 ambient-dried. 1,6 mm i.d. 250 µm Wall thickness	8
PLCL 60:40 freeze-dried. 1,6 mm i.d. 250 µm Wall thickness	4
PCLm	4
P(3HO-3HD)/PCL 75:25 No leached. 1,2 mm i.d. 500 µm Wall thickness	4
P(3HO-3HD)/PCL 75:25 Leached. 1,2 mm i.d. 500 µm Wall thickness	4
P(3HO-3HD)/P(3HB) 85:15 Leached. 1,2 mm i.d. 500 µm Wall thickness	4

According to the results obtained in the analysis of the regenerated nerves of the different implanted groups in the 6 mm gap, two principal materials were chosen by the Consortium for building a prototype to sustain regeneration in longer gaps: PLCL and P(3HO-3HD)/PCL 75:25.

Next, experiments in the 10 mm gap with a follow-up of 4 months were performed. Two different experiments were included in this gap. First, we measured the proven ability of the different hollow prototypes designed by the Consortium to sustain regeneration in a limiting 10 mm gap (2 cm in human), as well as stability of the NGC to fulfill the onset of structural degradation from 6 months after implantation and 2 years of time to be completely resorbed established by the Consortium. After that, the two internal structures selected as more promising, an extruded multichannel insert made of PCLm or electrospinning microfibers made of P(3HB):P(3HO) (50:50) (5µm and 8µm fibres), were included to provide the physical support required to achieve an axially guided regeneration among the nerve stumps.

The list of implanted groups and the percentage of regenerated animals in each case is summarized below.

Table 2: List of animals implanted in the 10 mm gap and number of regenerated nerves in each group.

Group	n	Regenerated animals
AUTOGRAFT	8	8
NEUROLAC-TW®	10	7
P(3HO-3HD)/PCL 75:25 leached. 1,5 mm i.d. 500 µm Wall thickness	8	7
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness	16	13
P(3HO-3HD)/PCL 75:25 leached. 1,5 mm i.d. 250 µm Wall thickness	8	3
P(3HO-3HD)/PCL 75:25 No leached. 1,5 mm i.d. 250 µm Wall thickness	8	6
PLCL 60/40 freeze-dried 500 µm wall thickness	8	6
PLCL 60/40 freeze-dried 250 µm wall thickness	8	2
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + PCLm	8	2
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + PCL HYPES	8	1
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + P(3HB):P(3HO) (50:50) Fibers 5 µm	6	1
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + P(3HB):P(3HO) (50:50) Fibers 8 µm	6	0

Task 5.2. *In vivo* assessment of prototypes in a critical gap.

Once the external hollow prototype was chosen (conclusions obtained from the first trial in the 10 mm gap), experiments in the long critical 15 mm gap were performed. These experiments were run in parallel to the second trial in the 10 mm gap (study of the internal structures in a limiting gap of 10 mm), in order to adapt the scheduled work to the limited time frame available in the project.

In this case, only hollow tubes of P(3HO-3HD)/PCL 75:25, non-leached and with a wall thickness of 500 µm were analyzed as external structure for the designed prototype.

The list of implanted animals and number of regenerated nerves in each group are summarized below.

Table 3: List of animals implanted in the 15 mm gap and number of regenerated nerves in each group.

Group	n	Regenerated animals
AUTOGRAFT	8	7
NEUROLAC-TW®	8	1
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness	8	0
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + PCLm	8	0
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + PCL HYPES	8	0
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + P(3HB):P(3HO) (50:50) Fibers 5 µm	6	0
P(3HO-3HD)/PCL 75:25 No leached. 1,8 mm i.d. 500 µm Wall thickness + P(3HB):P(3HO) (50:50) Fibers 8 µm	6	0

Key results:

According to the measurements performed in the regenerated nerves 4 weeks after surgery and repair, including area of the regenerated cable, neurofilament reactive area and fibronectin reactive area, the Consortium validated the following external materials:

PLCL 60/40 freeze dried and P(3HO-3HD)/PCL 75:25, both leached and non-leached.

Then, experiments in the 10 mm gap were performed. In this case, either a gold standard clinical procedure (autograft) or an FDA approved device (Neurolac-TW®), were compared with the selected NEURIMP-derived prototypes both hollow and implemented with an internal structure. Experiments were divided in two blocks.

In the first block, autograft, Neurolac and hollow external prototypes were analyzed. In a second attempt, and once the external material was chosen, tubes were implemented with internal structures.

Electrophysiological and histological results of the first 10 mm gap experiment (comparison between autograft, Neurolac and hollow external prototypes) were carried out during the following 4 months after injury and repair.

Onset of Compound Muscle Action Potentials (CMAPs) of target muscles (gastrocnemius, tibialis anterioris and plantar muscles) was earlier in the autograft group than in the NGC-repaired groups. These amplitudes were higher at 90 days post injury (dpi) compared to the NGC-repaired groups. However, the recording values of proximal muscles (gastrocnemius and tibialis anterior muscles) in the autograft tended to estabilize up to the final time-point (120 dpi), whereas in the NGC-repaired groups increased reaching similar values to the autograft.

Among the NGC-repaired groups, according to the percentage of animals that responded positively to this electrical stimuli and the values of the recorded signals, P(3HO-3HD)/PCL either leached or non-leached with 500 µm of wall thickness presented the higher scores.

These results were confirmed when analyzing the histological results. Although no significant differences were observed in terms of area of the regenerated cable, estimation of myelinated fibers and density/distribution of axons, qualitative differences were observed in terms of presence of Schwann cells accompanying regenerated axons. These qualitative differences included a better fasciculation of the regenerated axons indicative of more mature regenerated cables in the 500 µm wall thickness groups compared to the 250 µm wall thickness ones.

When comparing the results obtained in the P(3HO-3HD)/PCL, either leached or non-leached, of 500 µm wall thickness with Neurolac, slightly higher values in the percentage of regenerated animals after 120 dpi and electrophysiological tests were obtained. However, no significant differences were obtained analyzing histological data.

Regarding PLCL implanted prototypes, significant differences in the percentage of regenerated nerves and electrophysiological results were obtained comparing the tubes of 500 µm and 250 µm of wall thickness. Both tubes presented signs of fast degradation and robust breaking points. However, this effect seemed to have a larger effect on the 250 µm wall thickness group, thus compromising final results. In this case, degradation might occur prior to fibrin cable formation and nerve cable stabilization, impeding the correct regrowth of the regenerated axons and the final effective target reinnervation.

Electrophysiological results added to the analysis of the percentage of regenerated animals in the second attempt in the 10 mm gap, where we assessed the implementation of internal structures to hollow tubes of non-leached P(3HO-3HD)/PCL with 500 µm of wall thickness, revealed that the inner structures had an unexpected negative impact on the regeneration capacity of the operated animals.

Similar results were observed in the analysis of the regeneration capacity of implanted prototypes to sustain regrowth of damaged axons in critical 15 mm gaps. Despite of the efforts of the Consortium to

build a device to compete against autograft to become a real surgical alternative to repair limiting gaps in the clinic, the obtained results discard the possibility of using this device for long gap repair.

Although slight deviations occur during the course of the project, all the work was completed in the scheduled time frame.

Two milestones were described in the WP5 at the beginning of the project.

MS17 (Demonstration of the best performance of the final prototype to repair nerve gap lengths), was successfully completed by month 42, with the non-leached P(3HO-3HD)/PCL and 500 µm of wall thickness as final NGC prototype capable to sustain regeneration in limiting gaps (10 mm in the rat peripheral nerve, no critical). The results presented are slightly better than the Neurolac-TW® (FDA approved conduit).

MS18 (Prove the ability of the prototype to be an alternative to a nerve autograft) was completed by month 48. However, results obtained by the Consortium discard the possibility of using the designed prototype to be a real alternative to autograft to sustain regeneration in critical gaps, 15 mm in the rat sciatic nerve and thus over 3 cm in humans.

Work Package 6 Medical Devices regulations governing all processes within NEURIMP

The objective of this work package is to ensure that the NEURIMP INGC is designed and developed according to the relevant provisions of the Medical Device Directive (MDD) 93/42/EEC to demonstrate safety and performance in line with the intended use.

Task 6.1: Design control

When the device has not been developed in accordance with the applicable regulation and legal requirements, the process of valorisation will stagnate and the devices will not reach the marketplace. To ensure proper design control, a training is provided to create awareness on the ISO 13485 requirements and quality procedure is written describing the design and development process covering all sites and disciplines from the input Phase to the output Phase. A Design Traceability Matrix is established to document design decisions and a Design Freeze document is implemented to document the verification for each stage of the design process compliance with the input requirements.

Output of the design process is a Design History File, which is a structured compilation of all information related to realization of the final design of the product. On extranet, a filing structure is published to enable all partners to provide the required input documentation.

In the Description of Work it was included to perform on-site verification on the implementation of the design and development procedure Design control. It is decided not to audit each facility, but instead put efforts in training the partners on the requirements for writing protocols and reports, since it became evident that partners were not aware of the relevance of documenting this in the design process.

Task 6.2: Risk Management

Risk Management plays an important role in the development of Medical Devices and access to the market. For risk management, the harmonized standard ISO14971:2012 can be used as a framework to manage systematically the risks associated with the use of the device. A training is provided to introduce all partners with Risk Management in compliance with the harmonized standard. A procedure is written to translate the requirements in an implementation. A Risk Management Team is formed that ensures that all relevant areas of expertise are covered and a Risk Management Plan is drawn up.

With the Risk Management Team, face-to-face and Webex meetings are held to identify and evaluate the risks of the Nerve Guide Implant covering the whole life cycle of the device, from design concept to frozen design, and finally meeting the market requirements. An FMEA is prepared with the input of all partners and a Risk Management Report is written.

Task 6.3: Biological & safety studies

One of the objectives of the NEURIMP project is to search for the most favourable polymer for nerve regeneration in combination with mechanical, physiological and biodegradable properties. All partners of the NEURIMP consortium are experts in their own field and have a lot of knowledge on this subject, but there is limited awareness of the requirements for Regulatory Compliance. In Technical File submission, ISO 10993 must be addressed, either by means of testing or by providing a rationale with

evidence that the specific element of the ISO 10993 is not applicable or sufficiently covered by the available information. Furthermore, for biological safety evaluation according to the standards, it is required to test the final product after upscaling in the manufacturing setting and facilities of the product to be marketed. To create awareness, a training is provided to inform partners on the requirements.

Throughout the development of raw materials and production methods, the partners have performed a lot of material properties studies to ensure the biological safety and performance of the final design of the INGC.

In the third period, all evidence is gathered from the deliverable reports and presentations to summarize information available related to the biological safety of the NEURIMP INGC. Based on this information and available data from literature, risks related to the biological safety of the raw materials and production techniques are identified, all applicable parts of the ISO 10993-series are reviewed to select the most appropriate testing methods for the INGC.
The biological safety risk assessment in combination with available data and the requirements of the ISO 10993-series are used to write a biological evaluation plan. All data are combined into Deliverable report 6.3.

Task 6.4: Clinical evaluation

It is mandatory for all medical devices to conduct a clinical (data) evaluation to demonstrate conformity with the essential requirements of the Medical Device Directive (MDD) 93/42/EEC. The objective of task 6.4 was to provide a literature based clinical evaluation of comparable products in the market based on intended use.

Since the design of the device and the materials used are innovative, equivalence with competitor product (Neurolac) was claimed only for the hollow tube configuration of the NEURIMP INGC. The available clinical data and safety data for the Neurolac were analyzed. In addition, the gaps with respect to clinical data needed to place the device on the EU market were identified and discussed. Equivalence in intended use is used to detect the foreseeable risks related to the INGC.

A clinical evaluation training is provided and all partners are requested to provide input on the search criteria. With the clinical data obtained for Neurolac and the input provided by partners, clinical evaluation plan is written and a thorough and structured literature search and review are executed. This resulted in a Data Collection Report and Clinical Evaluation Report. Input of partners and an expert clinician are used to finalize the deliverable report 6.4.

Task 6.5: Technical documentation for future regulatory submission

The objective of task 6.5 is to prepare a Summary Technical Documentation (STED) that can be used for future regulatory submission (CE-mark) to accelerate future international regulatory approval and clearance for the product.

A training was provided to create awareness on the expectations related to a Summary Technical Documentation (STED)file. All information supplied by the partners was used to prepare Customer Requirements Specifications. The applicable standards are listed and used in the Essential Requirements Matrix to indicate how to establish the evidence of product safety and compliance to the state of the art. All technical information made available and the desired technical specifications as described in the DoW document are used to compile a Summary Technical Documentation Report (STED, deliverable

6.5) reflecting the current status to the design of the device. The report indicates gaps to be completed with the validation data after upscaling of the device to be marketed.

Key results:

Within WP6.2 we established a risk assessment with all team members and a risk management report is drawn up. Both documents are based on the current status of the product design and need to be verified after production process validation and need to be monitored after market release.

Within WP 6.3, the risks related to the raw material characteristics and production methods with additives are addressed. The preliminary biological safety results obtained, mainly generated in WP 3 and WP 5, demonstrate promising results. Since the standards require a full scope biological safety assessment on the finished device after packaging and sterilization, a biological safety plan is established.

The clinical evaluation executed in WP 6.4, which comprises of a clinical evaluation plan, a data collection report and a clinical evaluation report, is literature based with the purpose to describe the state of the art on nerve guide conduits. The NEURIMP INGC is compared with Neurolac, but the equivalence is limited to the intended use.

The clinical evaluation clearly demonstrates the need to develop a nerve graft with improved mechanical and physical properties, enabling to bridge nerve gaps that exceeds 40 mm and providing guidance for axons to grow. Since this is a novelty, equivalence to other products can not be claimed and therefore a Clinical Investigation will be required for the final design of the INGC after upscaling and and sterilization.

For WP 6.5 an inventory is made of all applicable (harmonized) standards. All elements expected in a Summary Technical documentation are addressed. Data already available are included in the report and the requirements for information to be added to the file is described. Therefore, the deliverable report resulting from WP 6.5 provides guidance in preparing a technical file for CE submission.

Work Package 7 Dissemination, IPR and Exploitation Plan

The main goals of WP7 were focused to 4 aspects: i) Identification of potentially conflicting IPR and the establishment of an IP strategy also taking into consideration market aspects and partners preferences, ii) Properly knowledge management, iii) development and coordination of dissemination and intellectual property activities and iv) conducting a market analysis.

These goals have been achieved performing 5 different tasks:

Task 7.1: IPR Aspects

In this task Histocell has leaded the study of the background IP and project goals to avoid infringing obvious 3rd party IP that would have to be licensed-in or would require significant changes of the product design as well as the definition of the strategy to assure the non-infringement of any of the identified patents have been performed.

Within the framework of the Freedom to Operate study, the partners involved (Histocell, Tekniker and Contipro) have contribute to search the possible interactions of existing patents with the medical device that is going to be developed in the NEURIMP project.

After analyzing the patents that may interfere with our development, the consortium is following the status of these patent applications and, considering the combination of biomaterial, design and manufacturing process selected during the project execution, those that obtained interaction scores above 10 have been considered as the most relevant for NEURIMP objectives framework. Next task was to analyze all their claims accepted and define a strategy to assure the non-infringement of any of the identified patents.

The objective of the refined IP strategy was identifying the most relevant strategies to protect NEURIMP nerve implant and adopt the most adequate IPR strategy taking in consideration market aspects and partner´s preferences.

For this purpose, actions as: Individual analysis of exploitable results; Assess relevance of existing rights concerning the implant for non-infringing some patents claims; Analysis of strategic FTO options have been analyzed or Selection of best FTO option for protect any of the results in base to partners exploitation, expectative were developed.

Task 7.2: Management of generated knowledge and external trends:

In this task Histocell has leaded the constitution of the Committee for Dissemination and Exploitation (CDE):

Representatives of the Committee

BODY	ENTITY	NAME	Email adress
Committee for Dissemination and Exploitation (CDE)	IK4-TEKNIKER (Dissemination Manager)	Ruth Díez	Ruth.diez@tekniker.es
	HISTOCELL (Leader)	Eva Gonzalez	egonzalez@histocell.com
	CONTIPRO	Pavlina Sobrova	Pavlina.sobrova@contipro.com
	VORNIA	Colm O'Dowd	Colm.odowd@vornia.com

The CDE has been involved in the monitoring and evaluation of the information that is generated in the project in order to classify as confidential or not. The partners inform to the CDE about the intention of

publication of the results of the project in order the CDE analyze the potential repercussion over the patent protection. The methodology and templates for these purpose has been developed.

TEKNIKER has ordered, a company specializing in IPR, the development of a technical-legal report on the patentability of a device for the regeneration of peripheral nerve.

A continuous analysis of results patentability has been done an as consequence:

- CONTIPRO and UOW have deeply analyzed the commercial exploitability of the combination of hyaluronic acid (HA) with polyhydroxyalkanoates (PHA). The analysis has revealed that the commercialization represents an industrial risk due to similar products in the market for encapsulation of lower price. Up to now, the application and therefore, the inventive step of this conjugates has not been demonstrated. CONTIPRO suggested continuing with the co-publication of the results due to the elevated cost of a patent and its exploitation.
- The UoW has filled a United Kingdom Patent Application (No. 1713985.8) about the use of PHA blends in conduits for nerve regeneration
- IK4-Tekniker, The University of Westminster, The University of Sheffield and the Hospital de Parapléjicos de Toledo filled a PCT patent application, which was submitted through the European Patent Office (EPO) on 28 Febr 2018. The invention with title: “Implantable Nerve Guidance Conduit for Nerve Repair” has the application number PCT/EP2018/054984.

Task 7.3: Market Analysis:

In this task a Market analysis has been performed leading by Histocell. The goal of market analysis was to reveal the needs of potential customers and identify the target customers, with respect to performance and position in the care and value chain as well as identify potentially competing products and approaches in the market. It was required to identify the most important unique selling properties (USPs) and to ensure that the development process is focused to achieve competitive advantage in its field of application.

Market analysis covered the criteria and impact of acceptance of the developed product by healthcare providers as a tool to treat peripheral nerve injuries and the investigation of this product’s potential to address additional fields of applications to broaden the applicability of the technology developed in the consortium within regenerative therapies.

An alignment of feature and performance requests with production, design, and intellectual properties (IP) restrictions have been performed to consider potential technical restrains in an early design phase. An estimation of COGS (cost of goods sold) under realistic market assumptions has given feedback to product design to consider cost effective production already in the development stage and establish a base for potential negotiations with producers or licensees

As result of task 7.3 developments the Deliverable 7.8. “Market report covering market size and potential and competitive environment was delivered in moth 30. The delivery date of this document was delayed 6 months in order to have NEURIMP Prototype fully defined and a more realistic idea of the unique selling proposition (USP) of NEURIMP medical device than in month 24.

Task 7.4.: Dissemination Stategy

Internal dissemination:

NEURIMP members of the consortium maintained an effective internal communication via email (internal electronic mailing lists), Webex teleconferences every 6 weeks, and face to face meetings every 6 months. During 2017 NEURIMP Webex meetings were held the 27th of January, 10th of March, 21st of April, 3th of June, 14th of July, 8th of September, 6th of October and 10th of November. Face to face meetings were placed the 28-29th of March in Hospital Nacional de Parapléjicos (Toledo), 13th and 14th of September in Stockholm and the project final meeting 4 and 5th of December in Eibar. After each Webex or face to face meeting, the minutes were written and shared with all the partners. Shared documents including project documents (administrative and technical), reports, minutes and publications are uploaded on NEURIMP website on the private area.

It is important to highlight the relevance of the Project Website for communication actions. NEURIMP website was built at the beginning of the project (www.neurimp.eu) and has been maintained by the project management team staff. The website aimed to ensure a rapid exchange and circulation of information between partners and stakeholders and has enabled the publication of the new advances to a wider audience.

External dissemination

NEURIMP website was constantly updated by TKN with public accessible information including events and papers of interests for Neurimp´s stakeholders. Moreover, website traffic was regularly checked via google analytics, to have an idea of the impacts of the dissemination performed and to promote new actions.

Due to the final Neurimp´s industrial orientation, the scientific dissemination of the results was carefully dealt avoiding the dissemination of project results that can interrupt exploitation strategy and intellectual property rights. Therefore, the results of only those materials and techniques, which were not established as candidates for the second phase of the project (M24) were disseminated with the publication of 3 papers.

From M37 to M48 NEURIMP partners actively collaborated in the project dissemination participating with oral (13) and poster presentations (7) in conferences and workshops at European and International level, and scientific publications (2).

Detailed information of the dissemination actions has been compiled in D.7.9.

Task 7.5. Exploitation

The committee for dissemination and exploitation got underway the first version of the plan (included in the deliverable 7.5) that has been regularly updated in accordance to the project progress. The NEURIMP roadmap from the development phase until market implantation has been discussed and next step for developing the Plan for Use & Dissemination of Foreground (D.7.9) has been detailed.

Once NEURIMP is finished, a strategy based on co-industrialisation or licensing to a healthcare supply manufacturer is envisaged as a previous step to entry into clinical phases and certification of the implant as a Class III Medical device.

Histocell and Contipro have taken advantage of the opportunity of learning more about IP management and use, attending to the WEBINAR entitled “IP Commercialisation and Licensing” that was organized by the European IPR Helpdesk on 5th of May.

Histocell has leaded the preparation of Final PUDF (Deliverable 7.9.), in which all actions performed by the consortium to comply with the initial plan have been compiled.

Two patents were filled between some of the NEURIMP's partners:

- The **UoW** has filled a United Kingdom Patent Application (No. 1713985.8) about the use of PHA blends in conduits for nerve regeneration
- **IK4-Tekniker** leaded the process of application for a patent protecting the selected biomaterials, geometrical design and manufacturing process. The strategy was agreed with the Assigness, extending the discussion to the Commercialization groups of all partners involved. The filing of a PCT patent application was done through the European Patent Office (EPO) on 28 Febr 2018. The invention with title: "Implantable Nerve Guidance Conduit for Nerve Repair" has application number PCT/EP2018/054984 and the owners of the invention are IK4-Tekniker, The University of Westminster, The University of Sheffield and the Hospital de Parapléjicos de Toledo.

Key results:

The outcomes of task 7.1 have been the Deliberable 7.2: "Initial FTO study based on background IP and project goals" and Deliverable 7.3:"Refined IP strategy".

In the document D.7.2, a summary of the claims of the analyzed patents is included, concerning only those device configurations, materials and manufacturing techniques that have been considered in the definition of work of the project.

In the document D.7.3, the most relevant strategies to protect NEURIMP nerve implant and adopt the most adequate IPR strategy taking in consideration market aspects and partner´s preferences has been compiled.

In task 7.2, all partners have contributed to analyze the results that some of the partners have intention of disseminate, and the strategy for patenting the final NEURIMP's solution has be defined.

The analysis of patentability of the results of the project has resulted in the submission of 2 patents:

- UoW has submitted a patent about the use of PHA blends for nerve regeneration (United Kingdom Patent Application No. 1713985.8)
- A PCT patent about the nerve guidance conduit was filled by TKN, UoW, USFD and HNP. The filing of the PCT patent through the European Patent Office (EPO) was done on 28 February 2018. The invention is entitled: "Implantable Nerve Guidance Conduit for Nerve Repair".

In task 7.3, the Deliverable.7.8: "Market report covering market size and potential and competitive environment" was delivered in month 30 (24th June 2016). This document includes:

- NEURIMP Road Map and main stakeholders involved from the development phase until market implantation has been defined.
- European, U.S.A and Global size market.
- Main Competitors list and products description.
- NEURIMP prototypes estimation cost.
- SWOT analysis of current project situation and Confrontation Matrix.

In task 7.4; Deliberables 7.5, 7.6, and 7.7: “Report on Dissemination Activities”, were delivered in month 18, 36 and 48 and these deliverable collected the dissemination activities carried out all project and show how Neurimp´s partners have been implicated and actively participated in them.
The dissemination activities and results were maintained, and even slightly increased, from the beginning of the project.

- Dissemination activities were actively performed during all the project period and Neurimp´s partners were implicated and actively participated in them. The dissemination activities and results were maintained, and even slightly increased, from the beginning of the project to the end and in the last year with more than 20 dissemination actions performed (Oral presentations, Posters, Leaflet handout, etc..)
- The project Website has been a powerful tool for partner communication and it has enabled the publication of the new advances to a wider audience.
- Due to the final Neurimp´s industrial orientation, the scientific dissemination of the results was carefully dealt avoiding the dissemination of project results that can interrupt exploitation strategy and intellectual property rights. However, during the last year of the project, 3 scientific papers have been published.

In task 7.5, a deep analysis of the exploitability of the project results has been done and as consequence the Final Plan for Use & Dissemination of Foreground (D.7.9) has been obtained and launched.

4. Potential impact and the main Dissemination activities and exploitation of results

4.1. Socio-Economic and Societal Implications

Importance of Transected Gap Length

Transected peripheral nerve injury (PNI) repair involves sizing up each patient's capacity to regain the use of their damaged nerve and the risk associated with the repair attempt. To be sure, every repair approach technique or device carries with it the potential for failure at a cost of life-long morbidity. This risk of morbidity is not confined to the nerve injury site alone since having a paralysis or loss of sensation leaves the patient more vulnerable to other risks such as falling or an inability to sense skin trauma. The risk of a poor outcome is greatest with transected nerve injuries, which was the focus of NEURIMP project. Compression type injuries where blood supply and cellular organization remain intact at some level carry with them a greater likelihood of recovery with repair or protection interventions. With each millimeter of a gap separation on a transected nerve there are associated greater repair challenges and more complicated treatment decisions.

For transected nerve injuries where the surgeon feels it would be risky to directly join the nerve-endings together with suture; there are various techniques and device options. A hollow tube or connector device may be placed as a simple conduit to grossly align the nerve endings in an attempt to allow for peripheral nerve re-generation without the stress of pulling the nerve endings together. It is generally accepted that the hollow tube method has limitations in terms of gap length, with the longer gap lengths having less efficacious outcomes when repaired by connector or hollow tube devices. For gap lengths beyond acceptable hollow tube lengths, the surgeon may elect to harvest nerve from elsewhere in the body and place it in the gap – this is called an autograft.

As a nerve tissue specific material, autograft provides well suited scaffolding for nerve re-generation. This technique is currently used for the longer gap repairs and is well received by the injured tissue area as it is the patient's own tissue (autologous), thus minimizing inflammation and scaring to the area while providing a re-generative optimal conduit. For large diameter nerve repairs, the surgeon may bundle smaller diameter nerve grafts together creating a larger conduit for the re-generation process to take place. But, the price of autograft is steep as the harvested nerve site is now rendered permanently damaged creating new life-long morbidity where there was none. At best, this is a trade-off of morbidities if, and only if, the autograft works. If the repaired site isn't able to re-generate then the patient is left with two injuries, one from the initial injury and the other iatrogenic (treatment induced) as a result of the autograft. Newer technologies, as those ones being developed in NERIMP project, strive to address the significant drawbacks of autologous tissue harvest as an alternative option.

Positioning of NEURIMP in Large-Gap Repairs:

The market opportunity offers an alternative to the autograph and allograft products avoiding the problems that these techniques carry:

- Autograft provides well suited scaffolding for nerve re-generation, but there are significant limitations in the use of nerve autografts, such as causing a second surgery site to harvest tissue

from the donor site, which is associated with donor site morbidity and loss of function. Therefore, the availability and the length of nerve that can be harvested are limited. Use of autografts is currently restricted to critical nerve gaps of nearly 5 cm length.

- Nerve allografts, which are decellularized and processed resulting in a surgical implant with the natural structural pathways to guide regeneration; However, no clinical studies have examined its efficacy for the treatment of sensory nerve defects and they require systemic immunosuppression in the patient for approximately 18 months.
- The advances made in hollow tube devices are significant, as shown by the number of commercially available nerve guides. The limitation appears to be in the length of the defect that these can treat (which is not longer than 3 cm) with the sophisticated materials and designs.

The overall force of substitute products is medium in the medical device industry because even though there are intended to substitute some products, the demand for the products in general is strong and growing. Therefore, the effect of substitute products on the market of medical devices could be described is moderate.

Attending to the large-gap repair needs, NEURIMP target was to increase the length of the defect that we can treat through the use of the current knowledge that has been accumulated mainly over the last decade. In this scenario, NEURIMP's value proposition is a new product for the treatment of large-gap transected nerves, offering a less invasive technique, with improved regeneration rates and a competitive price in the current market.

Currently, Dr. Xesco Soldado, member of the Advisory Committee and surgeon of peripheral nerve injuries, is handled the tubes developed in NEURIMP project and reporting its good elastic properties as well as its good performance for suturing as compared with the reference commercial tube Neurolac®.

4.2. Impact on the European Biomaterial Industries

As consequence of NEURIMP development 2 patents applications have been submitted and future patent licensing revenues are expected.

During the project, a high general advancement of knowledge has been acquired execution by the institutions involved and the 3 PhD students that have been trained in project topic.

The companies CONTIRPO and HISTOCELL are using the new knowledge to design and develop new scaffold for regenerative medicine and cosmetics that will allow the companies increase the products portfolio and the turnover in the next 3 years.

The research centers working in microfabrication will use this knowledge to generate new scaffolds for other supplication fields in medicine regenerative with different shapes and a certain control over the degree of porosity in terms of porous volume and porous size.

The companies participating in the project are taking advantage of the knowledge acquired to strengthen their position in the European market:

HISTOCELL is using the new knowledge acquired in NEURIMP to design and develop a new scaffold for application on cosmetics. The launching to the market of this new device for skin anti-aging will

allow the company increase the products portfolio and will impact on the turnover and the staff of the company that is expected to be increase with 4 new workers on the next 3 years.

VORNIA is using the new knowledge acquired in NEURIMP to aid in design and develop of synthetic bioresorbable polymers with improved properties for application in the fields of tissue engineering and regenerative medicine. Customisable materials with specialised properties can allow Vornia to provide the best portfolio of bioresorbable polymer products to device makers and may impact on the turnover and the staff of the company in the coming years.

CONTIPRO is using the new knowledge acquired in NEURIMP to broad their knowledge about copolymerization of HA with natural polymers. The impact of the project towards novel applications of its patented electrospinning technology have increased marketing and collaborations in Europe.

4.3. Impact on the high qualifies human resources in Europe:

During the project, a high general advancement of knowledge has been acquired execution by the institutions involved and the 3 PhD students that have been trained on project topic.

Vornia trained 1 PhD on novel technologies on the production of high-quality bioresorbable polymers for application in the medical field. Also, many of Vornia's general staff members acquired significant knowledge in terms of design, management, risk analysis and dissemination from time spent on the NEURIMP project.

UoW, trained 1 PhD on Nerve tissue engineering using blends of polyhydroxyalkanoates.

USF, trained 1 PhD on 3D In vitro analysis methods.

4. 4. New knowledge dissemination and communication:

A clear and target oriented dissemination strategy has been essential to enhance the impacts of the Neurimp project. Therefore, leading by the committee for dissemination and exploitation main objectives defined in the preliminary *Plan of Use and Dissemination of Foreground* (PUDF), have been reached.

The patent strategy defined in the initial PUDF has limited strongly the publication of most relevant research papers, which has been postponed until the NEURIMP’s developments are well protected. It means that most of the publishable results of the project will be published in 2018. In summary, currently 3 paper are already published and 2 more have been submitted, and it is expected that 3 paper more will be published during 2018.

During the 4 years of NEURIMP development, different types of dissemination and communication activities have been developed by the consortium partners.

- Thanks to the high presence of the partners in symposiums, congresses and conferences of international and European relevance, a big number of oral presentations (37) and posters (15) have been made for dissemination of NEURIMP goals and results as well as leaflets spreading.

- NEURIMP website was constantly updated with public accessible information including events and papers of interests for Neurimp´s stakeholders. Moreover, website traffic was regularly checked via google analytics, to have an idea of the impacts of the dissemination performed and to promote new actions.

- The consortium has taken advantage of social networks and the project has been present in facebook, linkedin and Twitter.

- Furthermore, in order to reach to the general audience, radio and press mass media were used.

4.5. Transference of new materials and technologies from the Universities and Research centers to the industries, patent licensing…

The two patent applications developed generate intellectual property rights over: a) the use of certain PHAs biomaterials for peripheral nerve injury, and b) the combination of a hollow tube and an inner structure based on either microchannels or fibers with a well-defined combination of biomaterials and geometries. These filled patents (UK and PCT) alongside the exploitation agreement between partners having generated the foreground will be used for discussion with target companies fabricating commercial nerve guidance conduits based on natural or synthetic polymers. A posterior agreement will be discussed for patent licensing.

The research centers working in microfabrication will use this knowledge to generate new scaffolds for other supplication fields in medicine regenerative with different shapes and a certain control over the degree of porosity in terms of porous volume and porous size.

The research centers and SME synthesizing PHAs biomaterials and its combination with synthetic polymers and Hyaluronic acid-based natural materials will take advantage of the biomaterials' up-scaling developed during the NEURIMP project. It will allow them to synthesize medium to large quantities of new biopolymers for analysis of its mechanical and thermal properties as well as *in-vitro* analysis of biodegradability, cytotoxicity or hemocompatibility. It will also help to be used for fabrication in new microfabrication processes and 3D printing protocols under different microfabrication setups.