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Design and fabrication of tubes-guided structure with electrical stimulation module for neural regeneration and in-vivo testing

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Abstract. The research work is proposing a new biointerface to permit functional recovering of peripheral nervous fascicles using repair surgery. One biointerface can be experimentally used for Lewis rats' sciatic nerve. Simple tubes-guide structures are already experimentally used to safely regenerate peripheral nerves in recent years. Using dedicated software, the main tubular structure has been designed and then it has been 3D printed. Two types of gold electrodes (2 mm and 4 mm long) wrap the tube-guided structure in order to allow electric signals application around the tubes-guided structure. A special compact Wi-Fi electronic module containing a low frequency oscillator is attached to this structure. This novel biointerface enables a programmable electric stimulation process that accelerates the nerves growth after repair surgery. Functional tests may be performed and their results are expected to show that the regeneration rate of the sciatic nerve of the rats increases.

Key-words: Electrical stimulation, nanoelectrodes, biointerface, peripheral nervous fascicles, tubes-guided.

1. Introduction

Despite the development of microsurgical techniques in the last years, peripheral nerve repair continues to be a difficult task for plastic surgeons and neurosurgeons, as the medical and functional recovery of patients is rarely complete [1]. Most peripheral nerve injuries can only be treated by reconstructive surgical procedures [2]. The high number of people ($>$ 1 mil each year) suffering peripheral nerve injury due to very different causes: accidents, stretch, cutting, penetrating traumas, etc. creates huge needs and expectations from both doctors and patients [3]. Most of the treatment methods are unsatisfactory after lesions in the peripheral nervous system. New methods are expected to fulfil the existing needs. Traumatic injuries of peripheral nerves are the major causes for morbidity and disability in Europe and have a considerably high social impact. In Europe it has been estimated that the incidence of peripheral nerve injuries derived from trauma is about 300,000 cases per year [4]. There are 20 million people suffering from peripheral neuropathy in the U.S. and approximately $150 billion is spent annually for health care to peripheral nervous system (PNS) injury patients, which shows that health care costs associated with these disorders constitute a major economic burden [5]. These injuries cause irreversible disabilities such as permanent loss of sensation and motor function in SCI. According to the 2014 report of National Spinal Cord Injury Statistical Center (NSCISC), less than 1% of SCI patients could completely recover after injury [6].

Experimental work in recent years has highlighted the importance of tub-guide using to regenerate peripheral nerves after repair surgery [1]. The medical caseload of recent years has shown that peripheral nervous system disorders are characterized by a very limited recovery of lost functions and are frequently associated with neuropathic pain [7]. In functional recovery an important factor is the time elapsed between the time of the nerve injury and the surgical repair [8]. Recent research in this area has shown that the use of MSC (mesenchymal stem cells) has important therapeutic effects in injuried nerves regeneration: replacement of damaged cells, secretion of many growth factors and cytokines, inflammatory action and contributes to increasing immunity [7].

Experimental studies that were performed on both animals [9] and humans [10] showed that using of tube-guide biointerfaces help to safely regenerate peripheral nerves of the human patients. Clinical trials conducted on animals and humans have shown that neuroma formation is prevented if tubular structures for nerve regeneration are used [7].

In many clinical trials it is shown that biointerfaces with tubular structures for nerves ensure "a favorable microenvironment for the regeneration process during the healing period" [7]. In some clinical trials, it has been shown that tube-guide biointerfaces of PLC are biocompatible with nerve cells and improve morphological and functional recovery of peripheral nerves [7–11]. In some clinical trials, they were used to fabricate tube-guide biointerfaces from polymers with high electrical conductivity (PVA loaded with electrical conductive materials (polypyrrole (PPy)) and it has been found that they have improved the regeneration of the peripheral nerves. These tube-guide biointerfaces have also allowed the use of electrical stimulation for the accelerated regeneration of peripheral nerves of the human patients [12]. "These strategies improved the nerve regeneration, since PVA with CNTs or PPy is a conductive biomaterial with a high electrical

conductivity" [13].

2. Design the tube-guided structure for 3D bioprinting

The tubular structure was designed using the CATIA software. The CATIA software modules is used for project management, electronic systems design, biological systems modelling, and so on [14]. CATIA also has an interface easy to work with and customizable menus. Of these, the following modules were used to design the tubular structure:

1. The Sketcher module - for drawing a sketch of a 2D profile (flat plane).

2. The Part Design Module - for design the 3D parts of the tubular structure (in space).

3. The Drafting module - tools for design the 2D and 3D industrial drawings, the quotation being done automatically/manually [14].

After the design of the model, the CATIA file has been transformed into a readable file for 3D printer. Designers and engineers use additive technologies to realize conceptual models and finished products. Doctors and surgeons use 3D printing to get some medical equipment for training or simulation [15, 16]. In our case, the DLP Printing Technology (Digital Light Processing) was used, which is an additive technology consisting on the use of ultraviolet light for the consolidation of liquid polymer materials. Initially, the 3D CAD model is transformed by the programme of the 3D printer into the cross sections (slices) of the final piece, and then the resulted data is sent to the installation. Under the action of ultraviolet light, the photosensitive liquid solidifies in successive layers. With the "Creation Workshop" software (Fig.1a) of the printer it is possible to view and positioning the piece on the work plane as well as the mirroring operations, scaling, rotating and the possibility to simulate the deposition of the layers during the fabrication of the object (Fig.1b). After the slicing operation, the work platform is submerged in the polymeric material of liquid state and the manufacturing process begins [15, 16]. These additive technologies are the manufacturing processes with a spectacular growing in the various industries with multiple advantages comparing with traditional fabrication methods. With 3D printing it is possible to generate some biocompatible parts with remarkable precision (1micron resolution) and performances which can be used in many medical applications.

The fabrication and integration of the tubular structure of the biointerface (Fig. 2) was made by 3D bioprinting (in vivo) with biocompatible gels (CELLINK pluronics, collagen, gelma). The geometry of the regenerative neural biointerface was customized on the internal structure of the nerves where they were implanted so as to enable each nerve fascicle to grow in separate channels within the neural biointerface. The tubular structure of the neural biointerface separate the specific nervous fascicles into the nerve.

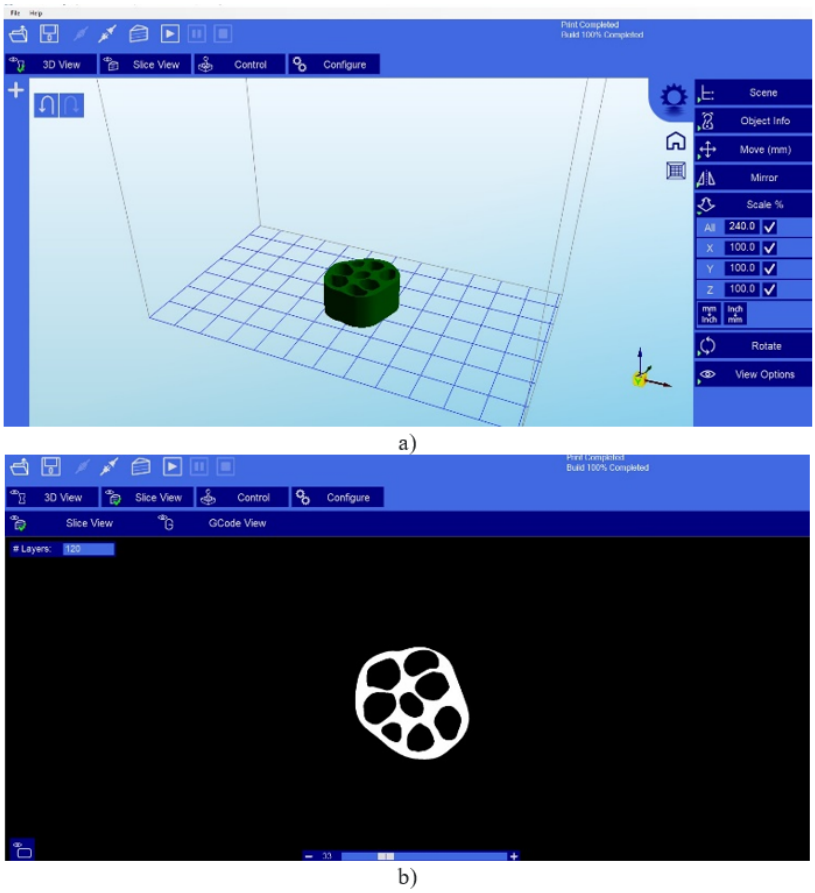

Fig. 1. Design the tubular structure using CATIA software: a) 3D view and b) 2D view.

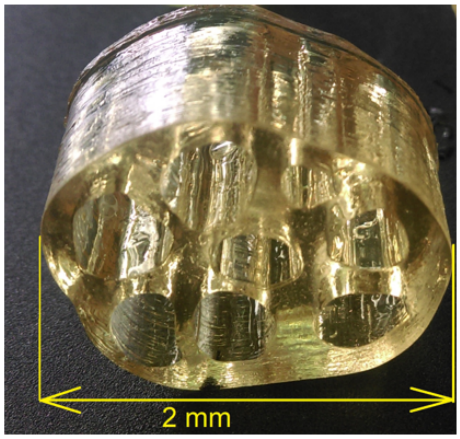

Fig. 2. The 3D bioprinted tubular structure of the neural biointerface.

3. Design the electronic module for electrical stimulation of the nerve

An electronic module equipped with gold electrodes has been used to electrically stimulate the nerve. Two types of electrodes were designed and fabricated: 2 mm and 4 mm (Fig. 3).

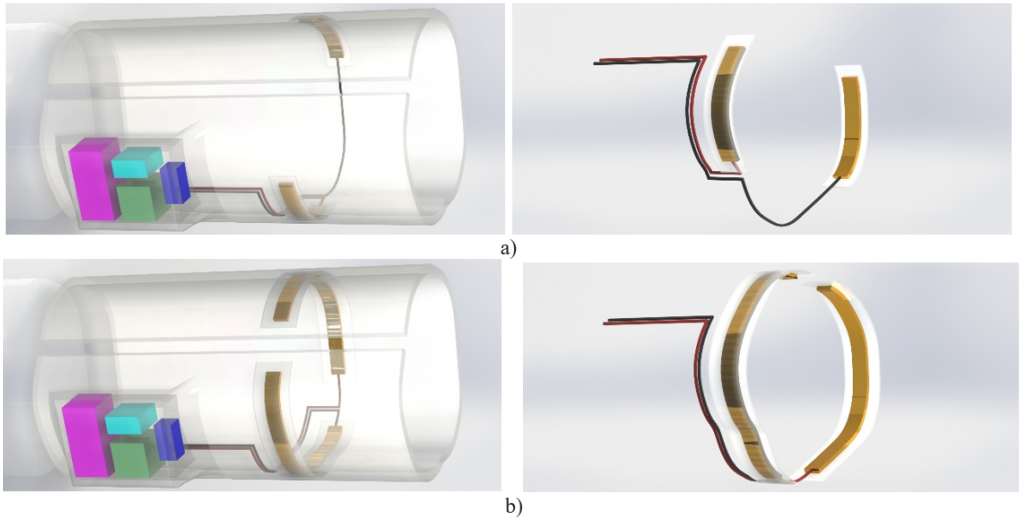

Fig. 3. Electric stimulation electrodes and Wi-Fi module before mounting on the nerve.

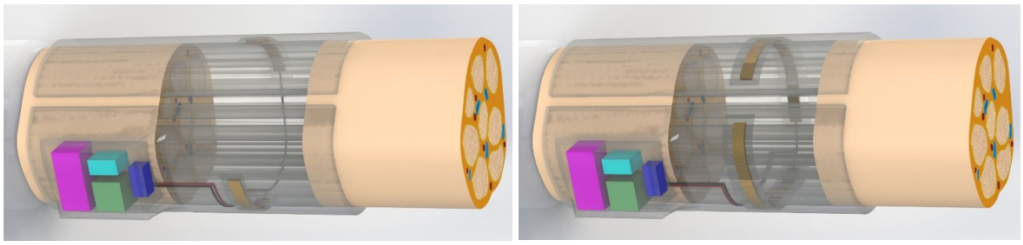

Fig. 4. Electronic module mounted on the nerve and equipped with two types of stimulation electrodes: a) 2 mm electrodes; b) 4 mm electrodes.

The block diagram of the electronic module is shown in Fig. 5 and it is based on previous experimental designs [16]. The module contains a programmable oscillator, SiT1534 Ultra-Small, Ultra-Low Power 1 Hz – 32.768 kHz as the main part of the circuit from SiTime [17], in the smallest market available footprint. The use of random signal generators was also analyzed and it was found that the functional scheme of the electronic module would become too complex.

The power supply uses an induction coil. The fabrication of the electronic module was made in a compact form and was encapsulated in a structure fabricated with biocompatible and absorbable materials. It was anchored to the sciatic nerve of the rat using a grip sleeve fabricated

with biocompatible materials. At the end of the regeneration process and after the complete functional restoration of regenerated axons, the electronic components (electrodes and Wi-Fi module) were removed from the peripheral nervous system of the rats by surgery. The implantation of the Wi-Fi module allowing electrical stimulation was minimally invasive, has very small dimensions and because of the wires replacement by this module the risk of infections was reduced significantly.

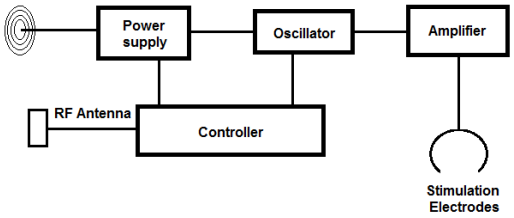

Fig. 5. The WIFI module block diagram.

4. Protocol for implanting and testing the neural biointerface in the sciatic nerve of rats

One biointerface can be experimentally implanted in the Lewis rats' sciatic nerve. The anesthesia protocol involve isoflurane induction and a cocktail of Xylazine and Ketamine in order to maintain sedation. An incision of the skin in the gluteal region is performed. The muscles are divided and the sciatic nerve is revealed. The nerve is cut 1 cm below the sciatic notch. The nerve stumps are photographed. Using a 3D bioprinter with a biodegradable polymer (Poly D, L – lactic-co-glycoic acid), the biointerface is designed to fit the nerves fascicular structure. In the study group the electrical stimulation module may be implanted, while in the control group is not. The wound is closed in anatomic layers using absorbable sutures. The module should be activated immediately after surgery. The rats are clinical assessed on daily basis. Functional tests are performed at 3, 6 and 12 weeks. The pin prick test (pinching the skin from knee to toes until movement is achieved; grade 0 – no movement, grade 1- above the knee, grade 2 – below the knee, grade 3 – distal to the ankle) and the toe-spread test (0 – no movement, 1 – any sign of mobility, 2 – abduction, 3 – extension) are enough to establish the degree of nerve regeneration. After 12 weeks, surgeries must be performed to recover the electrical stimulation modules. We should expect that the bio conductors are completely absorbed, while around the module a capsule of fibrous tissue is formed.

5. Electrical stimulation module for neural regeneration

Electrical stimulation must be used to accelerate the axons regeneration from the tubular structure of the biointerface: the circular positioning of the electrodes on the outer surface of the damaged nerve allows the electrical stimulation to be performed locally directly on the axons using for regeneration a low amplitude signal with an optimal frequency (20Hz) [19]. A SiT1534 Ultra-Small, Ultra-Low Power 1 Hz – 32.768 kHz Programmable Oscillator should be used. The frequency of signal generated by this circuit is factory programmable starting from 32.768 kHz

down to 1 Hz, with less than 20 ppm frequency tolerance. It has a very small footprint in chip-scale (1.5 x 0.8 mm2) and it is Pb-free, RoHS and REACH compliant. The DC voltage supply ranges from 1.5V to 3.63V, the circuit supports low-voltage battery backup from a coin cell or supercap, with a bias current less than 1 μA. In order to accelerate the axons regeneration in the tubular structure, the low-frequency electric stimulation (20 Hz) is used for 10 days. The 20 Hz frequency is selected due to the fact that according to other experimental findings, this is the mean frequency to generate action potential in motorneurons [19]. The results of the functional test should show that the regeneration rate of the sciatic nerve of the rats is accelerated in cases where electrical stimulation was used compared to those in which it was not used.

6. Conclusions

A tubular structure was firstly designed and then 3D printed in order to separate the specific nervous fascicles into the nerve. Gold electrodes wrapping this structure allow electric stimulation using a special compact Wi-Fi electronic module contains an ultra-Small and ultra-low power programmable oscillator that enables the low-frequency electric stimulation towards nerves‘ accelerated regeneration. Using such special designed biointerface, wholly externally supervised, power-supplied and controlled, infectious risks are significantly reduced. A biointerface can be experimentally implanted in the Lewis rats' sciatic nerve together with the electrical stimulation module in order to accelerate the natural nerves growth. The results of the functional test should reveal that the regeneration rate of the sciatic nerve of the rats increases.

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