3D-BIOPRINTING SYSTEM FOR FABRICATION OF ALGINATE MICROFIBERS

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The given work is devoted to the theme of 3D-printing of alginate microfibers with encapsulated stem cells which are used for the nerve regeneration. The motorized printing device is developed and tested. The appropriate software controls the device and allows to design the printing structures.

Some nerve injuries require repair in order to regain sensory or motor function. The primary indications for nerve repair or grafting are 1) an injury or continuity defect in a nerve, as a result of trauma, pathology, surgery, or disease, that cannot regain normal function without surgical intervention and 2) loss of normal neurologic function, resulting in anesthesia, paresthesia, dysesthesia, or paralysis, that cannot be corrected by nonsurgical treatment. In some nerve injuries (e.g., neurapraxia), the nerve regains sensory or motor function unless irreversible compression, neuroma (axonotmesis), or transection (neurotmesis) occurs. In more severe injuries, there may be significant loss of nerve substance (continuity defect), or a section of nerve may need to be removed to expose normal nerve tissue in preparation for nerve repair. Thus, nerve repair and nerve grafting procedures may be required to provide continuity between the proximal and distal portions of the nerve [3].

The idea of the project is to find out if alginate microfibers with encapsulated stem cells, placed between the endings of the damaged nerve, can help to stimulate the growth of the new neural tissue. It is supposed to print alginate microfibers with a defined diameter and length (fig. 1a). Then they should be twisted into a one bunch, imitating the nerve stricture (fig. 1b). The bunch of alginate microfibers holds together thanks to scaffold, produced by electro spinning method, which should be the same size as the damaged nerve (fig. 1c). Next this bunch could be put to the place of the nerve’s damage, connecting fractured nerve (fig. 1d). It is supposed that stem cells, encapsulated in fibres, should stimulate the growth of the new neural tissue under the influence of the neural electrical pulses. These fibres should “show” the direction of growth, so that after the repairation the nerve could function as it has been before.

Fig. 1. Method idea: a – alginate fibre, b – bunch of alginate fibres; c – fibres in scaffold; d – nerve’s repair

After analyzing of 3D bioprinting systems, the structure chart of the device called Alginate MicroPrinter (AMP) was designed (fig. 2). It includes a three-dimensional motion system based on three stepper motors, which changes the position of the nozzle and its rate of movement. The pump controls the flow rate of alginate hydrogel. The PC with the corresponding software sends commands, represented by G-Code, to the Motion controller, which decode them and make the motor work. The developed design of the printer is represented on fig. 3.
Fig. 2. Structure chart of the AMP-System

Fig. 3. The AMP-System
The printing space has such dimensions: 250mm x 202mm x 70 mm. According to the size of the nerve, this is more than enough to print microfibers. The resolution of printing is 2,5 μm.

A motion of the nozzle and a printing program are controlled by special designed software, which sends commands to the Motion Controller. As the Motion Controller the prototyping board Arduino Mega 2560 is used. Three IC motor drivers, which are connected to the board, allow controlling three stepper motors. The program for the Arduino board was designed in Arduino IDE. This program allows receiving coded motion commands from the PC software, calculating the new coordinates and sending signals to the motor drivers which make motors, as well as the nozzle, to move. At the same time an alginate solution flows through the nozzle, so that a microfiber is printed in the Chloride Calcium solution. Commands, sent to the controller, are represented by G-code, which is used to control 3D-printers and CNC machines.

The control of the AMP is performed by the corresponding software, which is designed in the Microsoft Visual Studio Community 2015 using Visual C# programming language (fig. 4). This software allows us to use several modes: Printing Mode, Optimization, Manual Control. The optimization mode provides an opportunity to control the printing program, the speed of the nozzle motion, the dimensions of the printed fibres. This optimization mode helps to determine the relation between the speed of the nozzle and the diameter of the printed fibres. The results of this optimisation are used to calibrate the default program. The default program is a mode in which allows choosing which alginate solution is used and which diameter of the microfiber is fabricated. Other parameters of the printing program for all situations are default (but can be changed by an authorized person).

In future the designed system is supposed to be improved to get the opportunity of printing any 3D-model with the alginate solution. This will allow to print parts of scaffolds and tissues of organs.

![Fig. 4. Software for AMP-system](image-url)
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