Incorporation of nerve growth factor-loaded microspheres into chitosan/polycaprolactone hybrid implants to enhance peripheral nerve tissue regeneration

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INTRODUCTION

OBJECTIVE

- Develop a new method for fabrication of implants intended for peripheral nervous tissue engineering
  - fast
  - sterile
  - customized implants (dimensions, composition)
- Implants:
  - in the form of hydrogel
  - structurally mimicking the extracellular matrix of the peripheral nervous system
  - able to release active agents in a spaciotemporal controlled manner

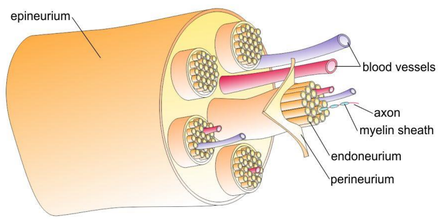


Figure 1. Structural organization of the peripheral nerve.

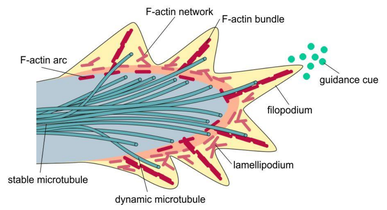


Figure 2. Structure of the growth cone.

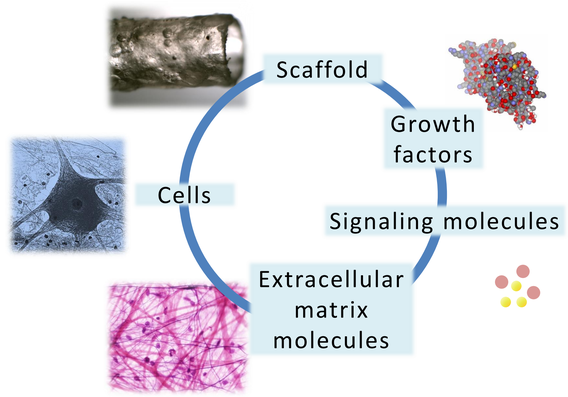


Figure 3. Components of implants for peripheral nervous tissue engineering.

METHODS AND RESULTS

IMPLANT FABRICATION

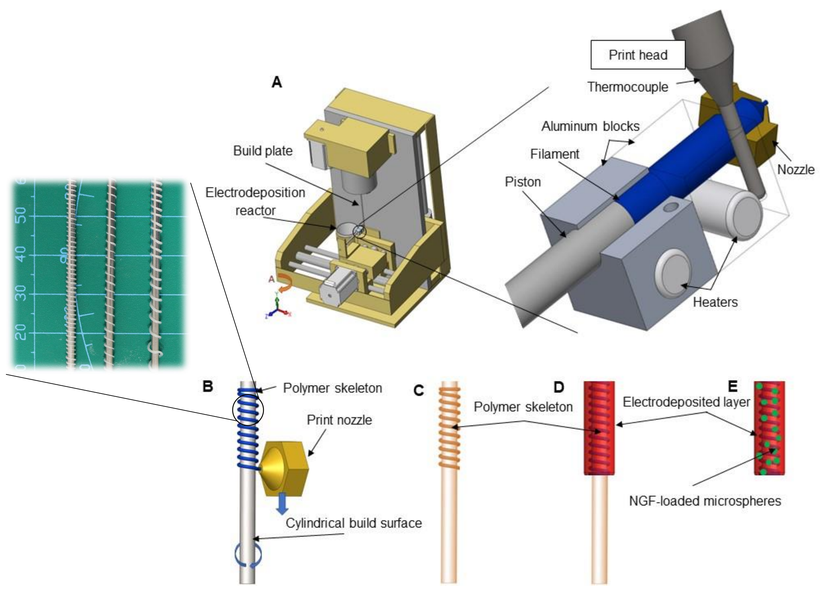


Figure 4. (A) Scheme of the apparatus based on polymer extrusion and electrophoretic deposition for fabrication of hybrid implants. Sequence of the manufacturing process: (B) PCL helix can be extruded with predefined geometry, constant or gradient pitch. (C) PCL helix is covered by dopamine. (D) Chitosan hydrogel deposit is obtained in the process of electrophoretic deposition. (E) NGF-loaded microspheres can be successfully adsorbed to the structure of implant.

Patent application sent to the Patent Office of the Republic of Poland: P. P. 428594 (2019).

STRUCTURAL CHARACTERIZATION

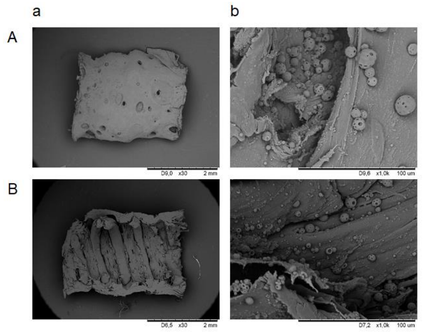


Figure 5. SEM images of implants. (A) Outer and (B) inner surface. (a) 30x and (b) 1000x magnification.

Figure 6. (A) The confocal images of mHippoE-18 cells grown on tested CH/PCL/NGF-μS implant. (B) Influence of implant and control biomaterial on axon elongation and of mHippoE-14 cells. (C) The light microscopy images of mHippoE-18 cells grown in the presence of tested CH/PCL/NGF-μS implant (right). Control cultures images in light microscope showing cells grown in medium alone (left) and cells grown in the presence of commercially available biomaterial serving as control (center). In the graph data are shown as a mean with error bars indicating standard deviation. Values with statistically significant differences are labeled by brackets and asterisks as follows: **** $p < 0.0001$. Data were compared using an ordinary one-way ANOVA followed by Tukey's multiple comparisons. CONT – control (cells w/o implant); BCS – commercially available biomaterial.

BIOLOGICAL PROPERTIES

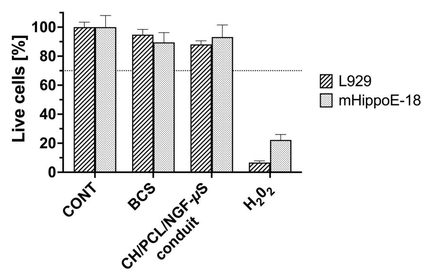


Figure 7. Results of cytotoxicity assay conducted for CH/PCL/NGF-μS implant and control biomaterial on L929 and mHippoE-18 cells. Data are shown as a mean viability ± standard deviation (n = 6) compared to cells grown in medium alone (control, n = 12). According to the ISO 10993-5:2009 standard the reduction of cell viability, in the presence of biomaterial, by more than 30% is considered a cytotoxic effect. CONT – control (cells w/o implant); BCS – control biomaterial.

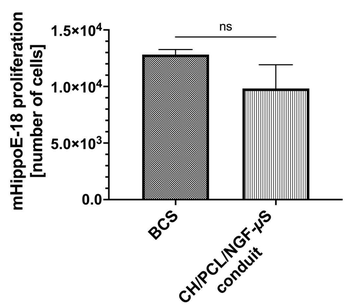


Figure 8. Results of proliferation assay conducted for CH/PCL/NGF-μS implant and control biomaterial on mHippoE-18 cells. Data are shown as a mean from three repeats with error bars that indicate standard deviation. Values with no statistically significant differences are labeled by ns: $p > 0.05$. Data were compared using Mann-Whitney U test. BCS – commercially available biomaterial.

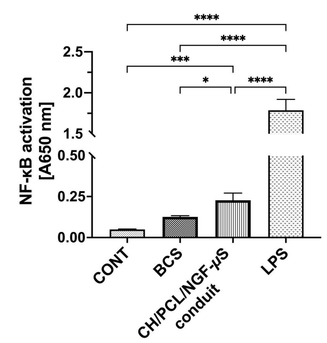
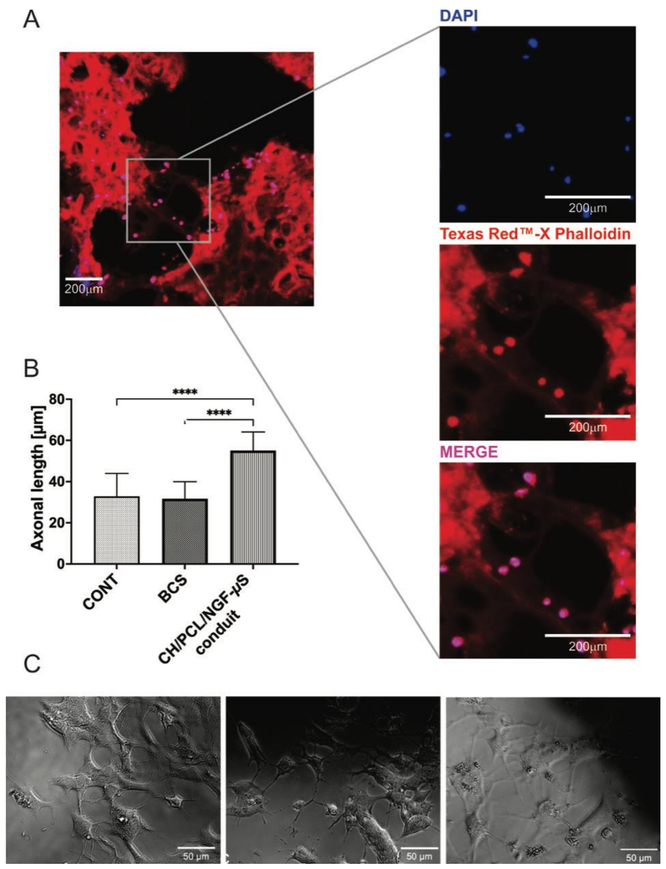


Figure 9. Results from interaction of CH/PCL/NGF-μS or control biomaterial with THP1-XBlue™ cells. Data are shown as a mean from three repeats with error bars that indicate standard deviation. Values with statistically significant differences are labeled by brackets and asterisks as follows: * $p < 0.05$, *** $p < 0.005$, and **** $p < 0.0001$. Data were compared using an ordinary one-way ANOVA followed by Tukey's multiple comparisons. CONT – control (cells w/o implant); BCS – commercially available biomaterial.



IN VITRO RELEASE STUDIES

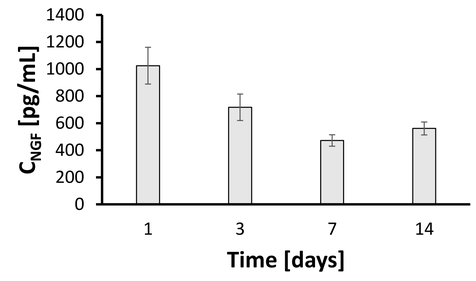


Figure 10. Cumulative release profile of NGF (pg/cm of CH/PCL/NGF-μS implant) over a 14-day period from CH/PCL/NGF-μS implant.

CONCLUSIONS

- New method for fabrication of implants intended for peripheral nervous tissue engineering was developed
- The possibility to change the shape of the extruded PCL structure is a valuable tool for incorporation of mechanical or biological cues that favor cell ingrowth, guidance, and correct targeting of axons
- As the developed automatic apparatus is designed to produce implants in a short time, the technology can be regarded as more effective compared to existing solutions
- Implants are cytocompatible, non-pyrogenic and due to controlled release of NGF stimulate the axonal growth of hippocampal cells
- The next studies will be focused on application of the received implants for appropriate animal models

ACKNOWLEDGEMENTS

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