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# Enhanced growth of neural networks on conductive cellulose-derived nanofibrous scaffolds

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ABSTRACT

The problem of recovery from neurodegeneration needs new effective solutions. Tissue engineering is viewed as a prospective approach for solving this problem since it can help to develop healthy neural tissue using supportive scaffolds. This study presents effective and sustainable tissue engineering methods for creating biomaterials from cellulose that can be used either as scaffolds for the growth of neural tissue in vitro or as drug screening models. To reach this goal, nanofibrous electrospun cellulose mats were made conductive via two different procedures: carbonization and addition of multi-walled carbon nanotubes. The resulting scaffolds were much more conductive than untreated cellulose material and were used to support growth and differentiation of SH-SY5Y neuroblastoma cells. The cells were evaluated by scanning electron microscopy and confocal microscopy methods over a period of 15 days at different time points. The results showed that the cellulose-derived conductive scaffolds can provide support for good cell attachment, growth and differentiation. The formation of a neural network occurred within 10 days of differentiation, which is a promising length of time for SH-SY5Y neuroblastoma cells.



## 1. Introduction

In modern society, recovery from spinal cord injuries (SCI) and neurodegenerative diseases (NDD), such as Alzheimer's disease and Parkinson's disease, accounts for one of the biggest global public health challenges in terms of the number of patients and the healthcare costs [1–3]. Neurons and synapses in the central nervous system are extremely limited for regeneration after substantial damage. The common current treatments include either invasive surgery or drugs that solely delay or temporarily ameliorate symptoms, and no permanent cure has yet been developed [4–6].

Tissue engineering can serve as a novel approach for improving the activity of nervous systems impaired by SCI and NDD. The neural tissue supported by tailor-made scaffolds can be used in different ways such as a replacement for injured tissue, as a mechanism of therapeutics delivery or as a drug screening model for the study of the degenerative neural disorders in vitro [7,8]. For in vitro studies, the main requirements for the support material are non-cytotoxicity and appropriate morphology. Biodegradability plays minor role since scaffold materials are not intended for transplantation, however the mechanical characteristics of scaffolds need to be suitable for neural tissue development and furthermore provide stability of structure throughout the necessary period of time. Non-cytotoxic scaffolds are able to attach cells without any detrimental impact [9]. Additionally, cell adhesion, migration, proliferation and differentiation, as well as oxygen diffusion and exchange of nutritive and metabolic substances, are greatly affected by external topography and internal porosity of a scaffold which should provide required space for cell development processes [10,11]. Furthermore, there is an important characteristic of scaffold material particularly concerning neural tissue. Neurons are capable of using relatively weak electrochemical signals in mV range for regulation of cellular functions. Electrical conductive scaffolds can help to transmit these essential signals between neurons, which has a positive influence on the development of neural tissue [12,13]. In earlier studies, carbon nanostructures with a wide range of conductivity values (mostly above 10 S $cm^{-1}$) showed a beneficial impact on the performance of neurons at both the single cell level and the neuronal network level [14–16].

Biopolymer-based scaffolds previously showed good results in tissue engineering applications [17]. However, an abundant biopolymer such as cellulose has been paid very little attention to this point as regards its use in engineering new scaffold materials with specific functional properties. Cellulose is a biocompatible polymer [18,19], and cellulose-based materials proved to be inexpensive and sustainable candidates

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for nerve regeneration as they were able to enhance the tissue-scaffold integration [20,21] and increase neural attachment and survival [22]. Yet, cellulose-derived scaffolds have a great potential for further improvements.

In this study, we introduce new cellulose-derived materials with the combination of essential properties needed for enhanced growth of neural tissue in vitro. Electrospun cellulose (EC) was used as a starting material to make conductive nanofibrous scaffolds, as illustrated in Scheme 1. Electrospinning allows production of nanofibrous material with suitable properties to mimic the neural extracellular matrix (ECM) environment including its structural, topographical and mechanical features [23–25]. Moreover, the EC was modified via two different methods to make the scaffold surface electrically conductive. In one method, the fibrous EC scaffold was functionalized with multi-walled carbon nanotubes (MWCNTs) while, in another method, it was transformed into carbon nanofibers (CNFs) via carbonization. Both conductive scaffolds are aimed to support adhesion, growth and differentiation of neural cells, which could be used in the development of a future disease screening model or a biomaterial for the regeneration of neural tissue in vitro.

## 2. Materials and methods

### 2.1. Production of scaffolds

#### 2.1.1. Electrospun cellulose

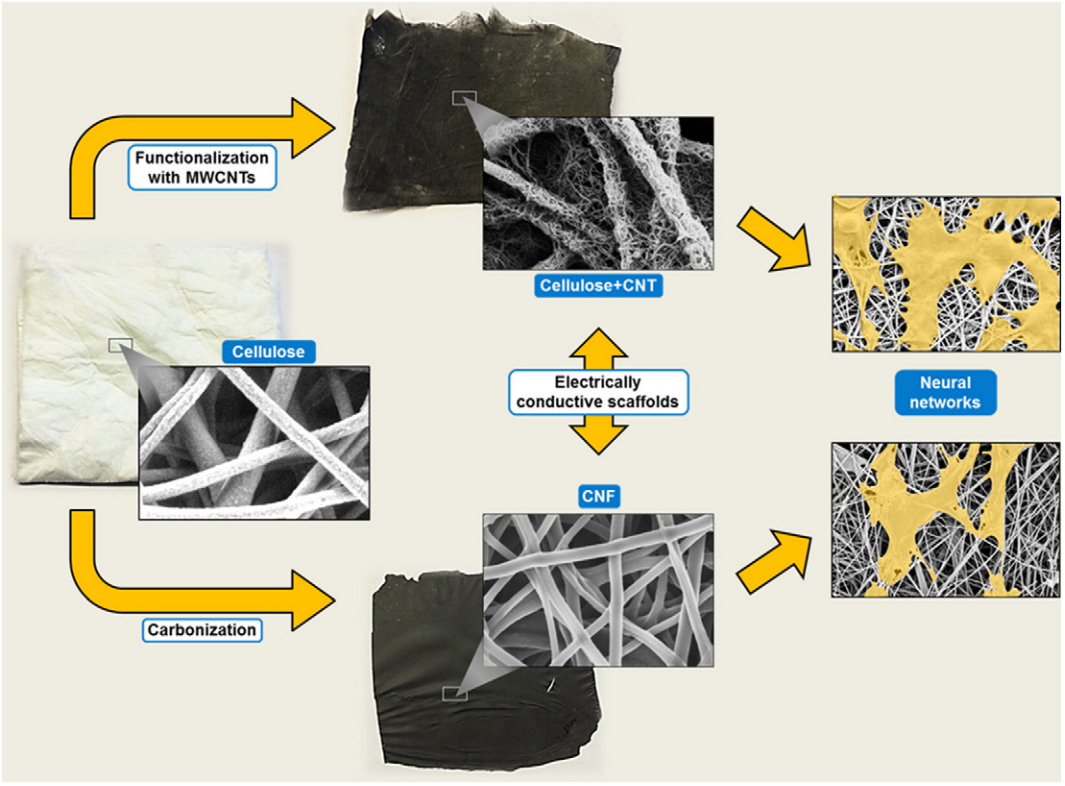
The mats of fibrous electrospun cellulose (EC) were produced by two consecutive steps of cellulose acetate electrospinning and subsequent deacetylation (cellulose regeneration). Cellulose acetate (17 wt.%, Mn = 30 kDa, Sigma Aldrich) solution in a 2:1 solvent ratio of acetone (99.9%, Sigma Aldrich) and dimethylacetamide (99.9%, Sigma Aldrich) was used for electrospinning, which was performed at 20 °C and a relative humidity of 65%. The solution was fed continuously by the syringe pump (10 ml, NE-1000) at a flow rate of 0.35 ml/h through a stainless steel needle with a blunt nozzle connected to a high voltage supply (Spellman's CZE1000R). The voltage between the needle and collector was 18 kV, and the distance was 15 cm. A cylindrical grounded collector with a diameter of 10 cm, rotating at 25 rpm, was used for collection of the fiber mats. After electrospinning, the fiber mats were dried at 80 °C for 24 h and then immersed in a 0.05 M NaOH ethanol solution overnight in order for cellulose regeneration to occur. The cellulose fiber mats were then washed thoroughly with deionized water to remove excessive NaOH and acetic acid.

#### 2.1.2. Functionalization with MWCNTs

First, multi-walled carbon nanotubes (MWCNTs, 30 mg, 0.2 wt.% concentration of CNTs in final dispersion, carbon purity + 95%, surfaced modified COOH, Nanocyl) were placed in Milli-Q (MQ) water (7 ml). The dispersion was heated to 90 °C in a water bath and stirred for 1 h in a tightly closed vial, followed by 20 min of sonication. Following this, cetyltrimethylammonium bromide (30 mg, Sigma, assay ≥ 98%) was added to the vial. The total volume of 15 ml was reached by an addition of MQ water. The solution was again heated to 90 °C in a water bath and stirred for 1 h in a tightly closed vial followed by 20 min of sonication. After heating, the dispersion was transferred to a centrifuging test tube and centrifuged for 20 min at 3500 rpm, and was then decanted over the precipitate into a separate container. Regenerated cellulose mats with a size of 5 × 5 $cm^2$ were immersed in the CNT dispersion and left for 96 h in a tightly closed container. Finally, cellulose samples containing CNTs were washed with MQ water and placed in a Petri dish for drying.

#### 2.1.3. CNF synthesis

Carbon nanofibers (CNFs) were produced via carbonization of regenerated cellulose samples in a quartz tube furnace (Tempress, Netherlands) in $N_2$ flow (1 l/min) by heating up to 800 °C at a heating rate of 5 °C/min. Samples were maintained at the highest temperature for 2 h and then cooled in flowing nitrogen.




**Scheme 1.** Illustration of the two fabrication methods of the cellulose-derived nanofibrous scaffolds for neural network development.