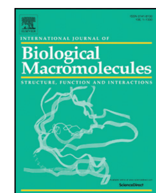
International Journal of Biological Macromolecules 119 (2018) 1195–1203

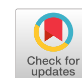
Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac



Fabrication, characterization and biocompatibility of collagen/oxidized regenerated cellulose-Ca composite scaffold for carrying Schwann cells



Wenli Song [a,1], Yuhua Zhao [a,1], Yadong Wu [b,*], Zhipeng Li [a], Hui Lv [c], Siyu Li [d], Yue Jiang [d], Chun Song [c], Fang Wang [b], Yudong Huang [b]

[a] Harbin Sport University, Harbin 150008, China
[b] MIIT Key Laboratory of Critical Materials Technology for New Energy Conversion and Storage, School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin 150001, China
[c] The First Affiliated Hospital of Harbin Medical University, Harbin 150007, China
[d] Harbin Medical University (Da Qing), Da Qing 163319, China

ARTICLE INFO

Article history:
Received 19 May 2018
Received in revised form 7 August 2018
Accepted 10 August 2018
Available online 12 August 2018

Keywords:
Schwann cells
Oxidized regenerated cellulose-Ca
Collagen
Composite
Biocompatibility

ABSTRACT

Schwann cell (SC) is the primary structural and functional part of the peripheral nervous system, and it plays a key role in the repair and regeneration of peripheral nerve. In order to develop a suitable scaffold for SC nerve tissue engineering, three kinds of scaffolds, including pristine collagen, pure oxidized regenerated cellulose-Ca (ORC—Ca) and collagen/ORC-Ca composite scaffolds, have been fabricated for carrying SC in this study. SC is then seeded on the scaffolds to form SC-scaffold nerve tissue engineering composites and evaluate their biocompatibility. The chemical and physical structure of the scaffolds are investigated by FTIR, NMR and SEM. The wettability of the collagen/ORC-Ca composite scaffold is close to that of pristine collagen, and the tensile strength of the composite scaffold (0.58 MPa) is better than that of pristine collagen (0.36 MPa). Cytotoxicity, cell proliferation, cell adhesion and western blotting assays are conducted to evaluate the biocompatibility and properties of different scaffolds. The results show that the three scaffolds exhibit no toxicity, and the proliferation rate of SC on the collagen/ORC-Ca composite scaffold is significantly higher than that of the other scaffolds ($p < 0.05$). The number of the adhesion cells on the composite scaffold (244.67 ± 13.02) is much more than that in the pure ORC-Ca group ($p < 0.01$). Furthermore, the expression of N-Cadheri and PMP22 proteins in the collagen/ORC-Ca composite scaffold is significantly superior to the other two scaffolds (both $p < 0.01$). Therefore, it could be concluded that the collagen/ORC-Ca composite is a promising candidate as a scaffold for carrying SC to form nerve tissue engineering composites in order to assist the peripheral nervous in the repair and regeneration.

© 2018 Published by Elsevier B.V.

1. Introduction

In sport training, peripheral nervous injury may happen to the athletes due to the axonal degeneration and segmental demyelination, which are caused by the nervous laceration and compression ischemia attributed to excessive training. Peripheral nervous injury could seriously affect the human's movement function, and even stop the normal exercise training [1]. Fortunately, the emergence of tissue engineering provides us a new direction to seek the ideal candidate for nerve tissue engineering composite [2–4]. With the rapid development of tissue engineering [5–8], the nervous repair has been converted from autologous nervous repair to tissue engineering nervous transplantation, which could overcome many defects brought by autologous transplantation [9,10]. Nerve tissue engineering composite can be used to repair the injury of the peripheral nerve and promote the regeneration of nerve [2,11,12], and it mainly contains the biological scaffold and the seed cells [13,14], protecting the axon and directs its growth [15–19].

Nowadays, some natural materials [20], synthetic polymer [21,22] and the bio-macromolecules have been employed to develop scaffolds that can direct the cell fate [23–25]. Collagen and cellulose are regarded as biodegradable materials, with high hydrophilicity and good biocompatibility [26–30]. They have been widely applied to prepare cell scaffolds [31], biological substitutes [32,33], drug controlled release agent in biomedical field and tissue engineering field [34–36]. Collagen could promote the growth and adhesion of different kinds of cells, leading the cells adhere onto the framework of scaffold to support the cells and make the tissue stable. However, its high degradation speed results in a bad performance for supporting [37,38]. Oxidized regenerated cellulose exhibits a slower degradation speed than that of collagen, and it also possesses a good mechanical property [39–43].

* Corresponding author at: School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin 150001, China.
E-mail address: wuyadong@hit.edu.cn (Y. Wu).
[1] Note: These authors contributed equally to this work

https://doi.org/10.1016/j.ijbiomac.2018.08.055
0141-8130/© 2018 Published by Elsevier B.V.

SC is evolved from precursor cell of neural crest and serves as the primary neuroglial cells of peripheral nervous system [44,45]. It wraps around the axon and forms myelin sheath of myelinated nerve fiber, in order to support and protect axon, maintain the operation and stabilization of microenvironment, participate in the nutrient metabolism of axon [46–49]. SC, after transplanted into the injured spinal cord, is able to form a permissive substrate and produce growth factors for the regeneration of axon [50–53].

In this study, we propose to design a composite scaffold for carrying SC for peripheral nerve regeneration. Three kinds of scaffolds, including pristine collagen scaffold, pure ORC-Ca scaffold, collagen/ORC-Ca composite scaffold, have been fabricated, tested and evaluated in our experiment. The obtained collagen/ORC-Ca composite exhibits good biocompatibility and could be used as a scaffold for carrying SC.

2. Materials and methods

2.1. Materials

Cellulosediacetate (CDA) (AR, Mn = 30,000) is obtained from Shanghai Chineway Pharmaceutical Technology Co., LTD., Shanghai, China. NO_2 (AR) is purchased from Summit Specialty Gases Co., Ltd., Tianjin, China. CCl_4 (AR), acetone (AR), $Ca(OH)_2$ (AR) and NaOH (AR) are received from ShuangShuang Chemical Co., Ltd., Yantai, China. Ethanol absolute (EtOH) (AR) is purchased from Fu Yu Chemical Co., Ltd., Tianjin, China. Ultra-pure water is purchased from Xinyue Chemical Co., Ltd., Weihai, China. Collagen is prepared from our laboratory. Hexafluoropropanol (HFP) is obtained from Sigma Aldrich, US. SC lines are obtained from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China. Mouse endothelial cells, bovine serum and penicillin are provided by Sanggon biotech Co., Shanghai, China. All the initial chemicals in the work are used without further purification.

2.2. Fabrication of the three kinds of scaffolds

2.2.1. Fabrication of pristine collagen scaffold

Collagen from bovine tendon is dissolved into HFP (w/v, 2/10) under constant stirring at ambient temperature. Until dissolve completely, the collagen solution is added into a syringe. The syringe is then placed onto a syringe pump with the speed of 0.6 mL/h, and the syringe tip is connected to a 50 kV voltage supply in order to charge the solution. The emitted collagen fibers are collected on a square conductive paper (length, 15 cm; width, 15 cm; distance, 11 cm). Finally, the pristine collagen scaffold is removed from the paper and freeze-dried at −55 °C for 15 h.

2.2.2. Fabrication of pure ORC-Ca scaffold

CDA is dissolved in acetone (w/v, 2/10) under constant stirring at ambient temperature overnight. 18 kV of voltage, 13 cm of distance and the 1.1 mL/h of flow speed is adopted for electrospinning. The obtained CDA membrane is detached from the collector and dried under vacuum at 60 °C for 10 h. Then, hydrolysis of CDA membrane for preparing cellulose is performed in 0.05 M NaOH/EtOH for 30 min at ambient temperature. Hydrolysis is terminated by thorough rinsing the membrane in distilled water, and then the hydrolyzed membranes are dried under vacuum at 60 °C for 10 h. Prior to oxidation, NO_2 is dissolved into CCl_4 to prepare 20% (wt) NO_2/CCl_4 oxidant solution, then hydrolyzed membrane is added into a round bottomed flask containing mentioned oxidant in a proportion of 1:42.6 (g/ml) (hydrolyzed membranes: oxidant). Stirring constantly, keep the reaction temperature at 19.5 °C and oxidation duration is 20 h. After the reaction, the membrane is rinsed three times with CCl_4, 50% (v/v) ethanol aqueous solution and 100% ethanol, respectively. The oxidized membrane is freeze-dried at −55 °C in vacuum for 48 h. Next, the oxidized membrane is immersed into 0.02 M $Ca(OH)_2$/ethanol solution, followed by shocking at 25 °C for 10 h. Finally, the prepared pure ORC-Ca scaffold is washed three times with 75% (v/v) ethanol aqueous solution and 100% ethanol, respectively, subsequent with frozen-drying at −55 °C in vacuum for 72 h.

2.2.3. Fabrication of collagen/ORC-Ca composite scaffold

The as-prepared pure ORC-Ca scaffold (thickness = ca. 0.1 mm) is used as the matrix to collect the emitted collagen fibers. The basic electrospinning parameters for the collagen fibers are the same as above, only the distance is changed to 8 cm, and each side of the pure ORC-Ca scaffold should be electrospun for 30 min, in which the mass ratio of collagen to ORC-Ca is 1:1. The obtained collagen/ORC-Ca composite scaffold is finally freeze-dried at −55 °C in vacuum for 24 h.

2.3. Characterization and test of the scaffolds

Fourier transfer infrared (FTIR) spectroscopy is obtained in the KBr pellet mixture form a Nicolet-Nexus 670 spectrophotometer. Solid state ^{13}C NMR spectra are recorded on Bruker Avance 400 WB spectrometer equipped with a 4 mm standard bore CP/MAS probe head (magnetic field 1/4 9.4 T, 13C frequency 1/4 100.12 MHz) at room temperature. The spinning rate and the contact time are 10.0 kHz and 3.0 ms, respectively. Each dried and powdered sample is scanned for 1000 times, recorded with 6 s recycle delay. Scanning electron microscopy (SEM) observation is obtained in a Hitachi S-4700 SEM. The wettability of the scaffolds is examined by dynamic water contact angle SL200KB measurement at room temperature. Tensile strength of the scaffolds was tested by using a Cmt8102 electric universal testing machine.

2.4. Cell culture

The SC lines are cultured in DMEM supplemented with 10% fetal bovine serum and antibiotics (100 mg/mL of streptomycin and 100 U/mL of penicillin) at 37 °C in a humidified incubator with 5% CO_2. Prior to the experiment, pure ORC-Ca scaffold, pristine collagen scaffold and collagen/ORC-Ca composite scaffold are cut into 1×1 cm^2 and sterilized under UV light for 30 min. Then, SC is seeded onto the three kinds of scaffolds, the growth of SC on the scaffolds is observed by using an inverted microscope.

2.5. Cytotoxicity assay

Pristine collagen scaffold, pure ORC-Ca scaffold, and collagen/ORC-Ca composite scaffold are cut into 1 cm × 1 cm and sterilized for 30 min under UV light. Then, the scaffolds are added into 10 mL of DMEM medium containing 10% fetal bovine serum and cultured at 37 °C in a humidified incubator with 5% CO_2 for 72 h. The leaching liquid is collected for later use. CCK-8 assay is used to evaluate the cell availability. Single cells suspensions of SC in 100 μL DMEM are added into a 96-well plate and incubated for 24 h at 37 °C under 5% CO_2. Then 50 μL of different scaffolds extracting solution and 50 μL DMEM are added into the 96-well plate and incubated for 24, 48 and 72 h, respectively. After that, the medium is removed, and then 100 μL of DMEM and 10 μL of CCK-8 solution are added into each well, which is incubated for additional 4 h. It is noted to avoid forming bubbles. The optical density (OD) of each well is measured by the enzyme-linked immunometric meter at 450 nm. Three parallel specimens are tested each time. Finally, the relative growth rate (RGR) is calculated, and the toxicity of the scaffolds could be evaluated according to the relative proliferation rate of cells.

2.6. Cell proliferation study

Firstly, three kinds of scaffolds are placed into a 12-well plate, then 1000 SC suspension per 100 μL in DMEM are seeded onto the scaffolds, and SC is directly seeded onto the well without any scaffold in the

Download English Version:

https://daneshyari.com/en/article/8326619

Download Persian Version:

https://daneshyari.com/article/8326619

Daneshyari.com