Journal of Fiber Bioengineering and Informatics 8:1 (2015) 133–142
doi:10.3993/jfbi03201513

Fabrication, Characterization and Biological Evaluation of PRGD/PDLLA/β-TCP Scaffold for Nerve Regeneration ⋆

Zhu Zhang [b†], Xiaopei Wu [a†], Yixia Yin [a,*], Zheng Zhao [a,*], Shipu Li [a]

[a] *State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan 430070, China*

[b] *Affiliated Hospital of Wuhan University of Technology, Wuhan 430070, China*

Abstract

A novel nerve repairing material PRGD/PDLLA/β-TCP was synthesized and characterized with Scanning Electron Microscope (SEM), Fourier Transform Infrared (FTIR) spectroscopy, and mass loss ratio. The effects of PDLLA or PRGD/PDLLA/β-TCP on viability and growth of Schwann Cells (SCs) were investigated by MTT assay and SEM. After implantation of different materials, histological assessment was performed. The results showed that, compared with PDLLA, PRGD/PDLLA/β-TCP materials displayed better biocompatibility, degradation property and less inflammatory reaction. Moreover, PRGD/PDLLA/β-TCP materials promoted the adhesion and proliferation of Schwann cells and exhibited better degradation performance than pure PDLLA. These results indicated that PRGD/PDLLA/β-TCP has a potential application in the fields of nerve regeneration.

Keywords: Schwann Cells; PRGD/PDLLA/β-TCP Composite; Implantation; Inflammatory Factors; Biocompatibility

1 Introduction

Numerous investigations have been accomplished toward scaffold materials for neural tissue engineering over the past 30 years [1–3]. Scaffold materials have a significant effect on peripheral and central nervous system regeneration. A variety of natural or synthetic polymers have been used for fabricating biodegradable nerve scaffold materials. After nerve tissue was repaired, the conduit will gradually degrade without inducing an inflammation [4, 5]. Commonly used natural and synthetic biodegradable polymers including collagen and hyaluronic acid,

⋆This work was supported by the National Basic Research Program (No. 2011CB606205), Natural Science Foundation of China (No. 51403168, 81190133), Natural Science Foundation of Hubei Province (No. 2013CFB354 and 2014CFB839), Central University Grant (No. 2013-IV-099) and Doctoral Research Fund of Wuhan University of Technology (No. 471-40120093).

*Corresponding author.

†Authors contributed to this paper equally

Email addresses: yinyx71@126.com (Yixia Yin), zhengzhao@whut.edu.cn (Zheng Zhao).

PDLLA [6], poly(L-lactide-co-glycolide) (PLGA), polyester urethane, poly-ε-caprolactone (P-CL) and poly(phosphoester) have been synthesized into conduits for conducting nerve growth [7, 8]. However, none of these polymers might be considered to be ideal nerve scaffold materials.

Currently, nerve scaffold materials is inclined to induce strong tissue reaction and aseptic inflammation, so these biodegradable nerve scaffolds hardly have the same repairing effects on nerve regeneration as autologous nerve transplantation.

Due to good biocompatibility, PDLLA and β-TCP have been approved to be applied in clinics by Food and Drug Administration (FDA). The PDLLA is used in animal experiments on nerve repair and shows good repairing effects [9, 10]. However, PDLLA degradation is acidic and easily causes aseptic inflammation in vivo, affecting its further application as nerve repairing material. β-TCP provides calcium ion which guides the direction of nerve axon growth cone regeneration and neutralize the acidity generated during PDLLA degradation [11]. Some literatures reported its regulation of inflammation by affecting inflammatory cytokines [12]. Furthermore, the PDLLA lacks bioactive signals existing in the Extracellular Matrix (ECM) for cell adhesion and proliferation. Partial sequences of protein polypeptide are known to promote cellular attachment and proliferation by providing anchorage points and thereby triggering signal transduction by activation of integrin receptors [13]. Therefore, the above problems can be overcame by introducing of partial sequences of protein polypeptide, such as ECM adhesion proteins and cell-binding peptides into PDLLA to enhance cell adhesion and proliferation. The Arg-Gly-Asp (RGD) sequence, found within many ECM proteins, has been the most extensively studied motifs and substrates and found widespread use in adhesion research [14].

In this research, we attempted to synthesize PRGD/PDLLA/β-TCP materials to promote the adhesion and proliferation of cell in nerve tissue regenerative process. We also studied their degradability and investigated cytocompatibility in vitro; the experiment of transplantation *in vivo* was conducted to detect histocompatibility and inflammation of materials to provide theoretical basis for the study and application of nerve repairing materials.

2 Experimental Methods

2.1 Acquisition of Cells

Newborn SD rats were sentenced by being soaked into 75% alcohol; sciatic nerve was removed under sterile conditions, cut into explanted tissues at the size of 1 mm^3 on clean bench, and then the explanted tissues were put into flask. The tissues were arranged at the distance of 1 cm. The flask was inverted and put into incubator at 5% CO_2 and 37 °C for 8 h; when the explanted tissues were firmly adhered to the bottom surface, turn over the flask and add a little culture medium. According to cell growth, culture medium was timely replenished. Generally, every 2-3 days, the culture medium was replaced with a new one. One week's culture of separated sciatic nerve explanted tissues, an observation of cell growth state was that the cells isolated from the marginal explanted tissues had covered the bottom surface. After abandoning culture medium, 0.25% trypsin was used for enzymatic digestion and separation, and differential adhesion for purifying Schwann cells.