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Design and optimization of a biodegradable porous zein conduit using microtubes as a guide for rat sciatic nerve defect repair

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ABSTRACT

Various degradable biomaterials have been used to bridge injured peripheral nerve defect; however, drawbacks such as poor mechanical properties, inappropriate degradation rate, and toxic degradation products continue to limit the application of them in nerve repair. Considering the unique properties of zein, such as its biocompatibility, biodegradability and ease of fabrication, we report the use of zein conduits to repair injured rat sciatic nerves with a 10-mm defect. Three-dimensional zein conduits were designed with/without pores, and with/without microtubes including in the lumen of conduits. Zein conduit with microtubes yielded satisfactory results in sciatic function index (SFI), proximal compound muscle action potentials, density of myelinated nerve fibres and myelin thickness, which were not inferior to autograft but slightly superior to the hollow conduit at the 4th month post-implantation. The conduits degraded almost completely within two months, which was shorter than the suggested period of four months. Thus, the use of a porous conduit with microtubes inside as the guidance may play important roles in successful repair. Notably, the regulatory body will more likely approve designs employing a single component, such as the natural polymer zein.

1. Introduction

Peripheral nerve injury is a common clinical issue and challenge to human health that can result from natural disasters, industrial injuries, traffic jams, war wounds and even some systemic diseases [1,2]. Grafts such as acellular allogeneic or xenogeneic tissues and artificial conduits must often be implanted when the nerve lesion has a long gap or defect [3]. Among various grafts, autografts are still regarded as the clinical gold standard to repair nerve injury. However, drawbacks such as the limited sources of donor tissue, extra incisions, and the need to sacrifice normal nerve tissue, which leads to the risk of neuroma, limit the use of autografts [4]. Thus, artificial nerve conduits were developed in the 1980s [5]. Initially, these conduits were non-degradable, thereby inducing chronic inflammatory responses and leading to poor functional recovery in the regenerated nerve, often requiring a secondary surgery to remove these materials [6]. Therefore, biodegradable conduits were subsequently developed.

Synthetic degradable conduits such as Neurotube® and Neurolac®, which are constructed from polyglycolic acid (PGA) and poly(DL-lactide-ε-caprolactone) (PLCL), have been approved by the Food and Drug Administration (FDA) and applied in the clinic. Nevertheless, Neurotube® has a high degradation rate, resulting in reduced mechanical properties, and Neurolac® has high rigidity, leading to joint inflexibility, foreign body responses, and severe swelling [7]. Natural conduits based on chitosan, collagen, alginate, silk and extracellular matrix components have been studied, and some have been used clinically. These natural materials not only stimulate cell adhesion and migration but also promote the proliferation and growth of cells [8,9]. Currently, some collagen conduits, such as Neurogen®, NeuroMatrix™ and Neuroflex™, are available commercially. Although Neurogen® is the most widely studied and applied product in the clinic, this compound undergoes a long degradation period of more than two years, which carries the risk of inducing nerve compression [7,10]. In comparison, the degradation period of collagen-based NeuroMatrix™ and Neuroflex™ is four to eight months [10]. However, clinical data indicate that these materials run the risk of cracking and tearing when penetrated by the suture needle [11]. A chitosan conduit, Reaxon® Plus, has also recently been approved by the FDA, but its degradation period is more than 77 weeks in rats, and the compressive mechanical

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property is low [12]. Chitosan conduits including PGA filaments have been used to repair nerve defects in the clinic, and more randomized clinical data are needed to support their medical application [13]. Considering the drawbacks of the currently used conduits, new materials with more suitable properties are welcomed.

The ideal nerve conduit should exhibit biocompatibility with a low host response, mechanical flexibility, good permeability, appropriate degradability, and the ability to promote nerve regeneration [14]. Zein is a natural polymer derived from corn, whose advantages include good biocompatibility, good biodegradability, ease of processing, wide resource availability, low cost and good mechanical properties [15]. Zein has been processed into films, microspheres, nanoparticles, gel, fibres and scaffolds for drug delivery, DNA transfection and tissue engineering applications [16–18]. However, the use of zein as a nerve conduit for peripheral nerve regeneration has not been reported. Our group has studied the potential of zein as a novel biomaterial for tissue engineering since 2002, and the first zein scaffold product, Resorbable Bone Substitute, has met the ISO 10993 requirements for biocompatibility according to the China State Food and Drug Administration of China (CFDA). Thus, we propose that zein can well fulfil the aforementioned requirements of an ideal nerve conduit.

Here, we use zein as a sole biomaterial, to study how the pore structure of the conduit wall and the use of microtubes to guide the conduit lumen affect repair. We designed a three-dimensional porous conduit with a controllable degradation time, which we used to bridge a 10-mm nerve defect in Sprague Dawley (SD) rats. The purpose of our study was to realize the complete degradation of the zein conduits and the intraluminal microtubes to achieve similar nerve regeneration and functional recovery to that of autografts.

2. Materials and methods

2.1. Fabrication of conduits and microtubes

Zein was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. The porous zein conduits were prepared using the dipping-leaching method. Different masses of sodium chloride were dissolved in deionized water to obtain different concentrations of sodium chloride solution (0%, 1%, 3%, and 10%, w/v). Then, 70% (v/v) ethanol solutions were prepared at each sodium chloride concentration. Zein powder was dissolved in each ethanol solution at a concentration of 150 mg/ml. Teflon tubes with an outer diameter of 1.5 mm were used as moulds for conduit fabrication. The moulds were immersed in the zein solution for 5 s and subsequently dried in the oven for 30 min. The temperature was 31 ± 2 °C and the humidity was 60 ± 5%. After dipping, the conduits were dried overnight under the same conditions. The salt was leached out in ultrapure water at different temperatures, including 60, 90 and 120 °C, for 30 min. Next, the conduits were soaked in fresh ultrapure water for another 30 min at room temperature. The zein conduits were freeze-dried and then cut into conduits of 12 mm in length. For *in vivo* X-ray imaging, medical grade $BaSO_4$ paticles were dispersed into zein solution at a concentration of 300 mg/ml, and the final content of $BaSO_4$ was nearly 30% (w/w) in conduits.

The dipping-leaching method was also used to fabricate the zein microtubes. Teflon microfilaments of 150 μm in diameter were used as the moulds for immersion. To obtain porous microtubes, a sodium chloride solution (30%, w/v) was prepared and subsequently used to prepare a 70% ethanol-sodium chloride solution. Next, the zein was dissolved in the 70% ethanol-sodium chloride solution at a final concentration of 150 mg/ml. The immersion and desalting processes were conducted as described above. Finally, the microtubes were cut into 8-mm segments.

Non-porous polylactic acid (PLA) conduits were also fabricated using the dipping method. PLA with a molecular weight of 100,000 Da was purchased from the Institute of Medical Device in Shandong, China. The PLA was first dissolved in dichloromethane and the particles of $BaSO_4$ were dispersed into PLA solution. The Teflon tubes described above were used as templates for immersion, followed by drying. This process was repeated until the thickness of the conduits reached 500 μm.

2.2. Morphological observation of the zein conduits and microtubes

The morphology of the zein conduits and microtubes were observed via fluorescence microscopy (IX71, Olympus Optical Co., Ltd., Japan) and field emission scanning electron microscopy (SEM, S-3400N, Hitachi, Japan and Ultra Plus, Carl Zeiss, Germany). Both the cross-section and outer surface of the zein conduits and the microtubes were observed.

2.3. Detection of the porosity of the zein conduits and microtubes

The porosity of the zein conduits and microtubes was characterized using the apparent densities method [19]. After freeze-drying, the weight of the zein conduit samples, including mass of dry samples (m_1), apparent mass of saturated samples (m_2), and mass of wet samples (m_3) was measured. The open porosity *Pa* and total porosity *Pt* were calculated according to the following equations:

$$Pa = (m_3 - m_1)/(m_3 - m_2) \times 100\% \quad (1)$$

$$Pt = (Dt - Db)/Dt \text{ and } Db = m_1 D_1/(m_3 - m_2) \quad (2)$$

Db, Dt and D1 represent the volume density of the conduits, the density of zein (1.22 g/cm^3) and the density of water at the testing temperature, respectively. In addition, the porosity and the pore distribution of conduits were also characterized by using a mercury intrusion porosimeter (AutoPore IV 9510, Micromeritics, USA).

2.4. Permeability of the zein conduits with and without microtubes

The permeabilities of the porous zein conduit (PC) and the porous zein conduit inserted with four microtubes (PCM) were evaluated according to the reference [20]. The glucose (10 mM, Mw = 180), lysozyme (1 mM, Mw = 14400) and bovine serum albumin (BSA, 1 mM, Mw = 66200) were added into PC and PCM. Then the conduits were sealed with silica gel and immersed into 1 ml phosphate buffered saline (PBS) in the oven with a temperature of 37 °C. The diffused glucose, lysozyme and BSA across the conduit wall were analysed by using a high performance liquid chromatography and bicinchoninic acid (BCA) protein assay kit (Beyotime Biotechnology, Nantong, China).

2.5. Testing the mechanical properties of the zein conduits

The mechanical properties of the zein conduits were tested using a universal testing machine (Z020, Zwick Roell, Germany). The conduits were incubated overnight in PBS at 37 °C prior to testing. The transverse compression test was performed on conduits with a length of 12 mm and inner diameter of 1.5 mm. A force was applied perpendicular to the longitudinal axis of the conduits at a rate of 1 mm/min. The compressive strengths of the conduits are reported as the compression load versus the displacement, calculated as the deformation ratio between the diameter of the compressed conduit and the initial conduit diameter [21]. Thus, we presented the load that conduits can withstand when the