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Preparation and degradation of chitosan-poly(*p*-dioxanone)/silk fibroin porous conduits

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ABSTRACT

Chitosan-poly(*p*-dioxanone) (CH-PDO) copolymers with various poly(*p*-dioxanone) (PDO) percentages changing from around 20 to 70 wt% were synthesized. The selected CH-PDO with the PDO content of around 45 wt% was blended with prescribed amounts of silk fibroin (SF) to build porous conduits that are potentially suitable for the applications in long-gap nerve repair. Some conduits were further crosslinked using genipin to ensure their required compressive strength and proper degradation rate. By optimizing processing conditions, crosslinked CH-PDO/SF conduits had an average porosity of around 70% in their porous wall, and the pore-size in the conduit wall presented a radial change within a region varying from around 10 to ca.100 μm. After being exposed to PBS degradation system for various periods up to 10 weeks, the weight loss of crosslinked conduits was less than 8 wt% and the pH of degradation media showed a small positive deviation from their initial pH of 7.4. After 10-week implantation in rabbits, most optimally crosslinked CH-PDO/SF conduits were able to maintain regularly tubal shape with a small decrease in their length and diameter and their *in vivo* degradation rate was predominately controlled by crosslinking and synergetically regulated by the content of SF and PDO in the conduits; and in addition, they showed well-defined ability to retain the compressive strength at a retention rate higher than 70% in comparison to their original strength in wet state. These results suggest that these newly developed CH-PDO/silk fibroin conduits have promising potential for long-gap nerve repair.

1. Introduction

Peripheral nerve injuries often occur due to tumor extirpation, traumatic injuries and some other reasons. One of commonly used clinical therapies for reconstruction of nerve gap is nerve autograft. Despite effectiveness of autografting, the use of autologous nerve grafts is limited by several disadvantages, including donor-site morbidity, limited availability and size mismatch [1]. In particular, in situations where the gap between the cut ends of the transected nerve is too long to permit connection by end-to-end tensionless suture or by autografting, artificial conduits are usually utilized to bridge the gap for the reconstruction of injured nerve [2]. To date, a variety of nerve conduits have been developed by using different materials and techniques [3]. Despite various morphologies and structures, the basic function of conduits is to provide temporary support for outgrowth of axons from the amputated nerve ends while preventing in-growth of fibroblasts into the nerve gap. In the cases of larger nerve gaps, for example, a gap length of around 30 mm or longer, increasing requirements for conduits are compulsory. It is generally accepted that suitable conduits for larger nerve gaps have to be strong enough to support nerve regeneration over a certain period of time while being degraded at proper rates because the reconnection of long nerve gap is time-consuming, and unsuitable conduits could collapse before accomplishment of never reconstruction [1,2,4]. Accordingly, selection of desirable materials is of importance for building satisfactory conduits used for the long-gap nerve repair.

Up to now, many synthetic polyesters, such as polyglycolide, polylactide, polycaprolactone and their copolymers, have been investigated for the fabrication of conduits [5,6]. These polyesters have mechanically strong characteristics in wet state, good processing properties and slow degradation rate. Despite of wide

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usability, they also have some intrinsic drawbacks including hydrophobicity, lack of reactive groups, neutral charge distribution and acidic degradation products [6–8]. As an alternative to synthetic polymers, increasing attention for desirable materials has been paid to certain natural polymers such as collagen, chitosan and silk fibroin [2,5].

Chitosan is one of the most abundant natural polymers next to cellulose and has demonstrated advantages such as biocompatibility, biodegradability, hydrophilicity, non-toxicity and cellular affinity and adhesion [9,10]. In particular, it is structurally similar to glycosaminoglycans (GAGs) that widely exist in many types of extracellular matrices (ECM), making it attractive for many biomedical applications [2,3,9]. Nevertheless, chitosan conduits commonly show brittle features and they often break when their two ends are sutured with epineurium of nerve stumps [9,11–13]. Furthermore, chitosan conduits have poor strength in wet state and fast *in vivo* degradation rate. Therefore, chitosan conduits are generally unsuitable for bridging longer nerve gaps because they may collapse early, and in turn, block the nerve regeneration [9,13].

Another natural polymer, silk fibroin (SF), has also been commonly used for nerve repair. SF is a fibrous protein and shows various advantages such as low inflammation response and thrombogenicity, promotion of cell adhesion and facilitation of three-dimensional colonization for many types of cells [14]. In addition, SF conduits show robust tensile properties in wet state and have significantly slow *in vivo* degradation rate compared to chitosan conduits [15,16]. However, pure SF conduits in dry state also have brittle characteristics, which is unfavorable for clinic operations. Therefore, SF is often used together with other polymers to improve strength and to extend degradation duration of resulting materials [14–17].

Poly(*p*-dioxanone) (PDO) is a biodegradable and biocompatible polyester with a low glass transition temperature (ca.-10°) [18]. In comparison to aforementioned polyesters, PDO has comparative strength while showing excellent toughness [19], which is especially favorable for enhancing mechanically weak and brittle biomaterials. In addition, PDO can be depolymerized, and the percent recovery of corresponding monomers can reach as high as 99.3% under conditions of reduced pressure and suitable temperatures [20], meaning that PDO can be highly recycled and function as a real low-carbon and environment-friendly polymer. Taking into account the merits of chitosan and PDO, chitosan/PDO complexes may serve as operational materials for constructing conduits in considering the mechanically strong and slowly degradable characteristics of PDO. Nevertheless, it is difficult to produce well miscible chitosan/PDO blends or composites because there are wide differences in the processibility of two components. Accordingly, grafting PDO onto chitosan could be an effective alternative to use them together.

In addition to the requirements on suitable materials, the external form and internal structure of conduits also play an important role in conduit functions. In spite of various structures, the interior of conduits usually appears to be single-lumen or multi-channels, and in many cases, the single-lumen conduits are usually stuffed with fibers, gels or others inside [4,21]. These fillers function as support for the attachment and migration of proliferated cells, and on the other hand, roughly guide the growth of regenerated axons. In the case of single-lumen conduits, the mechanical properties and degradation behavior of the conduits themselves will be of prime importance to the performance of conduits regardless of different fillers.

Nowadays, a variety of single-lumen conduits have been built by using various materials and methods. Nevertheless, there are still difficulties in achieving desired conduits that can effectively bridge long gaps while helping to regenerate nerves with satisfactorily function recovery. Engineering longer nerve having appropriately satisfied composition and properties practically similar to native never still remains a significant challenge [1,2,4,5,21,22]. In the present study, an attempt was made to fabricate a type of single-lumen conduits that can be further stuffed with certain fibers for the use in bridging long nerve gaps. Chitosan-PDO copolymers were synthesized and they were then blended with SF to build certain types of conduits in view of the advantages of chitosan-PDO and SF. To our knowledge, so far, no effort has been made to fabricate chitosan-PDO/SF conduits. It was expected that some optimized conduits with well-defined mechanical and degradation properties are able to endure *in vivo* degradation over a suitable period of time without early collapse. Some results in relation to the preparation and degradation of presently developed conduits were reported.

2. Experimental

2.1. Materials

Chitosan was supplied by Heppe Medical Chitosan GmbH. Viscosity average molecular weight and degree of deacetylation (DDA) of received chitosan were measured as $7.43(\pm0.16) \times 10^5$ and 94.3(±1.2)%, respectively, following reported methods [13]. 1,4-dioxan-2-one (DO) was purchased from Sigma–Aldrich, and it was dried using CaH_2 and distilled under reduced pressure prior to use. Hexafluoro-2-propanol (HFIP, 99.6%) was supplied by Fisher. Stannous octoate ($SnOct_2$), genipin and other reagents were purchased from Sigma–Aldrich and used as received.

SF was produced using Bombyx mori cocoons following the method described elsewhere [23]. The obtained silk fibroin solution was centrifuged at 7000 rpm for 10 min to remove impurity and dialyzed against distilled water for 3 days using membrane tube (MW cutoff: 3500). The resulting SF solution was concentrated to around 2.0 wt% and lyophilized for further use.

Chitosan-PDO copolymers were synthesized using a method similar to that described elsewhere [24]. In a typical procedure, a given amount of chitosan was introduced into a flask, and the flack was vacuumed and purged with nitrogen. A prescribed amount of DO was slowly injected into the flask while stirring (molar ratio of DO to chitosan:20), and the mixture was stirred at 60 °C for around 3 h. To this mixture, a $SnOct_2$ solution in DMSO was added (molar ratio of DO to $SnOct_2$:1000:1), and grafting PDO onto chitosan was conducted under nitrogen atmosphere at 100 °C for 48 h. The reaction was stopped by cooling the mixture in ice water, and the obtained products were extracted using dichloroethane in a Soxhlet apparatus for 48 h to remove the homopolymer. The resulting chitosan-PDO was washed with ethanol, water and lyophilized. The powdery chitosan-PDO product was further dried in vacuum at 40 °C until constant weight was reached. By mainly changing the molar ratio of DO to chitosan, chitosan-PDOs with various weight percentages of PDO were achieved.

2.2. Fabrication of conduits

Homemade molds were employed for building different conduits. Each mold was consisted of a polytetrafluoroethylene (PTFE) tube and a rod-like stainless-steel mandrel whose one end is fixed on a stainless-steel cap and the other end is equipped with a removable end-cap. A selected CH-PDO with PDO weight percentage of around 45 wt% were used for fabricating conduits in considering suitable processibility, compressive strength and degradation rate for the resulting conduits.

1.0 wt% CH-PDO solutions were produced by dissolving CH-PDO in a mixed aqueous solution containing 1.0% acetic acid