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Investigation of mechanical properties and degradability of multi-channel chitosan–polycaprolactone/collagen conduits

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ABSTRACT

Multi-channel chitosan–polycaprolactone (CH–PCL)/collagen conduits were fabricated for potential applications in long-gap peripheral nerve repair. CH–PCLs with various PCL percentages changing from around 30 to 45 wt% were used for the fabrication of conduits, and the collagen content in the conduits was controlled at about 8 wt% or less in order to ensure the required mechanical strength and degradation rate of the resulting conduits. The porosity and average channel diameter of the conduits with a dimension of around 5 mm in outer diameter and 30 mm in length were optimized as around 80% and 200 μm, respectively. Swelling index, compressive load, deformation recovery and bending stiffness of the conduits were measured, respectively. In vitro degradation measurements was conducted in phosphate buffer saline, and results revealed that the effect of collagen content on the degradation rate of the conduits was not significant until the degradation time reached around 6 weeks. After being implanted into rabbits for various durations, the degradation of the conduits appeared to be strongly dependent on the collagen content in addition to the dependence on the degradation time. After 10-week in vivo degradation, some conduits that contained 6 wt% or less content of collagen still showed shape integrity and required compressive mechanical properties, suggesting that the degradation rate of the conduits can be effectively regulated by the collagen content while the required properties for the conduits would be well maintained.

1. Introduction

Peripheral nerve injuries are very common cases in clinical practice. In situations where the nerve gap between the nerve stumps is too long to permit a repair by end-to-end tensionless suture, a device that can play a supportive role is usually required to bridge the gap for guiding the growth of regenerated nerve fibers [1]. In the case of a surgical repair of damaged nerves, the commonly used technique for bridging a long nerve gap usually involves nerve autograft. Despite effectiveness of autografting, the use of autologous nerve grafts is often limited by several inevitable disadvantages, including donor-site morbidity, limited availability and size mismatch to the injured nerve [2]. Therefore, artificial nerve conduits have been proposed as an alternative to repair injured nerves. To date, a variety of single-lumen nerve conduits have been built by using various materials and techniques [3]. Nevertheless, it is generally accepted that the hollow single-lumen conduits are usually inapplicable to the long-gap nerve repair, regardless of different microtopographies or functions, since nerve repair with the aid of single-lumen conduits may lead to inappropriate target reinnervation due to the dispersion of regenerated axons grown across the graft [4,5]. As an alternative to single-lumen nerve conduits, the multi-channel conduits have attracted increasing attention to peripheral nerve repair so far. In comparison to single-lumen conduits, several advantages correlated with multi-channel conduits have been mentioned [4–9]: (1) the conduit can provide large surface area for the synthesis of lamina basalis and for the attachment of the seeded cell; (2) the multi-channels orientated lengthways inside the conduits are capable of reducing the dispersion of axonal branches; and (3) the longitudinally arrayed multi-channels can also reduce the misdirection rate of re-grown axons or polyinnervation of various targets by different axonal branches originated from the same motor neuron.

Up to now, certain types of synthetic polymers, for instance, poly(lactic-co-glycolic acid) and polycaprolactone as well as some

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natural polymers, such as collagen and chitosan, have been used to fabricate multi-channel conduits [9]. Of natural polymers, chitosan has a great attraction for nerve repair due to its good biocompatibility, biodegradability, hydrophilicity, low cost as well as nontoxic and nonantigenic properties [10]. In addition, it is clear that the chemical structure of chitosan is closely similar to glycosaminoglycans that widely exist in many types of extracellular matrices, and thus, chitosan can serve as an ideal biomaterial for diverse biomedical applications [11]. To date, chitosan and its derivatives have been used for producing different types of conduits, including single-lumen conduits [12,13] and multi-channel conduits [14,15]. In spite of many successes, chitosan conduits in dry state usually show brittle characteristics so that they are likely to break during the implantation where the two ends of the conduits need to be well sutured with epineurium of nerve stumps [13–16]. Furthermore, chitosan conduits usually show poor mechanical strength in wet state and fast degradation rates under physiological conditions. Accordingly, chitosan conduits are frequently unsuitable for bridging long nerve gaps since their low mechanical strength together with fast degradation rate will possibly cause early collapse of the conduits and subsequent blockage of nerve regeneration. Some efforts have been made so far to improve the mechanical strength and degradation properties of chitosan-based nerve conduits [2,8,11].

Polycaprolactone (PCL) is a biodegradable, biocompatible and semicrystalline polymer. Although PCL has some advantages such as high mechanical strength, facile processability and acceptable permeability, it degrades very slowly due to its five hydrophobic $-CH_2$ moieties in each unit. As a result, PCL has not been utilized as frequently as other members of the aliphatic polyester family such as polyglycolide, polylactide or their copolymers [10,11,17]. In addition, high hydrophobicity and lack of cell recognition sites for PCL molecules also limit the cell affinity toward the surface of PCL-based devices [18].

In the case of long multi-channel conduits that are designed for bridging long nerve gaps, for example, a gap length of around 30 mm or even longer, increasing requirements for the conduits are compulsory. It is now widely recognized that the desirable conduits have to be strong enough to support the growth of the regenerated nerves over a required period of time while being degraded in vivo at proper rates because the repair process of long nerve defects is time-consuming, potentially over months, and unsuitable conduits could collapse before the repair is basically accomplished. In such circumstances, chitosan/PCL complexes may serve as apposite materials for constructing long multi-channel conduits because of the above mentioned merits associated with chitosan and PCL components. In fact, some efforts have already been made to directly blend chitosan and PCL together to achieve satisfactory chitosan/PCL complexes with improved properties [19–24]. Nevertheless, it is found that there is only very limited miscibility existed between chitosan and PCL components. Apart from directly blending them, grafting PCL onto chitosan backbone has been demonstrated to be an effective alternative to use them together, and some results have demonstrated that certain types of chitosan–PCL (CH–PCL) copolymers with proper compositional proportions show regulable characteristics in their mechanical strength and degradation rates, which is dependent on the proportion of two components [25,26].

In the present study, an attempt was made to fabricate a type of multi-channel conduits using selected CH–PCLs and a small quantity of collagen. Of them, CH–PCLs will serve as main component to provide mechanical strength and endurance against degradation for the conduits, and meanwhile, collagen will function as a component for mainly regulating the degradation rates of the conduits due to the fast in vivo degradation properties of collagen [27]. Although several studies have been conducted for CH–PCLs, little work has been done to construct multi-channel CH–PCL/collagen conduits so far. Therefore, the resulting multi-channel conduits will represent a new type of conduits that may act as desirable implants for the applications in peripheral nerve repair. Some results in relation to preparation of multi-channel CH–PCL/collagen conduits and measurements for the compressive and degradation properties of the conduits were reported.

2. Experimental

2.1. Materials

Chitosan with medium molecular weight was supplied by Aladdin Inc. To achieve highly deacetylated chitosan, the received chitosan samples were deacetylated in a 50 wt% NaOH solution for 2 h at 100 °C, and the alkali treatment was repeated once. Viscosity average molecular weight and degree of deacetylation of the resulting chitosan were measured as $2.31(\pm0.16) \times 10^4$ and 93.7(±1.9)%, respectively, following reported methods [28]. PBS packets, collagen I from calf skin, caprolactone and some biochemical reagents were purchased from Sigma–Aldrich and used as received. All other chemicals were of analytical grade and purchased from different companies in China.

2.2. Fabrication of multi-channel conduits

A certain number of homemade moulds were employed for building different types of multi-channel conduits. Each mould was consisted of a polytetrafluoroethylene (PTFE) tube and two well matched stainless steel end-caps. By changing the inner diameter, wall thickness and length of the PTFE tubes, and altering the concentration of mixtures and processing conditions, different multi-channel conduits with various parameters such as porosity, average channel diameter, outer diameter and length were produced.

CH–PCLs were synthesized using phthaloyl chitosan as intermediate following some reported methods [25,26]. Briefly, amino groups of chitosan were first protected by reacting chitosan with phthalic anhydride, and the resulting phthaloyl chitosan (PHCH) was then used as intermediate for subsequent synthesis of copolymers. Grafting caprolactone onto PHCH was carried out under N_2 in the medium of DMF and the obtained PHCH-g-PCL copolymers were deprotected by eliminating phthaloyl groups using hydrazine monohydrate. By mainly changing the ratio of PHCH to caprolactone, reaction time and the volume of the medium, CH–PCLs containing different weight percentages of PCL were synthesized. Selected CH–PCLs with various PCL contents changing from around 30 wt% to 45 wt% were used for fabricating multi-channel conduits in considering the required compressive mechanical strength and degradation rate for the resulting conduits.

The selected CH–PCLs were dissolved in 1% acetic acid solution to produce 1 wt% solution. To each CH–PCL solution, various amounts of 1 wt% collagen solution in 2% acetic acid were added slowly with stirring. The mixtures were then concentrated in different beakers at 40 °C with stirring using a water bath until they reached required concentrations. Afterwards, each mixture was slowly injected into a corresponding mould using a syringe equipped with a long needle. The PTFE tubes on different moulds were wrapped with some thermal insulating materials to reduce the heat transfer cross the wall of the PTFE tube. The filled moulds were then moved into a freezer in which they were frozen at a presetting temperature in a region changing from −20 °C to −10 °C for 48 h. After removal of two stainless steel end-caps, the samples inside the PTFE tubes were dried in a freeze-dryer at