DEVELOPMENT OF A NERVE SCAFFOLD USING A TENDON CHITOSAN TUBE

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INTRODUCTION

Because chitosan has useful biological properties such as enhancement of wound-healing and an anti-bacterial effect, it is frequently used as a drug delivery carrier and wound-healing agent. Although this polymer can be used in various shapes, its medical application is limited due to a low mechanical strength. To overcome the fragility of chitosan preserving its biocompatibility, we have developed a novel tendon chitosan tube as a nerve conduit. The chitosan tube has the ability to mold its cross section into various shapes, and the influence of the cross section on the peripheral nerve regeneration was examined.

Although many studies showed the effectiveness of laminin on nerve regeneration, laminin is very difficult to synthesize due to its large molecular weight and cannot be applied to human since it is a tumor inducing material. Therefore, several cell adhesion sites in laminin have been identified recently, and some of its functional sequences have been treated in a 1N NaOH aqueous solution at 100°C for 4 h to remove any including proteins. Subsequently, the samples were treated in ethanol of 95wt% at 95°C for 8 h (and named tendon chitin). Followed by deacetylation of the tendon chitin with a NaOH aqueous solution of 50wt% at 100°C for 8 h under a nitrogen atmosphere. This process was repeated 3 times to ensure complete deacetylation and repeatedly rinsed with distilled water to remove NaOH (and named tendon chitosan). And that chitosan particles of the tube aligned in a longitudinal direction. The mechanical property and enlargement of contact phase between tube surface and penetrating cells, such as Schwann cells and the growth cone of the regenerating nerve tissue was formed in the central part of the tube only.

MATERIALS AND METHODS

Preparation of the Tendon Chitosan

Tendons harvested from crabs (Macrocheira Kaempferi) were treated in a 1N NaOH aqueous solution at 100°C for 4 h to remove any including proteins. Subsequently, the samples were treated in ethanol of 95wt% at 95°C for 8 h (and named tendon chitin). Followed by deacetylation of the tendon chitin with a NaOH aqueous solution of 50wt% at 100°C for 8 h under a nitrogen atmosphere. This process was repeated 3 times to ensure complete deacetylation and repeatedly rinsed with distilled water to remove NaOH (and named tendon chitosan). And the tubes possessing a circular- (diameter of 2mm) and triangular-shaped cross section (equal sides of 2.1 mm length) were prepared. The chitosan tubes having a triangular cross section and introduced carboxyl groups, were reacted in laminin, CDPGYIGSR or CSARQKAASIKVAVSD for 3 h at 4°C. The adsorbed amount of laminin, YIGSR and IKVAV on the tube surface was 6.9+/-.1.4 µg/tube, 4.9+/-.1.3 µg/tube and 9.7+/-.2.2 µg/tube, respectively.

Implantation of a Chitosan Tube into the Nerve Defect

The right sciatic nerve of the male SD rats was exposed and bridge grafting of 15mm length was carried out with the chitosan tube. Chitosan tubes with respectively a circular or triangular cross section, as well as triangular tubes adsorbed with laminin, YIGSR or IKVAV were used. Twenty-one rats in each experimental group were operated. As a control, sciatic nerves of 15mm length, harvested from other SD rats, were grafted in a similar way in eight rats (isografts).

Three rats in each experimental group were sacrificed after respectively 1, 2, 4, 6 and 8 weeks. And three rats in the control group were also sacrificed after 8 weeks. Specimens were taken from the central part of the grafted tube in each group, and embedded in Epon 812 resin. Thin sections were prepared and stained with toluidine blue. Ultrathin sections were prepared for transmission electron microscopy (TEM). In five rats from each experimental and control group, electrophysiological evaluations were carried out on the hind limbs 12 weeks after implantation. Evoked muscle action potentials (M-waves) were recorded on the triceps muscle of the calf on both implanted and untreated sides. The latency ratio between the experimental and non-treated side of the same rat was compared. After recording of M-waves specimens were harvested from the central part of the grafted tubes and the sciatic nerves 10 mm distance of the distal anastomosed site, and embedded in Epon 812 resin. Photographs of thin sections of distal sciatic nerves were taken to determine the total area of myelinated axons per measured nerve tissue (percentage axon area). Analyses were carried out using the public domain Scion Image.

RESULTS

Properties of Tendon Chitosan

Electron microscopic observation of the chitosan tube revealed that chitosan particles of the tube aligned in a longitudinal direction. The tensile strength, elongation and Young’s modulus of the tube was 73.8+/-.15.1 (N), 12.8+/-.2.3 (%) and 21.1+/-.1.4 (MPa) respectively.

Histological observations of the grafted tubes

Prominent cell infiltration was observed both inside and outside the chitosan tube after 2-4 weeks. TEM observation revealed not only inflammation cell infiltration, but also macrophages attached onto the wall of the tube. The inner space of the tube showed abundant newly formed vessels. However, after 6 weeks, this active cell infiltration gradually disappeared. In the circular and triangular tube groups, nerve generation was observed in tubes after 6 weeks. In some cases within the circular tube group, remarkable stenosis of the inner space of the tube was observed. In the cases of which the inner space was preserved, regenerating nerve tissue was formed in the central part of the tube only. While in the triangular tube group, the inner space of the tube generally was preserved, and nerve tissue regenerated throughout the whole tube. This nerve regeneration was observed not along the tube wall, but rather in the central part of the wall. Both in the laminin and laminin peptide groups, nerve tissue regeneration along the tube wall was observed in many cases. Many non-myelinated axons and immature myelinated axons accompanied with Schwann cells started to associate, as early as from 4 weeks. In both the laminin and laminin peptide groups, nerve regeneration matched the control, as was observed after 8 weeks.

The axon area percentage

The percentage of axon area in the circular tube group was found lower than in any other group (p<0.05). There was a tendency that the percentage of axon area in the laminin peptide groups was larger than in the triangular group, and that the YIGSR group matched the laminin group (p<0.05). And the percentage of axon area in the laminin group was found equal only to that of the isograft group (p<0.05).

Evoked muscle action potentials

M-waves were obtained 5/5 rats in the laminin, YIGSR and isograft group, in 4/5 rats in the IKVAV group, 3/5 rats in the triangular group and 1/5 rats in the circular group. Although the latency ratio in the laminin peptide group matched the laminin group, both groups showed a higher latency ratio than the isograft group (p<0.05).

DISCUSSION

Histological findings suggest that a triangular shape of the tube’s cross section may benefit nerve regeneration due to its superior mechanical property and enlargement of contact phase between tube surface and penetrating cells, such as Schwann cells and the growth cone from the nerve ends. Percentages neural tissue also support the superiority of a triangular shape over the conventional circular type. Histological findings showed nerve tissue regeneration along the tube wall, not only for the laminin, but also for the laminin peptide groups, suggesting that these laminin oligopeptides may also serve effectively to nerve tissue extension. Furthermore, according the percentage neural tissue found in relation to evoked action potentials, the sequential treatments with first YIGSR and second IKVAV matched the effectiveness of total laminin to enhance nerve regeneration. Although this effect of laminin or laminin peptides adsorption on the inner wall of tube is substantial, it was also found, that it is not sufficient to enhance the growth of regenerating axons when compared to the action of an isograft. Certain humoral factors secreted by Schwann cells may be necessary in a co-operative effect with laminin or laminin peptides to attain results comparable to the isograft.

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