行政院國家科學委員會專題研究計畫　成果報告

不同交聯度之降解性神經管對再生神經功能恢復影響之評估

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Influence of cross-linking degrees for a biodegradable genipin-crosslinked gelatin guide on peripheral nerve regeneration

Abstract

We evaluated peripheral nerve regeneration using biodegradable genipin-crosslinked gelatin nerve conduits (GGCs) with three different cross-linking degrees around 24%, 36%, and 51%. Biocompatibility and biodegradability of the GGC and its effectiveness as a guidance channel were examined as it was used to repair a 10 mm gap in the rat sciatic nerve. From this pilot study, we conclude that GGCs with the mean cross-linking degree at 36% can ensure nerve regeneration with a more mature structure demonstrated by better-developed epineurial and perineurial organization and axonal development. Regenerated nerves in the GGCs with the mean cross-linking degree at 24% and 51% were less favorable, could be due to degradation material irritation and mild nerve compression, respectively.

Introduction

In our previous study, we successfully used a biodegradable genipin-crosslinked gelatin conduit (GGC) to bridge a 10 mm gap in the sciatic nerve of the rat [1]. The temporal and spatial progresses of cellular activity within the conduit are similar to those seen for experiments using biodegradable nerve guides reported in the literature [2-4]. However, the GGCs we used, which had a cross-linking degree of 50%, did not obviously degrade until 6 weeks of implantation. Since regenerating nerve fibers can grow across a 10-mm gap in the sciatic nerve of the rat within 4 weeks [5], is it necessary for the nerve guide to remain intact for a long period is still unclear. In the present study, we constructed GGCs with lower cross-linking degrees around 24% and 36%, assuming breaking down more rapidly compared to those at 50%. It was therefore the aim of this study to test GGCs with different cross-linking degrees in a sciatic nerve rat model. Their biological performance, such as the biocompatibility and the effectiveness as a guidance channel were evaluated by correlating morphometric and functional data of the regenerated nerve.

Materials and Methods

Fabrication of GGCs

A 10% (w/w) solution of gelatin in distilled water was prepared by magnetic stirring. A silicone rubber tube (1.96 mm OD) was used as a mandrel vertically dipped into the gelatin solution at a constant speed where it remained for 5 min. The mandrel was then withdrawn slowly and allowed to stand for 30 min for air-drying. The mandrel was rotated horizontally consistently to reduce variations in the wall thickness along the axis of the tube. To make different cross-linking degrees of the tubes, the gelatin-coated mandrels were immersed in 0.5% (w/w) solution of genipin for 1 (group 1), 3 (group 2), and 48 hr (group 3), respectively. Each coated mandrel was then rinsed twice with distilled water, dehydrated for 10 min with 95% of ethanol, and air-drying for 1 week. Finally, the GGCs were sterilized with 75% of ethanol for subsequent implantation.

Cross-linking degree of GGCs

Ninhydrin assay was used to evaluate the cross-linking degree of GGCs [11]. Ninhydrin was used to determine the amount of amino groups of each test sample. The test GGCs were heated with a ninhydrin solution for 20 min. After heating with ninhydrin, the optical absorbance of the solution was

recorded using a pectrophotometer at 570 nm using gelatin at various known concentrations as standard. The amount of free amino groups in the residual gelatin, after heating with ninhydrin, is proportional to the optical absorbance of the solution. The cross-linking degree of GGCs was then determined .

Biocompatibility of GGCs

Six adult Sprague-Dawley rats, weighing approximately 280-350 g were used to evaluate the biocompatibility of GGCs. For the insertion of the implants, incisions (0.5 cm in length) were made and GGCs with different cross-linking degrees were implanted subcutaneously on both sides of the rats. Each rat received 6 implants, which were removed upon sacrifice at various time points: 1 week, 4 weeks, and 8 weeks. The implants were then removed for histological evaluation. For histomorphometric evaluation, sections were stained with hematoxylin and eosin. The tissue reactions to the implants in the subcutaneous tissue were evaluated for uniformity and thickness of the foreign body capsule as well as the inflammation responses under optical microscopy

GGCs implantation

Thirty adult Sprague-Dawley rats underwent placement of GGCs with the three different cross-linking degrees were divided into 3 groups, which were removed upon sacrifice at 8 weeks. For each group, 10 rats were operated on. The animals were anesthetized with an inhalational anesthetic technique. Following the skin incision, fascia and muscle groups were separated using blunt dissection, and the right sciatic nerve was severed into proximal and distal segments. The proximal stump was then secured with a single 9-0 nylon suture through the epineurium and the outer wall of the GGCs (1.96 mm ID). The distal stump was secured similarly into the other end of the chamber. Both the proximal and distal stumps were secured to a depth of 2.5 mm into the chamber, leaving a 10-mm gap between the stumps. The muscle layer was re-approximated with 4-0 chromic gut sutures, and the skin was closed with 2-0 silk sutures.

Results

Physical characteristics of GGCs

The cross-linking index of GGCs, expressed as a percentage of free amino groups lost during cross-linking, was 24±7%, 36±3%, and 51±7% for groups 1, 2, and 3, respectively.

Biocompatibility of GGCs

At 1 week post-implantation, GGCs in all the three groups persisted maintaining their lumens and wall integrity. A delicate and thin fibrous tissue capsule was present surrounding the whole implant. However, the nerve guide group 1 became soft and had a more reactive inflammation cell infiltration as compared to those in groups 2 and 3. Specifically, abundant small round-shaped cells, i.e. monocytes and neutrophils aggregated at the interfaces between the materials and their surrounding tissues (Fig.1(A)). At the time point of 4 weeks, fibrous tissue capsules became thicker with a compact structure along with active neovascularization. Up to this time, the inflammatory reaction decreased remarkably (Fig. 1(B)). At 8 weeks, a chronic inflammation reaction was noted with macrophages and giant cells phagocytizing degraded parts at the edges of GGCs (Fig. 1(C)).

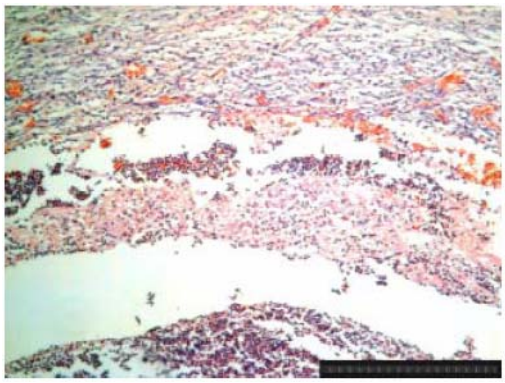

Fig.1(A)

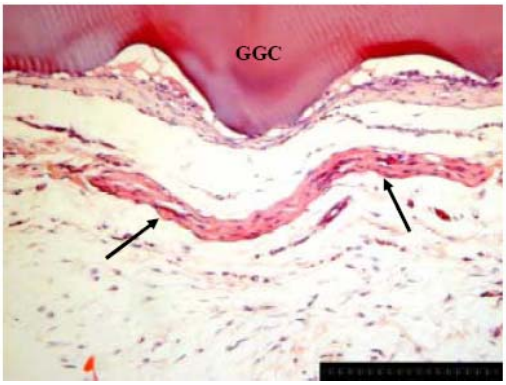

Fig. 1(B)

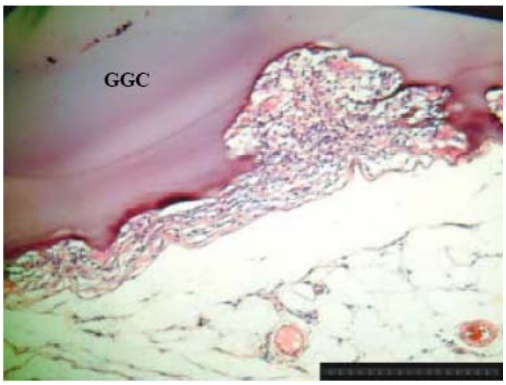

Fig. 1(C)

Fig.2(A)

Fig. 2(B)

Fig. 2(C)

Nerve regeneration

Macrographs of the GGCs with the three different cross-linking degrees are shown in Fig. 2(A)-2(C). In group 1, all the nerve guides had totally disappeared, exposing slender regenerated nerves inside. In group 2, all conduits were well integrated into the regenerating nerve tissue. As for the group 3, only minor degradation was seen for the nerve guides even after 8 weeks of implantation. For groups 2 and 3, fibrous tissue encapsulation was noted, covering all over the left GGCs and the parts of the nerve stumps in the tube openings. After trimming the fibrous tissue, cutting the wall of the tube, the regenerated nerve was exposed and then retrieved. Overall gross examination of the GGCs in the three groups all revealed 100% of nerve formation.

Discussion

In the present study, we found that all of the GGCs could support the nervous cell infiltration and subsequent tissue ingrowth during the regenerative processes, especially in the group with a mean cross-linking degree at 36%. In this group, all regenerated nerves had a mature structure, demonstrated by well-developed epineurial and perineurial organization and axonal development, whereas more or less the cables in the other two groups, especially the group with the lowest mean

cross-linking degree at 24% exhibited an irregular-shaped structure in which connective tissue occupied large portions of the endoneurial areas containing numerous Schwann cells and vascular cells only.

Conclusion

The present study shows that the physical and chemical characteristics of the GGCs can influence the quality and maturation of regenerated nerves: too low (24%) cross-linking degrees gave rise to more degradation materials evoking foreign body reaction; too high cross-linking degrees (51%) resulted in slow degradation of the conduit which could cause nerve compression. Both the tube configurations may hamper the growth of regenerating nerves in the guidance channels. From this study, we can conclude that of the three types tested, GGCs with a mean cross-linking degree at 36% could provide the most suitable environment for
regenerating nerves. However, more nerve guidance properties have to be investigated,
such as the lumen size and the wall thickness of the conduit to determine the exact influence of the GGCs on nerve regeneration.

References

[1] Chen YS, Chang JY, Cheng CY, Tsai FJ, Yao CH, Liu BS. An in vivo evaluation of a biodegradable genipin-crosslinked gelatin peripheral nerve guide conduit material. Biomaterials 2005;26:3911-8.

[2] Rodríguez FJ, Gómez N, Perego G, Navarro X. Highly permeable polylactide-caprolactone nerve guides enhance peripheral nerve regeneration through long gaps. Biomaterials 1999;20:1489-500.

[3] Itoh S, Takakuda K, Ichinose S, Kikuchi M, Schinomiya K. A study of induction of nerve regeneration using bioabsorbable tubes. J Reconstr Microsurg 2001;17:115-23.

[4] Bini TB, Gao S, Xu X, Wang S, Ramakrishna S, Leong KW. Peripheral nerve regeneration by microbraided poly(L-lactide-co-glycolide) biodegradable polymer fibers. J Biomed Mater Res 2004;68:286-95.

[5] Williams LR, Longo FM, Powell HC, Lundborg G, Varon S. Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: Parameters for a bioassay. J Comp Neurol 1983;218:460-70.

[6] Yao CH, Liu BS, Chang CJ, Hsu SH, Chen YS. Preparation of networks of gelatin and genipin as degradable biomaterials. Mater Chem Phys 2004;83:204-8.