行政院國家科學委員會專題研究計畫　成果報告

天然降解性神經管的開發與應用

計畫類別：個別型計畫
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執行單位：中國醫藥大學中國醫學研究所

計畫主持人：陳悅生
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計畫參與人員：章如瑛、杜寧漪

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中　華　民　國 94 年 10 月 5 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

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執行單位：中國醫藥大學放射技術系

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一、 中文摘要

應用神經導管橋接周邊神經已被證明是一理想修復方式，局部無瘢痕，並使神經再生的趨化特異性得以充分發揮。降解性神經導管避免了導管植入後，必須二次開刀取出的問題，臨床的後遺症較少。在眾多生醫材料中，利用經過交連後的膠原蛋白所製成的導管可降解、毒副作用低，為目前研究焦點之一，然而膠原蛋白的螺旋結構會引發生體排斥性而降低其生物相容性。學者們發現，明膠衍生自膠原蛋白，為膠原蛋白之二級結構破壞後的產物，其抗原性較膠元蛋白低。萃取自中藥梔子的天然交聯劑-綠梔子素的細胞毒性極小、對明膠的交聯度也十分優良。

本研究已明膠為基材、綠梔子素為交聯劑製造出 15 mm、重量 0.04± 10%、內徑 1.96 mm 交聯度 96+3%之降解性神經導管。神經導管接合術適用性評估顯示導管在 SD 大鼠體內可維持四週以上的機械支持強度，引導 10 mm 間距的神經再生功率達 100%產生旺盛血管新生作用；但有神經再生生長延遲及再生神經成熟度偏低的現象。

關鍵詞：梔子素；明膠；神經再生

Abstract

We evaluated peripheral nerve regeneration using a biodegradable nerve conduit, which was made of genipin-crosslinked gelatin. The genipin-crosslinked gelatin conduit (GGC) was dark blue in appearance, which was concentric and round with rough outer surface whereas its inner lumen was smooth. After subcutaneous implantation on dorsal side of the rat, the GGC only evoked a mild tissue response, forming a thin tissue capsule surrounding the conduit. 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. As a result, tube

fragmentation was not obvious until 6 weeks post-implantation and successful regeneration through the gap occurred in all the conduits at the three experimental periods of 4, 6, and 8 weeks. Histological observation showed that numerous regenerated nerve fibers, mostly unmyelinated and surrounded by Schwann cells, crossed through and beyond the gap region 6 weeks after operation. Peak amplitude and area under the muscle action potential curve both showed an increase as the function of the recovery period, indicating that the nerve had undergone adequate regeneration. Thus, the GGC not only can be effective aids for regenerating nerves but also can lead to favorable nerve functional recovery.

二、緣由與目的

A 10% (w/w) solution of gelatin (300 bloom number, Sigma #G2500) in distilled water was prepared by magnetic stirring. The gelatin-coated mandrel was then immersed in 1% (w/w) solution of genipin (Challenge Bioproducts Co., Taichung, Taiwan) for 24 hr for cross-linking. To allow fixation of the nerve tissue to the conduit, two small holes were drilled at both ends of the GGCs. Finally, the GGCs were sterilized with 25 kGy of γ-ray for subsequent implantation.

Thirty adult Sprague-Dawley rats underwent placement of GGCs, which were removed upon sacrifice at various time points: 4 weeks, 6 weeks, and 8 weeks. At each implantation time, 10 rats were operated on. 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. All animals were housed in temperature (22°C) and humidity (45%) controlled rooms with 12-hour light cycles, and they had access to food and water ad libitum

三、結果

GGCs were dark blue in appearance caused by the reaction between genipin and amino acids or proteins. SEM images of

GGCs in longitudinal section [Fig. 1(A)] and cross section [Fig. 1(B)] showed that the tubes were concentric and round with rough outer surface, whereas the inner lumen was smooth.

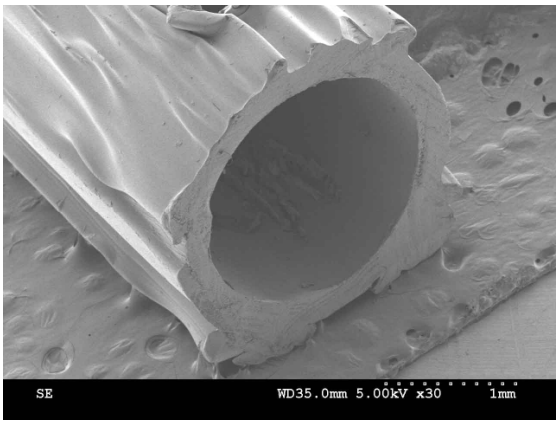

Figure 1(A)

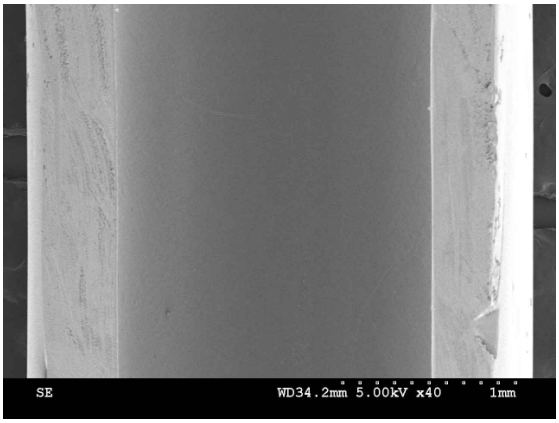

Figure 1(B)

Macrographs of the GGCs at different implantation periods are shown in Fig. 2(A)-2(B). Throughout the 8 weeks of experimental period, no nerve dislocation out of the GGCs was seen for all of the rats. Brownish fibrous tissue encapsulation was noted, covering all over the 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 at the three observation time points all revealed 100% of nerve formation in the tubes.

Figure 2(A)

Figure 2(B)

四、討論

Although the ideal material for a nerve guide has not been identified, successful materials must be biocompatible, which can inhibit the proliferation of fibroblasts and connective tissue surrounding the injured nerve. A wide range of materials has been developed for use as a nerve bridge, such as biological origin, i.e., arteries and veins or synthetic, i.e., various copolymers. Recently, application of combining herbal medicine and biomedical material science to nerve regeneration is a new approach.

In addition, we also found that genipin-fixed gelatin gel can be used as an extracellular matrix, providing a consistent and promoting effect on regenerating nerve. The gelatin is essentially denatured collagen, which has been specifically studied for use as wound dressing, adhesive, and absorbent pad in clinics. However, the gelatin must be cross-linked if it is willing to be used as a stable implant without losing its integrity.

Therefore, we selected the genipin, which is a naturally occurring and low-cytotoxic crossing agent, to fix the gelatin in the present study. Our aim was to test if the genipin-fixed gelatin can be used as materials to make conduits for nerve regeneration. As a result, we found that successful regeneration of nerves across the gap occurred in all of the GGCs even at the shortest experimental time point of 4 weeks. Macroscopic observations showed that unsatisfactory swelling or deformation of the GGCs was not seen. We believe that the stable dimensions of the GGCs, which could result from their heterocyclic crosslinking structure , played a critical role in contribution to the high success of nerve regeneration. The thin layer of surrounding fibrous tissue and minimal inflammation reaction also indicated that the GGCs were biocompatible. These results are not surprising since the gelatin has been shown a promising material for use in entubulization repair of nerve defects, and the genipin shown prominent neuritogenic activity in paraneurons such as PC12h cells .

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