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Implantation of a Matrigel-loaded agarose scaffold promotes functional regeneration of axons after spinal cord injury in rat

Sungmin Han [a, 1], Jee Youn Lee [b, 1], Eun Young Heo [d], Il Keun Kwon [d], Tae Young Yune [b, c, **], Inchan Youn [a, e, *]

[a] Biomedical Research Institute, Korea Institute of Science and Technology, 5, Hwarang-ro 14-gil, Seongbuk-gu, Seoul, 02791, Republic of Korea
[b] Age-Related Brain Diseases Research Center, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Republic of Korea
[c] Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, Seoul, 02447, Republic of Korea
[d] Department of Maxillofacial Biomedical Engineering and Institute of Oral Biology, School of Dentistry, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Republic of Korea
[e] Division of Bio-Medical Science &Technology, KIST School, Korea University of Science and Technology, 5, Hwarang-ro 14-gil, Seongbuk-gu, Seoul, 02791, Republic of Korea

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ABSTRACT

An agarose scaffold can be useful for supporting and guiding injured axons after spinal cord injury (SCI), but the electrophysiological signal of regenerated axon in scaffolds has not yet been determined. The current study investigated whether a Matrigel-loaded agarose scaffold would enhance the regeneration of axons after SCI. Moreover, the functional connectivity of regenerated axons within the channels of the scaffold was evaluated by directly recording motor evoked potentials. Our data showed that the agarose scaffold containing Matrigel can support and enhance linearly organized axon regeneration after SCI. Additionally, motor evoked potentials were successfully recorded from regenerated axons. These results demonstrate that an agarose scaffold loaded with Matrigel could promote the regeneration of axons and guide the reconnection of functional axons after SCI.

1. Introduction

Spinal cord injury (SCI) results in neuronal cell death and disruption of axonal pathways, which leads to the permanent loss of neurological functions below the injured site of the spinal cord. Extensive research has been conducted investigating the ability of various therapeutic approaches to promote axonal regeneration after SCI [1–3]. For example, the implantation of cells and growth factors can enhance the growth of damaged axons and stimulate both intrinsic and extrinsic neuronal growth states [3]. Anti-inflammatory drugs and rehabilitation training can help prevent secondary injury and reduce pain [4]. However, these methods have limited clinical use due to their lack of regenerative ability and directional organization of the axons.

Scaffolds derived from biomaterials are potential candidates for improving axonal regeneration by directional orientation along with their native organization after SCI. The scaffolds contain multiple linear channels for supporting and guiding injured axons, so they can act as a bridge between the proximal and distal stumps of the injured site, promoting axonal regeneration [4–6]. These scaffolds can be fabricated with precise linear channel arrays over lengths of several centimeters, and their mechanical properties closely match those of the normal spinal cord [7]. For example, long-tract sensory axons were regenerated though templated agarose scaffolds placed in spinal cord lesion sites [8]. Motor axon regeneration was also supported by agarose scaffolds [9]. These studies suggest that agarose scaffolds might can promote axonal regeneration and support the highly linear organization of axons after partial or severe SCI. However, axonal regeneration was evaluated by histological analysis to prove anatomical connectivity (i.e., axons penetration of the channels of the scaffold). Spinal cord white matter contains different axon tracts in different locations,

* Corresponding author. Biomedical Research Institute, Korea Institute of Science and Technology, 5, Hwarang-ro 14-gil, Seongbuk-gu, Seoul, 02791, Republic of Korea.
** Corresponding author. Department of Biochemistry and Molecular Biology, and Age-Related and Brain Diseases Research Center, School of Medicine, Kyung Hee University, Medical Building 10th Floor, Dongdaemun-gu, Hoegi-dong 1, Seoul, 130-701, Republic of Korea.
E-mail addresses: tyune@khu.ac.kr (T.Y. Yune), iyoun@kist.re.kr (I. Youn).
[1] Sungmin Han and Jee Youn Lee contributed equally to this work.

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and ascending and descending axons travel a long distance to convey various information of afferent and efferent activities, which was provided by electrophysiological signals called action potentials. Consequently, to validate functional recovery of the injured spinal cord, functional connectivity of the regenerated axons should be assessed electrophysiologically and anatomically.

The aim of present study was to investigate whether an agarose scaffold could functionally regenerate damaged corticospinal tract (CST) fibers that pass through the channels of the scaffold. The thoracic spinal cord containing CST was removed from adult rat, and an agarose scaffold was implanted into the cavity, and the channels of the agarose scaffold were loaded with Matrigel. Four weeks after implantation, the anatomical connectivity of the regenerated axons was assessed by histological and immunohistochemical analyses, and long-tract motor axons of the CST were evaluated by anterograde Fluorogold (FG) labeling. To evaluate the functional connectivity of the regenerated CST axons, motor evoked potentials were induced by ultrasound cortex stimulation and directly recorded from regenerated CST axons in the channels of the scaffold.

2. Materials and methods

2.1. Fabrication of templated agarose scaffold

Agarose scaffolds containing multiple linear channels were produced based on a previously described protocol with some modifications [10]. Fig. 1A shows the custom-made molds, which consisted of two side cap molds and a middle mold. Fiber bundles were composed of 20 stainless steel fibers 150-μm in diameter. Fibers were oriented parallel to the longitudinal axis with 300-μm inter-fiber distances. Agarose powder (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water at 100 °C on a hotplate, and a 3% agarose solution was prepared. The molds were assembled and preheated to 65 °C. The agarose solution was then slowly injected into the molds using a preheated syringe. After resting at room temperature for 10 min, the molds were soaked in 70% ethanol for 2 min and the gelled agarose scaffolds were separated from the molds (Fig. 1B). The scaffolds were immersed in 70% ethanol under UV light overnight, and rinsed with phosphate buffered saline (PBS; Lonza, Walkersville, MD, USA). The channels of the agarose scaffolds were filled with Matrigel (CORNING, NY, USA) in a vacuum chamber to remove air bubbles, and then the scaffolds were cured at 37 °C overnight.

2.2. Experimental animals and groups

Adult male Sprague-Dawley rats (230–250 g; Samtako, Osan, Korea) were used in this study. All animal experiments were performed in accordance with the Guidelines and Polices for Rodent Survival Surgery provided by the Animal Care Committee of the Kyung Hee University (permission number: KHUASP(SE)-15-006). The rats were divided into 3 groups: (i) laminectomy only, without injury (Sham); (ii) spinal cord dorsal column removed and non-grafted (SCI), in which the lesion cavity was left empty; (iii) spinal cord dorsal column removed with immediate implantation of an agarose scaffold into the cavity (SCI + Scaffold).

2.3. Spinal cord injury and scaffold implantation

Animals were anesthetized with chloral hydrate (500 mg/kg, intraperitoneal injection). Laminectomies were performed at T9-T10, and four superficial cuts were made in the dorsal surface of the spinal cord (approximately 1.5 mm rostral-caudal and 2 mm laterally centered at the midline) using fine scissors. The spinal cord tissue within the marked rectangle was removed by aspiration, and a hole with a depth of 1.5 mm was made (the dorsal CST was completely removed). The agarose scaffold was placed within the cavity (Fig. 1C). To stabilize the scaffold within the spinal cord, a specially made agarose thin film (2.2 mm × 2.2 mm x 50 μm) was

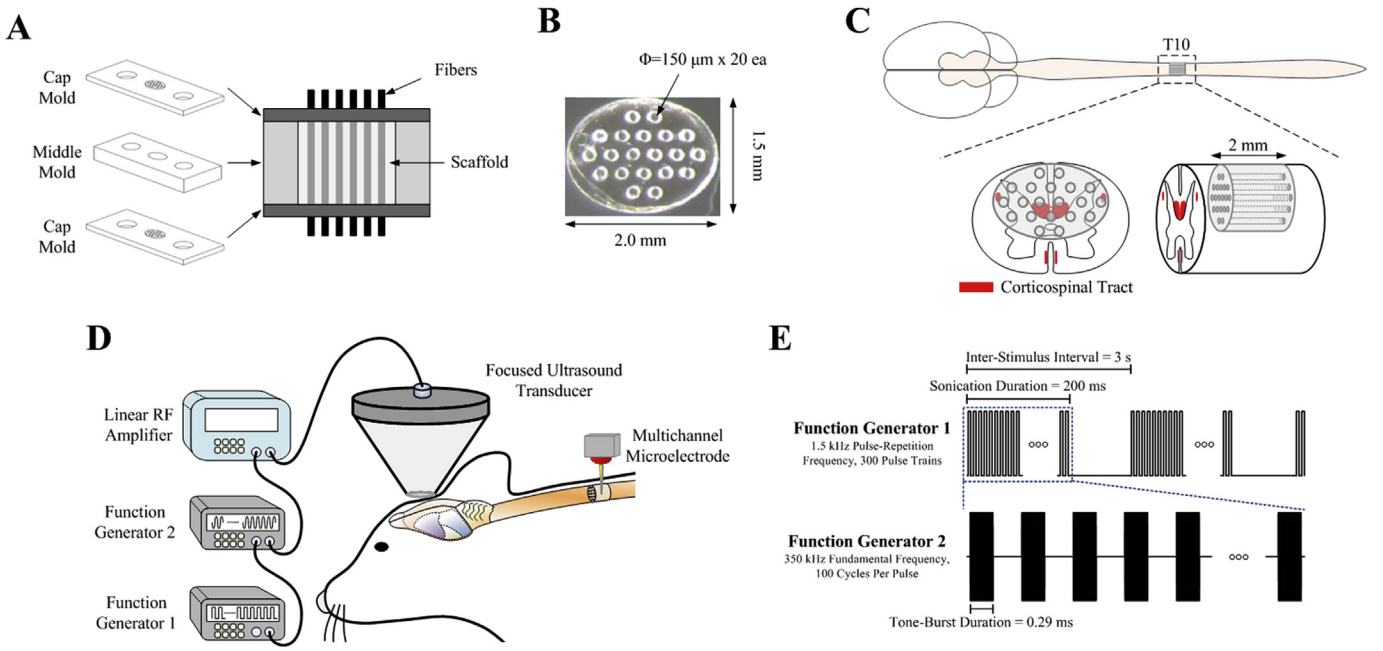

Fig. 1. Experimental setup for scaffold transplantation and ultrasound cortex stimulation (A) Preparation of scaffold architecture. The molds consisted of two cap molds and one middle mold, and 20 fibers were oriented parallel to the longitudinal axis. (B) Transverse view of a scaffold. Channels were 150 μm in diameter and the inter-fiber distance was 300 μm. (C) 2. Schematic of the injury of the rat dorsal column at T10 and the implantation of agarose scaffolds into the injury cavity. (D) The ultrasound transducer was located above the rat brain and a multichannel microelectrode was inserted into the scaffold channels. (E) The sonication parameters were set to a 350 kHz fundamental frequency, 1.5 kHz pulse-repetition frequency, 0.29 ms tone-burst duration, and 200 ms sonication duration.

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