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Platelet-rich fibrin membrane nerve guidance conduit: a potentially promising method for peripheral nerve injuries

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Peripheral nervous system injuries can cause partial or complete transection of the nerve, thereby lead to paralysis, poor or abnormal sensation, and painful neuropathies. Platelet-rich fibrin (PRF) is defined as a second-generation platelet concentrates with high regenerative potential.[1] Moreover, PRF is easy to prepare without chemical preprocessing, as inducing faster nerve repair is critical in the clinical use of nerve conduits for peripheral nerve repair. However, PRF membrane nerve conduit (PRF-NGC) has not been well studied. In this study, PRF-NGC was compared with autologous nerve grafts (ANG) and polyurethane (PUR) nerve conduit to investigate the potential effect of PRF-NGC on peripheral nerve repair. Regenerated nerves were analyzed by immunofluorescent staining and transmission electron microscopy. Measurements of wet weight and Masson's trichrome stained sections of gastrocnemius muscle were collected for deterministic quantification of sciatic nerve functional recovery.

All animal experiments involving female BALB/c nude mice (8-week-old) and Sprague-Dawley (SD) rats (weight, 180–220 g; Vital River Laboratory Animal Technology, Beijing, China) were approved by the Ethics Committee of the Animal Laboratory of the Plastic Surgery Hospital, Beijing, China. First, 5-mL blood sample from donor SD rats under general anesthesia was collected into a glass-coated tube without anticoagulant and centrifuged at 400×*g* for 10 min. Subsequently, fibrin clots in the middle layer were compressed into a membrane using a PRF box. Finally, PRF membrane was trimmed into sectioned slices (7 mm × 3 mm) and wrapped the 25-gauge syringe needles based on the orientation of the major axis. The lateral edges were discontinuously sutured with the use of 11-0 microsurgical sutures to prepare PRF membrane nerve conduits. A total of 24 nude mice were randomly divided into three groups ($n = 8$ in each group): the ANG group, the PRF-NGC group (PRF group), and the PUR conduit group (PUR group). In the ANG group, the 5-mm long sciatic nerve was repaired by rotating the resected nerve segment 180°, and then by suturing with 11-0 fiber sutures. In the PUR group and PRF group, we removed the 5-mm long sciatic nerve of each mouse, and each nerve stump was inserted 1 mm into the end of the conduits, which was sutured with 11-0 fiber sutures. Matrigel (BD Biosciences, USA) was injected into the 7 mm sterile PUR conduit (0.9 mm internal diameter) and PRF-NGC respectively. Immunofluorescent staining, transmission electron microscopy, assessment of gastrocnemius muscle atrophy, and statistical analyses were performed as previously described.[2]

Scanning electron microscope images revealed that fibrin matrix formed numerous pores on the surface and upon the cross-sections on the PRF membrane [Figure 1A]. PRF membranes were trimmed into slices and wrapped to form a nerve conduit [Figure 1B]. In degradation assay *in vitro*, PRF membranes were found to have maintained the original shapes for longer than 3 months, whereas the time for the complete biodegradation of conduits was 2 to 3 weeks *in vivo*. Twelve weeks post-surgery, the general appearance of regenerated tissue was smooth-surfaced, without infection and neuroma formation. No obvious adhesions with adjacent tissues were detected, indicating that PRF had good biocompatibility. Figure 1C demonstrated that the extraneural vascular system including main blood vessels, which run parallel to the proximal-distal direction of the regeneration process and massive levels of angiogenesis in smaller diameter on the regeneration tissue. Immunofluorescent staining showed that a network of longitudinal vessels within the PRF group segment, suggesting that vascular supply regenerated in both within extraneural and interneural vascular system [Figure 1D]. The features of myelinated nerve fibers and maturation

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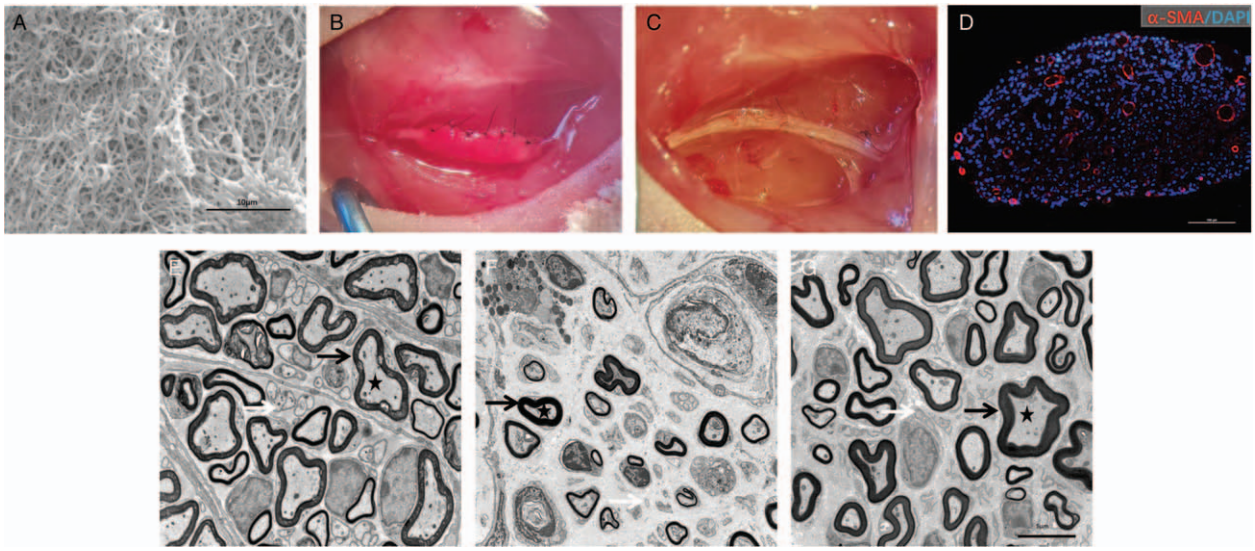

Figure 1: Microscopic pictures of cross-sections of the regenerated nerve and general photos. (A) Scanning electron microscope image of PRF membrane, scale bar: 10 μm. (B) The PRF membrane was wrapped to form a nerve conduit. (C) General observations at 12 weeks post-surgery. (D) Immunofluorescence staining of regenerated nerves. Anti-smooth muscle antigen staining (red), DAPI (blue), scale bar: 100 μm. (E–G) Transmission electron microscopy images in PRF group, PUR group, and ANG group, scale bar: 5 μm. Black arrows: myelin sheath. Black star: myelinated nerve fibers. White arrow: unmyelinated nerve fiber. DAPI: 4', 6-diamidino-2-phenylindole; PRF: Platelet-rich fibrin; PUR: Polyurethane; ANG: Autologous nerve grafts.

levels of regenerated nerves were examined by comparing transmission electron microscopy images (Figure 1E–G, Supplementary Figure 1, http://links.lww.com/CM9/A192). The number of myelinated fibers in the PRF group was significantly higher than what was observed in the PUR group ($P < 0.0001$), while myelinated fibers numbers were lower than in the ANG group ($P = 0.0013$). Similarly, our results regarding myelin thickness revealed that the mean value was largest in the ANG group (0.88 ± 0.25 μm), followed by the PRF group (0.63 ± 0.20 μm), and then the PUR group (0.32 ± 0.14 μm). All differences among groups were statistically significant ($P = 0.0012$; PRF *vs*. PUR, $P = 0.0038$; PRF *vs*. ANG, $P = 0.0129$). Also, the mean fiber diameter was significantly larger in the PRF and ANG treatment groups, while we did not identify any significant between the PRF and ANG treatment groups (PRF *vs*. PUR, $P = 0.0052$; PRF *vs*. ANG, $P = 0.1730$). The results from tests used to determine different degrees of gastrocnemius muscle atrophy via the measures of wet weight ratio and Masson trichrome staining that were observed in the three groups are shown in Supplementary Figure 2, http://links.lww.com/CM9/A193. The recovery of muscle atrophy in the PRF group was comparable to the ANG group.

In this study, we used a novel nerve conduit made of platelet-rich fibrin membrane successfully for bridging a 5-mm long sciatic nerve in nude mice. The histological, morphometric and functional findings revealed that the PRF-NGC treatment was significantly better than the PUR negative control group and at a level similar to the regenerated nerve in the ANG group (the positive control group). The number of myelinated fibers, fiber diameter, myelin thickness, and degree of muscle recovery in the PRF group were significantly superior to the PUR group. The results demonstrated that fiber diameter and degree of muscle recovery in the PRF group were not worse than the ANG group. However, fewer regenerated axons and a reduced level of myelin thickness were observed in the PRF group than the ANG group, which suggested that it was still less effective than the gold standard. Improvement in the rate of axonal regeneration by PRF-NGC was likely due to releasing growth factors that acted to speed up elongation. Nonetheless, one possible explanation is that PRF is composed of a biopolymer fibrin wherein nutrients and oxygen would have diffused into the regeneration nerve through a permeable PRF conduit before the tube had become vascularized. Interestingly, Cattin *et al*[3] demonstrated that blood vessels can be used as a guiding path to direct the migration of Schwann cells (SCs) after peripheral nerve injury. In our research, neovascularization occurred on the surface of regenerated nerve and transverse sections of the middle segment in the PRF treatment group. The newly formed blood vessels in the PRF conduit may also provide a scaffold for migrating SCs, and this potential aspect deserves to be consulted in further study. Previous research has reported that injecting PRF gel inside of a silicon tube group had positive improvement compared to silicon group for releasing nutrients.[4] However, fibrin components may also block anon extension and have negative effect since they can retain inside of nerve conduit for the first 2 to 3 weeks. Since PRF membrane is flexible with stretching property of 2 to 4 times and is easy to handle and suture.[5] This research used tubulized PRF membrane to form a nerve conduit to fully optimize the advantages of PRF.

In conclusion, PRF-NGC could serve as a growth factor-rich scaffold and enhance the formation of blood vessels in the regeneration tissue. Therefore, our novel findings suggest that better functional recovery may be achieved for treating clinical peripheral nervous system injury with PRF-NGC in the future.

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Conflicts of interest

None.

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