

BASIC SCIENCE REVIEW

# ULTRASTRUCTURE AND CELLULAR BIOLOGY OF NERVE REGENERATION

## ABSTRACT

Hippocrates provided the first written description of the peripheral nervous system (PNS), as early as the 4th century B.C., and later Herophilus identified nerves as such, distinguished them from tendons; he also traced nerves to the spinal cord. The traditional Hippocratic teaching of the time, however, doubted that nerve healing occurred. Through the subsequent centuries, several papers were written about the PNS but, without sufficient understanding of anatomy, physiology, and the regenerative capacity of the PNS, it is not difficult to comprehend the frustration that might have been encountered by surgeons in dealing with nerve injuries and their subsequent repair. This was probably the reason why nerve repair was rarely actually undertaken prior to the 19th century.

A plethora of studies on the PNS and its regeneration has been reported over the last 150 years and has provided us with current knowledge. It is important, before describing the most recent developments in the area of peripheral nerve regeneration, to briefly outline the major advances over the last century. Currently, the therapeutic approaches taken toward the patient with peripheral nerve injury change continuously. Sophisticated advances in technology, cellular and molecular neurobiology, and electron microscopy will doubtless optimize reconstructive strategies in treating nerve injury.

A greater awareness and understanding of the nerve ultrastructure, as well as the underlying mechanisms of the regenerative process and those factors detrimental to nerve regeneration, will assist in the successful repair of nerve injury. This paper reviews the cellular, biochemical, and ultrastructural elements of nerve injury and repair, and the rationale for current reconstructive strategies and techniques.

Peripheral nerve fibers show a far greater capacity for regeneration than do those in the CNS. This is one of the most incredible characteristics distinguishing the PNS from the CNS. This difference in the capacity for effective regeneration might, at least to some degree, be attributed to the differences in cellular organization between the CNS and the PNS. A review of the histomorphometric, cellular, biochemical, and genetic elements of peripheral nerve regen-

eration will be presented, in order to clarify the structural basis on which nerve regeneration can be promoted in the PNS.

Both myelinated and non-myelinated axons in the PNS are surrounded by Schwann cells which, in turn, are covered by basal lamina on the outer surface. Schwann cells in myelinated axons form their own internodal myelin sheath, which is separated from an adjacent internode by a node of Ranvier.



**Figure 1.** An electron micrograph of the node of Ranvier. The node is identifiable as a cleft between neighboring myelin sheaths. The axon at the node is covered by Schwann cell processes. The nodal axolemma is characterized by a dense subplasmalemmal undercoat. The basal laminae of neighboring Schwann cells are continuous at the node. ( $\times 5000$ )

Therefore, Schwann cells are aligned discontinuously along the axon, separated by nodes of Ranvier at both the proximal and distal ends of the internode (Fig. 1). The basal lamina however, is continuous between adjacent Schwann cells, even at the nodes. In contrast, unmyelinated fibers have continuous Schwann cell sheaths, with no gap or node between adjacent Schwann cells. Thus, all peripheral nerve fibers reside inside a continuous basal lamina tube. Following axotomy, the distal axonal segment gradually degenerates and eventually is phagocytized, a phenomenon known as Wallerian degeneration.<sup>1</sup>



**Figure 2.** An electron micrograph of regenerating growth cones (G) and Schwann cells (S) within the basal lamina tube, in the end-to-side neurorrhaphy model of the rat sciatic nerve. Growth cones extend into the space between the Schwann cell basal lamina (arrow) and the parent axon. The parent axon is degenerating, and Schwann cells begin to envelop the growth cone. Basal lamina covers both Schwann cells and regenerating growth cones. ( $\times 7500$ ).

Schwann cells lose contact with axons, then transiently proliferate, forming cell strands called Schwann-cell columns or bands of Büngner, within the basal lamina tube.<sup>2</sup>

Regenerating axons emerge at the nodes of Ranvier from the proximal stump of the lesion, with growth into the distal nerve segment (*i.e.*, band of Büngner), and extend as far as the target organs.<sup>3</sup> Subsequent to myelin debris removal by macrophages, the bands of Büngner are formed, and serve as the pathway by which regenerating axons grow through to their target in the PNS.<sup>4</sup> Certainly, some regenerating axons fail to follow the band of Büngner, and enter the connective tissue compartment, resulting in no further growth. The bands of Büngner thus provide the regenerating axons with a favorable environment for growth.

Since regenerating axons extend along the surface of Schwann cells and the inner surface of the basal lamina, it is possible that both Schwann cells and the basal lamina serve as a needed substrate for these regenerating axons. The basal lamina is an extracellular matrix that acts as a scaffold for epithelial and neural cells. It can be thought of as a "glue," with one side in contact with the cells, and the other side in contact with the surrounding connective tissue (Fig. 2). Regenerating axons grow vigorously along the inner surface of the Schwann-cell basal lamina, from which Schwann cells have been removed. Since the basal lamina contains a variety of adhesion molecules, it is possible that regenerating axons could utilize these molecules, located on the inner surface of the Schwann cell basal lamina, for attachment. On the other hand, Schwann cells express on their plasma membrane, the adhesion molecules belonging to the immunoglobulin superfamily and the cadherin superfamily, which are considered to participate in axon-Schwann cell adhesion.<sup>5</sup> Therefore, regenerating axons have a dual adhesion property, *i.e.*, attachment to the Schwann cell and attachment to the basal lamina. Schwann cells, associated with Wallerian degeneration, produce a variety of trophic factors. Even the basal lamina of Schwann cells is considered to have a trophic role, because it can retain trophic factors such as fibroblast growth factor, which binds to the heparin sulfate proteoglycan of the basal lamina.

Aside from environmental factors, growth cones and their dynamics are critical in understanding the mechanism of nerve regeneration (Fig. 3). Molecules that are presumed to be associated with the promotion of growth-cone extension include vesicle-associated proteins, protein kinase C, and tyrosine kinase; these will be discussed later.

Nerve regeneration in the CNS differs significantly. CNS tissue consists of densely interwoven glial cell processes. Glial cells have no basal lamina, except for some astrocyte processes which are in contact with the pia mater at the external surface,