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A therapeutic shock propels Schwann cells to proliferate in peripheral nerve injury

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Abstract:

Damage to the peripheral nervous system (PNS) is a prevalent issue and represents a great burden to patients. Although the PNS has a good capacity for regeneration, regeneration over long distances poses several difficulties. Several recent studies have addressed Schwann cells' limited proliferative capacity; however, a solution has yet to be found. Here, we examine the effects of extracorporeal shock wave therapy (ESWT) on Schwann cell isolation, culture, and proliferation rate. The study conducted demonstrated that Schwann cells treated with ESWT had significantly improved isolation, culture, and proliferative capacities. These findings represent a solution to a significant problem that hospitals and health-care providers face every year: how to treat long distance damage to the PNS with the limited proliferative capabilities of Schwann cells. Although these findings are promising, further studies must be conducted to address the molecular mechanisms by which ESWT alters Schwann cells and the potential implications for peripheral nerve damage and other prevalent illnesses. This study is a review article. Referred literature in this paper has been listed in the references part. The datasets supporting the conclusions of this article are available online by searching the PubMed. Some original points in this article come from the laboratory practice in our research centers and the authors' experiences.

Key words:

Culture, extracorporeal shock wave therapy, isolation, proliferation, purinergic signaling, Schwann cells

Extracorporeal Shock Wave Therapy as an Alternative Treatment for Peripheral Nerve Damage

Peripheral nerve lesions account for 300,000 medical cases every year in Europe, resulting in repeated hospitalization and a great burden to the health-care system and patients.[1] While the peripheral nervous system is capable of regeneration, multiple difficulties present with the process of regeneration over long distances, such as following proximal lesions, or nerve gaps. Although nerve autografts are at the forefront of treatment for peripheral nerve damage and tissue loss, they do not always result in adequate regeneration.[2] Specifically, damage to multiple nerves or long-distance nerve gaps challenge the limitations of autografting in terms of the amount of available donor tissue. Several researchers have attempted to find alternative treatments to assist in nerve regeneration including scaffolds-like artificial nerve guidance tubes and neurotrophic materials. Some of these alternatives are currently used in the clinic to treat nerve injuries; however, disagreements are ongoing regarding the effectiveness, proper use, and side effects of these treatments.[3,4] One of the major barriers to long-distance nerve gap repair is the restricted proliferation of Schwann cells.[5] These cells play a prominent part in peripheral nerve regeneration, participating in the elimination of axonal and myelin fragments, initiating proliferation, and aligning themselves to form the so-called bands of Büngner.[6] Following elongation of the axon along the bands of Büngner, Schwann cells begin the re-myelination of the new axon to conclude the regenerative process.

A recently developed method to facilitate a positive functional outcome during peripheral nerve regeneration is extracorporeal shock wave therapy (ESWT). ESWT was originally used in urology to disintegrate kidney stones;[7] however, both preclinical and clinical studies suggest ESWT would be an effective therapy in regenerative medicine such as to treat ischemic-induced tissue necrosis,[8] nonunion fractures,[9-11] or chronic wounds.[12,13] The shockwave produced is a sonic pulse, initially spiking to a positive peak of up to

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100 MPa in 10 ns and then falling to a negative amplitude of up to – 10 MPa. One total cycle of the shockwave lasts <10 μs. Biological reactions to the shockwave are believed to be caused by the high initial pressure, proceeded by a tensile force and mechanical stimulation.[14]

An investigation by Hausner *et al*.[15] demonstrated a new tactic of hastening nerve regeneration following peripheral nerve injury, while simultaneously using an autologous nerve graft. Extracorporeal shockwaves were directed at the injury site after the sciatic nerve was dissected and bridged in surgery. The results of this study showed animals treated with ESWT had significantly advanced functional recovery compared to the control.

Extracorporeal Shock Wave Therapy Facilitates Schwann Cell Proliferation and Improved Function

A recent study conducted by the LBI Trauma team studied the behavior and the regenerative capabilities of *in vitro* Schwann cells following ESWT. In this investigation, following the dissection of the rat sciatic nerve and treatment with ESWT, Schwann cells were harvested from tissue and cultured for 15 passages. When these nerves were treated *ex vivo*, there was found to be an immediate increase in extracellular adenosine triphosphate (ATP), resulting in multiple observed effects, beginning with an elevated Schwann cell yield following isolation. In the nerves treated with ESWT, there was a significant improvement in the culture quality, suggested in higher purity, the manifestation of regenerative phenotype markers, and the cell proliferation rate. On the other hand, the cells of the control group became increasingly senescent, demonstrated by a reduction in proliferation, elevation in P16INK4A expression, and lack of phenotype-specific markers. In summary, ESWT exhibited advantageous effects on Schwann cell isolation and culture.[16]

Further Studies Needed to Deepen Understanding of Extracorporeal Shock Wave Therapy and Overcome Limitations

Following peripheral nerve injury, Schwann cells are prompted to alter their phenotype from myelinated to multiplying and activated and constructing bands of Büngner, the substrate for developing axons. Countless investigations have demonstrated the significance of Schwann cells during the process of peripheral nerve regeneration,[17-19] but others have also highlighted the limitations associated with ESWT.[20-22] Specifically with long-distance injuries, there is a great demand for supportive Schwann cells expanded *in vitro*, such as those seeded on a tubular graft, due to the fact that autologous Schwann cells have limited proliferative capabilities and would not be able to construct bands of Büngner in a tube longer than 40 mm.[5] The limited proliferative capabilities of Schwann cells can also be seen *in vitro*, together with insufficient culture purity, a major problem with Schwann cell cultures. The study conducted by Schuh *et al.* is one of the first to display an improvement in culture purity and proliferation of *in vitro* Schwann cells following treatment with ESWT.[16]

Considering the beneficial effects of ESWT on the proliferative capacity of Schwann cells, it begs the question if hyperproliferative Schwann cells will cause harmful results such as schwannoma formation or excess proliferation following the stimulus. To test this, Schuh *et al.* performed a functionality investigation. In this study, Schwann cells were kept in a basic medium without any proliferation stimulating growth factors for 5 days. Those Schwann cells previously treated with ESWT were even more effected by the change of stimulus, as they not only halted proliferation but also there was a significant decrease in proliferation and an increase in myelin-associated phenotypic markers.[16] This swift response to the lack of mitogenic growth factors suggests the capacity of Schwann cells to adjust to the myelinating phenotype. Nonetheless, the effect of Schwann cells in an *in vivo* model has yet to be determined.

The results presented by Schuh *et al.* demonstrated a solution to the limited proliferative capacity of Schwann cells, a significant problem when they are used as a treatment for peripheral nerve damage. They showed that this limited proliferative capacity can be reversed using ESWT. Due to the fact that Schwann cells treated with ESWT can build a growth substrate for a longer time and at an increased rate, the effect *in vivo* would be twice as impactful.[16] Treatment of Schwann cells with ESWT not only would result in faster regeneration through stimulation of autologous Schwann cells as shown by Hausner and his fellow scientists[15] but also would enable reimplantation of numerous autologous expanded Schwann cells in a regenerative state.

One of the basic mechanisms that could explain the displayed results is the prolonged release of ATP. It is known that a variety of mechanisms are responsible for the excretion of ATP including ABC transporters and the vesicular secretion of ATP over pannexins/connexins.[23-25] The purinergic signaling that follows is essential, not only as a danger-associated molecular pattern but also in an assortment of cellular processes such as chemotaxis, proliferation, and differentiation and intensification of other stimuli.[26,27] This also encompasses the interactions between axons and Schwann cells. In particular, unmyelinating and immature Schwann cells convey signals to axons with extracellular ATP in a paracrine manner.[28,29] It has been suggested that glutamate and ATP exist in a positive feedback loop, in which one enhances the activity of the other.[30] The path of each Schwann cell is determined by stimulation of purinergic metabotropic p2Y receptors, neuronal activity, and the activity of ATP.[31-33] In addition, the stimulation of metabotropic glutamate receptors plays a role in determining the fate of Schwann cells.[34] The fact that purinergic signaling is considered a paracrine and an autocrine amplifier for other stimuli compounds the heightened proliferation of Schwann cells treated with ESWT in a medium with both pituitary extract and forskolin, proliferation stimulating factors. Furthermore, it is also suggested that adenosine, a byproduct of ATP hydrolysis, plays a part in altering histone-modifying proteins causing epigenetic modifications.[35] As a result, epigenetic alterations may be able to explain the heightened susceptibility to external signals and the extended phenotypic stability of Schwann cells demonstrated in the phenotypic switch experiment conducted by Schuh *et al*.[16]

In summary, the positive observations of increased culture purity, decreased expression of senescence-associated phenotypic markers following long cultivation periods, and increased proliferation rate without phenotype commitment

in Schwann cells treated with ESWT may be best explained by extracellular ATP activity. To deeply understand the underlying outcomes of ESWT on Schwann cells and the nerves, further studies must be conducted concentrating on epigenetic process, purinergic signaling, and mechanotransduction.

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Conflicts of interest

There are no conflicts of interest.

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