

AMERICAN SOCIETY FOR PERIPHERAL NERVE

Newsletter



Fall 2005

President's Message

Dear Colleagues,

I am looking forward to seeing all of you during the 15th Annual Meeting of the American Society For Peripheral Nerve in Tucson, Arizona on January 14-15, 2006. Our meeting format will combine a few sessions with the ASRM meeting, including instructional courses, panels, and an outstanding nerve paper session. The program chair, Ivan Ducic, M.D., has outlined an outstanding scientific program, including nine instructional courses, as well as a panel on the current management of complex peripheral nerve disorders presented by seven experts in this field. We have invited Ayan Gulgonen, M.D., from Istanbul, Turkey, who has unprecedented personal experience in treating thousands of traumatic peripheral nerve injuries. The strength and mission of ASPN is to keep the balance between clinical and basic science interests. We have fulfilled this mission by inviting Bruce Trapp, Ph.D., the Chairman of the Cleveland Clinic Foundation's Department of Neurosciences, who will share his insights regarding cellular and molecular mechanisms of myelination, demyelination, and dysmyelination. We are also looking forward to the lecture of invited speaker Richard Ransohoff, M.D., Professor in the Department of Molecular Medicine at the Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, who will be discussing the role of chemokines in neural inflammation.



The ASPN 2006 annual meeting will take place at the Loews Ventana Canyon Resort outside of Tucson, Arizona

Finally, I would like to share with you the news that we have opened the ASPN website – www.peripheralnerve.org. This website will facilitate exchange of information between our already-established membership, and will locate ASPN in the global network for active interactions with members of other neuroscience societies.

For all of the above reasons, I am encouraging you all to come and join us in Tucson, Arizona for the anniversary 15th meeting of the American Society For Peripheral Nerve.

Maria Siemionow, M.D., Ph.D., D.Sc.
President

From The Editor's Desk

The world has indeed become a small global village. Advances in communications have made our world small enough to allow instant sharing of information and news. Our peripheral nerve surgery profession has enjoyed the benefit of the instant communication between the far corners of the globe. This issue of the newsletter highlights the tremendous participation of peripheral nerve surgeons and researchers from around the world. One of the highlights of our next most exciting ASPN meeting in Arizona will be presentation by Dr. Ayan Gugonen from Turkey. We have an invitation to the members of ASPN from Professor Hanno Millesi to attend a special peripheral nerve surgery meeting to be held in Vienna, Austria in March 2006. Dr. Neumeister's article about his experience in Vietnam illustrates the wonderful learning experience that we get by teaching in other countries. Our society should extend its hands to the distant corners of the earth and should make an effort to welcome and embrace peripheral nerve surgeons from around the world. I would suggest sponsoring one or two international surgeons who can not afford the cost to attend our annual meeting. We all have learned a great deal from our international colleagues. We ought to return the favor.

Nash Naam, M.D.

Future Meeting Update – January 2006, Tucson, Arizona

The program for 15th annual meeting at the Loews Ventana Canyon Resort in Tucson will be held on Saturday and Sunday, January 14th and 15th, 2006. We will have exciting presentations since the quality of submitted abstracts was great. Using this occasion, I want to thank all of you who helped select abstracts for presentation since it was a lot of work. Also, it is my pleasure to inform you that the ASPN web site (www.peripheralnerve.org) is now available. This took a lot of hard work of many society members, in particular Dr. Paul Cederna. Although we did not manage to have abstract submission available via our own web site this year, it will be available for the 2007 meeting. I hope that our web site will also help attract more members to our society since information will be easier to access.

Our invited speakers this year include Dr. Richard Ransohoff who will be talking on ‘The Role of the Chemokines in the Neural Inflammation, Dr. Bruce Trapp, discussing Axon-Glial Interactions, while Dr. Ayan Gulgonen will presented his experience on “Nerve injuries in the Palm”. Beside that, two combined panels with ASRM will include Composite Tissue Allografts and Treatment of Difficult Nerve Problems, what combined with abstract presentations will certainly make the next meeting be a very good experience for all of us.

Volunteering in Vietnam Teaching and Learning

Volunteering one’s time and effort to perform surgery on medical missions can be described as rewarding, fulfilling, and to say the least extremely enlightening. Dr. Robert C. Russell, Dr. Seng Feng Jeng from Taiwan and I recently went to Ho Chi Minh City Vietnam and set up a microsurgical unit in the main hospital. We soon realized that two more descriptive words should be added to our vision of this mission. These words include: astonishing and brilliant. The purpose of the mission was to educate local surgeons and their residents on the indications outcomes and techniques of microsurgery. This mission would be followed by further trips to perform surgery with the local surgeons and patients in need of functional restoration through microsurgery. Other areas of Vietnam such as Hanoi and Danang have had similar microsurgical units developed in the past and know their local surgeons are performing hundreds of successful free tissue transfers on their own. Southern Vietnam, however, has been left with a void in this field of reconstruction and is in dire need of learning and practicing microsurgery. Dr. Phuong Lem, the head of plastic surgery at this public hospital in Ho Chi Minh City, was eager to have us initiate the microsurgical workshop to return within the year to perform surgery on patients.

I am looking forward to seeing you in Tucson and to enjoying an exciting and stimulating program suited to the diverse interests of our society.

Ivica Ducic, MD, PhD ASPN 2006 Program Chairman



The Resort surrounded by Coronado National Forest

The initial task is centered on a series of lectures on flap anatomy, physiology and dissection, further talks develop the principles of flap diversity, chimeric flaps and perforator flaps. Each of the free tissue transfer was outlined and described in detail. The surgeons and residents were given a written synopsis as well as the complete talks on a CD for their review and education. The afternoons were filled with dissections, applied anatomy and the recently described flaps. The Vietnamese surgeons would huddle around us with wide eyes and curious faces and every one of their jesters professed their enthusiasm and intrigue. On the third day, we set up an animal lab for practice microsurgical teaching. Three microscopes were brought over ahead of time as a gift from the hospitals from Springfield, Illinois. The microscopes were left in the Ho Chi Minh hospital for permanent use. Micrins gave the microsurgical instruments and Dr. Seng Feng Jeng supplied the microsurgical sutures. Throughout the lectures dissections and microsurgical practicing and practice on vessels, the language barrier was really an issue. This was in part due to the efforts of Dr. Phuong. To our amazement though, another interpreter was present. Dr. Chow was an orthopedic surgeon from the orthopedic hospital some 25 minutes away. Dr. Chow was able to translate our lectures and as we spoke in help in defining details of the cadaveric dissection. The interpretation in its own right was wonderful but the more astonishing feature of Dr. Chow was that he was already a self taught accomplished microsurgeon. Because of

the north / south politics Dr. Chow was not allowed to work in the public hospital. As a personal honor to him Dr. Chow wanted us to go to his orthopedic hospital to see his work and to evaluate the care of his patients. During the 2 hour break we made our way over to his hospital and there we walked the wards in amazement. The wards were immediately beside the parking lot (almost a part of the parking garage where hundreds of motor bikes were parked). Accompanied by his residents, Dr. Chow escorted us to each room, introduced us to the patients and proceeded to discuss each case. Here in lies the amazement, the cases he described were performed by himself. These cases included functioning gracilis muscle transfers for brachial plexus injuries, functioning serratus anterior muscle transfers to the forearm along with nerve grafts to the spinal accessory nerve and intercostals to restore flexor digitorum profundus function. We observed head and neck, lower and upper extremity and trunk reconstruction cases through a variety of free tissue transfer techniques. Dr. Chow also presented a number of patients who's heels were reconstructed with reverse sural artery flaps. Dr. Chow had accumulated over 300 successful reconstructions using the sural artery flap.

Following patient rounds we sat down with Dr. Chow and he discussed how he taught his residents microsurgery. Dr. Chow manufactures his own microsurgery sutures, sizes 9-0, 10-0 and 11-0 for which he also has a patent in Vietnam.

He has also written two books illustrating the techniques of microsurgery in the principles of flap dissection. We were also amazed to find that he has made his own microsurgical CD that is used by all residents in his hospital. Astonished by these accomplishments in his clinical expertise, we found it remarkable and disappointing that his talents could not be used in the public hospital which serves most of Ho Chi Minh City and the surrounding country side. The evil head of politics seems to be restrictive as there is still a history of North and South differences in Vietnam. We were unable to find out from either hospital the real impediment to allowing such an accomplished microsurgeon to teach other local surgeons his craft. It remains a shame that the local hospital could not utilize Dr. Chow's education and microsurgical skills. Dr. Russell, Dr. Seng Feng Jeng, and myself remain in awe at what we saw in the orthopedic hospital in Ho Chi Minh City. We give credit to Dr. Chow for everything that he has done. We also applaud Dr. Phuong in her attempts to gain microsurgical techniques for the use of the remaining patients that need free tissue transfers in Ho Chi Minh City.

Michael Neumeister, MD, FRCS(C)

What's New in Peripheral Nerve Surgery and Research

Retrograde Labeling in Peripheral Nerve Research: It is Not All Black and White

Ayato Hayashi, M.D., Arash Moradzadeh, M.D., Daniel A. Hunter, R.T., Thomas H. Tung, M.D., Terence M. Myckatyn, M.D., Susan E. Mackinnon, M.D.

INTRODUCTION

Retrograde labeling is quickly becoming the new gold standard to study peripheral nerve regeneration. This technique labels the distal axons and then follows their path proximally to their origin in the ventral horn or dorsal root ganglion. There are only a few papers that evaluate the various dyes and application processes of this technique⁽¹⁻⁴⁾. We discuss our experience with this temperamental but potentially beneficial technique for the identification of neuronal pathways and functional connection.

PROCEDURE

Lewis rats, Balb/c mice, CD-1 mice and GDNF over-expressing transgenic mice were utilized. Three dye application methods were employed: (1) either 3% Fluoro-Gold solution (FG), 3% Fast-Blue solution (FB), or 10% Fluoro-Ruby solution (FR) was applied to the cut end of the tibial nerve using a conduit (serving as a reservoir) for 2 hours, (2) an injection of one of the above three tracers was given directly into the gastrocnemius muscle or, (3) Fast-Blue crystal compound (FBC) was directly applied to the cut-end of the tibial nerve. Four or 6 days following application of the labeling agent, spinal cords were harvested and 40µm

longitudinal frozen sections were obtained. The labeled neurons within the spinal cord were then evaluated using fluorescent microscopy, and quantitative analysis was performed using Pax-It (version 6.4, MIS, Illinois).

APPLICATION TECHNIQUES

The conduit reservoir is the most commonly used dye application technique. The main problem we encountered was failed staining. We attribute this to the accumulation of blood and serum (dilution effect), change in nerve position, and or inadequate contact between the dye and the cut end of the nerve during the 2 hour exposure. The intramuscular technique was easier to perform and provided less chance for failed staining secondary to technical error. In the mouse model, the intramuscular technique worked as well as the conduit technique and the numbers of stained axons were consistent. In the rat model, only 10% of neurons stained in comparison to a matched rat evaluated with the conduit technique. We attributed this difference to the greater mass of the rat musculature, resulting in difficulty with injection and delivery of the labeling agent.

The muscle injection technique may result in dye leakage from the puncture site onto surrounding muscles resulting in false positive results. Care should be taken when using FG because of its ability to extensively diffuse into surrounding tissues; there has been a report that FG was found in motor neurons in the appropriate ipsilateral segment and also in adjacent ipsilateral and contralateral segments⁽⁴⁾. We used a 31-gauge Hamilton syringe to avoid leakage and confirmed the adequacy of the injection by looking for a color change in the injected muscle. The FBC provided reliable application to

the cut end of the nerve, with decreased staining failure rates in comparison to the conduit technique.

COUNTING NEURONS

The major difficulty encountered during the objective quantification of stained neurons was deciding which neurons to count. With FG especially, wide variability in staining intensity, neuron size and background staining resulted in poor interobserver reliability. To overcome this obstacle, a few techniques can be implemented. First, labeled profiles can be stratified in terms of soma size so that only profiles of a specific diameter are counted. Moreover, cell bodies can be counterstained with a nuclear stain such as Hoechst, or a cyanine dimmer and only counterstained profiles counted. Counting motor neurons using unbiased stereology methodology for the quantitation of morphometric parameters should be considered^(1,3,5).

DYE COMPARISON

The greatest number of stained neurons was observed with Fluoro-Gold. Advantages of using FG included a decreased likelihood of staining failure, resistance to fading, and the possibility of double labeling with other dyes. The major disadvantage was background over-staining and inconsistency in the intensity of stained neurons. Although prior work with FG has mentioned the possibility of neuronal toxicity resulting in cell body death and injection site necrosis⁽⁵⁾, none was observed in our study. Similar to prior publications⁽¹⁾, we found that FB stained fewer neurons than FG. When FB was applied via a conduit, a high rate of failed staining was observed. However when injected into the mouse muscle, FB staining was the most consistent, with reliable neuron counts. Conversely, the FR was inconsistent using the injection and conduit technique.

DISCUSSION

To help improve count reliability, all neurons are counted by the same individual based on predetermined criteria and the opposite side is stained as an internal control. On the control side we utilize double staining techniques to reliably

distinguish the control and experimental side during the final evaluation by injecting FB into the mouse muscle with the addition of a FR injection on the control side. In the rat model we do not double-stain, although this technique has been reported using successive application of dyes to the cut end of nerves⁽²⁾. Another modification may involve double staining using a nuclear stain to confirm that the fluorescent cell is indeed a neuron.

Retrograde labeling using fluorescent dyes has been one of the fundamental methods to prove the connectivity of the peripheral nerve to the spinal cord and for distinguishing motor from sensory neurons. Currently, in the mouse model we are utilizing muscle injection with FB, and in the rat model FBC to the cut end of the nerve. However, this technique will benefit from continued refinement and standardization to improve its utility.

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The State of Stem Cell Transplantation in the Treatment of Peripheral Nerve Injuries

Jason Koob, Arash Moradzadeh, M.D., Susan Mackinnon, M.D., Terence Myckatyn, M.D.

The successful reconstruction of a peripheral nerve defect depends upon the ability of regenerating axons to reinnervate denervated target muscle and the receptiveness of neuromuscular junctions to reinnervation. In the past fifteen years there have been many studies aimed at exploring the exciting idea of using neural stem cells to preserve denervated muscle and neuromuscular junctions until proximal regenerating axons can reach distal targets. Despite the interest in such treatment, there remain many unanswered questions which we will briefly address.

The number of transplanted cells in past studies has varied greatly from one million to 14.8 million cells [1,2]. A larger

number of cells at initial transplantation correlated with a greater number of transplanted cells surviving at later time points. However, Grumbles et al. demonstrated that the diameters of experimental axons were greater with fewer transplanted cells [1]. Thus, while the number of surviving transplanted cells increased with a larger number of initial cells in the transplant volume, the diameters of those cells diminished. In addition, only one study has evaluated the effect of injection of the transplanted cells immediately after transection versus a one week delay [1]. The results of this study indicated that the diameter of the transplanted axon was significantly greater in the delay group. Future studies will determine both the optimal number of cells, and time of injection of those cells, to maximize both transplant survival rate and cell diameter.

Different delivery systems have been utilized yet there is no accepted gold standard. Methods of delivery have included direct injection of transplant cells into the axotomized distal stumps or incorporation into a collagen gel matrix within a vein graft or conduit. The use of vein grafts might enable transplant volumes and cell numbers to be increased but the interactions of the vein graft material and the neural progenitor cells has been shown to produce hypertrophic fibrous tissue, with signs of chronic inflammation [4]. The optimal distance to inject transplanted cells in relation to the muscle must also be established. Should the cells be injected directly into the axotomized distal nerve stump, more distally approaching the denervated muscle, or should they be injected into the muscle belly in the vicinity of the neuromuscular junctions?

Stem cells are pluripotent, undifferentiated cells that can be induced to become any cell type. While neural progenitor cells can be used in the treatment of peripheral nerve injuries, *what degree of cellular differentiation before transplantation is optimal for reinnervation?* A variety of different progenitor cells, including E14 and E15 ventral spinal cord cells or bone marrow stromal cells induced to become neural cells, have been employed [1,3]. It is unknown if optimal reinnervation will occur with a differentiated, committed state or if a more plastic state, capable of responding to environmental cues *in vivo* will prove to be more advantageous. Future research will identify the differentiation of the transplanted cells into such cells as Schwann cells, motoneurons, fibroblasts, or myocytes.

Will the use of neural progenitor cells result in improved functional recovery? Several studies have recorded twitch force and electromyographic activity (EMG) from the denervated muscles but other methods such as gait analysis, muscle area, and retrograde labeling may help demonstrate functional reinnervation of the target muscle [5,6]. Future papers not only need to prove that neural progenitor cells can establish a functional connection with denervated muscle and preserve the neuromuscular junctions, but also explore the interactions between these transplanted neural progenitor cells and the regenerating host axons. *Will the presence of the neural progenitor cells, now differentiated into motoneurons and occupying the bands of Bungner in the distal nerve stump, inhibit the regenerating nerves extending from the*

Thank you

The American Society for Peripheral Nerve and its Council thank Small Bone Innovation, Inc for sponsoring the guest

Invitation Peripheral Nerve Surgery Meeting

Dear Colleagues

Everyone interested in peripheral nerve repair is also interested in finding the answer to on question: How can we

host proximal nerve stump? Do the neural progenitor cells need to be engineered with a suicide gene enabling the researcher to selectively eliminate these cells at some point after they have successfully reinnervated the target muscle? Several projects in our lab hope to investigate these issues.

Despite over a decade of research with neural progenitor cells as a hopeful treatment option for peripheral nerve injuries, the advances have been limited. While the clinical applications of stem cells as a means of preserving denervated muscle for delayed reinnervation in peripheral nerve injuries remains extremely promising, many of the above mentioned issues need to be addressed through further research.

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room key card program at the annual meeting and Synovis for hosting the ASPN website

improve our results? New knowledge on nerve regeneration may have a chance of application in the near future. Surgical technique can certainly be improved by technical details, by minimally traumatic exploration in accordance with anatomy and by creating a favorable environment for the nerve. New techniques such as end-to-side coaptation could be applied more successfully if the ongoing controversy could finally be resolved. Improved diagnosis by high resolution MRI may

change our approach to some problems such as neurolysis. Proper muscle balance seems to be important in achieving

sufficient strength for regenerating muscles. Particularly in brachial plexus surgery, the prospective application of reconstructive surgery is gaining increasing importance in contrast to the retrospective application of palliative surgery. Moreover, physiotherapy is offering new modes of treatment

Further Development of Reconstructive and Cell Tissue-engineering Technology for Treatment of Complete Peripheral Nerve Injury in Rats

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In this work we evaluated the efficacy of biodegradable composite co-polymer guiding neurotube, based on tissue-engineering technology, for the treatment of complete peripheral nerve injury where the nerve defect is significant. The right sciatic nerve of 12 three-month-old rats was completely transected and peripheral nerve segment was removed. A 2.2-cm biodegradable co-polymer neurotube containing viscous gel (NVR-N-Gel) with survival factors, neuroprotective agents and Schwann cells was placed between the proximal and the distal parts of the transected nerve for

and increasing understanding of cerebral function and is providing a basis for new thoughts on motor and sensory re-education. All these topics and problems associated with peripheral nerve repair should be discussed at our meeting here in Vienna. It is therefore my pleasure to cordially invite you to Vienna to participate and to contribute your own thoughts on this fascinating topic.

Meeting dates: March 24-26, 2006

Information: www.medacad.org/pns2006

I look forward to welcoming you in Vienna.

Hanno Millesi, M.D.

reconnection a 2-cm nerve defect. The proximal and distal parts of the nerve were fixed into the neurotube using 10-0 sutures. Ultrasound observation showed growth of the axons into the composite neurotube 2 months after the surgery. Electrophysiological study indicated compound muscle action potentials in nine out of 12 rats, 2-4 months after peripheral nerve reconstructive surgery. The postoperative follow-up (up to 4 months) on the operated rats that underwent peripheral nerve reconstruction using composite co-polymer neurotube, showed beginning of re-establishment of active foot movements. The tube was dissolved and nerve showed complete reconnection. Histological observation of the nerve showed growth of myelinated axons into the site where a 2-cm nerve defect replaced by composite co-polymer neurotube and into the distal part of the nerve. In conclusion: (1) an innovative composite neurotube for reconstruction of significant loss of peripheral nerve segment is described; (2) a viscous gel, containing survival factors, neuroprotective agents and Schwann cells served as a regenerative environment for repair. Further investigations of this reconstructive procedure are being conducted

VISIT WWW.PERIPHERALNERVE.ORG FOR 2006 ANNUAL MEETING INFORMATION

For a complete listing of the events taking place during the 2006 ASPN Annual Meeting and to register on-line please visit our new web site at www.peripheralnerve.org. Registering for the annual meeting is easier than ever with our new online registration system. We are also offering a joint fee along with the AAHS (American Association for Hand Surgery) and the ASRM (American Society for Reconstructive Microsurgery) for those interested in attending all three organizations meetings. We look forward to seeing you in Tucson!