ACTA
ORTHOPAEDICA
et
TRAUMATOLOGICA
TURCICA

Author's translation
Acta Orthop Traumatol Turc 2008;42(2):130-134

# The role of creating a biological membrane in expediting nerve regeneration

## *Periferik sinir tamirinde biyolojik membranın sinir iyileşmesini hızlandırıcı etkisi*

**Tackin OZALP, Alain-Charles MASQUELET[1]**

*Celal Bayar University, Medical Faculty, Department of Orthopedics and Traumatology;*
*[1]Service de Chirurgie Orthopédique, Traumatologique et Reconstructrice de l'Appareil Locomoteur, Hôpital Avicenne, France*

**Amaç:** Sinir rehber kanalları, kesik iki sinir ucu arasındaki boşluğu köprülemek amacıyla kullanılan doğal ya da sentetik tübüler yapılardır. Biyolojik bir membran oluşturmak bir rehber kanalın ucuz ve kolay bir yolu olabilir. Bu çalışmada biyolojik membranın sinir iyileşmesini hızlandırıcı etkisi araştırıldı.

**Çalışma planı:** Ağırlıkları 200-250 gr arasında değişen 20 adet erişkin erkek Wistar albino sıçan 10'arlı iki gruba ayrıldı. Her iki grupta da median sinir kesisiyle 5 mm'lik bir defekt oluşturuldu. İlk grupta, defekt diğer üst ekstremite median sinirinden alınan bir parça ile greftlenirken, ikinci grupta defekte bir silikon implant yerleştirildi ve her iki uca anastomoze edildi. Beş hafta sonra silikon implant çıkarıldı ve oluşmuş olan membran içine diğer üst ekstremiteden alınan median sinir grefti uç uca anastomoz yöntemiyle dikildi. Kasların fonksiyonlarındaki geri dönüş Bertelli ve Mira tarafından önerilen testle değerlendirildi. Güç ölçümlerine, her iki grupta da greft konduktan beş hafta sonra başlandı ve 12 hafta boyunca sürdürüldü.

**Sonuçlar:** İkinci grupta sinir rejenerasyonunu gösteren kas gücü geri dönüşünün birinci gruba göre çok daha hızlı gerçekleştiği görüldü. İkinci grupta ortalama 10. haftada sinir iyileşmesinde tam geri dönüş gözlenirken, birinci grupta 12. hafta sonunda iyileşme oranı %90.2 idi ($p<0.05$).

**Çıkarımlar:** Günümüzde otojen greft uygulaması hala altın standart olan yöntemdir. Biyolojik membran sinir iyileşmesini hızlandırmaktadır. Ayrıca, sadece iki ucun açılması yeterli olduğundan, diseksiyon daha sınırlı olmakta, ameliyat kolaylaşmakta ve süresi kısalmaktadır. Bu avantajları nedeniyle diğer tekniklere iyi bir seçenek olarak düşünülebilir.

**Anahtar sözcükler:** Biyoprotez; önkol/inervasyon; median sinir; mikrocerrahi; sinir rejenerasyonu; protez implantasyonu/yöntem; sıçan; silikon.

**Objectives:** Nerve guidance channels are natural or synthetic tubular conduits used to bridge the gap between the nerve stumps. Creation of a biological membrane may be a simple and cheaper way to obtain a nerve guidance channel. The goal of this study was to examine the role of a biological membrane in expediting nerve regeneration.

**Methods:** Twenty adult male Wistar albino rats weighing 200 to 250 g were divided into two groups equal in number. All the animals underwent median nerve dissection to create a 5-mm gap. In the first group, the defect was repaired with a graft obtained from the contralateral median nerve, while in the second group, a silicon implant was sutured and anastomosed between the stumps. After five weeks, the silicon implant was removed and a nerve graft taken from the contralateral median nerve was anastomosed inside the neoformed biological membrane. Recovery of muscular function indicating nerve regeneration was assessed by the prehension test proposed by Bertelli and Mira. In both groups, measurements were started after five weeks of grafting and continued for 12 weeks.

**Results:** Rats in the second group exhibited an accelerated recovery and nerve regeneration compared to the first group. Nerve regeneration was completed at 10 weeks in the second group, whereas the recovery rate was 90.2% at 12 weeks in the first group ($p<0.05$).

**Conclusion:** The use of autogenous grafts is still the gold standard in nerve repair. This biological membrane not only expedites nerve regeneration, but also facilitates surgery and reduces operating time because it requires small incisions at the two ends. Considering these advantages, it may prove to be a good alternative to other techniques.

**Key words:** Bioprosthesis; forearm/innervation; median nerve; microsurgery; nerve regeneration; prosthesis implantation/methods; rats; silicones.

**Correspondence / *Yazışma adresi:*** Dr. Taçkın Özalp. 1786/1 Sok., No: 1/7, 35540 Karşıyaka, İzmir.
Phone: +90236 - 232 31 33 Fax: +90236 - 237 02 13 e-mail: tackino@yahoo.fr

**Submitted / *Başvuru tarihi:*** 26.04.2006 **Accepted / *Kabul tarihi:*** 26.02.2008

Nerve guidance channels are natural or synthetic tubular conduits used to bridge the gap between the nerve stumps. To assure a direct way for proximal stump regeneration, to obtain a biologic exchange between the nerve and the proximity across the channel porosity, to reduce the fibrous tissue infiltration, to form a conduit for neurotrophic factors secreted by damaged nerve ends are the aims of the studies but use of these channels are expensive and require more investigations.[1] To form a biological membrane can be a simple and cheap way to obtain a channel.

In this study, a flexible, silicon implant was used to serve as a template around which a new sheath created on the median nerve of the rats on a parallel idea of using the Hunter prosthesis in tendon reconstruction; and the role of a biological membrane in expediting nerve regeneration was examined.[2]

## Materials and methods

This study was realized in the "Ecole de Chirurgie Anatomy Laboratory, Microsurgery Section (Paris, France)" and the permission of the Ethical Committee was obtained. Twenty adult male Wistar albino rats weighing 200 to 250 gr. were used in the study. The rats were divided into two groups of ten.

Recovery of muscular function was used to assess the nerve regeneration after the nerve injury. For this recovery, a prehension test proposed by Bertelli and Mira was realized. This test related to the instinctive behavior of the animal evaluates easily the nerve regeneration and the reinnervation of the muscles. It was benefited by the finger and wrist flexors innervated by the median nerve for the test. With an injured median nerve the rat can not flex its fingers and its forefeet. The force measurements were done with an electronic balance with a fixed cage above. The rat, suspended by the tail, tried to catch the railing with the fingers. The strength of traction exercised on the tail was gradually increased until the moment the animal releases its grip, and the negative value seen on the balance was recorded. To avoid false measures, the contrelateral leg was denerved with a segmental resection of the nerve, as it was indicated by Bertelli and Mira.[2] In all two groups, we made previously a measure of initial strength by the right leg, after resection of a segment of the left median nerve. The measures were done weekly during twelve weeks. In the first group, the defect obtained with the resection of 5 mm. of the median nerve was repaired with a graft obtained from the contralateral median nerve. A two staged surgical technique was used for group two. In the first stage a silicone implant was placed in the 5 mm.gap of median nerve and anastomosed to the both ends. Five weeks later the silicon implant was removed and a median nerve graft taken from the other upper extremity was end-to-end anastomosed in the membrane formed. A seven mm. silicone implant with the same width of the nerve taken from a Hunter tendon prosthesis (Wright Medical Technology, Arlington, TN, ABD) was side-to-side sutured to the proximal and distal stubs overtaking of 1 mm. of every end with a 10/0 monopropylene suture (Ethylon, Ethicon, USA). All of these procedures were realized under microscope magnification.

In the first group, measurements were started after five weeks of grafting, in the second group, after the replacement of the silicone implant by graft and continued for 12 weeks. For statistical evaluation, the one way ANOVA test was used to compare the force measurements of the rats; $p<0,05$ value was statistically significant. SPSS 10.0 was used for the statistical analysis.

### Surgical technique

The rats were anaesthetized with intramuscular10 mg/kg of xylazin (Rompun, Bayer, Turkey) and 70 mg/kg of ketamine (Ketalar, Eczacıba_ı, Turkey). The section was made on a three cm. segment from the axilla to the elbow on the ventral side in the first group. The nerve was tracked down in the axillary region, followed up to the elbow where it is practiced a five mm. section. We used the same technique for the contralateral median nerve. The grafting was realized with a same length nerve graft taken from this side in the same session. For second group, after the segmental resection the implementation of a seven mm. silicone stalk of length overtaking of 1 mm. of every end of the split nerve was made (Fig.1). The goal was to keep the proximal and distal stubs in the membrane which would be neoformed. In the fifth week we opened the biological membrane on both sides and the graft taken from the other side was sutured instead of the silicone implant (Fig. 2). The skin was sutured with 3/0 polyglactin (Vicryl, Ethicon, USA).

## Results

No animal was lost during the study. In the second group the muscular force recovery indicating the nerve regeneration was much speedier according to the

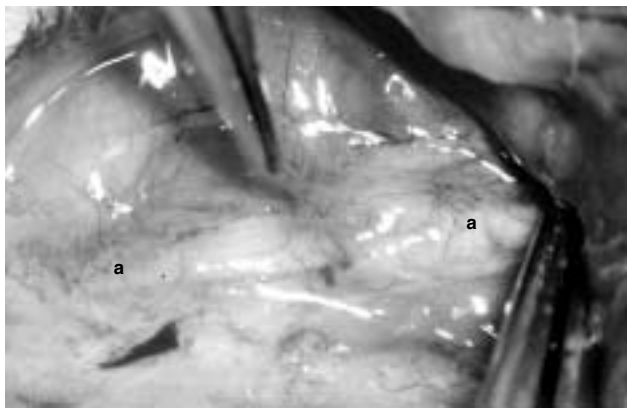


**Figure 1.**The silicone implant in the biological membrane.
a: Proximal and distal nerve ends.

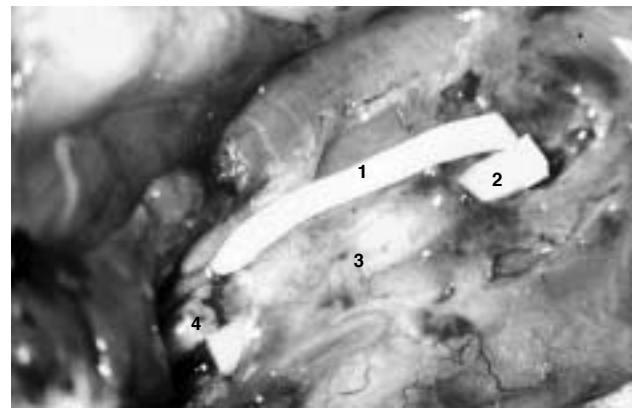


**Figure 2.** Grafting after the formation of the membrane.
1: Autograft; 2: Silicone implant; 3: Biological membrane; 4: Nerve stub.

first group. Nerve regeneration was completed at 10 weeks in the second group, whereas the recovery rate was 90.2% at 12 weeks in the first group ($p<0.05$). The force measurement values according to the time of the two groups were demonstrated on figure 3.

## Discussion

The repair of the defective peripheral nerves is not always successful. Autogenous graft application is the most used method. But because of the limited possibility of the donor nerves, scar tissues, loss of sensation and sometimes the painful neuromas on the donor sites, the diameter incongruity between the recipient nerve and the graft caused to search of other possibilities of repair.[1]

The concept of the use of tubes guides for the repair of the nervous losses of substance is not recent. In 1880, Gluck tried a tubulisation by using the bone; Payr in 1890 a steel tube. In 1891, Bungner reported the repair of a nerve by a blood vessel. In 1915, Kirk realized a fascial bypass for the repair of the loss of substance. In 1982 Chiu et al. published their works showing the success of the nervous regeneration with a conduit formed by a femoral venous graft. The Spanish biologist Ramon y Cajal, put in evidence the importance of the chemical factors secreted by the distal stub of the nerve who assuring a neurotrophisme. In 1944, Weiss adduced that to have a conduit in contact was more important than the neurotrophisme. But, after 1980, it was demonstrated that this last one was also important as guidance channels. Also the silicon tubes were used on the end-to-end anastomosis like the synthetic biomaterials on the defective nerves and it was reported good results.[1,3,4]

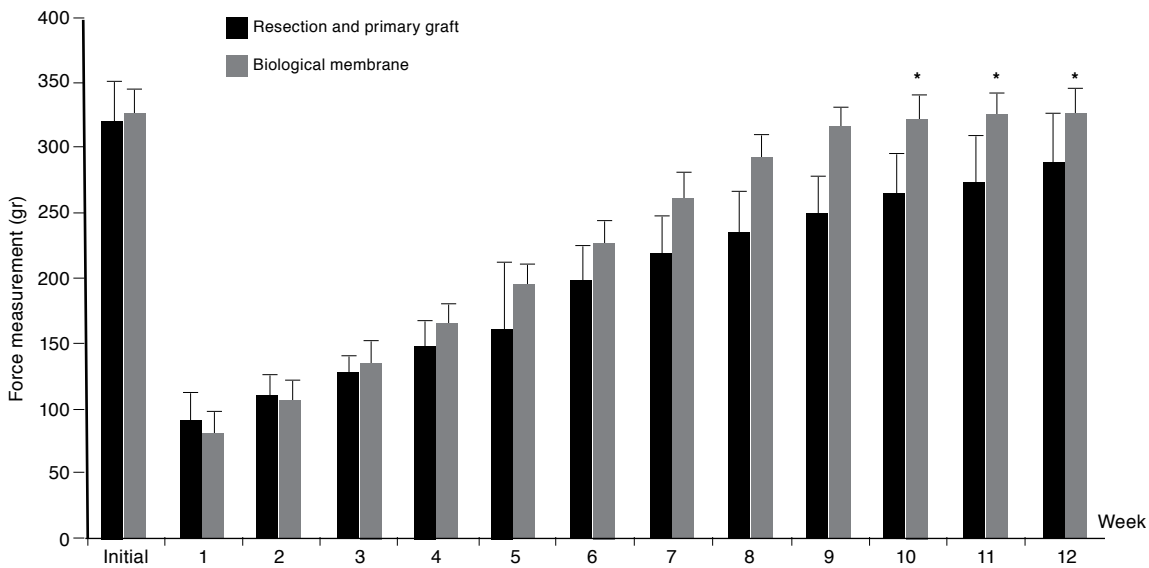


**Figure 3.** The force measurements of the groups according to time (mean ± SD).

All of these works are made to eliminate the difficulties of the autogenous graft application. To obtain a material with the appropriate length and diameter allowing the osmosis of the growth factors from the neighborhood through the porosity of the channel but blocking the passage of the inhibiting factors needs an advanced technology and this obligation can restrict their use economically. Moreover, all of these materials can be used for the short defects (< 3 cm.).[1,5,6] The success rate diminishes while the defect size enlarges.[7] But at the moment, the autogenous nerve graft is still considered as the "gold standard".

The formation a biologic membrane on the periphery of an autogenous nerve graft idea was arised from the Masquelet's work of the rapid consolidation of the bone grafts with a biologic membrane in the repair of the bone defects.[8] In the Masquelet's method, the gap between the two ends of the bone is supplemented by the bone cement to obtain a synovial membrane like structure, and bone graft is applied in the membrane formed by the reaction of foreign body after five weeks. The neoformed membrane inhibit the graft resorption and augment the revascularization and corticalization.[8]

The goal of this study was to obtain a protective sheath accelerating the nerve regeneration but not only a biological membrane replacing the autogenous graft. This membrane was developed by a silicone implant. This silicone implant is an inert material; consequently the rate of an allergic reaction is very low. Lundborg et al.[9,10] in their study compared the microsurgical end-to-end anastomosis and the silicon tube method with a 3-5 mm. gap and They did not find a difference. We did not find out a study in which the synthetic and biological (e.g. vein graft) were used together. The created median nerve defect of 5mm. was grafted in this study. But this 5 mm. defect on the rats can be correspond to greater defects in human race. We think that in case of primary repair possibility is low and a graft is required, the use of a two staged operation with a biological membrane can accelerate the nerve regeneration and is more advantageous in comparison with the primary nerve grafting.

The membrane formed by foreign body reaction is very rich of capillary vessel and contain vascular endothelial growth factor and transforming growth factor ß.[11] These factors stimulate the angiogenesis and accelerate wound repair. In this study, the membrane neoformed markedly accelerate the nerve regeneration. In the second group the recovery of the antebrachial flexor muscles innervated by median nerve was accomplished at tenth week, whereas in the second group it was not completed at the end of the twelveth week. One can inform that this technique accelerate the nerve regeneration between 25 to 30 percent. Its disadvantage is the need for a two staged operation.

The biological membrane forms a direct way between both stubs of the injured nerve. In the large soft tissue defects when a secondary nerve repair is planned, this sheath facilitates the operation and decrease the operation time especially for the vast nerve defects. During the second operative procedure for nerve grafting the soft tissue dissection is difficult due to the fibrous tissue augmentation. With this technique, especially in the large defects, the both sides of the membrane is opened with little incisions instead of one large incision; the graft can be easily passed by the tunnel formed, in consequence this procedure reduce the dissection and the operating time. To diminish in addition the operating time, it is possible to fix some guidance sutures on both sides of the membrane.

This is a pioneering work featuring the benefits of the biological membrane. To determine by the time the neurotrophic factors present in the membrane and to practice this technique on the large defects can increase its importance.

## References

1. Hudson TW, Evans GR, Schmidt CE. Engineering strategies for peripheral nerve repair. Clin Plast Surg 1999; 26:617-28.
2. Bertelli JA, Mira JC. The grasping test: a simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods 1995;58:151-5.
3. Kurklu M, Demiralp B, Kirdemir V, Komurcu M, Ulas UH, Oztas E, et al. Comparison between the distraction and grafting methods in the treatment of peripheral neural defects: an experimental study in rabbits. [Article in Turkish] Acta Orthop Traumatol Turc 2005;39:163-71.
4. Wang KK, Costas PD, Jones DS, Miller RA, Seckel BR. Sleeve insertion and collagen coating improve nerve regeneration through vein conduits. J Reconstr Microsurg 1993; 9:39-48.
5. Battiston B, Geuna S, Ferrero M, Tos P. Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurgery 2005;25:258-67.

6. Lundborg G, Dahlin L, Dohi D, Kanje M, Terada N. A new type of "bioartificial" nerve graft for bridging extended defects in nerves. J Hand Surg [Br] 1997;22:299-303.
7. Stanec S, Stanec Z. Reconstruction of upper-extremity peripheral-nerve injuries with ePTFE conduits. J Reconstr Microsurg 1998;14:227-32.
8. Masquelet AC. Muscle reconstruction in reconstructive surgery: soft tissue repair and long bone reconstruction. Langenbecks Arch Surg 2003;388:344-6.
9. Lundborg G, Rosen B, Dahlin L, Holmberg J, Rosen I. Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up. J Hand Surg [Br] 2004;29:100-7.
10. Lundborg G. The tube concept in nerve repair. Tech Hand Up Extrem Surg 1997;1:120-4.
11. Pelissier P, Masquelet AC, Bareille R, Pelissier SM, Amedee J. Induced membranes secrete growth factors including vascular and osteoinductive factors and could stimulate bone regeneration. J Orthop Res 2004;22:73-9.