Basic Medical Research

The effect of amnion membrane on the function and histopathology of the sciatic nerve in nerve crush injury in sprague dawley rat

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ABSTRACT

Introduction: The aim of this study is to evaluate the validity of the Amniotic Membrane for nerve regeneration in rat sciaticvnerve crush injury, both functionally and histologically.

Methods: In this study, data was obtained from 18 healthy Sprague Dawley rats. The intervention given was a unilateral right side sciatic nerve crush injury by pinching with a forcepand, and then the rats were observed with different methods: Group I (control group): 9 rats with damaged nerve left untreated with amniotic membrane. Group II: 9 rats, with damaged nerve treated with amnion membrane. The evaluation was performed over 21 days post-operatively for both functionally and histologically.

Results: There were significant difference in functional parameters evaluated by walking track analysis between Group I and Group II at day 5, 7, 14, and 21 ($P<0.05$). Histological features of Group II, after 21 days, showed that there was complete nerve regeneration and reconnection. Histological results showed that there was significant difference between Group I and Group II for axonal degeneration ($P=0.0024$), nerve regeneration ($P=0.016$), and lower inflammation reaction ($P=0.016$). However, there was no significant difference in nerve diameter ($P=0.23$).

Conclusion: It can be concluded from the results of this study that the application of amniotic membrane in crush injury nerve damage gave better outcome, both functionally and histologically, compared to the damaged nerve that was left untreated with amniotic membrane and this could be used as an alternative technique to improve nerve healing in nerve crush injury

ABSTRAK

Pendahuluan: *Studi ini ditujukan untuk mengevaluasi kemampuan membran amnion merangsang regenerasi saraf pada tikus yang mengalami sciatic nerve injury, ditinjau dari fungsi dan histologinya.*

Metode: *Dalam studi ini, data diambil dari 18 Tikus Sprague Dawley sehat. Intervensi sciatic nerve injury diberikan pada sisi kanan tikus menggunakan forcepand, lalu diobservasi dengan metode yang berbeda: Kelompok I (kontrol), 9 tikus dengan cedera saraf tanpa intervensi, dan Kelompok II, 9 tikus dengan cedera saraf yang mendapat intervensi membran amnion. Evaluasi fungsi dan histologi dilaksanakan setelah 21 hari pasca-operasi.*

Hasil: *Perbedaan signifikan dapat dilihat dari cara berjalan tikus pada kelompok I dan II pada hari ke-5, 7, 14 dan 21 ($P<0.05$). Gambaran histologi pada kelompok II terlihat setelah 21 hari, saraf yang rusak teregenerasi dan terkoneksi sempurna. Gambaran histologi memberikan hasil signifikan terhadap degenerasi axonal ($P=0,0024$), regenerasi saraf ($P=0,016$), dan reaksi inflamasi ($P=0,016$). Namun tidak ada perbedaan signifikan dalam diameter saraf ($P=0,23$).*

Kesimpulan: *Penggunaan membran amniotik pada kasus cedera saraf dapat memberikan hasil yang baik, ditinjau dari fungsi dan histologi, dibandingkan dengan kelompok tanpa intervensi, dan dapat digunakan sebagai alternatif dalam meningkatkan penyembuhan saraf pada kasus cedera saraf.*

Keywords: Peripheral nerve injury; Amniotic membrane; Nerve Crush Injury

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INTRODUCTION

Peripheral nerve injury is usually caused by accidental trauma, acute compression, or iatrogenic injury that can result in temporary or permanent neuropraxia. These kinds of injury can seriously affect the quality of life. The axonal damage affects the healing time of crush injury since it causes Wallerian degeneration. In the absent of axon damage, the healing process is faster. The use of amniotic membrane for wound healing is widely applied with satisfying result. It decreases fibrosis and scarring, decreases inflammation, has anti adhesive and non-immunogenic features, and also produces stem cell substrate (Dobreva, 2010). The most prominent factor inhibiting the nerve healing is fibrosis or scar on distal end stump regeneration caused by overwhelmed inflammation (Meng, 2011).

METHODS

Two groups of healthy Sprague Dawley rats weighing approximately 250 gr (n=9) were kept and fed according to the standard of LPPT Gadjah Mada University, Jogjakarta. The amniotic membrane was produced by Tissue Bank Unit of Dr. Soetomo General Hospital, Surabaya.

Each group was given different treatment: lesion on the right hind limb of the rats in Group I were given by clamping with surgical clamp, while for rats in Group II lesion on the right hind limb of the rats were given with surgical clamp and then amnion membrane scaffold was also provided.

We evaluated the function with walking tract analysis on day 1, 3, 5, 7, 14, and 21 after surgery in each group. Below is the figure of the walking tract box and the test tool to evaluate the function (Figure 1).

Figure 1. Walking Track Box.

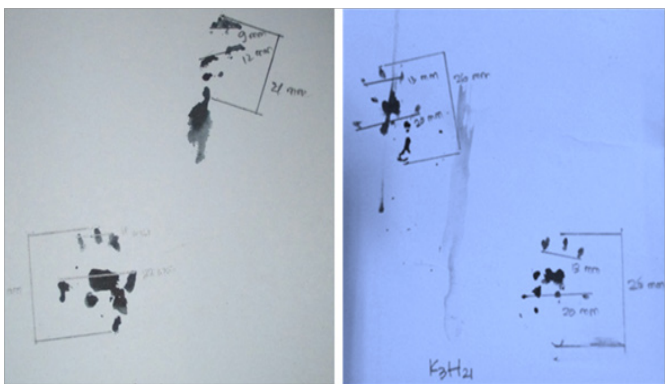
The rat's feet were stained with ink, as below (Figure 2).

Figure 2. Staining the rat's feet

And then the rats were placed in the tool and they were let to walk through the box to obtain the foot prints.

Figure 3. The foot print is being taken

The analysis of the rat's foot print including: the print length (PL) is the distance between the heel and the 3rd finger, the toe spread (TS) is the distance between the first toe and the 5th toe, the intermediate toe spread (ITS) is the distance between the 2nd toe and the 4th toe, and TOF is the distance between the closest toes (Sarikcioglu et al., 2008) as can be seen in the figures below.



(a) (b)

Figure 4. Walking Track of the Control Group at day 21 (a), and the Treatment Group at day 21 (b)

Another feature evaluated was the histologic picture of the nerve at day 21 after the surgery. The specimens were fixed by 10% formalin, dehidrated through a series of ethanol, and embeded in paraffin. The specimens were then sectioned into 4 micron-sizes and stained with Hematoxylin–Eosin and Malory staining. HE staining was conducted to evaluate the continuity of the damaged sciatic nerve, the angiogenesis, and the inflammation reaction caused by foreign body in the tissue. Malory staining was performed to evaluate the formation of colagen fibers.

RESULTS

Walking Tract Analysis Result

Below is the presentation of the data obtained:

Table 1. The average measurement of rat's foot print on Walking Track Box

	Group 1						Group II					
	H1	H3	H5	H7	H14	H21	H1	H3	H5	H7	H14	H21
EPL	32	32	32	32	32	32	34	34	34	34	34	34
NPL	32	32	32	32	32	32	34	34	34	34	34	34
ETS	3	3	3	4	6	9	3	4	9	12	24	24
NTS	23	23	23	23	23	23	24	24	24	24	24	24
EIT	2	2	2	3	4	6	2	3	6	7	15	15
NIT	15	15	15	15	15	15	15	15	15	15	15	15
TOF	65	65	65	65	65	65	63	63	63	63	63	63

Table 2. Calculation of Sciatic Nerve Functional Index in Control Group and Treatment Group at day 1, 3, 5, 7, 14, and 21.

	Group I						Group II					
	H1	H3	H5	H7	H14	H21	H1	H3	H5	H7	H14	H21
Tikus I	-88,91	-88,91	-88,91	-85,6	-83,3	-81,2	-88,9	-88,9	-77	-67,76	-8,8	-8,8
Tikus II	-88,89	-88,89	-88,89	-85,6	-83,3	-81,2	-88,93	-88,93	-76,03	-66,13	-8,8	-8,8
Tikus III	-88,9	-88,9	-88,9	-85,6	-83,3	-81,2	-88,91	-88,91	-76	-66,03	-8,8	-8,8
Tikus IV	-88,89	-88,89	-88,89	-85,6	-83,3	-81,2	-88,9	-88,9	-77	-67,76	-8,8	-8,8
Tikus V	-88,91	-88,91	-88,91	-85,6	-83,3	-81,2	-88,93	-88,93	-76,03	-66,13	-8,8	-8,8
Tikus VI	-88,9	-88,9	-88,9	-85,6	-83,3	-81,2	-88,91	-88,91	-76	-66,03	-8,8	-8,8
Tikus VII	-88,91	-88,91	-88,91	-85,6	-83,3	-81,2	-88,9	-88,9	-77	-67,76	-8,8	-8,8
Tikus VIII	-88,89	-88,89	-88,89	-85,6	-83,3	-81,2	-88,93	-88,93	-76,03	-66,13	-8,8	-8,8
Tikus IX	-88,9	-88,9	-88,9	-85,6	-83,3	-81,2	-88,91	-88,91	-76	-66,03	-8,8	-8,8
Rerata	-88,906	-88,906	-88,906	-85,6	-83,3	-81,2	-88,91	-88,91	-76,34	-66,64	-8,8	-8,8

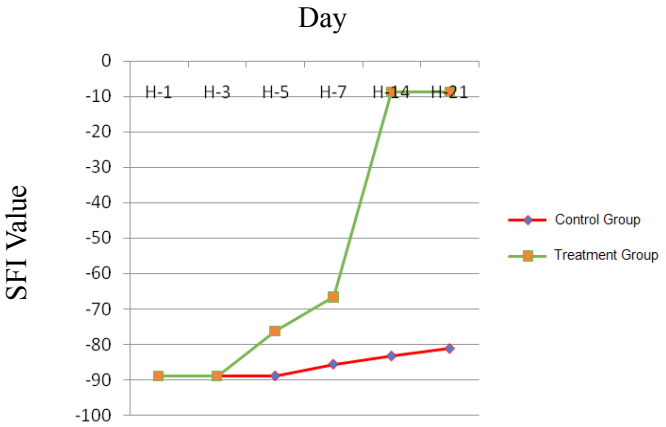
Table 3. T-test results of mean SFI in Control Group and Treatment Group at day 1, 3, 5, 7, 14, and 21

Control Group	Mean ± SD	Treatment Group	Mean ± SD	P
H1	-88.89± 0.12	H1	-88.91± 0.02	0.206
H3	-88.9± 0.01	H3	-88.92± 0.01	0.13
H5	-88.9± 0.01	H5	-76.34±0.57	0.01
H7	-85.60± 0.00	H7	-66.64±0.97	0.01
H14	-83.3± 0.00	H14	-8.8±0.00	0.00
H21	-81.2± 0.00	H21	-8.8±0.00	0.00

There is no significant result between the Control Group and the Treatment Group at day 1 and day 3 ($p > 0.05$), SFI value for both groups are -88, which indicates that the sciatic nerve function is bad. However, the results are significant at day 5, 7, 14, and 21 ($p < 0.05$) as can be seen in the graphic below.

The graphic below illustrates the SFI mean values of the Control and the Treatment Groups. The value for day 1

is almost –100. The improvement begins to show after day 3. At day 14, the SFI value in the Treatment Group becomes -8,8 and remains unchanged until day 21. This value shows that the function is almost normal. On the other hand, although there is a small improvement in the Control Group, the improvement is still below -80. This indicates that there is no improvement in function.



Graphic 1. SFI mean values for Control Group and Treatment Group at day 1, 3, 5, 7, 14,and 21.

Histology Result

HE staining was performed to evaluate degeneration process, including nerve diameter, cell condition, and axon parts. The analysis, based on Ciu et al., 2008 was performed by evaluating the shrunken nerve diameter (ND). The figures below show HE staining for the Control Group and the Treatment Group at day 21:

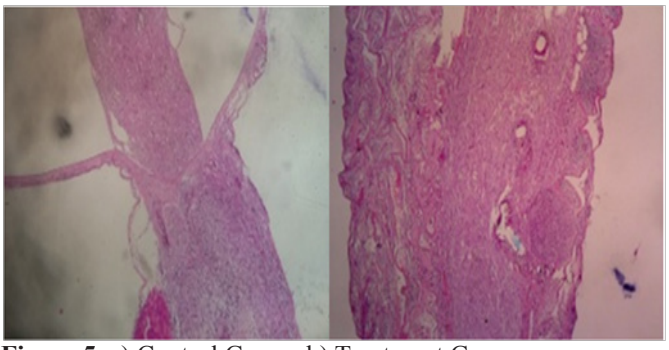


Figure 5. a) Control Group, b) Treatment Group

Table 4. t-Test result for ND (Nerve Diameter) of the Control Group and the Treatment Group at day 21 based on Chiu et al., 2008

Control Group	Mean ± SD	Treatment Group	Mean ± SD	P
H21	1.33 ±0.58	H21	0.68 ±0.58	0.23

Statistically, there is no significant difference between the Control Group and the Treatment Group in nerve diameter.

Table 5. t-Test result for *Axonal degeneration* (AD) of the Control Group and the Treatment Group at day 21 based on Chiu et al., 2008

Control Group	Mean ± SD	Treatment Group	Mean ± SD	P
H21	2.33 ±0.58	H21	0.68 ±0.58	0.024
	(a)		(b)	

Table 5 shows that there is significant T test result in the axon degeneration, where the Control Group is worse than the Treatment Group.

Table 6. t-Test result for Nerve regeneration (NR) of the Control Group and the Treatment Group at day 21 based on Chiu et al., 2008

Control Group	Mean ± SD	Treatment Group	Mean ± SD	P
H21	1.67±0.58	H21	3.00±0.58	0.016

Table 6 shows that there is significant t-test result in nerve regeneration, where the Treatment Group gives better result than the Control Group.

Figure 6 below presents the result of HE staining in the evaluation of inflammation process in the Control Group and the Treatment Group at day 21.

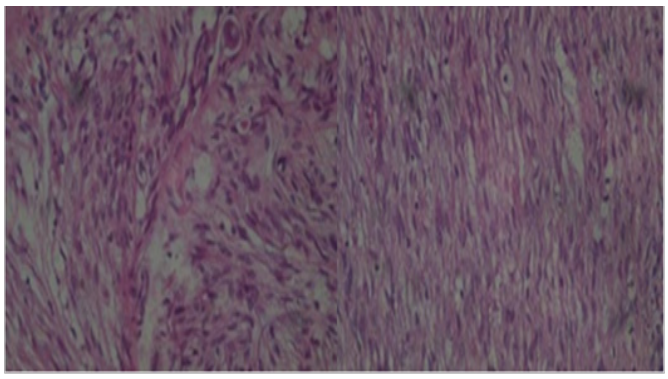


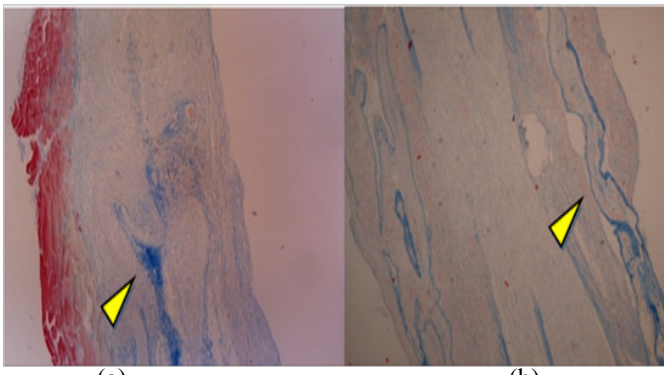
Figure 6. The image of inflammation process in the Control Goup (left) and the Treatment Group (right) at day 21.

Table 7 shows that there is significant t-test result in inflammation process, where the control group is categorized as moderately-severe and the treatment group as moderate.

Table 7. Mean and t-test values of inflammation in control grup and treatment group at day 21

Control Group	Mean ± SD	Treat-ment Group	Mean ± SD	p
H-21	3.00 ± 0.00	H-21	1.67 ± 0.57	0.016

Trichrome Massons Staining



(a) (b)

Figure 7. Trichrome Massons staining was performed to show the collagen fiber. The staining was taken at day 21. In Control Group (a), the colagen fiber is seen in the middle of the axon (yellow arrow). While in the Treatment Goup (b), the collagen fiber is in the border of the axon (yellow arrow), which is the amnion membrane collagen fiber.

DISCUSSION

When the peripheral nerve is damaged, it has the potential to regenerate. But it might experience growth disturbance, creating a mechanical barrier to axon growth and damaging the normal function. Consequently, the functional recovery and regeneration don't always happen, especially in neurotmesis. This condition will decrease the function and reduce the quality of life, and may cause huge psychosocial and economic burden for the patient and the community.

Many biological features of amniotic membrane are useful to increase growth factor, thus can mediate tissue healing. Some of those features are: antiadhesion, antibacterial, low immunogenicity, antiinflammation, and anti-scar. It is also easily available with affordable production, storage, and utilization cost.

This experiment showed better SFI result was achieved in the treatment group 12th weeks after operation with amnion membrane application compared to the Control Group. The result was seen from the walking track analysis and standard method to evaluate nerve healing after sciatic nerve injury. This result was supported by Mohammad et al., 2000, Forootan, 2011 and Meng et al., 2011. Meng et al., 2011 showed that SFI improvement was significant in 6 and 10 weeks after operation with application of amniotic membrane. Mohammad, et al. reported that the healing of sciatic nerve injury was higher in 2 to 4 weeks in the Treatment Group, while Forootan, et al. demonstrated that SFI improved in 12 weeks after operation.

CONCLUSION

Amniotic membrane application in crush injury-type peripheral nerve injury, shows to be beneficial in the regeneration and function regain. The application of amniotic membrane can reduce the formation of fibrous tissue in the peripheral nerve regeneration.

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