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Effectiveness Of Chicken Embryo Amniotic Membrane In Preventing Post Laminectomy Epidural Fibrosis Development – A Rat Laminectomy Model

Tavuk Embriyosu Amnion Zarının Post Laminektomi Epidural Fibrozis Gelişimini Önlemedeki Etkinliği (Rat Laminektomi Modeli)

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ABSTRACT

Objective: Amniotic membranes have been shown to enhance nerve regeneration and reduce perineural fibrosis after surgery. This study aimed to elucidate the effect of chicken amniotic membranes on preventing epidural fibrosis after laminectomy.
Method: In this study, 20 adult male Wistar albino rats weighing 300±50 g were used. The rats were randomly divided into two groups, each containing 10 rats. Group 1 (Control group, n=10): Rats underwent 3-distance laminectomy + primary closure. Group 2 (Experimental group, n=10): Rats underwent 3-distance laminectomy + chicken embryo amniotic membrane application followed by primary closure.
Results: In the group where the chicken embryo amniotic membrane was placed, the degree of macroscopic fibrosis varied between stage 1 and stage 3; 70% (n=7) of the subjects developed scar tissue that could be easily dissected in the dura mater (Rydell stage 1), two subjects (20%) developed scar tissue that caused structural deterioration in duramater and caused serious dissection difficulties but could be dissected (Rydell stage 2), while only one subject (10%) had scar tissue that was adherent to the dura mater and could not be dissected (Rydell stage 3). The difference between the groups was significant (p =0.001). Histopathologically, group 1 showed grade 2 and 3 scar tissue development, while group 2 showed grade 0 to 3 scar development, with a significant difference (p = 0.001).
Conclusion: The study results show that chicken embryo-derived amniotic membrane can be implanted as a physical barrier to reduce epidural fibrosis without affecting wound healing and causing infection the surgical area.

Keywords: Epidural Fibrosis, Failed Back Surgery Syndrome, Chicken Embryo Amniotic Membrane.

ÖZET

Amaç: Amniotik membranın, epitelyal hücrelerde vaskularizasyonu engelleyerek, inflamasyonu azaltarak ve apoptozisi baskılayarak periferik sinir cerrahisi sonrası sinir rejenerasyonunu artırdığı ve perinöral fibrozis gelişimini azalttığı gösterilmiştir. This study aimed to elucidate the effect of chicken amniotic membranes on preventing epidural fibrosis after laminectomy.
Metod: Bu çalışmada 300±50 g ağırlığında 20 erişkin erkek Wistar albino rat kullanıldı. Ratlar randomize olarak her biri 10 adet rat içeren 2 gruba ayrıldı. Grup 1 (Kontrol grubu, n=10): Sıçanlara 3 mesafe laminektomi + primer kapama işlemi uygulandı. Grup 2 (Deney grubu, n=10): Ratlara 3 mesafe laminektomi + tavuk embriyosu amnion zarı uygulaması yapıldıktan sonra primer kapama işlemi uygulandı.
Bulgular: Tavuk emriyosu amniyon zarı yerleştirilen gruptaki deneklerde ise, makroskopik fibrozis derecesinin stage 1 ila stage 3 arasında değiştiği, deneklerin %70'inde (n=7) duramaterde kolayca diseke edilebilen skar dokusu (Rydell stage 1) geliştiği, 2 denekte (%20) duramaterde yapısal bozulmaya neden olan ve ciddi diseksiyon zorluğuna neden olmasına rağmen diseksiyonu mümkün olabilen skar dokusu (Rydell stage 2) gelişirken, sadece 1 denekte (%10) duramatere yapışık ve diseke edilemeyen skar dokusu (Rydell stage 3) olduğu görüldü. Gruplar arasındaki farkın anlamlı olduğu tespit edildi (p =0.001). Histopatolojik olarak değerlendirildiğinde, grup 1'de 2 ve 3. derece skar dokusu gelişimi görülürken grup 2'de histopatolojik olarak 0 ila 3. derece arasında skar gelişimi olduğu, gruplar arasında anlamlı fark ortaya çıktığı görüldü (p =0.001).
Sonuç: Çalışma sonuçları, tavuk embriyosu kaynaklı amniyotik membranın yara iyileşmesini etkilemeden ve cerrahi alanda enfeksiyon gelişimine neden olmadan epidural fibrozisi azaltmak amacıyla fiziksel bir bariyer olarak implante edilebileceğini göstermektedir.

Anahtar Kelimeler: Epidural Fibrozis, Başarısız Bel Cerrahisi Sendromu, Tavuk Embriyosu Amniyon Zarı.

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INTRODUCTION

Lumbalgia, which ranks second among the reasons for consulting a doctor after upper respiratory tract problems worldwide, is seen especially in middle-aged individuals who comprise a large part of their active working lives. It creates a severe burden on the health system when the cost of diagnosis and treatment is considered, as well as significant loss of labor (1). There are many reasons for the etiology of lumbalgia. Approximately 85% of lumbar disc herniations (LDH), which constitute 2-3% of these reasons, are treated with medical or conservative approaches, while surgical methods are required in 15% of patients (2). It has been shown that the cause of failed back surgery syndrome (LFS), defined as the increase or continuation of complaints such as back and leg pain after surgery, is epidural fibrosis (EF) (adhesive arachnoiditis) in 6-24% of patients. The literature has reported that various sequelae may occur due to the pressure exerted by the fibrotic reaction on the surrounding structures (3). In addition, this situation may cause patients to receive analgesic support for a long time after surgery and even to undergo surgery again, which leads to severe deterioration in quality of life (4).

Considering that 250.000 patients are operated on for LDH every year in the United States (USA) alone, it is evident that a large number of people around the world are at risk of EF (5). Various agents such as contractures gel, benzothiazole polylactic acid membrane, carboxymethyl cellulose, fibrin glue, glucocorticoids, methyl methacrylate, spongostan, ADCON-L, urokinase, polylactic acid membrane, sodium hyaluronate hydrogel membrane, polytetrafluoroethylene membrane, vicryl mesh have been used experimentally and clinically to prevent fibrosis (6, 7). However, despite experimental and clinical studies, no tangible success has been achieved in preventing fibrosis. It has been shown that the human amniotic membrane increases nerve regeneration after peripheral nerve surgery and reduces perineural fibrosis development by preventing vascularization in epithelial cells, reducing inflammation, and suppressing apoptosis (8). However, ethical obstacles to obtaining human-derived amniotic membranes, high material costs, and risks of infectious diseases are the most significant obstacles to their widespread use. No study using chicken amniotic membrane to prevent epidural fibrosis has been conducted to date. Chicken embryo amniotic membrane is an easily obtained, cheap, and accessible material, and it does not require ethical committee approval to get it sacrificed until the second trimester. Within the scope of this research, we aimed to elucidate the effect of chicken amniotic membranes on the prevention of epidural fibrosis after laminectomy.

METHOD

Twenty adult male Wistar albino rats weighing 300±50 g were used in the experiment. The study followed the ethical rules (Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals, 8th edition, 2011, The National Academies Press, Washington D.C.) The Animal Local Ethics Committee was granted from our institution with protocol number 16.

To ensure environmental adaptation, the rats were fed with standard pellet feed (Bil-Yem Lt., Ankara, Turkey) in a laboratory environment at 22 ± 2°C room temperature, 60±5% humidity, and periodically (12 hours of darkness, 12 hours of light) under white fluorescent light for 10 days. The rats were given feed and drinking water ad libitum throughout the experiment. The rats were randomly divided into two groups, each containing 10 rats, and to prevent study bias, they were numbered and placed in separate cages by an academician who would not participate in the experimental and analysis phase of the study.

Study Groups

- Group 1 (Control group, n=10): Rats underwent 3-distance laminectomy + primary closure.
- Group 2 (Experimental group, n=10): Rats underwent 3-distance laminectomy + chicken embryo amniotic membrane application followed by primary closure.

Surgery

Thirty minutes before the operation, a single dose of 20 mg/kg cefazolin sodium (Cefazolin, Bilim İlaç, İstanbul-Türkiye) was administered intraperitoneally (IP) to the rats for prophylaxis. After general anesthesia was achieved by intraperitoneal administration of 50 mg/kg Ketamine hydrochloride (Ketalar flk, Eczacıbaşı, İstanbul, Turkey), the rats were fixed to the operation table with plasters from their legs. The operation area was prepared for the surgical procedure with povidone-iodine scrub (Medica brush; 4% chlorhexidine soap, Medica BV, Netherlands) and povidone-iodine (Poviod: 10% polyvinylpyrrolidone-iodine complex, Saba, Turkey) solution. The L1 and L4 vertebral levels were determined before the operation area was covered with sterile green. The lumbar fascia was opened and lateralized with an automatic retractor after passing the skin and subcutaneous tissues with a 3 cm midline incision.

The motorized drilling method was used, which had the advantages of being a fast and easy laminectomy, not causing pain to the experimental animals, and having a short procedure duration. Before laminectomy, the area was prepared by stripping the paravertebral muscles. The L3-L5 spinous processes were found and excised with a 1 mm wide hand motorized drill, and L3-L5 laminectomy was performed (Figure 1).

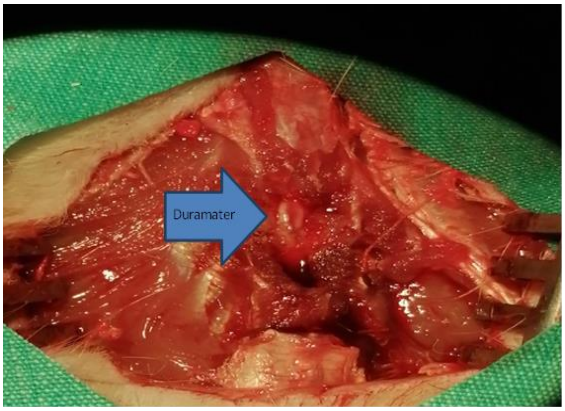

Figure 1. Macroscopic view of the laminectomy area. The area indicated by the arrow is the section area. Microscopic view of the laminectomy area. The area indicated by the arrow is the Duramater.

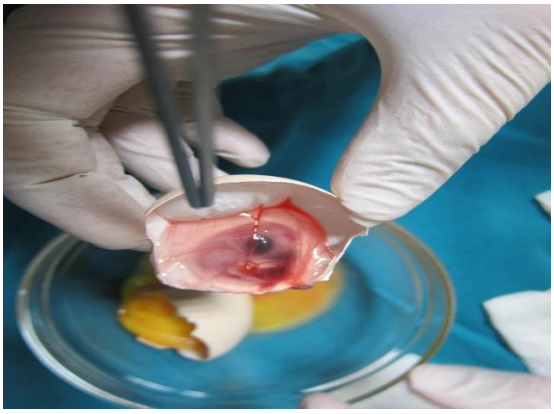

Figure 2: After removing the chicken embryo, the amniotic membrane is peeled off, and the amniotic membrane is separated from the embryo by the manual separation technique—stage 1.

After the ligamentum flavum and epidural fat tissue were removed and the dura mater was exposed, the area was irrigated with physiological serum after bleeding control. While the primary researcher performed these procedures for each rat, the assistant researcher performed the chicken embryo amniotic membrane preparation procedure (Figure 2). The method can be briefly summarized as follows: 36 fertilized chicken eggs incubated for 10 days in an incubator (Broodtech brand) providing 37Cº degrees and 50% humidity were removed from the incubator when the animal experiment was to be performed.

Our preliminary studies determined that amniotic membrane isolation was challenging up to the first 8 days due to prematurity and after the 12th day due to post-maturation. Since it was seen that the optimal period for isolation was the 10th day, the 10th day was noticed as the most efficient day of isolation. It takes 5 minutes to isolate the amniotic membrane from a single embryo. Again, in the preliminary studies, it was seen that embryo-containing eggs remained viable for 6 hours after being removed from the incubator. Still, the egg removal times for each rat were kept constant to see the optimum effect (Figure 3).

In the other groups, the procedures were carried out in the same way as the study group until the stage of placing the amniotic membrane in the field. The fascia was sutured in all animals with 5/0 polyglactone and the skin with 4/0 silk. After the subjects were allowed to recover at room temperature

at 28°C in the post-procedure period, the loss of lower extremity strength was checked with a walking test.

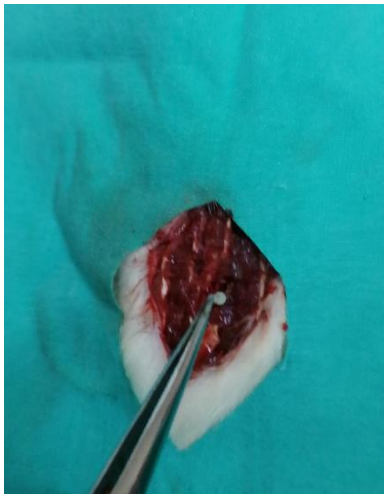

Figure 3: In the experimental group, the amniotic membrane isolated by the assistant researcher was applied to the laminectomy area for each rat during the application.

The subjects were fed free food and water for six weeks. The study's primary investigator evaluated their daily general health status and neurological examinations during this period. At the end of the sixth week, the rats were euthanized by giving a lethal dose of IP Thiopental Sodium (Pentothal Sodium, Abbott, Italy). The area was cleaned sterilely, and the skins were opened. Macroscopically, wound healing was seen and noted. The vertebral columns that underwent surgery in the previous session were removed en bloc for histopathological examination to determine the level of fibrosis.

The groups were coded so that the pathologist who performed the histopathological evaluation of the study and the statistician who performed the statistical assessment of the results were blinded to the study. All histopathological examinations were performed by a pathologist who was blinded to the study protocol.

Histopathological Evaluation

The vertebral column removed en bloc was fixed in 10% buffered formalin for 2 days. Then, it was decalcified in 10% formic acid for 2 days. Three 2 mm thick sections (proximal, middle, distal) were taken from the laminectomy area transverse to the vertebral column. These samples were placed in cassettes and washed in running water for 3 hours. After routine follow-up (13 hours), four μm thick sections were taken from the paraffin-embedded tissues, and Hematoxylin-Eosin and Masson's trichrome stains were applied. All preparations were examined under an Olympus BX51 light microscope (Olympus Corp., Tokyo, Japan). Dural fibrosis was staged according to He et al. (9). (Table 1). Fibroblasts and inflammatory cells were counted from 3 areas in each specimen at X400 magnification, one from the middle and the other from the laminectomy area. Fibroblast and inflammatory cell densities were averaged and graded as follows: grade 1, less than 100 fibroblasts or inflammatory cells per 400 × field; grade 2, 100–150 fibroblasts or inflammatory cells per 400 × field; grade 3, more than 150 fibroblasts or inflammatory cells per 400 × field (9).

Table 1. Histopathological grading criteria of scar tissue

Degree	The width of the scar tissue
0	There is no scar tissue in the duramater
1	A thin fibrosis between the scar tissue and the duramater Tapes
2	Involving 2/3 of the laminectomy defect Adhesions present
3	Disseminated scarring: more than 2/3 affected laminectomy defect and/or scar tissue nerve If it reaches to its roots

Statistical Analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 26.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage for categorical data and mean and standard deviation for continuous data were given as descriptive values. For comparisons between groups, the "Independent Sample T-test" was used for two groups, and the "Pearson Chi-Square Test" was used to compare categorical variables. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

The two groups' medians of the Rydell stage, scar degree, and fibroblast-inflammatory cell density were statistically significantly different (Table 2). When the subjects were evaluated macroscopically according to the Rydell criteria of the pathological specimens, it was seen that none of the subjects in the control group were Rydell stage 1. While 1 of the subjects in the control group (10%) developed scar tissue that caused structural deterioration in the dura mater and caused serious dissection difficulties but could be dissected (Rydell stage 2), the remaining nine subjects (90%) were observed to have scar tissue that was attached to the dura mater and could not be dissected (Rydell stage 3).

When the subjects in the group where the chicken embryo amniotic membrane was placed were evaluated regarding the macroscopic structure of the developing scar tissue, a significant difference was observed between them and the control group ($p < 0.001$). When the group was evaluated within itself, it was seen that the degree of macroscopic fibrosis ranged from stage 1 to stage 3, and 70% of the subjects (n=7) developed scar tissue that could be easily dissected in the dura mater (Rydell stage 1), two subjects (20%) developed scar tissue that caused structural deterioration in the dura mater and caused serious dissection difficulties but could be dissected (Rydell stage 2). In comparison, only one subject (10%) had scar tissue that was attached to the dura mater and could not be dissected (Rydell stage 3) (Table 2).

Table 2. Comparison of groups in terms of fibrosis and inflammatory change.

Variables	Group 1 (n=10)	Group 2 (n=10)	Test Statistics	p-value
Rydell evre	3(2-3)	1(1-3)	6,500	***<0,001****
Degree of scarring	3(2-3)	1(0-3)	10,000	***0,001****
Fibroblast- inflammatory cell density	3 (2-3)	1(1-3)	14,000	***0,002****
[a]: Mann-Whitney U test statistic. *: Statistically significant p-value Values are presented as median (Minimum-maximum).				

When the fibrotic tissue that formed was evaluated histopathologically, it was seen that there was a 2nd and 3rd-degree scar tissue development in group 1. At the same time, there was a histopathological scar development between 0 and 3rd degree in group 2, and there was a significant difference between the groups ($p = 0.001$) (Table 2). When the groups were analyzed within themselves, adhesions were observed in 30% of the subjects in group 1. In comparison, more than 2/3 of the laminectomy defect was affected, and scar tissue developed extending to the nerve roots in 70% of the subjects.

In group 2, only one subject was found to have more than 2/3 of the laminectomy defect affected and/or severe scar tissue developed extending to the nerve roots. In contrast, 20% of the subjects did not develop any scar tissue, five subjects developed fibrosis in the form of thin fibrous bands between the operation area and the dura mater, and two rats were observed to have adhesions covering 2/3 of the laminectomy defect.

Finally, inflammatory changes at the tissue level were evaluated in our study. For this purpose, fibroblast/inflammatory cell density distribution was assessed. In Group 1, 70% of the subjects had >150 fibroblasts/lymphocytes at X400 magnification in each specimen, while 30% had 100-150 fibroblasts/lymphocytes at X400 magnification. In Group 2, 70% of the subjects had <100

fibroblasts/lymphocytes at X400 magnification in each specimen, while 20% had 100-150 fibroblasts/lymphocytes at X400 magnification in each specimen, and 10% had >150 fibroblasts/lymphocytes at X400 magnification. The groups significantly differed regarding inflammatory changes at the tissue level (p =0.002).

Table 3. Comparison of groups in terms of clinical outcomes.

Variables	Group 1 (n=10)	Group 2 (n=10)	p-value
Wound dehiscence (n,%)	0 %0	0 %0	-
Wound infection (n,%)	0 %0	0 %0	-
[a]: Mann-Whitney U test statistic. [*]: Statistically significant p-value Values are presented as numbers (%).			

Table 3 shows the changes in the surgical area in rats in our study. When the surgical field was examined regarding incision problems, there was no problem with infection or incision healing in both groups.

DISCUSSION

Approximately 185.000–200.000 lumbar spine surgeries are performed annually in the United States to treat a variety of clinical conditions, including spinal stenosis, spondylolisthesis, LDH, and discogenic back pain. BBCS, a condition characterized by persistent pain and varying degrees of functional disability after lumbar spine surgery, may occur in 13–61% of patients after surgery. Causes of unsuccessful surgery include inadequate surgical decompression, recurrent disc herniation, lumbar instability, extensive epidural scarring, and inadequate fusion (10). Postoperative epidural scarring may cause extradural compression or dural tension, resulting in recurrent radicular pain and physical impairment. Intraspinal hemorrhage after laminectomy is a scaffold for fibroblast migration from the periosteum and paraspinal muscles and wound healing (11).

The granulation tissue resulting from fibroblastic activity transforms into a dense fibrotic scar called the "postlaminectomy membrane." EF, which is mainly caused by the erector muscle, can cause back pain in the postoperative period by causing compression or traction on the dura mater or nerve roots, depending on the severity of the fibrosis. Moreover, it can cause difficulties during the dissection phase, increased surgical time, bleeding, and an increased risk of surgical complications in revisional surgical procedures. There is no effective treatment after the scar tissue has developed. Although fibrotic tissue can be excised and the compressed nerve roots can be released during revisional surgery, fibrosis tends to reoccur after secondary surgery (12). Therefore, developing a treatment modality that reliably reduces fibrosis without causing side effects is necessary. In the literature, many treatment modalities have been developed for treating EF after postlaminectomy. Although the effectiveness of these methods has been investigated in both experimental and clinical settings, unfortunately, no method has been effectively used for this purpose. One of the most promising methods used for this purpose and in terms of treatment effectiveness is using a human-derived amniotic membrane. The amniotic membrane consists of an epithelial cell layer on a basement membrane. Von Versen-Höynck et al. (13) have shown that the amniotic membrane inhibits neovascularization, reduces inflammation, prevents infection development, and reduces postoperative adhesions. In addition, some studies have reported that amniotic membranes have been used successfully in conditions such as non-healing skin ulcers and severe ocular surface disease. Studies also report that it has been used successfully to reduce surgical adhesions in abdominal head and neck surgery (14–16). However, perhaps the most critical feature of the amniotic membrane is that it is an immune-privileged tissue that does not cause immune reactions and rarely causes immunological rejection. Therefore, assuming that the amniotic membrane is suitable for reducing postoperative EF in vertebral surgery is not unreasonable.

Tao et al. (17) used amniotic membrane implantation to reduce epidural adhesions after laminectomy in dogs. This study used The amniotic membrane in several forms, and its effects were tested. In the first group (FAM), the material prepared by freeze-drying the amniotic membrane under a vacuum of 0.2 Torr for 24 hours was applied to the laminectomy area. In the second group (CAM), the material to be used was obtained by dipping the amniotic membrane in 0.25% glutaraldehyde (GA) solution and then

incubating it at room temperature for 1 hour and then washing it with phosphate-buffered saline solution to remove residual GA. Finally, the obtained materials were sterilized with ethylene oxide for implantation and then applied to the laminectomy site. The control and autologous free fat (AF) graft groups were the other two. This study reported no inflammatory reaction in the operation area in the samples taken at postoperative 1, 6, and 12 weeks after FAM and CAM implantation. The researchers who reported that a small number of inflammatory cells (lymphocytes, neutrophils, and histiocytes) were observed in the area adjacent to the dura mater and peripheral nerve roots thought that this situation was due to the immune-privileged cell properties of the amniotic membrane (17).

One of the most original studies on the subject is the study by Subach et al. (18). This study evaluated the effect of dehydrated human amniotic/chorion membrane (dHACM) on developing fibrosis in soft tissue scar formation in the epidural space in humans undergoing posterior lumbar instrumentation. In 5 cases with no previous history of spinal surgery and undergoing transforaminal lumbar interbody fusion (TLIF) with posterior instrumentation, after placement of the fusion device, bone graft material, and posterior stabilization devices, the dHACM (AmnioFix, MiMedx, Marietta, GA) was placed to fit the dura exposed by decompression. After adequate fusion development was documented radiologically, the instruments placed in the patients were removed. The surgeon scored the patients regarding ease of dissection in the epidural space and width of adhesions, and a tissue piece adjacent to the epidural space was examined histopathologically. When the results were evaluated regarding surgical dissection, it was stated that the dissection was easily removable in 4 out of 5 patients (80%), and the tissues could be safely separated with blunt dissection. Only one patient reported that they felt the need for sharp dissection during dissection. In the histological evaluation of the patients, it was reported that minimal fibrosis and fat infiltration were observed (18).

In our study, while 70% of the subjects in the control group had severe inflammatory cell accumulation (>150 fibroblasts/lymphocytes at X400 magnification), 30% had moderate inflammatory cell accumulation (100-150 fibroblasts/lymphocytes at X400 magnification). One of the critical points was that there were no subjects in this group with no inflammatory cells or mild accumulation. In the experimental group, 70% of the subjects had mild inflammatory cell accumulation in each specimen (<100 fibroblasts/lymphocytes at X400 magnification), 20% had moderate inflammatory reaction (100-150 fibroblasts/lymphocytes at X400 magnification), and 10% had severe reaction (>150 fibroblasts/lymphocytes at X400 magnification). It was observed that there was a significant difference between the groups in terms of inflammatory changes at the tissue level ($p = 0.002$). Fibroblasts migrate to the operation site from the paraspinal muscles, LF, posterior longitudinal ligament, and annulus fibrosis. Tao et al. (17) reported that in the CAM group, the number of fibroblasts continued at low levels for a long time due to the optimal preparation of the implanted material and the intact mechanical barrier properties. In contrast, in the FAM group, the material could not prevent fibroblast migration due to the deterioration of its properties after 6 weeks (17). Recently, Cunningham et al. (19) evaluated the preventive effects of a double-layered, chorion-free amniotic patch (DLAM; ViaShield®, Globus Medical Inc., Audubon, PA, USA) on EF in a sheep laminectomy model. This study reported that less fibroblast accumulation was observed in the DLAM-treated group at the tissue level compared to the control group at the 4th and 10th weeks. As is known, fibroblasts are a critical part of the postoperative healing process. However, excessive fibroblast infiltration in the surgical area, secreting various growth factors such as transforming growth factor β1 (TGF-β1), triggers matrix formation, increases scar tissue development, and may compromise the natural integrity of the surrounding tissue, which may lead to persistent pain, paresthesia, and neurological deficits (20). In our study, a significant difference was observed in the number of fibroblasts in the experimental group compared to the control group. However, in our research, fibrosis development was evaluated 6 weeks after the first operation, which is consistent with the period when the FAM group lost its effect in the studies of Tao et al. (17). Therefore, we believe that optimum information was obtained regarding evaluating the effectiveness of the biological material used in our research.

Recently, Cunningham et al. (19) evaluated the preventive effects of a double-layered, chorion-free amniotic patch (DLAM; ViaShield®, Globus Medical Inc., Audubon, PA, USA) on EF in a sheep laminectomy model. This interesting study evaluates the subject from a broad perspective as a whole, in addition to the development of fibrosis, by assessing the levels of proinflammatory, antiangiogenic, and immune-modulatory cytokines in the medium. The study showed that the medium contained moderate to high levels of immunomodulatory cytokines GDF-15, TIMP-1, TIMP-2, and angiostatin in the DLAM group. At the same time, a significant difference was reported between the group and the control group. As is known, GDF-15 exhibits anti-inflammatory effects by inhibiting macrophage activation and reducing leukocyte migration to the damaged area (21). TIMP-1 and TIMP-2 modulate matrix metalloproteinase activity, which can reduce neovascularization and cause anti-angiogenic effects. Finally, angiostatin, an inhibitory cytokine specific to endothelial cells, is one of the most common cytokines in the amniotic membrane structure. Angiostatin is thought to block angiogenic signaling pathways triggered by cytokines such as FGF-2 through its anti-angiogenic effects (22). On the other hand, Cunningham et al. (19) reported that proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and TNF-β were not detected in the DLAM group. Furthermore, they noted that this group had high anti-inflammatory cytokine interleukin-1ra (IL-1ra) levels. In this way, they reported that applying the amniotic membrane was beneficial in controlling inflammation by causing low and anti-inflammatory cytokine levels. Unfortunately, our study did not evaluate the effects of chicken embryo amniotic membrane on cytokine levels.

Literature data show that the amniotic membrane contributes to its ability to function as an optimal protective barrier thanks to the basement membrane architecture and immunomodulatory cytokines found in its structure (23, 24). No adverse problems, such as wound infection and skin separation, were observed in any subject in either group. The material was thought to be used safely when evaluated from this perspective.

CONCLUSION

Chicken embryo-derived amniotic membrane reduced scar development at the histopathological level and contributed to wound healing at the cellular level by reducing fibroblast-inflammatory cell density. As a clinical observation, it was determined that chicken embryo-derived amniotic membrane application did not show foreign body reaction in the environment, did not cause infection development, and did not cause incision-related problems. The study results clearly showed that chicken embryo-derived amniotic membrane can be implanted as a physical barrier to reduce epidural fibrosis without affecting wound healing or causing infection in the surgical area. The results prove that chicken embryo-derived amniotic membrane is a promising biomaterial for future clinical applications.

DESCRIPTIONS

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AI Statement

The authors used AI and AI-assisted Technologies (Grammarly and MS Word Editor) in the writing process. These technologies improved the readability and language of the work. Still, they did not replace key authoring tasks such as producing scientific or medical insights, drawing scientific conclusions, or providing clinical recommendations. The authors are ultimately responsible and accountable for the contents of the whole work.

Competing interests

The authors declare that they have no competing interests.

Consent for Publication

The original article is not under consideration by another publication, and its substance, tables, or figures have not been published previously and will only be published elsewhere.

Data Availability

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethical Declaration

The study followed the ethical rules (Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals, 8th edition, 2011, The National Academies Press, Washington D.C.) The Animal Local Ethics Committee was granted from our institution with protocol number 16.

REFERENCES

1. Maharty DC, Hines SC, Brown RB. Chronic Low Back Pain in Adults: Evaluation and Management. Am Fam Physician. 2024;109(3):233-244.

2. Zileli M, Oertel J, Sharif S, Zygourakis C. Lumbar disc herniation: Prevention and treatment of recurrence: WFNS spine committee recommendations. World Neurosurg X. 2024;22:100275. Published 2024 Feb 9. doi:10.1016/j.wnsx.2024.100275

3. Stevens S, Agten A, Timmermans A, Vandenabeele F. Unilateral changes of the multifidus in persons with lumbar disc herniation: a systematic review and meta-analysis. Spine J. 2020;20(10):1573-1585. doi:10.1016/j.spinee.2020.04.007

4. Alver S, Ciftci B, Celik EC, et al. Postoperative recovery scores and pain management: a comparison of modified thoracolumbar interfascial plane block and quadratus lumborum block for lumbar disc herniation. Eur Spine J. 2024;33(1):118-125. doi:10.1007/s00586-023-07812-3

5. Webb CW, Aguirre K, Seidenberg PH. Lumbar Spinal Stenosis: Diagnosis and Management. Am Fam Physician. 2024;109(4):350-359.

6. Yue WWS, Touhara KK, Toma K, Duan X, Julius D. Endogenous opioid signalling regulates spinal ependymal cell proliferation. Nature. 2024;634(8033):407-414. doi:10.1038/s41586-024-07889-w

7. Shen F, Ding J, Wang Y, et al. Topical hemostatic agents in spinal surgery. Spine J. 2024;24(6):933-946. doi:10.1016/j.spinee.2024.01.003

8. Alves APDS, Teixeira RJM, Silva RMD, Canevari RA, Sant'anna LB. Amniotic membrane modulates MMP9 and MMP12 gene and protein expression in experimental model of the hepatic fibrosis. An Acad Bras Cienc. 2024;96(4):e20231086. Published 2024 Oct 28. doi:10.1590/0001-3765202420231086

9. He Y, Revel M, Loty B. A quantitative model of post-laminectomy scar formation. Effects of a nonsteroidal anti-inflammatory drug. Spine (Phila Pa 1976). 1995;20(5):557-580. doi:10.1097/00007632-199503010-00010

10. Costa F, Oertel J, Zileli M, Restelli F, Zygourakis CC, Sharif S. Role of surgery in primary lumbar disk herniation: WFNS spine committee recommendations. World Neurosurg X. 2024;22:100276. Published 2024 Feb 23. doi:10.1016/j.wnsx.2024.100276

11. Schönnagel L, Camino-Willhuber G, Braun S, et al. Association Between Osteoarthritis Burden and Intervertebral Disk Degeneration in Patients Undergoing Lumbar Spine Surgery for Degenerative Lumbar Spondylolisthesis. Spine. 2024;49(7):478-485. doi:10.1097/BRS.0000000000004847

12. Xie H, Wang B, Shen X, et al. MMC controlled-release membranes attenuate epidural scar formation in rat models after laminectomy. Mol Med Rep. 2017;15(6):4162-4168. doi:10.3892/mmr.2017.6531

13. von Versen-Höynck F, Syring C, Bachmann S, Möller DE. The influence of different preservation and sterilisation steps on the histological properties of amnion allografts--light and scanning electron microscopic studies. Cell Tissue Bank. 2004;5(1):45-56. doi:10.1023/b:catb.0000022276.47180.96

14. Li C, Yao L, He F, Hua K. Transplantation of acellular amniotic membrane seeded with adipose-derived mesenchymal stem cells in a rat model of intrauterine adhesion. Ann Med Surg (Lond). 2024;86(8):4463-4474. Published 2024 Jul 1. doi:10.1097/MS9.0000000000002249

15. Maljaars L, Gudde A, Oosthuysen A, Roovers JP, Guler Z. The Regenerative Capacity of Tissue-Engineered Amniotic Membranes. ACS Appl Bio Mater. 2024;7(3):1441-1448. doi:10.1021/acsabm.3c00765

16. Huang X, Yang X, Huang J, et al. Human amnion mesenchymal stem cells promote endometrial repair via paracrine, preferentially than transdifferentiation [published correction appears in Cell Commun Signal. 2024 Jun 13;22(1):326. doi: 10.1186/s12964-024-01706-7]. Cell Commun Signal. 2024;22(1):301. Published 2024 May 31. doi:10.1186/s12964-024-01656-0

17. Tao H, Fan H. Implantation of amniotic membrane to reduce postlaminectomy epidural adhesions. Eur Spine J. 2009;18(8):1202-1212. doi:10.1007/s00586-009-1013-x

18. Subach BR, Copay AG. The use of a dehydrated amnion/chorion membrane allograft in patients who subsequently undergo reexploration after posterior lumbar instrumentation. Adv Orthop. 2015;2015:501202. doi:10.1155/2015/501202

19. Cunningham BW, Seiber B, Riggleman JR, Van Horn MR, Bhat A. An investigational study of a dual-layer, chorion-free amnion patch as a protective barrier following lumbar laminectomy in a sheep model. J Tissue Eng Regen Med. 2019;13(9):1664-1671. doi:10.1002/term.2920

20. Choi HJ, Kim KB, Kwon YM. Effect of amniotic membrane to reduce postlaminectomy epidural adhesion on a rat model. J Korean Neurosurg Soc. 2011;49(6):323-328. doi:10.3340/jkns.2011.49.6.323

21. Fraser JF, Cuttle L, Kempf M, Phillips GE, Hayes MT, Kimble RM. A randomised controlled trial of amniotic membrane in the treatment of a standardised burn injury in the merino lamb. Burns. 2009;35(7):998-1003. doi:10.1016/j.burns.2009.01.003

22. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Prog Mol Biol Transl Sci. 2017;147:1-73. doi:10.1016/bs.pmbts.2017.02.005,

23. Arends F, Nowald C, Pflieger K, Boettcher K, Zahler S, Lieleg O. The biophysical properties of Basal lamina gels depend on the biochemical composition of the gel. PLoS One. 2015;10(2):e0118090. Published 2015 Feb 17. doi:10.1371/journal.pone.0118090

24. Maan ZN, Rennert RC, Koob TJ, Januszyk M, Li WW, Gurtner GC. Cell recruitment by amnion chorion grafts promotes neovascularization. J Surg Res. 2015;193(2):953-962. doi:10.1016/j.jss.2014.08.045