Anti-adhesive Effect of Hyaluronate in a Rabbit Laminectomy Model

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Background: Postlaminectomy dural adhesion is a common cause of recurrent symptoms. Hyaluronic acid–based gel has been reported to reduce the incidence of postoperative adhesion in the peritoneal cavity; however, its effect on preventing postoperative scar formation at laminectomy sites is not yet known. The purpose of this study was to evaluate the anti-adhesive effect of hyaluronic acid–based gelatin after laminectomy, using a rabbit model.

Methods: Twelve adult New Zealand rabbits underwent two-level lumbar laminectomy, and were randomly assigned to one of two groups of six rabbits each. The treatment group received hyaluronic acid–based gelatin treatment and the control group was untreated. Rabbits were sacrificed 8 weeks after treatment. Peel-off testing and histological analysis were performed to assess the tenacity and the extent of adhesion formation.

Results: No significant difference was observed in the neurologic performance between the two groups. The tenacity in the treatment group was significantly reduced compared to that of the control group (3.17 ± 0.75 vs. 4.33 ± 0.52, respectively; $p = 0.016$). Histological analysis showed significantly less scar tissue formation in the treatment group, with a larger subarachnoid space and greater distance between the dura and scar tissues. The amount of fibroblast cells also was significantly smaller in the treatment group than in the control group (3078 ± 313.68 vs. 3742 ± 455.65, respectively; $p = 0.042$).

Conclusions: No serious adverse events were reported, and no difference was found in the incidence of complications between the treatment and control groups. The findings suggested that hyaluronic acid–based gelatin may be effective for preventing postlaminectomy dural adhesion in rabbits.

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Key words: adhesives, hyaluronate, laminectomy, rabbits, scarring

At a Glance Commentary

Scientific background of the subject

Hyaluronic acid has been widely used in numerous clinical applications. It has been used to treat knee osteoarthritis by injecting it into the joint. It can act as an anti-adhesion barrier in preventing postoperative scar tissue formation in various surgical procedures such as myomectomy and laparotomy.

What this study adds to the field

Hyaluronic acid–based gel is effective in decreasing the tenacity of adhesion between the dura and scar and the amount of scar tissues after laminectomy in a rabbit model, which can be considered as a potential anti-adhesion barrier in preventing postoperative adhesion formation.

Postoperative fibrosis after spinal surgeries such as discectomy and laminectomy can sometimes cause neurologic side effects due to direct compression of the nerves by the surrounding structures or by the formation of adhesion between tissues, tethering the nerve roots and dural sac. Previous studies have investigated the close

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relationship between postoperative fibrosis and recurrent radiculopathy or low back pain after a successful discectomy. However, there is no well-defined evidence to support the fact that postoperative fibrosis is the major cause of physical impairment when no bony or other pathologic tissue was detected during the revision surgery.[1,2] Although some patients may consider undergoing subsequent surgeries for relief of their intolerable symptoms, the outcome is often unpredictable and the rate of complications such as iatrogenic nerve root injury or dural tear is relatively high. It is difficult for spine surgeons to confidently predict the benefits of such surgeries, which may also lead to legal problems. In addition, due to further degeneration at the surgical level or in the nearby areas, postoperative fibrosis can become more complicated and require longer surgeries, causing increased bleeding and risks associated with anesthesia, especially for elderly patients.[3,4]

Controlling fibrosis formation has been an important issue in successful spinal surgery. Many materials reportedly have the potential to prevent postoperative peridural adhesion or fibrosis, including physical materials such as barrier gels, Silastic® sheets (Dow Corning Corp., Midland, MI, USA), and absorbable foams, chemical materials such as nonsteroid anti-inflammatory drugs (NSAIDs), pharmaceuticals, and steroids, and biologic materials such as hyaluronate membranes, cellulose mesh, and free and pedicle fat grafts.[5-10] However, the effects of these methods and materials remain questionable due to several extraneous and confounding variables in the experimental designs and various anti-adhesive agents.

The theory of fibrosis formation has been well studied and is described as growth of the fibrous connective tissue into the surrounding hematoma after laminectomy. To prevent hematoma infiltration, the anti-adhesive materials must fill the space, thus providing a tamponade effect, preventing hematomatous material from entering the epidural space. Hyaluronic acid (HA) is one of the anti-adhesive materials that have a predicable half-life, stable quality, and elastic configuration. In addition, its biocompatibility and efficacy in the prevention of postoperative adhesions in general surgery or gynecologic procedures have been studied for many years. Also, it has been widely used in the treatment of degenerative knee joint disease and cosmetics, and poses no hazards to patients.[11,12] However, HA's effect on the prevention of postlaminectomy scar formation has not yet been tested. The purpose of our study was to examine the effects of HA on the prevention of postlaminectomy fibrosis formation and adhesion, using a rabbit model and histological analysis, gross observation, and peel-off testing.

METHODS

Materials and animals

The HA-based gelatin that was used in this study was Synvisc® (hylan G-F 20; Genzyme Biosurgery Corp., Ridgefield, NJ, USA). It is a mixture containing hylan A fluid, hylan B gel, and saltwater, with a high molecular weight of 6000 kDa. Hylans are made from sodium hyaluronate.

Animals for the experiment were obtained from the Laboratory Animal Center, Chang Gung Memorial Hospital. The principles and procedures of animal care and use were followed and approved by the Institutional Animal Care and Use committees of Chang Gung Memorial Hospital. A total of 12 adult New Zealand rabbits (weighing 2.8-3.2 kg each) were randomly divided into a treatment and a control group, each with 6 rabbits. A two-level lumbar laminectomy was performed in all rabbits. The treatment group was then treated with HA-based gelatin and the control group was untreated.

Surgical procedure

The surgical procedure was similar to that of human spinal laminectomy. Briefly, after general anesthesia, the surgical site was shaved and prepared. A 3 cm posterior longitudinal incision was made at the level of the fourth and fifth lumbar vertebrae, using meticulous hemostasis. Then, the soft tissue was dissected subperiosteally. The lumbar vertebral segments were exposed, and a total laminectomy was performed at L4-L5, using a power burr and a lamina punch, leaving the dura mater intact [Figure 1A and B].

For the treatment group, the dural theca was exposed after the laminectomy and the topical HA (Synvisc) was applied, so that it entirely covered the exposed spinal cord and roots [Figure 1C]. In contrast, the control group received no additional procedure. The fascia layer was closed with 2-0 VICRYL™ suture (Ethicon, Inc., Blue Ash, OH, USA) and then the skin was closed with 3-0 nylon suture. No iatrogenic dural or root lesions and no postoperative infection and neurologic deficit were noted. All rabbits were sacrificed 8 weeks after laminectomy.

Gross observation and peel-off testing

After euthanasia, blunt dissection was performed at the laminectomy site, and the residual lamina and pedicle containing the dural tube were all removed, exposing the spinal cord and surrounding scar tissue. The amount of scar tissue was graded by gross observation and scored on a scale from 1 to 3, where 1 ("mild") indicated scarring that affected less than 25% of the surgical field, 2 ("medium") indicated scarring that affected about 25-50% of the surgical field,

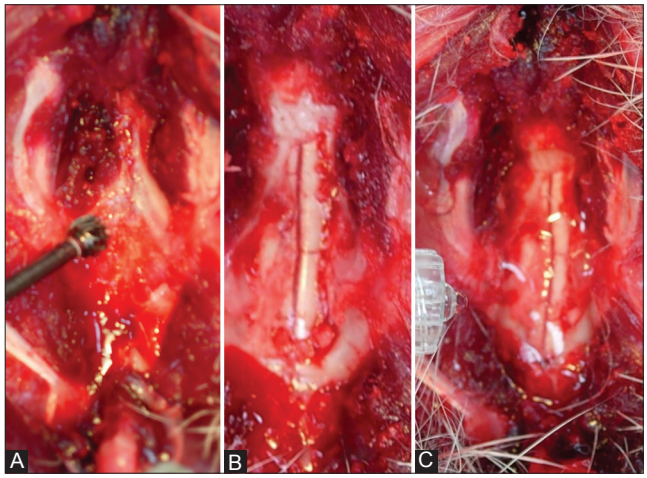

Figure 1: Surgical procedure. (A and B) A L4–L5 total laminectomy was performed with a power burr and a lamina punch, so that the dura mater remained intact. (C) Topical HA was immersed slowly to entirely cover the exposed spinal cord and roots.

and 3 ("large") was given where scarring was seen in more than 50% of the surgical field.[11]

Using forceps, the scar tissue was manually peeled off; the tenacity of adhesion between the scar tissue and dura was evaluated, and the difficulty of reopening the operated site was also taken into consideration. A six-level scoring system (grades 0-5) was used to indicate the difficulty of reopening the surgical site. With Grade 0, there was no adhesion between the dura and scar tissue. Grade 1 indicated very slight adhesion to the dura mater, and the tissue could be easily detached without applying manual force. With Grade 2, there was some adhesion to the dura and the tissue could be easily detached by moderate traction. With Grade 3, less than 50% of the operated area had adhesion and could be detached by strong traction. In Grade 4, more than 50% of the operated area had adhesion and could be bluntly detached by strong traction. Grade 5 indicated severe adhesion that could not be detached without disruption of the dura mater, unless sharp dissection was used.[12,13]

The grading systems of gross observation and peel-off testing were based on a random selection of rabbits. The assigned group and treatment status were revealed to the observer only after the scoring was done.

Histological examination

The tissue samples at the laminectomy site were harvested after the rabbits were sacrificed. The samples were fixed in formaldehyde solution, immersed into ethylenediaminetetraacetic acid (EDTA) for decalcification, and embedded in paraffin. The sections were deparaffinized in xylene and rehydrated through graded ethanol washes, followed by staining with standard hematoxylin and eosin. All sections were evaluated by the same histologist, who was blinded to the group and study method. The extent of adhesion (dura/root involvement score) was graded according to the method of He *et al*.[6] In Grade 0 adhesions, the dura mater was free of scar tissue. In Grade 1 adhesions, only thin fibrosis bands between the scar tissues and the dura mater were observed. In Grade 2 adhesions, continuous adhesion was observed but made up less than two-thirds of the laminectomy defect. In Grade 3 adhesions, scar adhesion was large and involved more than two-thirds of the laminectomy defect, and/or extended to the nerve roots.

The scar tissue consistency and inflammatory response were graded using the scoring system proposed by Miyamoto *et al*.[8] These researchers used a 4-point scoring system. A score of 0 indicated loose connective tissue with small collagen bundles, the presence of highly vascular adipose tissue, with moderate macrophage and inflammatory cell activity. A score of 1 indicated connective tissue density, edges of defect with evidence of new bone formation, and mild macrophage and inflammatory cell activity. A score of 2 was used for dense connective tissue and/or fibrocartilage, absence of adipose tissue, avascular tissue, and absence of macrophage and inflammatory cell activity. A score of 3 indicated dense collagenous connective tissue, absence of adipose tissue, avascular tissue, and absence of macrophage and inflammatory cell activity. In addition, fibroblasts were counted (×400 magnification), and the results from the two groups were compared.

Statistical analysis

Mann–Whitney U test was used to compare the values of each parameter between the control and treatment groups. Numerical data were presented as the mean plus or minus the standard deviation (Mean ± SD), while categorical data were expressed as absolute frequencies using SPSS software. A $p < 0.05$ was considered statistically significant.

RESULTS

Surgical outcome

All 12 rabbits were smoothly ambulatory after surgery without obvious neurologic deficits. The fascia, muscle, and skin around the surgical levels healed well in both groups, and no infection or discharge from the wound was noted at sacrifice.

Gross observation and peel-off testing

Gross observation showed that scar tissue occurred in the center of the laminectomy defect, and no residue of HA was found in either group, 8 weeks after laminectomy. In the control group, all scored 3 because a large amount of scar tissue formation was found in the laminectomy site. In

the treatment group, three scored 3 and the remaining three scored 2 for medium scar tissue formation. Wounds treated with HA showed a relatively smaller amount of scar tissue in comparison to untreated wounds ($p = 0.056$). Comparison of adhesion in both groups is shown in Table 1.

In manual peel-off testing, the mean of the scores was 4.33 ± 0.52 for the control group and 3.17 ± 0.75 for the treatment group. There was a significant difference in the tenacity of adhesion between the scar tissue and dura in the two groups ($p = 0.016$).

Histological analysis

The mean of the "dura/root involvement score" for the extent of scar adhesion was 2.00 ± 0.63 in both groups. However, the score for the scar tissue consistency and inflammatory response was slightly lower in the treatment group, with a mean of 1.67 ± 0.52, whereas the mean was 2.33 ± 0.52 in the control group ($p = 0.056$).

Furthermore, the histological results showed that the harvested tissue samples covered with HA seemed to have a larger subarachnoid space, a greater distance from the surface of the dura to scar tissue, and a smaller number of inflammatory cells in the scar tissue at the laminectomy site [Figure 2].

The density of fibroblast cells was significantly less in the treatment group with a mean of 3078 ± 313.68 cells than in the control group with a mean of 3742 ± 455.65 cells ($p = 0.041$).

DISCUSSION

Postoperative fibrosis and adhesion are normal components of the healing process. Fibrosis and adhesion are the result of an inflammatory reaction caused by the organization of the fibrin matrix, and these steps are necessary in tissue healing.[14] However, in cases of marked postoperative peridural fibrosis, fibrosis and adhesion can cause tethering of nerve roots and subsequent neurologic symptoms. It is also known that peridural scar adhesion is one of the causes of persistent radiculopathy.[4] In a randomized prospective study, Ross *et al.*,[2] demonstrated a strong correlation between scar adhesion and postoperative pain. Patients with extensive peridural scar adhesion were 3.2 times more likely to experience recurrent radicular pain than the patients with less extensive scarring. In addition to persistent postoperative symptoms, peridural fibrosis may also pose greater risks, including iatrogenic dura injury and nerve root injury; they often have more difficulties during surgery, longer operation times, and more bleeding when revision surgery is needed.

There have been several mechanisms proposed to explain the presence of postlaminectomy dural adhesion. While Key *et al.*,[15] suggested that epidural fibrosis may come from the annulus fibrosis of the disc, LaRocca *et al.*,[16] concluded that fibrosis originates from the posterior invasion of fibroblasts, extending from erector spinae muscle to the dura and then grows into the hematoma. Songer *et al.*,[17] provided evidence that the adhesion resulted from replacement of hematoma with epidural fat during surgery and that this tissue was then replaced by dense fibrotic tissue. Regardless of the exact mechanism for peridural scar adhesion, it seems to be the consequence of a series of processes involved in wound healing. The imbalance of fibrin deposition and fibrinolysis imposes a high risk of massive peridural adhesion, and hematoma attracts fibrin, which is deposited around the infiltration of the hematoma space.

Holtz *et al.*,[18] proposed a mechanism that could possibly reduce the formation of fibrosis. This involved the following steps: (1) reduction of the initial inflammatory reaction and exudation, (2) inhibition of the coagulated exudates, (3) promotion of removing fibrin, (4) separation of fibrin by a physical barrier, and (5) inhibition of fibro-

Table 1: Comparative results of analyzing adhesion between the two groups

	Control group			Treatment group			
	Mean	SD	Median	Mean	SD	Median	*p* value
Scar tissue amount	3.00	0.00	3	2.50	0.55	3	0.056
Scar tissue tenacity	4.33	0.52	4	3.17	0.75	3	0.016
Dura/root involvement score	2.00	0.63	2	2.00	0.63	2	1.000
Scar tissue consistency	2.33	0.52	2	1.67	0.52	2	0.056

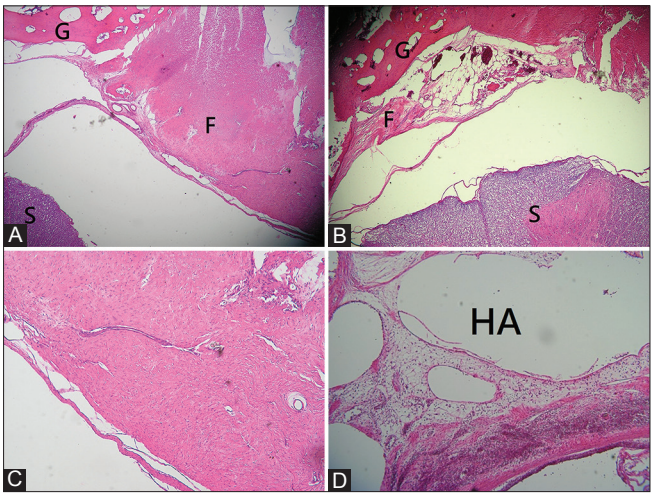

Figure 2: Histologic studies of the laminectomy site indicated significantly less scar tissue formation, a larger subarachnoid space, greater distance from the surface of dura to scar tissues, and fewer inflammatory cells: (A) control group; (B) experimental group; (C) dense fibrous tissue seen in the control group (magnified from a); (D) gelatin-containing vacuolated spaces in the epidural space and surrounded by loose connective tissue in the experimental group (F, fibrous tissue; G, bone tissue; HA, gelatin-containing vacuolated spaces; S, spinal cord).

blastic proliferation. There are two main ways to reduce initial inflammation: (1) decreasing the tissue injury by using minimally invasive procedures[19] and (2) inhibiting the inflammation pathway by using NSAIDs and steroids; however, this approach can increase the risk of infection.[20]

The true relationship between neurologic impairment and peridural fibrosis formation is unclear. It has been estimated that peridural fibrosis or adhesion affects as many as 24% of patients with failed back surgery syndrome.[2] The fibrotic tissue can restrict the nerve root, limiting its elasticity and thus making it more sensitive to motion, as can be seen during physical examination. Another alternative is that the tissue may occupy the peridural space and make it susceptible to spinal stenosis by ligament hypertrophy or disc bulge. For all these reasons, the amount of scar tissue and its degree of "stickiness" or adhesion should be minimized as much as possible. Most anti-adhesion agents provide a barrier to hematoma by avoiding the contact with the dura mater and nerve root, keeping the fibrotic tissue away from the neurologic organs. An ideal anti-adhesive agent also needs to be effective, biocompatible, and easily applicable, as well as predictably absorbable.[9] Several candidate anti-adhesive materials and methods have been investigated, including low-dose external beam radiation,[5] mechanical barriers such as fat,[21,22] polylactic acid,[23] Avitene® (Davol, Inc., Warwick, RI, USA), and Gelfoam® (Pfizer, Inc., New York, NY, USA),[24] and pharmacological agents such as anti-inflammatory agents, antioxidants, anticoagulants, and fibrinolytics.[25]

Results of our study show that HA-based gelatin is an effective and safe anti-adhesion material that can be applied *in vivo* without any significant adverse effects in an adult rabbit laminectomy model. It has been used as an anti-adhesive material in other anatomic fields, including the intraperitoneal cavity,[25-27] for post-surgery tendon adhesion,[28] and for many years, it has been successfully used for strabismus surgery.[29] HA, a natural extracellular matrix that resides in the human body, has an anti-adhesive effect and acts as a mechanical barrier that separates the injured tissues from the scar tissues by occupying the space and, thus, keeping the hematoma away from the dura mater long enough to have potential benefits for preventing postoperative peridural fibrosis.[30]

Adhesion formation is an early phenomenon that occurs primarily within 36 h of surgery, after hematoma formation. Anti-adhesion barriers must maintain their position on the injured site for at least this amount of time to be effective for preventing adhesion.[31] Hill-West *et al*.,[32] showed that HA was not effective in the prevention of postsurgical abdominal adhesion; however, their findings were contradicted by the results of other studies. These inconsistent results may be due to the fact that in some cases, the surgical area is greater than the volume of the HA material. In addition, because HA is rapidly absorbed, it may not remain in the peritoneum long enough to form an effective mechanical barrier. As a result, when HA is used as an anti-adhesive agent in abdominal surgery, it is reasonable to consider using it along with a more durable carrier. However, in the case of posterior spinal surgery, the surgical field is more confined to the epidural space and is thus easier to position a barrier from hematoma. As such, half-liquid type HA can infiltrate the surrounding area of the spinal cord and form an adequate space occupied not only as a barrier but also as a tamponade to stop the bleeding. Because of this, an additional carrier for HA may not be needed during spinal surgery.

Fibroblasts may play an important role in the formation of peridural fibrosis originating from the perivertebral muscles and bloodstream. Blocking the migration of fibroblasts to the surgical field is a theoretically applicable measure to prevent peridural fibrosis. However, when Kasimcan *et al*.,[7] evaluated the number of fibroblasts between treatment and control groups, they found no significant difference in the amount of fibroblasts. In our study, there were significantly fewer fibroblasts in the treatment group than in the control group. We hypothesized that since HA is a more biologically inert material, it triggers less inflammatory response than did the bioresorbable material used in Kasimcan *et al*.'s study.[7] A more detailed comparison of the effectiveness of HA with other anti-adhesion materials is needed.

HA-based gels have been used as promising anti-adhesive agents in numerous experimental studies. In our study, HA was associated with a reduction of scar tissue, as well as the degree of tenacity between scar tissue and dura mater. HA was absorbed gradually and presented with no adverse effects on local soft tissue healing or on neurologic performance. No potentially serious adverse events were observed in the test animals, and complication rates were similar in the treatment and control groups. Moreover, compared to the control group, the use of HA-based gelatin in the treatment group made no difference in healing of the skin, subcutaneous tissue, or muscle, with one exception: inhibition of peridural fibrosis formation and the adhesion adjacent to the dura at the laminectomy site.

This preliminary study had several limitations. First, the sample size was relatively small. Second, the grading systems for the amount of scar tissue formation, the tenacity between the scar tissue and dura mater, the extent of adhesion, and the scar tissue consistency were relatively subjective, unlike other analyses such as magnetic resonance imaging or quantitative testing, which present more precise images or quantitative results. Third, the tissue samples were harvested at only a single time-point, which is 8 weeks after surgery. The sampling period of anti-adhesive agents in an animal model is usually 4–8 weeks. Anti-adhesion materials are usually absorbed and degraded within 4 weeks. Lalountas *et al*.,[33] and Park *et al*.,[30] compared the effectiveness of different adhesion barriers such as cellulose film and hyaluronan in the prevention of postoperative adhesion

formation in rats. The samples were harvested and analyzed 2 weeks and 3 weeks after laparotomy. Brzezicki *et al*.,[34] also indicated that a significant difference in the extent of scarring between a laminectomy and sham group could be observed 6 weeks after surgery. Furthermore, Kato *et al*.,[35] demonstrated that it was not possible to quantitatively evaluate the degree of adhesion at 24 weeks after surgery in a rabbit model, because new bone growth was so significant at the laminectomy site in all groups. For all these reasons, we decided to design a study to evaluate the effect of HA in a rabbit laminectomy model, 8 weeks postoperatively. However, future analysis should involve a larger sample and longer observation period. Maturation of scar tissue may occur over a longer period, which might also alter the final results. Finally, the surgical procedure in the current study was limited to laminectomy, excluding discectomy. In the rabbit model, discectomy involves the disruption of annulus fibrosis and the release of material from the nucleus pulposus, which more closely mimics human surgery. It may affect the degree of adhesion and possibly the effectiveness of the anti-adhesion property of HA, which is less reliable in the separation of the anterior aspect of the spinal cord. Furthermore, some authors suggest that some hemostatic characteristics and cytokine inhibitor mixtures can increase scar prevention by decreasing the amount of hematoma and thus lessening the chemotaxis of fibrin. Greater attention to the study design will be needed to develop this product.

Although this was an animal study, it also provides useful information about the effects of hyaluronate in the prevention of postoperative peridural scar adhesion after laminectomy. Nonetheless, further studies should be conducted to determine if HA is harmful to normal tissue, that is, to find whether it results in an increased rate of infection or limits tissue healing. Further clinical trials of the use of HA-based gels should be performed to confirm its effects in human subjects.

Conclusions

Based on the results of our preliminary study in rabbits, we found that HA-based gelatin is effective for decreasing the tenacity and amount of adhesion between the dura and scar at the laminectomy site. Thus, it can be considered as a potential adhesion barrier for preventing postoperative scar tissue formation.

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