A total laminectomy is the most popular and effective operation for spinal diseases such as spinal stenosis. However, this treatment has some limitations. Generally, the bone defects caused by the laminectomy regenerate as fibrous tissue. This healing process includes four phases: the development of a hematoma, the formation of granulation tissue, scar formation and the generation of a post-laminectomy membrane [1]. This post-laminectomy membrane may eventually lead to a secondary vertebral canal stenosis and result in dural sac and nerve root compression, gradually leading to the recurrence of the preoperative symptoms. A laminectomy also damages the integrity of the posterior arch, reducing the surface area of bone to which muscle can attach. Additionally, the facet joint...
excision may reduce the lumbar mechanical strength. Collectively, these limitations can cause instability of the lumbar. In 1982, Hazlett et al reported that small joint resections in the lumbar were related to lumbar instability [2]. As a result of instability, degeneration of the lumbar may accelerate.

In recent years, the problem of lumbar instability because of a total laminectomy has attracted widespread attention. However, all operational methods that strive to repair or replace the vertebral lamina [3,4], or reduce the resection, typically result in residual symptoms because of incomplete decompression. Thus a second operation is frequently required. In the 1970s, micromorselized bone was first used in artificial hip joint replacement revisions [5]. Since then, many studies have demonstrated its osteogenic potential [6–8]. More recently, micromorselized bones have been used for the treatment of bone defects, bone nonunion, spinal fusion and other diseases. However, when the morselized bones have been used separately, the structure has often been loose and difficult to fit securely within the defect [9]. In vertebral column surgeries, there is a risk of the morselized bone falling into the spinal canal and oppressing the nerve tissue. Thus, to optimize the use of micromorselized bone, it is necessary to identify other materials that can complement and securely shape the micromorselized bone.

Early in the 1990s, Songer et al [10] proposed the “three-dimensional theory”, suggesting that semi-liquid materials were most suitable for preventing extradural fibrosis. Some clinicians have used polymer sodium hyaluronate [11], fibrin gel [12] or chitosan [13] to cover the spinal dura, and they affirmed their positive effects in hemorrhage control and spinal dural adhesion prevention after an operation in the vertebral canal. Polylactic acid (PLA) materials have good biocompatibility and biodegradability, and the degradation products are known to participate in metabolism. These materials have been widely applied in drug delivery, ophthalmology, as surgical sutures, in fracture internal fixation and in tissue engineering [14–16], and they are now considered one of the most important biodegradable medical materials [17–21]. PLA adhesion prevention gel (PLA gel) in which the main ingredient is polymer-D, L-lactic acid (5–100%), polyethylene glycol (8–80%), glycolic acid (0–80%) and caprolactone (0–80%) with molecular weight 40–200 kDa, proteins, polysaccharides, vitamin K and pyrrolidone. PLA gel is a transparent, colorless, gelatinous semi-liquid at room temperature, with a degradation period of 2 months. Twenty local, healthy wolf dogs were provided by the Laboratory Animal Center of the First Affiliated Hospital of Harbin Medical University (15 males and 5 females, 12–15 kg, aged 20–25 months).

Treatments
Anesthesia was performed with a 3% pentobarbital sodium intravenous injection (1 mL/kg). After computed tomography (CT) examination, tissues were separated layer by layer until the L1, L3 and L5 acanthus and vertebral laminae were revealed. Laminectomies were conducted, forming three operative defects (2.5 × 1.0 cm). Next, we selected the larger acantha and vertebral lamina bone, removed the periosteum and cartilage and then made micromorselized bone (average particle size = 195 μm) using self-made bone milling equipment. Sixty segments were randomly divided into four groups (A–D; Table 1).

Group A (n = 16) was treated with a composite of micromorselized bone and PLA gel. After thorough hemostasia and flushing at the operative site, 0.2 mL of
PLA gel was spread on the back of the dura, with the film forming after 5–10 seconds. Next, 0.8 g of micromorselized bone was spread over the PLA gel evenly, and the defective vertebral lamina section was coated with 0.2 mL PLA gel. Group B ($n=16$) was treated with pure micromorselized bone. The micromorselized bone (0.8 g) was spread over the dura and throughout the defective vertebral lamina section thoroughly. Group C ($n=14$) was treated with PLA gel only. The PLA gel (0.2 mL) was spread on the back of the dura and throughout the defective vertebral lamina section. Group D ($n=14$) was the blank control group. After thorough hemostasia and flushing at the operative site, the defect was closed with sutures.

**Observation parameters**

After the operation, mental status, vital signs, muscle strength, muscle tension and motion capability of the wolf dogs was observed. CT examination was performed before the operation and postoperatively for all groups at 4th and 8th weeks. Both preoperative and postoperative CT images of Groups A and B were processed by Image Pro Plus software (version 6.0; Media Cybernetics, Inc., USA), and the cross-sectional areas of the spinal canal were measured. The dogs were euthanized at 4th and 8th weeks, respectively, and the treated segments were harvested and dissected equally into two parts. One half was used for general observations and the evaluation of scar tissue formation. Independent physicians not connected with this study performed the anatomical inspection of the spinal canal cross-section using an operative microscope. The measurements included graded evaluations of the toughness and peridural adhesion according to the revised Rydell and Balazs Standard [22]. Grade 0 was without obvious adhesion between the dura and the scar; Grade 1 exhibited scattered and thin adhesion between the dura and the scar, but easily separable; Grade 2 showed extensive and compact adhesion between the dura and the scar, they could be separated with force while keeping the dura complete; Grade 3 demonstrated severe adhesion between the dura and the scar, with separation destroying the dura. The second half of the harvested segment was formalin-fixed, paraffin-embedded and stained with hematoxylin and eosin. The specimens were histologically examined in order to observe newly generated tissues and symphysis.

**Statistics analysis**

Spinal canal cross-sections and peridural adhesion are presented as the mean ± standard deviation. Statistical analysis was performed by SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and data were analyzed by a least significant digit $t$ test based on a multiple comparison ($p=0.05$). A covariance analysis based on the average comparison of random group single factors was performed, followed by an additional least significant digit $t$ test ($p=0.05$).

**RESULTS**

On the second day after the operation, all dogs were able to drink and eat and were without fever, cachexia or agitation. Dogs were able to stand, walk and shake tails. One week later they could run and jump.

**CT examination of spinal canal cross-sections**

At 4th week, a newly generated callus was observed on both sides of the defective vertebral lamina sections, with some gaps existing between the two sides of the newly generated calluses in Groups A and B. Vertebral lamina collapse and vertebral canal stenoses were not seen in Group A. The callus was thick and expanding with an irregular shape in Group B. At 8th week, all of the defective vertebral lamina sections were connected by newly generated calluses. In Group A, the interior and the exterior lamina were clear and the lamina edge

| Table 1. Distribution of vertebral laminae treatments |
|---------------------------------|----------|----------|----------|----------|----------|
| Group A | Group B | Group C | Group D | Total    |
| L1      | 6       | 9       | 2        | 3        | 20       |
| L3      | 7       | 4       | 4        | 5        | 20       |
| L5      | 3       | 3       | 8        | 6        | 20       |
| Total   | 16      | 16      | 14       | 14       | 60       |

Group A = Combination of polylactic acid gel and micromorselized bone; Group B = micromorselized bone; Group C = polylactic acid gel only; Group D = no treatment.
was smooth. In Group B, the defective vertebral lamina sections were covered by newly generated thick calluses. Soft tissues were observed in the operative regions, the subarachnoid cavity was open, and the outline of the dural sac was smooth in Group C. The adhesion shadow behind the dura had entered the spinal canal deeply in Group D (Figure 1).

**Comparison of spinal canal cross-sections preoperatively and postoperatively**
The change in the spinal canal cross-sections of specimens in Group B was significantly greater at 4 and 8 weeks as compared with those in Group A ($p<0.05$). In Groups A and B, there were no obvious differences between the 4-week and 8-week time points ($p>0.10$; Table 2).

**General inspection and grading of scar tissue**
At 4th and 8th weeks, the PLA gel could not be detected in Group A, and the shape of the newly generated vertebral lamina grew well with a clear outline and a smooth interior surface, while adhesion with soft tissue did not exist or was minimal. In Group B, a thickened vertebral lamina formed and was conglutinated with the dura, and cartilage tissue was found in the middle region of the newly generated vertebral lamina. Some micromorselized bones and soft tissues entered the spinal canal in specific segments. At 4th week, in Group C, we observed what appeared to be degradation remnants of the PLA gel behind the dura, and at 8 weeks, the dura was in contact with soft tissues, but separated easily upon dissection. In Group D, the adhesion between the dura and the scar was extensive and difficult to separate at 8th week. The newly generated bony vertebral lamina was not evident in Groups C or D at both 4th and 8th weeks (Figure 2).

According to the least significant digit $t$ test, the degree of adhesion of Groups A and C was much lower compared with Groups B and D at 4th and 8th weeks, respectively ($p<0.01$). However, the differences in adhesion between Groups A and C, and those between Groups B and D were not as distinct ($p>0.10$; Table 3).

**Histological observations**
In Group A, at 4th week, the non-absorbed micromorselized bone was surrounded by gel remnants, fibroblasts and osteoblasts. At 8th week, more mature and circuitous bone trabecula structures and a clear medullary cavity were observed. The structure of PLA had mostly disappeared and was replaced with fibrous tissue and newly generated bone (Figure 3).

In Group B at 4th week, most of the micromorselized bone was absorbed and surrounded by osteoblasts.

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**Table 2. Changes in spinal canal cross-sectional areas ($n=8$ per group)***

<table>
<thead>
<tr>
<th>Time</th>
<th>4th week</th>
<th>8th week</th>
<th>F value</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>$-0.19\pm4.46$</td>
<td>$23.45\pm7.70$</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>$-0.16\pm12.77$</td>
<td>$17.55\pm7.58$</td>
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<td></td>
</tr>
</tbody>
</table>

*Data presented as mean ± standard deviation. Spinal canal cross-sectional areas were significantly increased in Group B at 4 and 8 weeks as compared with those in Group A at the respective time points ($p<0.05$). In Groups A and B, there were no obvious differences between the 4th and the 8th week ($p>0.10$).
**Figure 2.** General inspection of lumbar cross-sections taken at 4th and 8th week postoperatively: (A) Group A at 4th week, gaps between the two sides of newly generated calluses were found; (B) Group A at 8th week, the shape of the spinal canal in the cross-section was intact and no gaps were observed; (C) Group B at 4th week, the newly formed vertebral lamina contained an inordinate structure with a connection between the ends of the residual vertebral lamina, some micromorselized bone and soft tissue had entered the spinal canal in specific segments; (D) Group B at 8th week, a thickened vertebral lamina formed and had conglutinated with the dura closely, cartilage tissue was observed in the middle region of the newly generated vertebral lamina; (E) Group C at 4th week, a few particles were found behind the dura and were considered to be degradation remnants of the polylactic acid gel; (F) Group C at 4th and 8th weeks, there was no evidence of a newly generated vertebral lamina, and the dura could be easily separated from contact with the posterior soft tissues; (G,H) Group D at 4th and 8th weeks, there was no evidence of a newly generated vertebral lamina and adhesion with the dura was extensive.

**Figure 3.** Histological sections of Group A at 4th and 8th weeks (hematoxylin and eosin staining; original magnification, A–D = 200×; E and F = 20×): (A) At 4th week, the non-absorbed micromorselized bone was surrounded by gel remnants and fibroblasts (short black arrow); (B) chondrocytes (short white arrow) and (C) osteoblasts (long black arrow) were observed in proximity to the micromorselized bone; (D) newly generated bone clusters penetrated the remaining micromorselized bones and fibroid polylactic acid; (E) at 8th week, the clearance between the dura and the newly generated posterior vertebral lamina were obvious (black arrowhead); (F) bony union was apparent between the host vertebral lamina and the newly generated lamina.
At the host bone edges of the vertebral lamina, cartilage and fibrous tissue as well as osteoblasts, were apparent. The newly generated vertebral lamina attached to the dura tightly. At 8th week, there were more mature bone trabeculae in the central region of the defect (Figure 4).

At 4th week in Group C, the PLA remnants were scattered throughout the fibrillar connective tissue filling the defect site. At 8th week, most of the PLA had degraded and was absorbed, replaced by mature fibrous tissue. The orientation was in good order and the surface adjacent to the dura was smooth (Figures 5A and 5B).

In Group D at 4th week, fibrous and chondrified bones were observed in the vertebral lamina, and the defect area was replaced with fibrous connective tissues. At 8th week, a large amount of fibrous tissue comprised the defect area with tight adhesion between the scar tissue and the dura (Figures 5C and 5D).

**DISCUSSION**

Autologous micromorselized bone possesses the capabilities of bone regeneration, inductivity and conductivity. It is popularly used for the treatment of bone defects, bone nonunion and spinal fusion. Previous studies [23,24] have revealed a number of reasons for these advantageous characteristics. First, micromorselized bone has a large surface area, which may allow it to release more growth factors and thus exert greater bone conductivity. Second, distortions of bone grafts that are induced by conformance or elasticity can stimulate bone regeneration. Additionally, the enlarged surface area enhances the ability of cells to absorb nutrients, thus encouraging cell survival. Moreover, the porosity of the micromorselized bone supports the growth of tiny blood vessels. All of these factors may accelerate and strengthen the ability of micromorselized bone to stimulate bone regeneration.

However, when implanted alone, micromorselized bone creates an irregular shape behind the dural sac and can lead to many risk factors associated with a lower efficacy of bone regeneration. These include bone loss, as well as hematoma and blocking or adhesion of the implant to the spinal canal. PLA gel transforms into a semi-solid film upon exposure to tissue fluid and adheres to contact surfaces with great affinity. Additionally, the degradation time can be customized. These characteristics are unmatched by any other adhesion prevention products [25]. In this study, with the exception of adhesion prevention, the PLA gel also played a role as a flexible carrier of micromorselized bone. During the operation, we found that the PLA gel was able to contain micromorselized bone and adhere to the back of the dura with flexibility, allowing fluctuation with the pulsation of cerebrospinal fluid. Thus this semi-solid material offered an ease of manipulation better than standard rigid materials, which have less plasticity and are difficult to fix within the defect.

At the interface between the PLA gel and the micromorselized bone, the gel resembled a three-dimensional scaffold after transforming into a semi-solid film, while keeping in contact with the micromorselized bone. With the degradation of the PLA gel or the absorption of micromorselized bone, many interspaces were formed, providing growth space and a framework for newly generated bone, as well as providing space for the migration of cells and the growth of capillary vessels.

PLA gel can play an important role in avoiding bone loss and maintaining progenitor cells and growth factors within the defect site. Gradually, due to the dynamic equilibrium that formed between the pulsation of the front dural sac and the compression of the fibrous connective tissues, a proper radian of newly formed vertebral lamina developed in group A. Additionally, the anatomic confirmation of the spinal column largely resumed; thus the skeletal structure of the newly generated vertebral lamina appeared

<table>
<thead>
<tr>
<th>Time</th>
<th>Group A (n = 8)</th>
<th>Group B (n = 8)</th>
<th>Group C (n = 7)</th>
<th>Group D (n = 7)</th>
<th>F value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th week</td>
<td>0.86 ± 0.69</td>
<td>2.71 ± 0.49</td>
<td>0.43 ± 0.79</td>
<td>2.57 ± 0.53</td>
<td>23.647</td>
<td>0.000</td>
</tr>
<tr>
<td>8th week</td>
<td>0.43 ± 0.53</td>
<td>1.71 ± 0.49</td>
<td>0.57 ± 0.53</td>
<td>2.29 ± 0.49</td>
<td>21.591</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Data presented as mean ± standard deviation. The degree of adhesion in Groups A and C were significantly less than those in Groups B and D (p < 0.01). The difference in adhesion between Groups A and C, and those between Groups B and D were not significant (p > 0.10).
more mature than that of Group B at 8th week. We speculate that the regenerated vertebral lamina treated with a combination of PLA gel and micromorselized bone can be expected to confer stability to the spinal column and prevent peridural adhesion as a long-term outcome. Further research on ligament structure will confirm whether the newly generated vertebral lamina can provide an attached bed for soft tissue growth and enable ligament regeneration.

These results indicate that the combination of autologous micromorselized bone and PLA gel can prevent scar adhesion effectively and reconstruct a defective vertebral lamina with good molding after a laminectomy.
REFERENCES


自體微小顆粒骨
－聚乳酸複合物修復全椎板切除術後的犬椎板

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硬膜外粘連、錶發椎管狹窄和腰椎不穩是全椎板切除術的併發症。本研究利用自體微小顆粒骨—聚乳酸凝膠複合物來修復全椎板切除術後的椎板損傷，評估其骨發生、椎板重建和防粘連的能力。20 隻犬拍攝腰椎 CT 後進行 L1・L3・L5 節段的全椎板切除，得到的 60 個手術節段分為四組：A 組，用自體微小顆粒骨—聚乳酸複合物填充損傷（n = 16）；B 組，用微小顆粒骨填充損傷（n = 16）；C 組，用聚乳酸填充損傷（n = 14）；D 組為空白對照組（n = 14）。4 週和 8 週後進行 CT 檢查和組織學觀察，AB 兩組發現了椎板的重建，但是 B 組新形成的骨結構外形較之 A 組不甚規則。B 組椎管橫截面變化比 A 組顯著（p < 0.05）。粘連程度評分顯示 A 組和 C 組粘連程度較其他兩組輕（p < 0.05）。以上發現為椎管狹窄的治療提供了一種更有效的策略。

關鍵詞：自體微小顆粒骨，骨重建，聚乳酸（PLA），椎管狹窄症，全椎板切除術
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