Development of new biodegradable hydrogel glue for preventing alveolar air leakage

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Objective: Air leakage is a frequent complication during lung surgery. A new hydrogel glue was created by mixing aldehydized dextran and ϵ -poly(L-lysine), and its feasibility as a surgical sealant was evaluated in comparison with that of conventional fibrin glue.

Methods: Bursting pressure after application of each glue to 30 × 30-mm pleuroparenchymal defects was evaluated in two groups of 14 beagle dogs. Biodegradability and histotoxicity of the glues were evaluated in another 6 dogs with 15-mm circular pleuroparenchymal defects. Adhesions, infections, and histologic changes were observed on scheduled days for 6 months.

Results: The mean bursting pressure after application was 38.4 ± 4.6 cm H₂O for the new glue and 32.1 ± 4.5 cm H₂O for fibrin glue ($P = .02$), the former providing more effective sealing of pulmonary air leakage than the latter. Macroscopically, no adhesions or infections were observed in areas of glue application. About 90% of the new glue degraded within 3 months, but complete disappearance was not observed by 6 months. On the other hand, the fibrin glue was replaced by white pleural tissue at 4 weeks. Histologically, the new glue was covered by one layer of mesothelial cells at 2 weeks and completely covered by thick fibrous tissue at 4 weeks. Inflammatory reaction was minimal around the residual glue after 3 months. Although the new glue degraded more slowly than did the fibrin glue, the biocompatibility of the new glue was sufficient for clinical use.

Conclusion: Our new hydrogel glue demonstrates a strong sealing effect, with good biocompatibility, and has potential usefulness as an adhesive in lung surgery.

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Postoperative air leakage is the most frequent complication after lung surgery. Although it is usually not life-threatening, management of air leakage requires chest tube drainage, necessitating longer hospitalization. Standard surgical methods using sutures or staples have a disadvantage of causing further trauma to the lung tissue. Therefore, various tissue sealants have been applied to prevent air leakage after lung surgery.¹⁻⁵

Among these sealants, fibrin glue is widely used. Various application techniques have been developed to enhance the adhesive properties of fibrin glue, such as rubbing and spraying, which enhance the sealing effect.^{6,7} However, some reports have indicated that the use of fibrin glue does not reduce the duration of chest-tube drainage or hospitalization.^{8,9} In fact, thoracic surgeons have often found that the

Figure 1. Chemical structure and appearance of new-glue. New-glue is composed of two solutions of aldehyded dextran and ϵ -poly(L-lysine) (A). After the two solutions are mixed, gel formation proceeds on the basis of Schiff base formation. An equal volume of each solution is stored in the separate cylinders of a dual syringe device at 4°C until use (B).

use of fibrin glue alone often fails to prevent air leakage from pulmonary parenchymal defects during surgery and that postoperative leakage tends to recur. Furthermore, the industrial production of fibrin glue has an inherent disadvantage in that the basic source material is human blood, and thus complete prevention of infectious contamination is difficult. Therefore, new synthetic sealants have been developed and their efficacy has been investigated in animal models.¹⁰⁻¹⁸

Recently, a new biodegradable hydrogel glue (new-glue) was developed, and its favorable properties as a sealant were reported.¹⁹ New-glue comprises two aqueous solutions of aldehyded dextran and ϵ -poly(L-lysine). After the two solutions have been mixed, gel formation proceeds on the basis of Schiff base formation. In the body, new-glue is degraded mainly by hydrolysis. It has a number of favorable characteristics, such as high bonding strength, high flexibility, and low cytotoxicity. Furthermore, the onset time of gel formation and the *in vitro* degradation speed of the hydrogel can be controlled arbitrarily by variation of the selected components.

The aim of this study was to evaluate the efficacy of the new synthetic hydrogel glue when applied to pulmonary tissue. The sealing effect of new-glue was evaluated by measuring the air leakage pressure in pleuroparenchymal defect models in comparison with that of conventional fibrin glue. In addition, *in vivo* degradability and histotoxicity were examined for assessment of biocompatibility.

Materials and Methods

Preparation of new-glue

New-glue is a hydrogel adhesive prepared by mixing two kinds of liquid of aldehyded polysaccharide and ϵ -poly(L-lysine), which form a Schiff base.¹⁹ The chemical structure and crosslinking are described in Figure 1, A. In brief, solution A is composed of 20 w/w% aldehyded dextran with a molecular weight of 75K. The aldehyde groups are introduced by conventional periodate oxidation of dextran (Malaprade oxidation). Solution B is composed of 10 w/w% ϵ -poly(L-lysine) with a molecular weight of 4K containing 2 w/w% succinic anhydride. Solution B is dyed blue (brilliant blue FCF; Wako Pure Chemical Industries, Ltd, Osaka, Japan) at a concentration of 50 ppm for visualization. After sterilization with a syringe filter (0.2- μ m pore size), 2 mL of each solution is stored

Figure 2. Pleuroparenchymal defects for evaluation of glue sealing effect and histotoxicity. Large pleuroparenchymal defects (30 × 30-mm square, 1-mm deep) were created with scalpels and electrocautery for the evaluation of sealing effect (A). Circular pleuroparenchymal defects (15 mm in diameter) were created with scalpels, and hemostasis was obtained by pressure with a sponge for evaluation of histotoxicity (B).

in each cylinder of a dual-syringe device (Mini-Dual Syringe; Plas-Pak Industries, Inc, Norwich, Conn) at 4°C until use (Figure 1, B). The onset time and end time of gel formation at 25°C are about 10 and 60 seconds, respectively.

Animals and Anesthesia

The Kyoto University Animal Experimentation Committee approved the experiment, and all the surgical procedures and euthanasia were performed in accordance with the National Institutes of Health animal care guidelines. Twenty adult beagle dogs weighing approximately 8.0 to 12.0 kg were used for this study. After premedication with atropine sulfate at 0.03 mg/kg, the dogs were anesthetized by intramuscular injection of ketamine hydrochloride at 15 mg/kg and xylazine at 7 mg/kg. After intratracheal intubation with a Broncho-Cath endobronchial tube (Mallinckrodt Medical, St Louis, Mo) for 1-lung ventilation, mechanical ventilation at a respiratory rate of 14 breaths/min and a tidal volume of 25 mL/kg was started (50% oxygen, 50% nitrous oxide mixed with 1% halothane) to allow maintenance of anesthesia by inhalation during surgery. The airway pressure was sustained at 10 to 15 cm H₂O. During 1-lung ventilation, tidal volume was maintained so that it did not exceed an airway pressure of 15 cm H₂O. Ampicillin sodium at 50 mg/kg was administered intravenously just before the operation.

Assessment of Sealing Effect of the Glues

Fourteen beagle dogs were used as subjects to evaluate the sealing effect of the glues. The animals were placed in the left decubitus position, and a right thoracotomy was performed. A pleuroparenchymal defect was created in the upper lobe with scalpels and electrocautery for hemostasis (coagulation 60 W, ACUTOR SK-2, Acoma Medical Industry Co, Ltd, Tokyo, Japan), with the lung fully inflated at a positive airway pressure of 15 cm H₂O. The defect size was adjusted to be 30 × 30-mm square and 1-mm deep (Figure 2, A). The lung was immersed in normal saline solution, and the air leakage pressure at which bubbles first formed was measured. The following two methods were used to compare the sealing effect of the glue after application.

Rubbing of new-glue (7 dogs). New-glue (0.1 mL/cm²) was dropped onto the pleuroparenchymal defect area and rubbed gently for 10 seconds. After application, the glue surfaces were kept at rest by 1-lung ventilation for 2 minutes.

Rubbing and spraying of fibrin glue (7 dogs). The fibrin glue used in this study was a commercially available type (Bolheal;

Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan). This fibrin glue consists of two components. Solution A is a protein concentrate consisting of fibrinogen, plasma fibronectin, factor VIII, and plasminogen, reconstituted in aprotinin solution. Solution B is thrombin reconstituted in calcium chloride solution. For the rubbing and spraying method,^{6,7} solution A was dripped and gently rubbed onto the pleuroparenchymal defect area. Then, both solutions (0.2 mL/cm²) were sprayed simultaneously onto the rubbed surface as a mixed aerosol using a Bolheal spray set (Chemo-Sero-Therapeutic Research Institute). After application, the glue surfaces were kept at rest with 1-lung ventilation for 5 minutes.

The lung was again inflated after immersion in normal saline solution, and airway pressure was monitored. The airway pressure at which onset of air leakage was confirmed by observation of bubbles was defined as the bursting pressure in this study. When major air leakage in the sealing area was still observed at 15 cm H₂O because of destruction of the sealant after the air leakage test, the glue was reapplied. After these procedures, the thoracic cavity was thoroughly lavaged, a 10F chest tube was placed, the wound was closed in layers, and the animals were allowed to recover. The chest tube was intermittently monitored for air and fluid output until disappearance of air leakage. Thoracic radiographs were taken at 3 days and 1, 2, 4, 8, 12, 16, 20, and 24 weeks after the operation to evaluate the inflation of the lung. The animals were humanely killed by intravenous administration of an overdose of pentobarbital 1, 2, 4, 8, 12, 16, and 24 weeks after the operation for gross observation of adhesion and infection.

Assessment of Glue Biodegradability and Histotoxicity

Another group of 6 beagle dogs was used to evaluate the biodegradability and histotoxicity of the glues. After thoracotomy, two circular pleuroparenchymal defects with a diameter of 15 mm were created in the upper lobe of the right lung (Figure 2, B). Each site was resected with scalpels, and hemostasis was obtained by pressure with a sponge to avoid a thermal injury caused by electrocautery.²⁰ Each pleuroparenchymal defect was sealed by new-glue or fibrin glue as in the previous technique. After these procedures, the chest was closed in layers, and the dogs were allowed to recover, except for 1 dog that was used to evaluate the penetration of the glues after application. The animals were humanely killed by intravenous administration of an overdose of pentobarbital 1 hour after application and 2, 4, 8, 12, and 24 weeks after the

Figure 3. Bursting pressure before and after application of new-glue and fibrin glue. There were no significant differences in mean air leakage pressure after creating the pleuroparenchymal defects ($P = .82$). After application, new-glue showed better sealing effect than fibrin glue ($P = .02$).

operation to evaluate the biodegradability and histotoxicity of the glues.

The whole right lungs were reinflated with 10% formalin and immersed in the same solution. After fixation, each defect site was resected, embedded in paraffin, sectioned, and subjected to hematoxylin and eosin, Masson trichrome, and elastica–van Gieson staining.

Statistical Analyses

The data shown represent mean values $\pm$ standard deviation. The unpaired t test was used to compare different treatments and determine the P value.

Results

Throughout the experiment, all the animals survived uneventfully until they were humanely killed, and there was no change in body weight exceeding 1 kg.

Sealing Effect of the Glues

The mean air leakage pressure after creation of the pleuroparenchymal defects was 7.7 ± 0.9 cm H₂O for the new-glue animals and 8.1 ± 0.7 cm H₂O for the fibrin glue animals, the intergroup difference being nonsignificant ($P = .82$). Severe air leakage was observed at 15 cm H₂O from the whole of the resected area in all animals. The mean bursting pressure after application of the glues was 38.4 ± 4.6 cm H₂O for new-glue and 32.1 ± 4.5 cm H₂O for fibrin glue (Figure 3). New-glue showed better sealing effect than that of fibrin glue ($P = .02$). Three animals treated with fibrin glue underwent glue reapplication because most of the glues had peeled off during lung inflation. In all animals, it was possible to remove the chest tube on

the day after the operation, and chest radiographs showed inflation of the lung on each scheduled day. Macroscopically, severe adhesion of the right whole lung was observed in 1 animal treated with each glue, resulting from injury of the intercostal arteries during chest tube insertion. Except in these 2 animals, no adhesions or infections were observed in the areas of glue application.

Macroscopic Observations of Glue Biodegradability

In the 6 animals used for assessment of glue biodegradability and histotoxicity, no adhesions or infections were observed in the application area for both new-glue and fibrin glue. Macroscopically, new-glue was dyed brown, owing to the Maillard reaction, and was covered by a thin white membrane at 2 weeks after the operation (Figure 4, C). It was still clearly present and transparent at 4 weeks (Figure 4, E) but was considerably lightened at 2 months (Figure 5, A). About 90% of the new-glue degraded within 3 months, but some scattered residual glue was still obvious (Figure 5, C). Degradation of new-glue had progressed further by 6 months, but complete disappearance was not observed at this time (Figure 5, E). On the other hand, a small amount of whitish fibrin glue was obviously present at the center of the application area at 2 weeks (Figure 6, C). At 4 weeks, the application area was covered by whitish colored pleura (Figure 6, E). After 2 months, it was difficult to distinguish the application area from the surrounding normal pleura, except for the presence of a hollow.

Histologic Study of Glue Biodegradability and Histotoxicity

Histologically, new-glue had penetrated into the lung parenchyma to a depth of about 400 μ m at 1 hour after application (Figure 4, B). At 2 weeks, new-glue was covered by one layer of mesothelial cells. Infiltration of foreign body giant cells, lymphocytes, and fibroblasts was observed around the glue. No necrotic tissue or hemorrhage was evident (Figure 4, D). At 4 weeks, new-glue was completely covered by thick fibrous tissue with decreased numbers of histiocytes and fibroblasts and an increased amount of collagen fibers (Figure 4, F). Degradation of new-glue had progressed further by 2 months (Figure 5, B), and the glue had mostly disappeared from the lung surface by 3 months (Figure 5, D). At 6 months, there was no inflammatory reaction around the small amount of residual glue and no fibrotic change in the regenerated pleura and lung parenchyma (Figure 5, F). On the other hand, fibrin glue had penetrated into the lung parenchyma to a depth of about 300 μ m at 1 hour after application (Figure 6, B) and at 2 weeks was encapsulated by a thick fibrocellular layer. Infiltration of histiocytes, lymphocytes, and fibroblasts was observed around the glue (Figure 6, D). At 4 weeks, the application area had been replaced by thick fibrous tissue with slight infiltration of lymphocytes (Figure 6,

Figure 4. Macroscopic and histologic appearance at 1 hour and 2 and 4 weeks after application of new-glue. New-glue, which was dyed blue, was rubbed on the defect area (A) and penetrated into the deep lung parenchyma (B). At 2 weeks, the glue had turned brown, owing to the Maillard reaction, and was covered by a thin white membrane (C), consisting of one layer of mesothelial cells. Foreign body giant cells and fibroblasts were observed around the glue (D). At 4 weeks, the glue was still evident (E) and was completely covered by thick fibrous tissue (F) (hematoxylin and eosin stain; original magnification $\times 100$; scale bar shows 200 μm).

F). After 2 months, the lung surface was completely covered by a thick fibrocellular layer, which gradually thinned within 6 months.

Discussion

Sealant materials for preventing air leakage must be sufficiently strong to remain sealed when the lung is inflated to pressures of up to 30 to 40 cm H₂O.⁵ For a sealant spread on pulmonary tissue, flexibility and compliance are desirable qualities to accommodate the physiologic expansion and contraction of the lung. Such a material should also bond rapidly to the lung tissue and remain unaltered by underlying blood or moisture. Furthermore, the sealant should be locally nonirritating, systemically nontoxic, lacking in antigenicity, bacteriostatic, and slowly soluble in body fluids. Although fibrin glue has been widely used, it carries a risk of virus infections. It has been reported that more than 20% of uninfected persons become infected with human parvovirus B19 through the use of fibrin sealant during thoracic surgery.²¹ Therefore, it has been recommended that the use of fibrin glue should be limited to healthy adults for whom it is inevitably required until human parvovirus B19-free products become clinically available.

This is the first report of the use of a newly developed hydrogel glue in vivo. Nakajima and associates¹⁹ have reported some favorable properties of new-glue as a tissue adhesive for medical applications. New-glue has no risk of transmitting infectious materials of human or animal origin, because human plasma and animal-derived components are not used as its source. It is biodegradable and ready to use because no dissolution time is required. Furthermore, it has high flexibility and a bonding strength 4 times higher than that of fibrin glue. Although necrotic changes in the lung parenchyma after the use of gelatin resorcinol-formaldehyde-glutaraldehyde glue⁴ have been reported, the cytotoxicity of aldehydized dextran and ϵ -poly(L-lysine) is about 1/1500 that of formaldehyde or glutaraldehyde, or less.

In this study, new-glue was recognized to possess some desirable properties as a lung sealant. First, its bursting pressure was significantly higher than that of fibrin glue when applied by rubbing and spraying. Previous experimental studies documenting the effectiveness of fibrin glue and other tissue adhesives for sealing air leakages were performed on relatively small pulmonary defects.^{4,5,10-16} Clinically, however, large pleuroparenchymal defects are often created by segmentectomy, synechotomy, or interlobar tran-

Figure 5. Macroscopic and histologic appearance 2, 3, and 6 months after application of new-glue. At 2 months, new-glue had considerably lightened (A), and inflammatory reactions around it were slight (B). At 3 months, about 90% of the glue had degraded (C), and scattered fragments of residual glue were evident (D). By 6 months, complete disappearance of the glue had not occurred (E), and no inflammatory reaction was observed around the slight amount of residual glue (F) (hematoxylin and eosin stain; original magnification $\times 100$; scale bar shows 200 μm).

section. The mean air leakage pressure of 38.4 cm H₂O used in our large pleuroparenchymal defect model would be within the range of the increase in airway pressure caused by coughing. Furthermore, the appearance of seal breakage was pinhole-like for new-glue but extensive for fibrin glue. Fibrin glue often peels off after the lung is inflated because of its low bonding strength and low flexibility. The use of new-glue would shorten the period of chest tube drainage. Second, the time required for onset of new-glue gel formation was about 10 seconds, which allowed sufficient time to rub the glue into the surface of the lung parenchyma. This is in contrast to the immediate gel formation by fibrin glue, making it impossible to rub the mixed solutions into the lung surface. Third, new-glue had sufficient flexibility, which is an indispensable characteristic for a lung sealant. Fourth, neither adhesion nor infection was observed around areas where new-glue had been applied.

Previously, we had adopted another conventional animal pleuroparenchymal defect model to evaluate the biodegradability and histotoxicity of glues. However, this model had two major problems. First, the glues, especially fibrin glue, peeled off during measurement of the seal breaking pressure. Reapplication of the glues for air sealing led to an increased glue volume, making it difficult to evaluate the biodegradability of

the glues accurately. Therefore, we used separate animal models for measurement of bursting pressure and for evaluation of glue biodegradability. Second, thermal injury caused by electrocautery for hemostasis, such as destruction of alveoli and severe infiltration of fibroblasts in the lung parenchyma, was inevitable, making it difficult to evaluate the histotoxicity of the glues accurately. To overcome these problems, we²⁰ established a new method for creating a uniform pleural defect model for studies of lung sealants, and we applied this animal model in the present experiment to evaluate the histotoxicity of the glues.

Histologically, penetration of new-glue into the lung parenchyma was almost equivalent to that of fibrin glue. One layer of mesothelial cells covered new-glue by 2 weeks. Infiltration of inflammatory cells around the glue was more than that around fibrin glue. Inflammatory reactions were reduced by 4 weeks, and about 90% of new-glue had degraded on the lung surface by 3 months after application. The normal structure of the pleura and lung parenchyma had recovered by 6 months. Fibrin glue, on the other hand, showed rapid degradation and had completely disappeared by 4 weeks.

The histologic findings indicated that air leakage would not recur after 4 weeks, because by that time

Figure 6. Macroscopic and histologic appearance at 1 hour and 2 and 4 weeks after application of fibrin glue. Fibrin glue was applied by the rubbing and spraying method (A) and penetrated into the lung parenchyma (B). At 2 weeks, glue with a whitish appearance was clearly evident at the center of the application area (C) and was encapsulated by a thick fibrocellular layer. Infiltration of lymphocytes and fibroblasts was observed around the glue (D). At 4 weeks, the application area was covered by whitish colored pleura (E), which was thickened with fibrous tissue and slight infiltration of lymphocytes (F) (hematoxylin and eosin stain; original magnification $\times 100$; scale bar shows 200 μm).

new-glue or fibrin glue had been completely covered by thick fibrous tissue. Therefore, absorption of a lung sealant by tissues after 4 weeks may be desirable, because any remnant material might induce an unfavorable foreign body reaction such as inflammation or infection. An in vitro experiment showed that new-glue was degraded in phosphate-buffered saline within about 4 weeks.¹⁹ However, new-glue did not disappear completely from the lung surface until 6 months after application, even though it had become covered by one layer of mesothelial cells by 2 weeks. Differences in the fluid environment surrounding the glue might influence the rate of bioabsorption. However, new-glue seemed to have sufficient biocompatibility for clinical use during the absorption process, because there was only a minimal inflammatory reaction around the area of application.

Conclusions

Our new biodegradable hydrogel glue had sufficient sealing properties for preventing air leakage from large pleuroparenchymal defects. New-glue was significantly superior to fibrin glue as a sealant in this beagle model. It showed slower in vivo degradation and a slightly more inflammatory reaction in the early stage than did fibrin glue, although the normal lung

structure had recovered without fibrosis by 6 months. We believe that new-glue may be an alternative to fibrin glue for the control of air leakage during lung surgery.

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References

- Allen MS, Wood DE, Hawkinson RW, Harpole DH, McKenna RJ, Walsh GL, et al. Prospective randomized study evaluating a biodegradable polymeric sealant for sealing intraoperative air leaks that occur during pulmonary resection. *Ann Thorac Surg.* 2004;77:1792-801.
- Belboul A, Dernevik L, Aljassim O, Skrbic B, Radberg G, Roberts D. The effect of autologous fibrin sealant (Vivostat) on morbidity after pulmonary lobectomy: a prospective randomised, blinded study. *Eur J Cardiothorac Surg.* 2004;26:1187-91.
- Wain JC, Kaiser LR, Johnstone DW, Yang SC, Wright CD, Friedberg JS, et al. Trial of a novel synthetic sealant in preventing air leaks after lung resection. *Ann Thorac Surg.* 2001;71:1623-8.
- Nomori H, Horio H, Morinaga S, Suemasu K. Gelatin-resorcinol-formaldehyde-glutaraldehyde glue for sealing pulmonary air leaks during thoracoscopic operation. *Ann Thorac Surg.* 1999;67:212-6.
- Macchiarini P, Wain J, Almy S, Darteville P. Experimental and clinical evaluation of a new synthetic, absorbable sealant to reduce air leaks in thoracic operations. *J Thorac Cardiovasc Surg.* 1999;117:751-8.
- Morikawa T, Katoh H. Improved techniques of applying fibrin glue in lung surgery. *Eur Surg Res.* 1999;31:180-6.

7. Kawamura M, Gika M, Izumi Y, Horinouchi H, Shinya N, Mukai M, et al. The sealing effect of fibrin glue against alveolar air leakage evaluated up to 48 h; comparison between different methods of application. *Eur J Cardiothorac Surg.* 2005;28:39-42.
8. Wong K, Goldstraw P. Effect of fibrin glue in the reduction of postthoracotomy alveolar air leak. *Ann Thorac Surg.* 1997;64:979-81.
9. Fleisher AG, Evans KG, Nelems B, Finley RJ. Effect of routine fibrin glue use on the duration of air leaks after lobectomy. *Ann Thorac Surg.* 1990;49:133-4.
10. Nomori H, Horio H, Suemasu K. Mixing collagen with fibrin glue to strengthen the sealing effect for pulmonary air leakage. *Ann Thorac Surg.* 2000;70:1666-70.
11. Otani Y, Tabata Y, Ikada Y. Sealing effect of rapidly curable gelatin-poly (l-glutamic acid) hydrogel glue on lung air leak. *Ann Thorac Surg.* 1999;67:922-6.
12. Ono K, Ishihara M, Ozeki Y, Deguchi H, Sato M, Saito Y, et al. Experimental evaluation of photocrosslinkable chitosan as a biologic adhesive with surgical applications. *Surgery.* 2001;130:844-50.
13. Feito BA, Rath AM, Longchampt E, Azorin J. Experimental study on the in vivo behaviour of a new collagen glue in lung surgery. *Eur J Cardiothorac Surg.* 2000;17:8-13.
14. Herget GW, Kassa M, Riede UN, Lu Y, Brethner L, Hasse J. Experimental use of an albumin-glutaraldehyde tissue adhesive for sealing pulmonary parenchyma and bronchial anastomoses. *Eur J Cardiothorac Surg.* 2001;19:4-9.
15. Kobayashi H, Sekine T, Nakamura T, Shimizu Y. In vivo evaluation of a new sealant material on a rat lung air leak model. *J Biomed Mater Res.* 2001;58:658-65.
16. Ranger WR, Halpin D, Sawhney AS, Lyman M, Locicero J. Pneumostasis of experimental air leaks with a new photopolymerized synthetic tissue sealant. *Am Surg.* 1997;63:788-95.
17. Tsuda T, Nakamura T, Yamamoto Y, Teramachi M, Kiyotani T, Lee YH, et al. Prevention of postoperative air leakage from lungs using a purified human collagen membrane-polyglycolic acid sheet. *Ann Thorac Surg.* 1999;68:339-42.
18. Kjaergard HK, Pedersen JH, Krasnik M, Weis-Fogh US, Fleron H, Griffin HE. Prevention of air leakage by spraying vivostat fibrin sealant after lung resection in pigs. *Chest.* 2000;117:1124-7.
19. Nakajima N, Sugai H, Tsutsumi S, Hyon SH. Self-degradable bioadhesive. *Key Eng Mater.* 2007;342-343:713-6.
20. Araki M, Tao H, Sato T, Nakajima N, Hajime S, Hyon SH, et al. Creation of uniform pleural defect model for the study of lung sealants. *J Thorac Cardiovasc Surg.* 2007;134:145-51.
21. Kawamura M, Sawafuji M, Watanabe M, Horinouchi H, Kobayashi K. Frequency of transmission of human parvovirus B19 infection by fibrin sealant used during thoracic surgery. *Ann Thorac Surg.* 2002;73:1098-100.