In Vitro Comparison of Two Widely Used Surgical Sealants for Treating Alveolar Air Leak

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Abstract

Background Controversies surrounding the efficacy of sealants against alveolar air leak (AAL) are abundant in the literature. We sought to test the widely used sealants, TachoSil (Takeda Pharmaceutical Company Limited, Osaka, Japan) and BioGlue (CryoLife Europa Ltd., Surrey, United Kingdom) in an in vitro model.

Materials and Methods After creation of a focal superficial defect (40 × 25 mm) in swine lungs ($n = 40$), AAL was assessed with increasing inspired tidal volume (TVi). Upon sealant application in a randomized order, AAL was assessed in the same way until sealant burst.

Results At TVi = 400, 500, 600, and 700 mL, BioGlue achieved sealing in 19, 19, 16, and 14 tests, while TachoSil sealed in 19, 14, 4, and no test, respectively. The maximally tolerated pressure of BioGlue was higher than TachoSil (40.3 ± 3.0 vs. 36.0 ± 4.9 cm H_2O, $p = 0.003$). Cohesive and adhesive failures were found in 10 and 1 tests of BioGlue, respectively, while all burst failures of TachoSil were adhesive. Concerning elasticity, TachoSil allowed more expansion of the covered defect than BioGlue (6.3 ± 3.9 vs. 1.4 ± 1.0 mm, $p < 0.001$).

Conclusion The tested sealants demonstrated high sealing efficacy. While BioGlue was superior in resisting higher ventilation pressure, TachoSil possessed better elasticity.

Keywords
- experimental
- lung
- air leak
- sealant

Introduction

Alveolar air leaks (AAL) are common sequelae of lung surgery, particularly in pleural decortication, dissection of firm pleural adhesion, and division of incomplete fissures. They are associated with increased postoperative morbidities, delayed removal of chest tubes, and prolonged hospital stay.[1–3] In the surgical practice, the conventional closure techniques including suturing and stapling might not always be feasible, especially in video-assisted thoracic surgery (VATS) procedures. In the last two decades, various surgical sealants have been developed and widely practiced in lung surgery as adjuncts to conventional repairs.[4] The recent survey reveals that many surgeons choose surgical sealants based on individual experience and preference.[4] This is believed to, at least partly, be attributed to the limited data comparing the sealing efficacy of different sealants.

The sealing efficacy indicated by producers is usually determined by means of the modified industrial standard test method for burst pressure strength of surgical sealants (e.g., American Society for Testing and Maerials F 2392–04), which does not conform to the surgical reality. Databases on animal experiments are lacking, perhaps due to the ethical concerns.[3] The prospective, randomized clinical trials assessing sealants in treating AAL demonstrated very inconsistent results.[1,2] More importantly, the primary end points usually

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used in these trials (viz., the length of hospital stay and the duration of chest tube) are dependent on various factors besides significant AAL, limiting their reliability.

Recently, our group has developed an in vitro lung model assessing the sealing efficacy of various sealants.[5] Two widely used sealants—a human thrombin-fibrinogen sponge (TachoSil; Takeda Pharmaceutical Company Limited, Osaka, Japan) and an albumin-glutaraldehyde glue (BioGlue; CryoLife Europa Ltd., Surrey, United Kingdom)—were tested by means of this model. The results are hereafter reported.

Materials and Methods

Experimental Protocol

Lungs were freshly excised from German Landrace pigs (around 80 kg) without preference in gender, which were euthanized in a local slaughterhouse. Within 2 hours after harvest, the lungs were dissected along the trachea until the tracheal bifurcation was reached. The lower lobe was selectively intubated, followed by inflation through manual ventilation. The fully bloated lower lobe was subsequently immersed in warm water to ensure its impermeability. After being connected to the ventilation machine (Evita, Dräger, Lübeck, Germany), the lower lobe was ventilated in a volume-controlled mode with a positive end-expiratory pressure of 5 cm H_2O, I:E ratio of 1:2 and a ventilation frequency of 12/min. The lower lobe was fully inflated when inspired tidal volume (TVi) $\geq$ 400 mL. Overinflation was observed, if TVi $\geq$ 800 mL. To create a standardized superficial parenchymal lesion, a rectangle measuring 40 $\times$ 25 mm was first marked with a marker pen on the fully inflated lung lobe. Using a small, conic headed drill, the defect was created by carefully applying pressure to the marked site, starting at the edges and advancing toward the middle of the designated area (►**Fig. 1**). Surgical knots were then tied on the cranial and caudal edge of the lesion. Starting ventilation at TVi = 300 mL, TVi was increased by 100 mL in steps until a maximal inspiratory pressure (Pmax) of 40 cm H_2O was reached. Following each increase in TVi, the expiratory tidal volume (TVe), resistance, compliance, as well as Pmax, mean inspiratory pressure (Pmean), and plateau inspiratory pressure (Pplat) were recorded after five cycles. AAL was calculated as the difference between TVi and TVe.

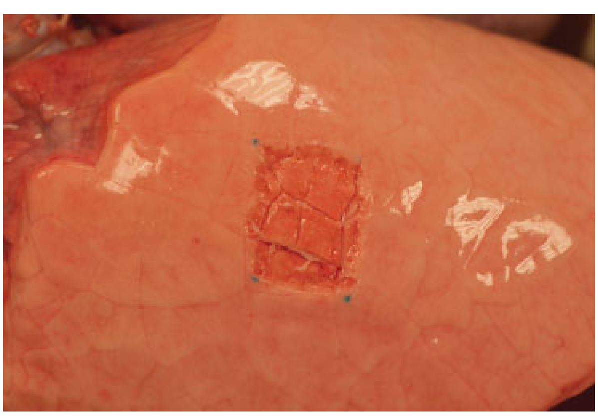

Fig. 1 A superficial lung defect was created in a previously marked area of 40 $\times$ 25 mm on the inflated lower lobe.

Thereafter, TachoSil and BioGlue were applied in a randomized order ($n = 20$ in each group) according to the usage guide, respecting a safety margin of 1 cm to all sides. TachoSil sponge (9.5 $\times$ 4.8 cm) was shortly humidified in isotone saline and subsequently placed on the pleural defect of inflated lung lobe. Using a moist bandage, mild pressure was applied for a period of 5 minutes to the sealant sponge to ensure full adhesion according to the usage guide of the producer. As to BioGlue, pleural defect was dried before the sealant application. In a slow and steady manner, 2 mL liquid sealant was then applied on the pleural defect through the primed applicator tip, resulting in an even coating. Before the tests, a full 2 minutes was given to allow BioGlue to achieve the full strength according to the usage guide. The lower lobe was then ventilated again with TVi rising slowly from 100 mL. Commencing at 400 mL TVi, the same parameters as before sealant application were measured after every increase in TVi by 100 mL. The distance between the two sutures was measured to evaluate the elasticity of sealants.

Air leak at the site of pleural defect was assessed in submersion tests and graded following the Macchiarini scale as grade 0 (no leak), grade 1 (countable bubbles), grade 2 (stream of bubbles), and grade 3 (coalesced bubbles).[6] Sealing was considered, if no bubble was visible after five cycles of ventilation (grade 0). Sealing failure was determined once air bubbles were observed (grade 1 or higher). If sealing failure occurred, Pmax of the last ventilation cycle was determined as the maximally tolerated pressure (MTP). The sealing failure was furthermore categorized into adhesive or cohesive failure. Adhesive failure was considered, if the failure occurred at the interface between sealant and parenchymal defect. Cohesive failure was defined as the failure within the sealant. Application failure was considered, when cohesive or adhesive failure of sealant was observed before starting the test at 400 mL TVi. The results were adjusted by two investigators independently. Any disagreement would be arbitrated by a third investigator.

Finally, the lung specimens containing the attached sealant were resected and fixed with 10% formalin. The specimens were further embedded in paraffin, and processed to obtain sections for hematoxylin-eosin staining. The tests were performed under room temperature and moderate air humidity. The experimental procedures have been established in the previous study.[5]

Statistical Analysis

The normality of variables was tested by the Kolmogorov–Smirnov one-sample test. Descriptive statistics are presented as mean $\pm$ standard deviation in case of normal distribution. Categorical variables are expressed as percentages. Continuous data were compared using Student *t*-test. Multiple linear regression analysis was used to determine the ventilation parameters associated with AAL. Statistical significance was assumed if $p < 0.05$. All statistical evaluation was performed using SPSS (version 16.0 for Windows; SPSS, Inc., Chicago, Illinois, United States).

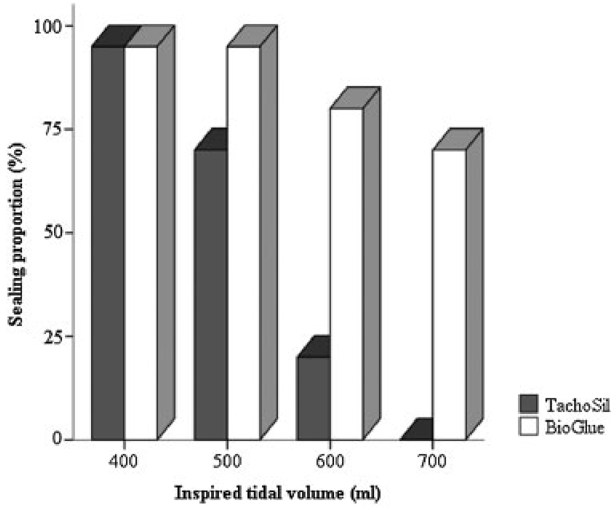

Fig. 2 Sealing proportion of TachoSil and BioGlue with ascending TVi.

Results

A total of 40 consecutive tests were performed in the present experiment. In the assessment before sealant applications, AAL increased linearly with ascending TVi. Among the recorded ventilation parameters, Pmax was the only parameter predictive of AAL in the multiple linear regression analysis ($p < 0.001$).

Sealant application failure occurred once in each group. There was no disagreement on the adjustment of air tightness in the submersion tests between the investigators. At TVi = 400, 500, 600, and 700 mL, BioGlue achieved sealing in 19 (100%), 19 (100%), 16 (84.2%), and 14 (73.7%) tests, while TachoSil sealed in 19 (100%), 14 (73.7%), 4 (21.1%), and no (0%) tests, respectively (p = NS, $p = 0.04$, $p < 0.001$, $p < 0.001$). ►**Fig. 2** demonstrates the sealing proportion in the two groups. The MTP of BioGlue was 40.3 ± 3.0 cm H_2O, significantly higher than that of TachoSil (36.0 ± 4.9 cm H_2O, $p = 0.003$). Cohesive and adhesive failures were found in 10 and 1 tests in the BioGlue group respectively, while all burst failures of TachoSil were adhesive. Concerning elasticity, TachoSil allowed more expansion of the covered lung defect than BioGlue (6.3 ± 3.9 vs. 1.4 ± 1.0 mm, $p < 0.001$, ►**Fig. 3**). Hematoxylin-eosin staining of the sealed lung specimen showed the TachoSil sponge and the BioGlue foam attaching densely to the underlying lung surface (►**Fig. 4**).

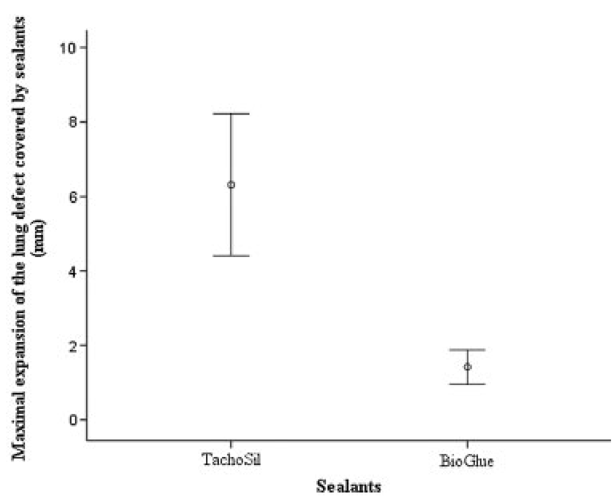

Fig. 3 Expansion of the lung defects covered by TachoSil and BioGlue.

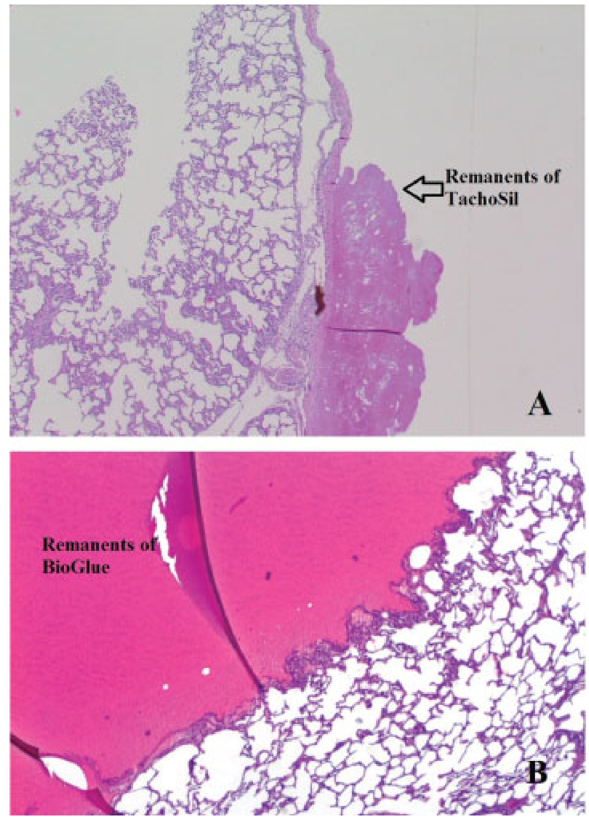

Fig. 4 Photomicrograph of histological sections showed the sealants layer attaching densely the underlying lung tissue (A: TachoSil, B: BioGlue).

Discussion

In treating AAL, surgical sealants are an important element in the armamentarium of thoracic surgeons. However, the multitude of clinical trials assessing the efficacy of various sealants demonstrated inconsistent results and presented often limited validity due to small sample size and inherent flaws of study design.[1,5] In addition, there are very few comparative clinical trials in this regard. As more and more sealants with different properties are available, questions have arisen concerning the purported overall clinical benefits. In this respect, it is pivotal to compare the sealing efficacy of different sealants and find the most effective one.

In our study, both TachoSil and BioGlue presented strong sealing efficacy in treating AAL. The MTP of these two sealants are close to the upper limit of the commonly applied ventilation pressure (40 cm H_2O). When compared with TachoSil, BioGlue demonstrated significantly higher MTP, thus the superior sealing efficacy. Moreover, adhesive burst failure was found only in 1 out of 19 tests with BioGlue, while all burst failures of TachoSil sponges were adhesive. BioGlue consists of the bovine serum albumin (BSA) as the active chemistry and the glutaraldehyde as the connector molecule. The aldehyde groups of glutaraldehyde react with the amine groups of BSA and those present in the extracellular matrix and cell surface, resulting in strong cross-linking after 2 to 3 minutes.[7] In contrast, TachoSil is a thin collagen patch sponge coated with dried human fibrinogen and human thrombin.[8] Upon application, fibrinogen is activated by thrombin and converted into fibrin monomers, which subsequently form a highly concentrated and stable fibrin clot.[9] According to our results, the adhesive strength of fibrin clot, which holds the TachoSil sponge tightly to the wound surface, appears lower than that of the cross-links in BioGlue. This finding is consistent with the results of the previously published in vitro experiments. Pedersen et al created pleural defect with a diameter of 10 mm in harvested porcine lungs and assessed the sealing efficacy of diverse surgical sealants including TachoSil and BioGlue in submersion tests. The recorded burst pressure of TachoSil (35 cm H_2O) was lower than that of BioGlue (55 cm H_2O).[10] Of interest is also the in vitro experiment of Carbon et al, in which a piece of porcine lung tissue containing a standardized defect with a diameter of 10 mm was clamped in the flange over the top of a pressure chamber.[11] Pressure was applied in the chamber till the sealant material was lifted off or destroyed. In this lung model, TachoSil achieved a burst pressure of 51.2 cm H_2O (50.2 hPa).

Another interesting finding of our study is the marked elasticity of TachoSil. This result is consistent with other clinical and experimental studies.[12] In a multicenter, prospective, and randomized study of 189 patients undergoing lobectomy, Lang et al tested the efficacy and safety of TachoComb (Takeda Pharmaceutical Company) as the processor of TachoSil for air leak treatment. They reported that the elastic properties of TachoComb was especially suitable for sealing of dynamically expanding systems like lung tissue.[8] In addition, Izbicki et al used TachoComb for treating diffused parenchymal bleeding and/or air leaks in 52 patients and parenchymal lesions in 12 procines.[12] They found that "the high elasticity of the fleece does not disturb lung extension movement." In contrast, BioGlue presented a very rigid nature. Good elasticity allows surgical sealants to adapt the movement of the lung surface after application. This biomechanical property is particularly important, if the lung is trapped due to chronic inflammatory process of the lung and pleura. In decortication surgery for re-expansion of the residual lung parenchyma, removal of the peel from the underlying parenchyma results almost inevitably in superficial tears and AAL.[13] A rigid sealant like BioGlue might prevent the re-expansion and worsen the stretchability of trapped lung. This disadvantage would be more apparent, if the longstanding resorption time of BioGlue (2 years) is taken into account.[14] Comparatively, TachoSil will be reabsorbed 4 to 5 months after application. In this patient subpopulation, TachoSil with its high sealing efficacy and marked elasticity appears to be the sealant of choice. In view of the higher MTP, BioGlue might be considered in favor over TachoSil for treating AAL in patients with underlying lung disease, which requires high inspiratory pressure. For instance, in patients with cystic fibrosis, surgical pleurodesis as an effective treatment for large and recurrent pneumothorax presents a relative contraindication for lung transplantation later.[15] Successful air leak sealing by topical application of BioGlue has been reported in cystic fibrosis patient.[16]

In our in vitro model, porcine lungs were used due to the similarity in design and biomechanical properties to human lungs.[11] The experimental procedures were constructed to simulate the real surgical and clinical setting of lung surgery. The MTP of TachoSil determined in our tests is comparable to that in the previously published animal experiment, suggesting the reliability of the present model.[3] Recently, Pedersen et al compared sealing efficacy of six different surgical sealants including TachoSil and BioGlue by means of another in vitro lung model.[10] They fixed harvested porcine lungs in a Plexiglas chamber filled with isotonic saline. After sealant application on deflated lungs, the lungs were ventilated with incremental peak airway pressure and air leaks were assessed by submersion tests. Their results demonstrated a median burst pressure of 35 cm H_2O in TachoSil group. Compared with our model, some issues may have complicated their study to the disadvantage of TachoSil. First, TachoSil was applied to deflated lungs, whereas the manufacturer recommends that lung should be inflated during application. The subsequent lung re-inflation and stretch may have impaired the bonding between fibrin sponge and lung surface. It is also worth noting that the time from lung harvest to experimentation averaged as long as 24 hours. The resulted degradation of proteins, especially the factor XIII being essential for the cross-linking of fibrin strands, may also have weakened the sealing efficacy of TachoSil.[9]

As VATS has been widely adopted and practiced in lung surgery, air leak sealing by means of topical sealant application through trocars has become a feasible approach.[16] On the contrary, prolonged air leaks are still one of the major complications after VATS major lung resections and result in prolonged hospital stay.[17] This illustrates the need for further improvement of air leak management during VATS procedures. Surgical sealants permitting a precise application to target sites thorugh trocars provide further advantage in these aspects. In the surgical practice, TachoSil sponge can be rolled and delivered through trocar easily. As liquid sealant, BioGlue can also be applicated thoracoscopically using a delivery tip extention.

Despite the strong sealing efficacy of TachoSil and BioGlue demonstrated in our in vitro tests, caution should be taken for potential side effects of these materials. While concern has been arised about the potential risk for transmission of bloodborne diseases by TachoSil and BioGlue derived from human or bovine blood plasma,[2–4,14] there have been report on the cytotoxic effects of BioGlue in in vitro and in vivo examinations.[18] Moreover, animal experiment has demonstrated that BioGlue reinforcement of aortic anastomoses impairs vascular growth.[19] In addition, Klimo et al found a strong association between the use of BioGlue and postoperative wound complications in their pediatric neurosurgical practice due to triggered acute pyogenic and chronic granulomatous inflammatory response.[20]

As one of the limitations of the present experiment, a certain variation in the size of ventilated procine lower lobes could not be totally avoided. To minimize this confounding feature, the lungs were harvested from the pigs in almost the same weight (around 80 kg). We did not observe marked difference in this regard during the tests. The lower lobes were fully inflated when TVi was 400 mL or higher in all tests. In addition, the randomization of the applied sealants might also contribute in reducing this bias. The authors recognize that the sealant applications were not blinded for the assessment of air tightness and the measurement of elasticity in the present experiment. It may have resulted in information bias, which was certainly minimized by randomization of the sealants. Finally, the observation bias might have arisen due to the inevitable subjectiveness in the judgment of air bubbles, even though it was performed by two investigators independently. Nevertheless, the statistic analysis revealed significant results in various aspects. The present experiment could make a positive contribution for the evidence-based sealant use in lung surgery.

In conclusion, our in vitro tests demonstrated the high sealing efficacy of TachoSil and BioGlue in treating AAL. While BioGlue was superior in resisting higher ventilation pressure, TachoSil possessed better elasticity. It is recommended to perform adequately powered and well-designed prospective clinical trials before a systematic sealant use.

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