Sealing effectiveness of a novel NHS-POx based patch: experiments in a dynamic *ex vivo* porcine lung

Bob P. Hermans[1]^, Wilson W. L. Li[1], Edwin A. Roozen[2], Daniël I. M. van Dort[1], Jort Evers[1], Erik H. F. M. van der Heijden[3], Stefan M. van der Heide[1], Harry van Goor[2], Ad F. T. M. Verhagen[1]

[1]Department of Cardio-Thoracic Surgery, Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands;
[2]Department of General Surgery, Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands;
[3]Department of Pulmonology, Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands

Contributions: (I) Conception and design: BP Hermans, WWL Li, EA Roozen, DIM van Dort, H van Goor, AFTM Verhagen; (II) Administrative support: BP Hermans, DIM van Dort; (III) Provision of study materials or patients: BP Hermans, DIM van Dort, EA Roozen; (IV) Collection and assembly of data: BP Hermans, DIM van Dort, EA Roozen, J Evers; (V) Data analysis and interpretation: BP Hermans, DIM van Dort, EA Roozen, WWL Li, H van Goor, AFTM Verhagen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Bob P. Hermans, BSc. Department of Cardio-Thoracic Surgery, Radboud University Medical Center, Radboud Institute for Health Sciences, Geert Grooteplein Zuid 10, 6525 GA, Nijmegen, The Netherlands. Email: Bob.Hermans@Radboudumc.nl.

Background: Sealants are used to prevent prolonged pulmonary air leakage (PAL) after lung resections (incidence 5.6–30%). However, clinical evidence to support sealant use is insufficient, with an unmet need for a more effective product. We compared a novel gelatin patch impregnated with functionalized polyoxazolines (NHS-POx) (GATT-Patch) to commercially available sealant products.

Methods: GATT-Patch Single/Double layers were compared to Progel®, Coseal®, Hemopatch® and TachoSil® in an *ex vivo* porcine lung model (first experiment). Based on these results, a second head-to-head comparison between GATT-Patch Single and Hemopatch® was performed. Air leakage (AL) was assessed in three settings using increasing ventilatory pressures (max =70 cmH$_2$O): (I) baseline, (II) with 25 mm × 25 mm superficial pleural defect, and (III) after sealant application. Lungs floating on saline (37 ℃) were video recorded for visual AL assessment. Pressure and tidal volumes were collected from the ventilator, and bursting pressure (BP), AL and AL-reduction were determined.

Results: Per sealant 10 measurements were performed (both experiments). In the first experiment, BP was superior for GATT-Patch Double (60±24 cmH$_2$O) compared to TachoSil® (30±11 cmH$_2$O, P<0.001), Hemopatch® (33±6 cmH$_2$O, P=0.006), Coseal® (25±13 cmH$_2$O, P=0.001) and Progel® (33±11 cmH$_2$O, P=0.005). AL-reduction was superior for GATT-Patch Double (100%±1%) compared to Hemopatch® (46%±50%, P=0.010) and TachoSil® (31%±29%, P<0.001), and also for GATT-Patch Single (100%±14%, P=0.004) and Progel (89%±40%, P=0.027) compared to TachoSil®. In the second experiment, GATT-Patch Single was superior regarding BP (45±10 *vs.* 40±6 cmH$_2$O, P=0.043) and AL-reduction (100%±11% *vs.* 68%±40%, P=0.043) when compared to Hemopatch®.

Conclusions: The novel NHS-POx patch shows promise as a lung sealant, demonstrating elevated BP, good adhesive strength and a superior AL-reduction.

Keywords: Air leak; lung sealant; lung surgery; biomaterials; *ex vivo*; experimental

^ ORCID: 0000-0001-8586-063X.

Introduction

Pulmonary air leakage (PAL) is an important concern for patients undergoing pulmonary resections. Prolonged PAL (pPAL), defined as PAL lasting longer than 5 days, affects 5.6–30% of patients (1). pPAL is the main reason for delayed chest tube removal, prolonged hospital stay and readmissions (2-4), while resulting in more complications including empyema (5), pneumonia (6,7) and mortality (8). Furthermore, 4.8% of patients with pPAL require additional interventions (extra chest tubes, bronchoscopy and reoperation) (6). In an attempt to prevent these postoperative consequences, various sealants have been developed for intraoperative use (9).

There is a broad variation in surgical management of air leaks. A recent study found significant between-hospital variation for pPAL incidence in the Netherlands (2.6–19.3% adjusted for case-mix), and also important variation regarding intraoperative PAL management, including sealant use (10). Available evidence supporting intraoperative sealant use in this setting is controversial at best, highlighted by a recent survey in which only 42% of surgeons believed enough evidence exists to support their sealant use (11). This is in line with a Cochrane review on this subject published in 2010 (12), that does not recommend the routine use of sealants due to unclear benefits, for example on length of hospital stay. However, this study pooled multiple products with varying modes of action into one analysis, but head-to-head comparisons between sealants are scarce (12,13). Considering some sealants might be more effective than others (14), a comparison of existing and novel products is warranted, to further substantiate sealant selection and use in current surgical practice. Furthermore, there is an unmet need for a single-component and more effective surgical sealant capable of sealing PAL in wet and dynamic biological environments, as stated by Brunelli (11).

To this end a novel patch based on porcine derived gelatin impregnated with synthetic polymers, may hold promise as a lung sealant. The gelatin-based patch contains poly (2-oxazolines) functionalized with N-hydroxysuccimide ester (NHS-POx) and nucleophilically activated polyoxazoline (NU-POx) (GATT Technologies BV, Nijmegen, The Netherlands) (15-18). After wet tissue contact, the reactive NHS side-chains will form covalent bonds with amines present on tissues, blood, NU-POx and the gelatin carrier itself. The polymers are further functionalized using hydroxyl groups for increased hydrophilicity to ensure adequate blood and water uptake, acting synergistically with coagulation mechanisms. This results in a strongly adhesive and cohesive polyoxazoline hydrogel with good sealing properties. Positive results have been obtained for hemostatic applications *in vivo* (15,17,18) and clinical trials are currently being performed to assess hemostatic efficacy on liver bleeding (ClinicalTrials.gov Identifier: NCT04819945 and NCT05385952).

In this study, we aim to investigate the aerostatic properties of this novel NHS-POx based patch in an *ex vivo* porcine lung model compared to other commonly used lung sealants, by measuring bursting pressure (BP), air leak (AL) reduction and conformability to the lung surface. While the ARRIVE reporting checklist is designed to improve the reporting of *in vivo* studies, we decide to follow the ARRIVE 2.0 reporting checklist in this *ex vivo* study considering many applicable items for better reporting in the study design, statistical analysis and results (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1821/rc).

Methods

Study setup

We performed an *ex vivo* study in a dynamic porcine lung model. First, a multigroup comparison was undertaken evaluating the novel patch applied in two ways (GATT-Patch Single and GATT-Patch Double) compared to Progel® (C. R. Bard, Inc., Murray Hill, New Jersey, USA),

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Highlight box

Key findings

• The novel gelatin patch impregnated with functionalized polyoxazolines exhibits good adhesive strength, better reduction of air leaks and elevated bursting pressures in a dynamic *ex vivo* porcine lung model.

What is known and what is new?

• Current lung sealants are insufficient for prevention of prolonged pulmonary air leakage, and better products are needed.

• The novel lung sealant patch is promising for further research and development, as compared with currently used products (TachoSil®, Hemopatch®, Progel®, Coseal®).

What is the implication, and what should change now?

• The novel patch shows promise as a lung sealant due to favourable mechanical properties. Further *in vivo* safety and efficacy studies should now be performed, in preparation for clinical use.

Coseal® (Baxter International Inc., Deerfield, Illinois, USA), Hemopatch® (Baxter International Inc., Deerfield, Illinois, USA), and TachoSil® (Takeda Pharmaceutical Company Limited, Tokyo, Japan). Sample size (N=10 per group) was chosen based on previous reported experiments (14). GATT-Patch Single/Double and Hemopatch® groups were randomized (Microsoft Excel, Aselect function) and blinded to the allocated group until the sealant was handed to the researcher, to mitigate unconscious influences on lesion creation. All GATT-Patches were provided by the manufacturer to the researchers, and all control products were commercially ordered.

Secondly, following this multigroup comparison, we performed a randomized head-to-head comparison of the novel GATT-Patch Single compared to the best product with the same mode of application (Hemopatch®). This experiment was undertaken in a similar randomized and blinded fashion after a formal sample size calculation (power =0.8, two-sided alpha =0.05, N=10 per group).
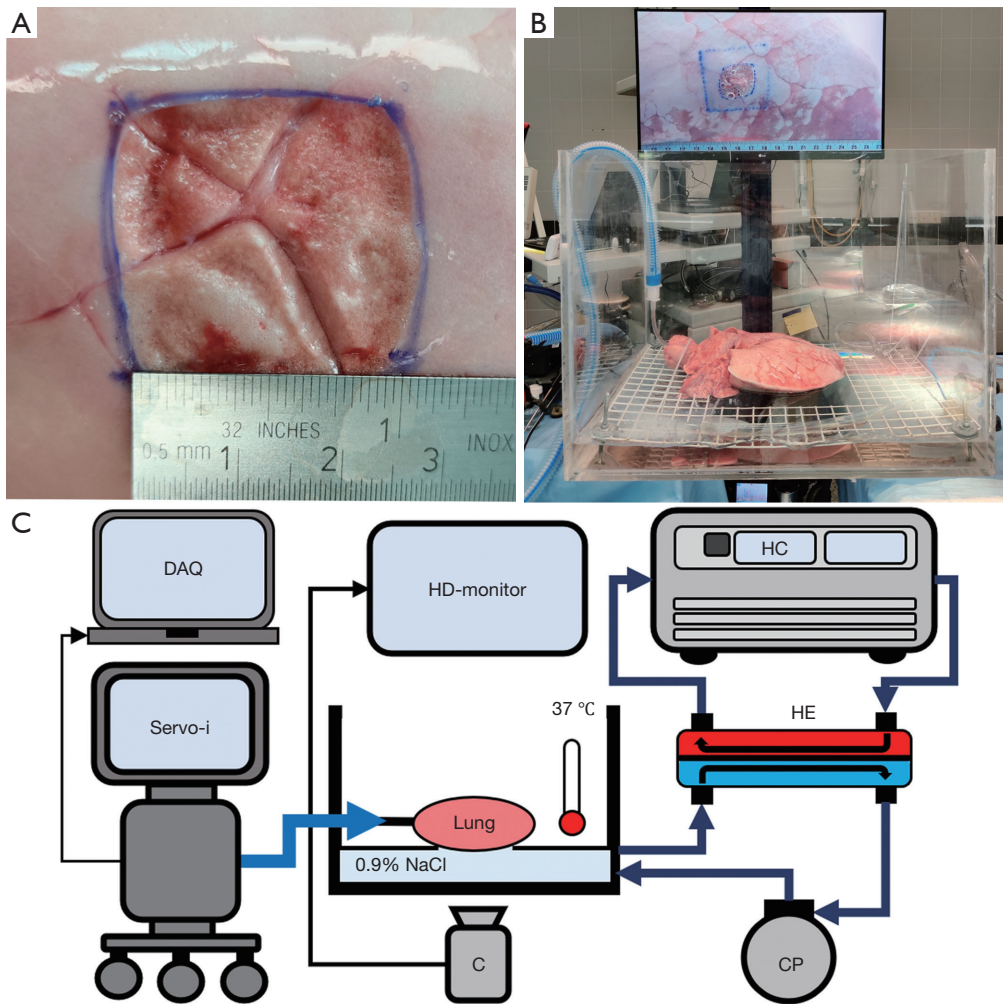
Porcine lung model

No live animals were involved in experimental procedures, and all experiments were performed on *ex vivo* porcine lung tissue obtained from a slaughterhouse. Lungs from domestic pigs, used for human consumption and weighing between 90–100 kg, were excised in the slaughterhouse, and put on ice as soon as possible for transport. All excess tissue was removed and sutures were used to prevent blood leaking in the setup. Caudal lobes were suctioned and selectively intubated (6.0 mm endo-tracheal tube). Lungs were inflated manually, followed by clamping of the tube. Airtightness was ensured before use. Lobes with poor compliance or persistent atelectasis (as visually assessed) were rejected. Rewarming of the included porcine lungs was started approximately fifteen minutes before measurements.

Specimens were examined in a clear container containing 10 L 0.9% saline (NaCl) kept at 37 ℃. A camera positioned under the container and a ruler positioned at water level were used to record PAL and surface area expansion (SAE) (*Figure 1* and *Video 1*). Airway pressures were recorded from the mechanical ventilator (Appendix 1), and synchronized to the video data by pressing on the lung (visible on both datafiles). Tidal volumes were noted from the mechanical ventilator at the end of each measurement cycle to calculate AL.

Study protocol and outcome measures

Study outcomes were collected during three separate measurement cycles: (I) before and (II) after creating a standardized superficial pleural lesion, and (III) after sealant application. All study outcomes and definitions are presented in *Table 1*. To ensure standardized study measurements, porcine lung specimen were ventilated under increasing pressures with pressure control ventilation [initial settings: respiratory rate 12/min, inspiratory:expiratory ratio 1:2, positive end-expiratory pressure (PEEP) 5 cmH$_2$O and pressure above PEEP 5 cmH$_2$O]. Each measurement cycle, the plateau ventilatory pressure (Pplat) was re-started at the initial settings and increased with 5 cmH$_2$O every 90 seconds. Pressure was increased until a maximum of 40 cmH$_2$O for cycles (I) and (II) and 70 cmH$_2$O or upon reaching of grade III leakage [*Table 1*, Macchiarini scale (19)] during cycle (3). These measurement cycles were conducted as follows: (I) baseline SAE measurements were performed after marking a 5 cm × 5 cm area at static lung inflation of 5 cmH$_2$O PEEP on a dorsal non-atelectatic segment of the caudal lobe and measuring 'Baseline SAE', 'Lung compliance' and 'Baseline AL'. The SAE is defined as the percentual increase of this previously marked area at 40 cmH$_2$O with respect to 5 cmH$_2$O as a measure for lung surface expandability (see *Figure 1B*); (II) Defect AL measurements were performed after creating a standardized superficial pleural lesion (2.5 cm × 2.5 cm, *Figure 1A*) using a sanding wheel (Dremel Lite 7760) and tweezers/scissors at static lung inflation of 10 cmH$_2$O PEEP. The lesion was made at the minimum depth required to remove the superficial pleura and expose the underlying parenchyma. Measurements included 'Baseline leaking pressures' [using the Macchiarini scale (19)] and 'Defect AL'; (III) BP measurements were performed after sealant application, applying all patches at 5 cmH$_2$O PEEP static lung inflation and gels on a deflated lung (*Table 2*). In case of atelectasis following sealant application, 15–25 cmH$_2$O PEEP was administered for 5–10 s. Measurements included 'BP', 'AL-reduction', 'Mode of failure' and 'expansion area change (EAC)'. BP was defined as the pressure setting at which the first bubbles were visible (i.e., grade I AL), and for values higher than 70 cmH$_2$O, 75 cmH$_2$O was noted as an off-scale value. EAC is the measure of sealant conformability to the lung, defined as the proportion SAE post sealant application with respect to SAE pre sealant application (*Table 1*).

Figure 1 Overview of *ex vivo* porcine lung model. (A) Standardized pleural defect (25 mm ×25 mm), made at static lung inflation pressure of 10 cmH$_2$O. (B) Experimental setup. (C) Schematic of experimental setup. DAQ, data-acquisition setup; Servo-i, mechanical ventilator; HD, high definition; NaCl, physiological saline solution; C, camera; HC, heater-cooler; HE, heat-exchanger; CP, centrifugal pump.
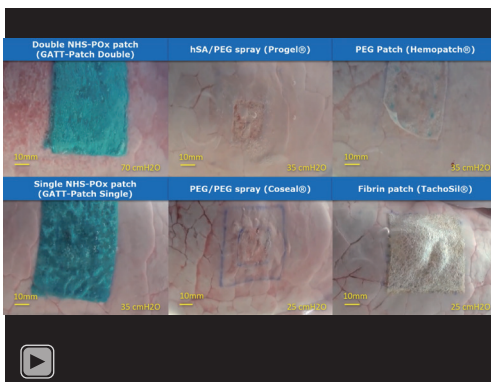
Video 1 Failure mechanisms of all sealant groups in the first experiment.

Recorded video data was used for independent assessment of leaking pressures and BP by two investigators, noting the timepoints when different leaking grades occurred in the videos (*Table 1*), followed by reaching consensus and synchronization of the corresponding pressure values. Assessors were blinded to the assigned group for the baseline videos, but not for the sealed samples due to obvious differences in product appearance.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (Armonk, New York, USA; IBM

Table 1 Data collection and processing

Variable	Definition
Measurement cycle 1: baseline SAE	
Baseline SAE	Surface area at 40 cmH$_2$O/surface area at 5 cmH$_2$O (ImageJ, version 1.53a, Wayne Rasband)
Baseline AL	Calculated at Pplat =40 cmH$_2$O, as "12x(TVi-'expiratory tidal volume') (read from the mechanical ventilator user interface)
	AL measured during 'Baseline SAE' measurements was subtracted from 'Defect AL' and 'BP measurements' to correct for circuit leakage and bias flow
Lung compliance	TVi at 40 cmH$_2$O/35
Ischemic time	Time from arrival at laboratory until start of experiments
Measurement cycle 2: defect AL	
Baseline leaking pressures	Macchiarini scale (grades: I = countable bubbles, II = stream of bubbles, III = coalesced bubbles) (16), assessed by two investigators
Defect AL	See 'Baseline AL'
Surface and water temperature	Measured before sealant application
Measurement cycle 3: BP measurements	
Sealed leaking pressures	See 'Baseline leaking pressures'
	BP = pressure at which first AL occurred (grade I)
AL-reduction	BP measurements AL: see 'Baseline AL'
	100%x [(Defect AL–BP measurements AL)/Defect AL], capped at 0 and 100% (calculated at Pplat =40 cmH$_2$O). Always noted as 100% if BP >40 cmH$_2$O
EAC	Sealed SAE: see 'Baseline SAE'
	EAC = Sealed SAE/Baseline SAE
	EAC =100% indicates perfect conformability of the sealant to lung expansion, EAC <100% indicates reduced SAE after sealant application compared to baseline (Appendix 1)
Mode of failure	Adhesive failure (leakage between sealant and lung; *Figure 3D*)
	Cohesive failure (leakage through the sealant; *Figure 3F*)

SAE, surface area expansion; AL, air leak; Pplat, plateau ventilatory pressure; TVi, inspiratory tidal volume; BP, bursting pressure; EAC, expansion area change.

Corp). Kolmogorov-Smirnov test was used for assessment of data distribution. Non-normally distributed data and normally distributed data were presented as median ± interquartile range (IQR) or mean ± standard deviation (SD) respectively. For the first experiment, Kruskal-Wallis test (non-parametric) or analysis of variance (parametric) were used. Bonferroni correction was used during post-hoc analysis (pairwise). For the second experiment, Mann-Whitney-U test (non-parametric) or independent samples *t*-test (parametric) were used. Fischer's exact test was used for categorical data. Missing data was analyzed by pairwise

exclusion. Tests were performed two-tailed and the null-hypothesis was rejected if P<0.05.

Results

First experiment

We performed 60 measurements on 60 lungs, 10 for each group of sealants. Baseline characteristics were similar between groups (*Table 3*). Four measurements had missing or incomplete video/pressure data, due to technical errors (GATT-Patch Double and Single, TachoSil® and Coseal®).

Table 2 Modes of application

Sealant	Application
TachoSil® (4.8x4.8 cm)	Saline gauzes: 3-minute compression
Hemopatch® (4.5x4.5 cm)	SBB lung priming
	Dry gauzes: 2-minute compression
GATT-patch single (5x5 cm)	SBB lung priming
	Diluted SBB gauzes: 2-minute compression + 60 mL diluted SBB irrigation
GATT-patch double (5x5 cm)	SBB lung priming, apply first patch
	Apply second patch perpendicularly with 60 mL SBB irrigation between patches
	Saline gauzes: 2-minute compression + 60 mL saline irrigation
Coseal® (2 mL)	Spray marked area, dry 60 seconds
Progel® (2 mL)	Spray marked area, dry 120 seconds

SBB, sodium-bicarbonate buffer (pH 8.3–8.5); diluted SBB, SBB with saline (1:2).

One measurement had missing tidal volumes for baseline AL measurements (GATT-Patch Double).

GATT-Patch Double (60 ± 24 cmH$_2$O) showed significantly higher BP compared to Progel® (33 ± 11 cmH$_2$O, P=0.005), Coseal® (25 ± 13 cmH$_2$O, P=0.001), Hemopatch® (33 ± 6 cmH$_2$O, P=0.006) and TachoSil® (30 ± 11 cmH$_2$O, P<0.001), but no significant differences in BP compared to GATT-Patch Single (40 ± 10 cmH$_2$O) (P>0.99). Between GATT-Patch Single, Progel®, Coseal®, Hemopatch® and TachoSil® no significant differences in BP were found (*Figure 2A*, *Table 3*). AL-reduction was significantly better for GATT-Patch Double compared to Hemopatch® (P=0.010) and TachoSil® (P<0.001). GATT-Patch Single (P=0.004) and Progel® (P=0.027) showed significantly better AL-reduction compared to TachoSil® (*Figure 2B*, *Table 3*).

The EAC was significantly better for Coseal® ($98\%\pm13\%$) compared to TachoSil® ($80\%\pm11\%$, P=0.023), GATT-Patch Single ($77\%\pm9\%$, P=0.005) and GATT-Patch Double ($76\%\pm9\%$, P=0.004) (*Table 3*). Cohesive failure was observed more often for GATT-Patch Double, GATT-Patch Single, Progel® and Coseal®, while adhesive failure was seen more frequently for Hemopatch® and TachoSil® (P<0.001) (*Table 3*, *Figure 3*, *Video 1*). Interestingly, different failure mechanisms were observed: progression until grade III leakage was generally more gradual for GATT-Patch Double, GATT-Patch Single, Progel® and Coseal®, requiring one or more pressure intervals to reach grade III leakage. For Hemopatch® and TachoSil®, grade III leakage was usually reached in the same pressure interval where first leakage occurred (*Table 3*).

Second experiment

Twenty measurements were performed on 20 lungs, 10 for each group. Despite adequate randomization, baseline AL was found to be significantly higher in the GATT-Patch Single group compared to the Hemopatch group (1.5 ± 0.8 *vs.* 0.8 ± 0.5 L/min, P=0.03). Other baseline characteristics were not significantly different (Table S1). Despite the larger baseline AL, BP for GATT-Patch Single (45 ± 10 cmH$_2$O) was significantly higher compared to Hemopatch® (40 ± 6 cmH$_2$O, P=0.043) (*Figure 4A*). AL-reduction also reached statistical significance (P=0.043) (*Figure 4B*). Hemopatch® showed only adhesive failure and GATT-Patch Single only cohesive failure (P<0.001). EAC measurements were not performed due to extensive adhesive failure of Hemopatch®.

Discussion

This *ex vivo* experimental study demonstrated favorable mechanical properties for the novel NHS-POx based lung sealant (GATT-Patch). The patch exhibited excellent adhesive characteristics to the lung parenchyma, withstanding repetitive lung expansions in a wet and dynamic *ex vivo* lung environment without tearing or debonding until pressures of at least 30–40 cmH$_2$O.

**Table 3** First experiment baseline characteristics and outcomes

Characteristics	Patch sealants				Spray sealants		P value
	GATT-Patch double	GATT-Patch single	Hemopatch®	TachoSil®	Progel®	Coseal®	
Baseline characteristics							
Sample characteristics							
Ischemic time (minutes)[†]	238±141	233±105	119±66	215±158	213±102	226±164	0.32
Left lower lobe, n [%]	8 [80]	9 [90]	5 [50]	7 [70]	6 [60]	9 [90]	0.33
Physical characteristics							
Surface temperature (℃)[†]	31.2±1.2	31.8±1.6	31.9±1.3	31.7±2.2	33.0±1.0	31.7±1.2	0.15
Water temperature (℃)[‡]	37.2±0.5	37.2±0.3	37.3±0.3	37.2±0.5	37.2±0.5	37.1±0.2	0.49
Baseline SAE (%)[‡]	152±13	149±25	153±18	148±20	153±35	151±15	>0.99
Lung compliance (mL/cmH$_2$O)[‡]	24±7	23±7	27±2	26±7	24±6	26±5.0	0.30
Leakage characteristics							
Baseline leaking pressures							
Grade I (cmH$_2$O)[‡]	10±5	13±11	10±5	15±5	10±1	13±5	0.15
Grade II (cmH$_2$O)[‡]	15±3	18±15	15±1	20±10	18±5	15±5	0.44
Grade III (cmH$_2$O)[†]	23±6	26±8	23±5	24±8	23±6	22±5	0.84
AL							
Amount (L/min)[‡]	1.1±0.6	0.6±1.5	1.2±0.8	0.7±1.4	1.1±1.4	0.9±0.9	0.64
Percent of TVi (%)[‡]	10.7±6.2	5.9±11.2	9.6±7.9	6.7±9.6	10.2±12.2	7.9±6.3	0.62
Experiment outcomes							
Sealed leaking pressures							
Grade I (cmH$_2$O)[‡]	60±24	40±10	33±6	30±11	33±11	25±13	<0.001*
Grade II (cmH$_2$O)[‡]	60±24	45±15	33±6	30±11	33±15	30±13	<0.001*
Grade III (cmH$_2$O)[‡]	65±21	50±29	35±6	30±20	40±19	40±25	<0.001*
AL-reduction (%)[‡]	100±1	100±14	46±50	31±29	89±40	90±58	<0.001*
Cohesive failure, n [%]	8 [89]	9 [90]	3 [30]	2 [20]	8 [89]	10 [100]	<0.001*
EAC (%)[†]	76±9	77±9	92±13	80±11	88±14	98±13	0.001*

*, P value indicate statistical significance (P<0.05). [†], mean ± standard deviation (SD); [‡], median ± interquartile range (IQR). SAE, surface area expansion; AL, air leak; TVi, inspiratory tidal volume; EAC, expansion area change.

Importantly, in the second head-to-head comparative experiment, GATT-Patch Single showed improved performance compared with a commercially available patch used for lung sealing with a similar application method (Hemopatch®).

Interpretation of results

The potentially higher BP which can be achieved with the NHS-POx patch may be of clinical relevance. It has been suggested that the BP of a sealant should be high enough to withstand pressures during coughing (14) or
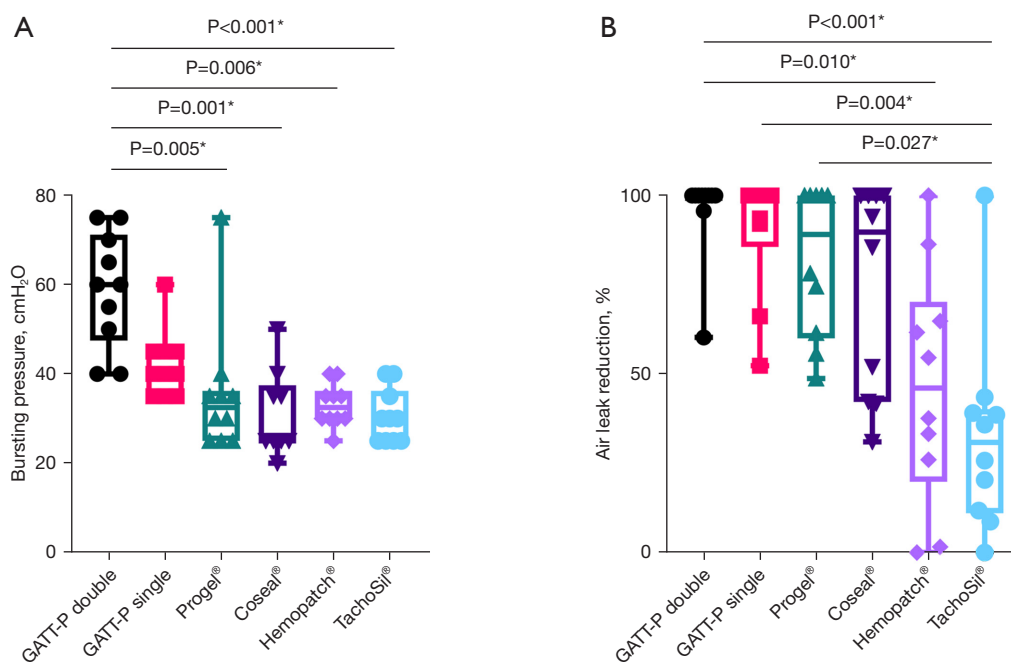
Figure 2 First experiment main results. (A) Bursting pressure; (B) air leak reduction. Presented as median, interquartile range and range. Statistical analysis is performed using a Kruskal-Wallis test (two-tailed) and P values are adjusted using the Bonferroni correction in post-hoc analysis. *, statistically significant at P<0.05. GATT-P, gelatin patch impregnated with N-hydroxysuccimide ester functionalized poly (2-oxazolines).

during positive-pressure recruitment maneuvers (up to 40 cmH$_2$O) (20,21). Physiologically, intra-thoracic pressures during coughing can reach up to 408 cmH$_2$O (22) and up to 71 cmH$_2$O in the post-thoracotomy state (23). Pedersen suggested that the actual pressures on the lesion might be lower due to pleural apposition, relieving transpulmonary pressures during coughing (14). In this context, we hypothesize that different modes of failure could make some sealants more effective than others. We observed that TachoSil® and Hemopatch® generally lose their sealing capacities after bursting due to lower adhesive strength. Progel®, Coseal®, GATT-Patch Double and Single remain effective in reducing the AL even after reaching BP and take one or more pressure-intervals to reach higher leakage intensities, due to the strong adhesive properties to the entire parenchymal lung defect (*Table 3*) (24).

As described previously, a lung sealant should have high conformability to the lung surface, to prevent debonding when re-expanding the lung (24). We hypothesize this is especially true for gel-based sealants, as they cannot be applied to a slightly inflated lung with active leakage (air bubble formation). We observed tears leading to sealant

failure for Coseal® and Progel® under increasing lung expansions, which may render these sprays less suitable for coverage of large areas. Patch sealants might not require as much conformability compared to gel sealants, being applicable to a slightly inflated lung surface with an active leak (counterpressure with gauzes). We observed successful applications at PEEP =5 cmH$_2$O for the TachoSil®, Hemopatch® and GATT-Patch groups. Debonding was not observed in the GATT-Patch groups during lung expansions due to strong adhesive properties. Thus, the experimentally measured lower EAC compared to Coseal® might not be of clinical importance.

Ease of use is an important benchmark for a sealants usability in the operating room. GATT-Patch Single has a similar mode of application compared to Hemopatch® and TachoSil®. However, GATT-Patch Double might be more difficult to implement in a clinical setting, especially minimally invasive surgery. In this case, a sprayable sealant might be preferable, which may also be better suited on irregular surfaces such as stapler lines, due to mechanical interlocking. Consequently, different modes of application are required depending on the surgical scenario.
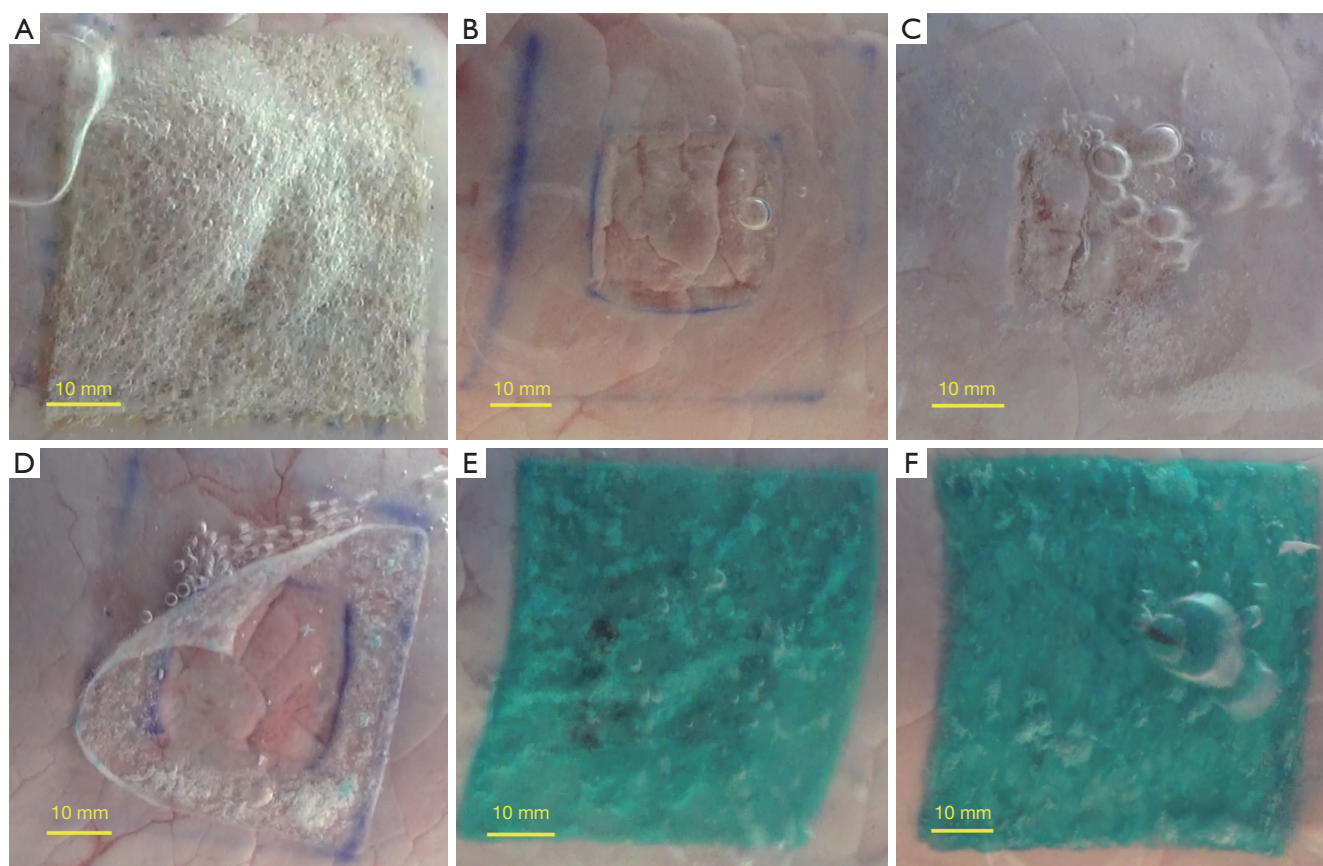
Figure 3 Modes of failure (adhesive or cohesive failure) (A) TachoSil® (adhesive), at 30 cmH$_2$O ventilatory pressure. (B) Coseal® (cohesive), at 50 cmH$_2$O. (C) Progel® (cohesive), at 40 cmH$_2$O. (D) Hemopatch® (adhesive), at 40 cmH$_2$O. (E) GATT-Patch Single (cohesive), at 70 cmH$_2$O. (F) GATT-Patch Double (cohesive), at 55 cmH$_2$O. Note: all lesions (25 mm × 25 mm) were made at 10 cmH$_2$O, patches were applied at 5 cmH$_2$O and gels on a deflated lung.

Before clinical use of GATT-Patch as a lung sealant, safety outcomes for implantation in the thoracic cavity need to be assessed *in-vivo*, including inflammatory properties, influences on wound healing, adhesion formation with the parietal pleura and biodegradation. Roozen *et al.* has shown that the histological results of GATT-Patch for intra-abdominal implantation were similar to control patches, showing complete biodegradation within 4–6 weeks (17). However, no significant conclusions could be drawn with respect to adhesion formation (17). Chemically, the amide and thioester bonds formed due to NHS reactivity are hydrolysable, and the NHS-POx degradation products have been shown to be effectively renally excreted in a rat model (16). Porcine-derived gelatin, constituting the patch-carrier for NHS-POx, is already being used in other devices such as SURGIFLO® Hemostatic Matrix

(Ethicon Inc. Raritan, New Jersey, USA), Gelfoam® and Gelita TUFT-IT® and may be cleared in 3 weeks (17). A theoretical downside to this material is the potential for antigenicity and allergic reactions, but this is very rare (25). Finally, GATT-Patch is being investigated in multi-center clinical trials of liver surgery (ClinicalTrials.gov Identifier: NCT04819945 and NCT05385952), suggestive of a promising safety profile in humans.

Comparison to literature

The extent of intra-operative PAL has previously been used to predict the occurrence of pPAL. Kim found an AL percentage of inspiratory tidal volume (TVi) >9.5% is predictive of pPAL, while Brunelli showed this for AL >500 mL/min (26,27). Furthermore, a recent consensus
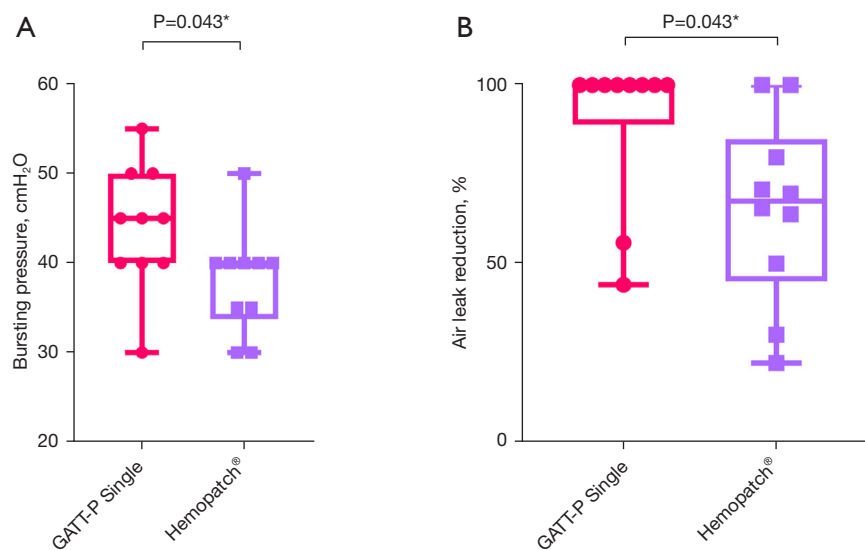
Figure 4 Second experiment main results. (A) Bursting pressure; (B) air leak reduction. Presented as median, interquartile range and range. Statistical analysis is performed using a Mann-Whitney U test (two-tailed). *, statistically significant at P<0.05. GATT-P, gelatin patch impregnated with N-hydroxysuccimide ester functionalized poly (2-oxazolines).

survey has proposed that AL >400 mL/min is classified as severe (28). Therefore, the amount of AL observed in our study as a result of the created pleural lesions in porcine lungs (median 0.6–1.2 L/min at Pplat =40 cmH$_2$O, *Table 3*) seems clinically relevant.

In previously published *ex vivo* experiments, defect AL wasn't always determined, making interpretation of those results more troublesome. Pedersen and Zang/Bures found higher BP for TachoSil® (median =35 cmH$_2$O, range =30–55 cmH$_2$O and mean =36.0 cmH$_2$O, SD =4.9 cmH$_2$O, respectively) when compared to our finding (median 30, range, 25–40 cmH$_2$O) (14,29). This may be due to differences in defect used. Fibrin glues, commonly used in thoracic surgery (9), were also investigated by Pedersen. However, Tisseel® and Evicel® both performed significantly worse when compared to TachoSil®, and were therefore not included in our study (14).

In current literature, comparative clinical studies evaluating effectiveness between sealants are scarce. In a recent systematic review (13), only 2/21 included studies compared one sealant to another. In these two randomized clinical trials (RCTs) [BioGlue *vs.* Vivostat (30) and Tisseel *vs.* Vivostat (31)], no significant differences were found in postoperative PAL duration. A more recent RCT not included in this systematic review compared TachoSil and Neoveil, showing no significant differences (32). Since 2018, a new clinical trial has been started (ClinicalTrials.

gov Identifier: NCT03450265), aiming to compare Hemopatch® to TachoSil® in a non-inferiority study. Based on a favourable trend in AL-reduction seen in our study (*Figure 2*), the capabilities of Hemopatch® for intraoperative AL management and preventing pPAL may be superior when compared to TachoSil® clinically, but results of this trial have to be awaited.

Limitations

For AL quantification, the tidal volumes displayed by the ventilator were used, and corrected for baseline deviations which could be caused by system leaks or bias flow. The last breath of each pressure setting was used, to ensure the measurement was not influenced by resolving atelectasis (overestimation) or escaping trapped air (underestimation). However, despite the use of similar AL measurements in previous studies (26,27,29), the most optimal air leak quantification approach remains to be validated.

With our SAE measurements, we attempted to integrate a conformability measurement in the BP setup. Due to possible influences by tears forming in the sealant (Progel® and Coseal®), debonding of the sealant (Hemopatch®) and camera angle, results should be interpreted cautiously (Appendix 1). The method used by Yamaoka may have advantages in this perspective, not being influenced by different tearing/debonding characteristics (24).

Difficulties were observed while applying Coseal® with the standard applicator, leading to inhomogeneous coverage, and higher BP may be reached with a more homogenous layer (e.g., dedicated spray set). Due to cohesive failure in Progel® and Coseal® groups, a thicker layer may lead to higher BP. Due to adhesive failure in TachoSil® and Hemopatch® groups, higher BP may be obtained with a larger adhesive overlap around the defect.

In the first experiment, randomization was not performed in the Progel® and Coseal® groups due to the limited shelf life of the prepared syringes (4 mL) and not in the TachoSil® group using the same allocation scheme. Influence of possible unconscious biases are expected to be low, due to the demonstrated statistically similar baseline measurements in the first experiment. Sample sizes were not large enough to detect all significant differences. Due to a significantly larger air leak in the GATT-Patch Single group in the second experiment, the actual effect size between GATT-Patch Single and Hemopatch may have been underestimated.

Recommendations for future research

While the porcine lung has been found to be comparable to the human lung (33), *ex vivo* results should be interpreted cautiously, as they may not translate accordingly to *in-vivo* situations (e.g., due to pleural mechanisms, coagulation, immune response, physiological breathing). The NHS-POx patch mode of application should be further optimized to improve usability in a clinical setting while maximizing mechanical cohesive strength (e.g., by creating a thicker single-layer patch). Before clinical use can be established, safety outcomes for implantation of the GATT-Patch in the thoracic cavity need to be assessed *in-vivo*. Fundamentally, mechanisms of sealant failure and BP should be further studied in relation to critical moments such as coughing or recruitment maneuvers, to establish threshold values for product development.

Conclusions

In conclusion, the novel NHS-POx based patch shows promise as a lung sealant due to favorable mechanical properties, demonstrating elevated BP and good adhesive strength to the lung. The gradual leakage pattern may provide superior reduction of an AL, especially in challenging clinical scenario's such as coughing or mechanical recruitment. The mode of sealant application should be optimized, and further *in-vivo* research is required to validate our findings and establish a safety profile for intra-thoracic implantation.

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1821/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1821/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-1821/coif). ER is employee of GATT Technologies and HG was a Scientific Advisor for GATT Technologies B.V until 31 December 2021, but not in relation to lung sealing technology. BH received funding and study materials through the institution for conduction of the study from GATT-Technologies B.V. The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. No live animals were involved in experimental procedures, and all experiments were performed on *ex vivo* porcine lung tissue obtained from a slaughterhouse.

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Appendix 1

Data-acquisition setup

Pressure data is retrieved from the mechanical ventilator (SERVO-I, Maquet Critical Care) through a RS232 link connected to a laptop using the ServoTracker software (version 4.1, Maquet Critical Care). The analog output of this software (-5 to +5V, sample rate 200/s) was then routed through two input-output devices (NI USB-6229 and NI USB-6210), which was then recorded into the analog input recorder application in Simulink (Matlab version R2020a) and oversampled at 1,000/s. Before this signal was saved, it was gained (g =40) and put through a discrete filter (f =50 Hz bandstop) to attain a clean signal in cmH_2O.

Conformability measurements

Surface area expansion calculation

For the surface area expansion (SAE) measurements, marked area (baseline) or sealant covered area (after sealing) of screenshots taken at airway pressures of 5 and 40 cmH_2O were calculated using ImageJ (version 1.53a, Wayne Rasband). The ruler was used to determine the scale in the program. In the case of extensive debonding (mainly in Hemopatch® group), the SAE of the remaining adhesive portion was measured, while tears within sealants (Progel®/Coseal®) were included in the SAE measurements. SAE was calculated as (surface area at 40 cmH_2O)/(surface area at 5 cmH_2O) and the area expansion change (EAC was calculated as (sealed SAE)/(baseline SAE). An EAC of 100% indicates perfect conformability of the sealant to the lung, while <100% indicates a reduced expansion after sealing compared to baseline.

Surface area expansion examples

Examples of how a SAE and consequent EAC measurement was performed. First, at ventilatory pressure settings of PEEP =5 cmH_2O and Pc =35 cmH_2O, screenshots were taken at 5 cmH_2O and 40 cmH_2O (*Figure S1A,S1B*). Then, using ImageJ (version 1.53a, Wayne Rasband), the scale was determined using the ruler and the baseline areas and subsequently sealed areas were calculated. As can be seen in *Figure S1*, these measurements may be influenced by: tears in the sealant, as was mainly seen in the Progel® and Coseal® gels (*Figure S1C,S1D*) and debonding of the sealant, as seen in Hemopatch® (*Figure S1F*). In the case of debonding, only the adhesive area was measured. In the case of tears, the entire area was still measured. Also, the lung was positioned manually above the camera and the exact angle between camera and measured surface was not measured, so some unknown factor of optical distortion may be of influence on these measurements.
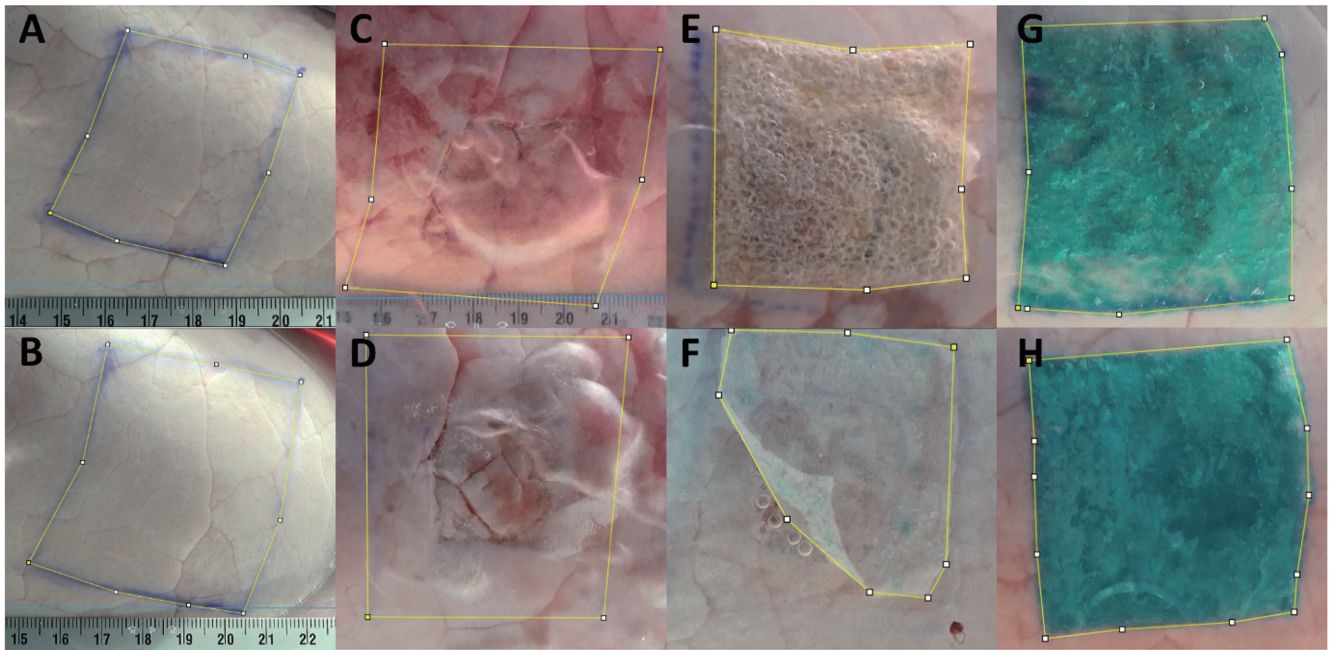
Figure S1 Surface area expansion measurement examples. (A) Baseline surface area measurement at 5 cmH$_2$O; (B) Baseline surface area measurement at 40 cmH$_2$O; (C) Coseal® example with tears; (D) Progel® example with tears; (E) TachoSil® example; (F) Hemopatch® example with debonding; (G) GATT-Patch Single example; (H) GATT-Patch Double example.

Table S1 second experiment baseline characteristics and outcomes

Characteristics	GATT-Patch Single	Hemopatch®	P value
Baseline characteristics			
Sample characteristics			
Ischemic time (minutes)[†]	173±104	180±130	0.90
Left lower lobe (n, %)	8 (80%)	7 (70%)	>0.99
Physical characteristics			
Surface temperature (℃)[†]	31.9±1.7	32.1±1.2	0.70
Water temperature (℃)[‡]	37.3±0.1	37.3±0.2	>0.99
Baseline SAE (%)[‡]	154±23	157±7	0.53
Lung compliance (mL/cmH$_2$O)[†]	25±4	24±6	0.47
Leakage characteristics			
Baseline leaking pressures			
Grade I (cmH$_2$O)[‡]	15±10	20±3	0.08
Grade II (cmH$_2$O)[‡]	20±8	25±11	0.11
Grade III (cmH$_2$O)[b]	23±11	30±11	0.32
AL			
Amount (L/min)[†]	1.5±0.8	0.8±0.5	0.03*
Percent of TVi (%)[†]	12.4±5.1	7.1±3.9	0.02*
Experiment outcomes			
Sealed leaking pressures			
Grade I (cmH$_2$O)[‡]	45±10	40±6	0.04*
Grade II (cmH$_2$O)[‡]	45±10	40±6	0.04*
Grade III (cmH$_2$O)[‡]	50±11	40±6	0.002*
AL reduction (%)[‡]	100±11	68±40	0.04*
Cohesive failure (n, %)	10 (100%)	0 (0%)	<0.001*
Adhesive failure (n, %)	0 (0%)	10 (100%)	<0.001*

*, P value indicate statistical significance (P<0.05). [†], mean ± standard deviation (SD); [‡], median ± interquartile range (IQR). SAE, surface area expansion; AL, air leak; TVi, inspiratory tidal volume.