WHITE PAPER

PROGEL™
Pleural Air Leak Sealant

In vitro evaluation of physical characteristics for lung applications, as compared to other surgical sealants.

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2. C. R. Bard, Inc. (Davol – Neomend)

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Abstract

Objectives: Sealants are commonly used to manage intraoperative air leaks in lung surgery. While only one sealant is FDA-approved for sealing lung leaks, selection of a specific sealant by surgeon is often arbitrary. *In vitro* modeling comparing important physical characteristics of five clinically available agents was performed to aid physicians in optimal sealant choice.

Methods: Tests performed on BioGlue®, Coseal®, DuraSeal™, Tisseel™ and PROGEL™ Pleural Air Leak Sealant (PALS) included preparation time, gel time, percent swelling in Phosphate Buffered Saline (PBS) solution (at 37°C) after 24 and 48 hours, degradation time (disintegration in 37°C PBS solution agitated water bath), burst strength (burst pressure on Days 0 to 7 post-application), and elasticity (Young's modulus derived from elongation).

Results: Sealant preparation time was as follows: BioGlue® (2m5s), Coseal® (2m48s), DuraSeal™ (4m21s), Tisseel™ (18m0s) and PROGEL™ PALS (3m35s). PROGEL™ PALS (14.26s) gelation time was comparable to Tisseel™ (14.42s), significantly less than BioGlue® (16.83s) ($P < 0.05$), and significantly greater than DuraSeal™ (0.91s) ($P < 0.05$). PROGEL™ PALS (17.80%) weight change at 24 hours was significantly less than Coseal® (295.60%) and DuraSeal™ (162.90%) ($P < 0.05$), and significantly greater than BioGlue® (17.80%) ($P < 0.05$). PROGEL™ PALS (97.11%) weight change at 48 hours was significantly less than Coseal® (446.00%) and DuraSeal™ (189.80%) ($P < 0.05$), and not significantly different than BioGlue® (21.30%). PROGEL™ PALS (22.67%) swelling diameter change at 24 hours was significantly less than Coseal® (67.04%) and DuraSeal™ (36.00%) ($P < 0.05$), and significantly greater than BioGlue® (6.06%) ($P < 0.05$). PROGEL™ PALS (28.43%) swelling diameter change at 48 hours was significantly less than Coseal® (81.30%) and DuraSeal™ (41.19%) ($P < 0.05$), and significantly greater than BioGlue® (8.13%) ($P < 0.05$). Mean time to degradation was 10 days for Coseal®, 15 days for PROGEL™ PALS, 28 days for Tisseel™, and 46 days for DuraSeal™. BioGlue® did not degrade over a three month observation period. PROGEL™ PALS (77.24%) elongation was not significantly different than Coseal® (62.35%), DuraSeal™ (121.86%) and BioGlue® (63.76%). PROGEL™ PALS (0.026 kPa) Young's modulus was significantly less than BioGlue® (11.91 kPa) ($P < 0.05$), and not significantly different than Coseal® (0.082 kPa) and DuraSeal™ (0.029 kPa). Tisseel™ could not be tested as it sheared during clamping in the test fixture. At t=0, PROGEL™ PALS (160.60 mmHg) burst strength was significantly greater than Coseal® (71.25 mmHg), Tisseel™ (13.98 mmHg), BioGlue® (115.47 mmHg) and DuraSeal™ (70.16 mmHg) ($P < 0.05$). At Day 1, PROGEL™ PALS (131.77 mmHg) burst strength was significantly greater than Coseal® (33.81 mmHg), Tisseel™ (17.42 mmHg), and DuraSeal™ (33.62 mmHg) ($P < 0.05$). At Day 2 and 4, PROGEL™ PALS (Day 2: 114.55 mmHg; Day 4: 64.95 mmHg) burst strength was significantly greater than Coseal® (Day 2: 17.68 mmHg; Day 4: 8.66 mmHg) and DuraSeal™ (Day 2: 39.25 mmHg; Day 4: 34.63 mmHg) ($P < 0.05$). At Day 7, PROGEL™ PALS (4.78 mmHg) burst strength was significantly less than BioGlue® (95.94 mmHg) ($P < 0.05$), and not significantly different than Coseal® (2.20 mmHg) and DuraSeal™ (17.11 mmHg). Tisseel™ was unable to be evaluated beyond Day 1 due to material degradation.

Conclusions: Commonly used surgical sealants possess markedly different chemical and physical characteristics that may affect their efficacy as adjuncts to stapling and sutures. The objective of this *in vitro* evaluation was to provide a comprehensive comparative assessment of multiple important sealant physical characteristics to educate users. From this assessment, PROGEL™ PALS demonstrated high burst pressure between t=0 through Day 4 post-application. PROGEL™ PALS also demonstrated several other physical characteristics including gelation, swelling, degradation rate, adhesiveness, and elasticity within the optimal range for deployment and function in relation to other commercially available products evaluated. Data suggest that PROGEL™ PALS possesses a unique combination of strength, compliance and tissue adhesion that may contribute to the proven clinical efficacy of this FDA-approved lung sealant.

Abbreviation List: ASTM = American Standard of Test Methods; FDA = US Food and Drug Administration; IFU = instructions for use; mmHg = millimeters of mercury (pressure measurement); PBS = Phosphate Buffered Saline; POALs = postoperative air leaks; PALS = pleural air leak sealant; PEG = polyethylene glycol; PTFE = polytetrafluoroethylene; US = United States

In vitro evaluation of physical characteristics for lung applications, as compared to other surgical sealants.

Introduction

Thoracic surgeons often utilize intraoperative sealants to reduce or eliminate air leaks of the lung, in an effort to improve postoperative outcomes when suturing or stapling is ineffective or not feasible (Bennet *et al.* 2003). Sealant use has improved postoperative leak outcomes by reducing or preventing air leakage (Bennet *et al.* 2003). In lung surgery the visceral pleura can become violated by staples/sutures, and sealants can mechanically or chemically interact with lung tissue to effectively suppress air leakage. Studies have demonstrated that despite new operative techniques and tools, approximately 58% of patients develop postoperative air leaks (POALs) following surgery (Okereke *et al.* 2004), and the incidence of prolonged air leaks has been demonstrated in up to 15.6% of patients (Brunelli *et al.* 2004). These complications increase morbidity and mortality risk, and generate high economic costs (Handy *et al.* 2010). As a result, sealants that decrease surgery-associated postoperative complications and/or hospitalization, can help directly benefit patients while also lowering the overall cost of care (Allen *et al.* 2004).

The lung tissue and function present unique challenges for the creation and formation of an ideal surgical sealant. For example, sealants and adhesives have been used on the surface of the lung to reduce postoperative air leaks, but if an agent is unable to expand and contract with the lung throughout the respiratory cycle, it is unlikely to maintain an effective seal (Azadani *et al.* 2009). Although studies have evaluated adhesives with respect to hemostatic efficacy, biocompatibility and safety, few have studied the mechanical properties of surgical sealant/glues for thoracic application (Berchane *et al.* 2008, Garcia *et al.* 2004). The ideal lung sealant requires the following characteristics: flexibility, adhesiveness, acceptable degradation period, biocompatibility, ease of use, and cost effectiveness.

A variety of plasma-based and synthetic sealant materials are commercially available. These agents include fibrin glues, cyanoacrylates, gelatin-resorcinol cross-linked with formaldehyde or glutaraldehyde, collagen, gelatin and polyurethane-based adhesives, polysaccharides, and polyethylene glycol (PEG)-albumin crosslinked hydrogel (Kobayashi *et al.* 2001, Preul *et al.* 2003, McDermott *et al.* 2004, Spotnitz *et al.* 2012). The clinical value of surgical sealant materials depend on their biocompatibility, toxicity, ease of use, application technique and their mechanical and physical characteristics (Artzi *et al.* 2011). The biophysical characteristics, including gel formation kinetics, burst pressure strength, adherence, elasticity, and degradation time can vary between sealants/adhesives considerably (Artzi *et al.* 2011, Shazly *et al.* 2008, Kjaergard *et al.* 2000), yet the physical and mechanical characteristics that influence clinical efficacy of commercially available surgical sealants have not been systematically assessed. While all these sealants are utilized in the thoracic surgical setting, only Progel™ PALS is FDA-approved, thus we compared the *in vitro* physical characteristics of BioGlue®, Coseal®, DuraSeal™, and Tisseel™, to Progel™ PALS.

BioGlue® (Cryolife, Inc., Kennesaw, GA, USA) is bovine serum albumin/glutaraldehyde tissue glue indicated for hemostasis in large vessel repair within the US, and for general surgical applications in Europe (BioGlue® IFU). Coseal® (Baxter Healthcare Corporation, Westlake Village, CA, USA) is a synthetic sealant hydrogel (two synthetic polyethylene glycols (PEGs), a dilute hydrogen chloride solution and a sodium phosphate/sodium carbonate solution), indicated for peripheral vascular reconstruction in both the U.S. and Europe (Coseal® IFU). DuraSeal™ (Covidien Plc, Mansfield, MA, USA) is a synthetic sealant hydrogel (PEG) with trilysine amine, indicated for spinal and cranial dural sealing in the U.S. and Europe (DuraSeal™ IFU). Tisseel™ (Baxter Healthcare Corporation, Westlake Village, CA, USA) is a human thrombin and fibrinogen combined with bovine aprotinin, indicated for hemostasis in cardiopulmonary bypass and splenic injury and as a colostomy surgery sealant in both the U.S. and Europe (Tisseel™ IFU). Progel™ PALS (C. R. Bard, Inc. (Davol – Neomend), Irvine, CA, USA) is a PEG-human serum albumin, and is the only FDA-approved product for sealing pleural air leaks adjunctive to suture or staple use within the U.S. (Progel™ PALS IFU). We report here an *in vitro* comparison of these five sealants with respect to clinically-relevant physical characteristics, including preparation time, time to gel formation, swelling, degradation time, burst pressure, and elasticity.

Materials and Methods

Methods for sealant reaction rate, mechanical testing, sealant water uptake, and burst pressure testing have been previously described by Campbell *et al.* 2005. All samples were prepared according to manufacturer's instructions for use and tests were performed on multiple of each of the following sealants: BioGlue®, Coseal®, DuraSeal™, Progel™ PALS (pleural air leak sealant), and Tisseel™. The following tests were performed: 1) preparation time, 2) liquid to gel transition time, 3) swelling percentage size and weight change at 24 and 48 hours, 4) degradation time, 5) burst strength/pressure over 7 days, and 6) elasticity. Data presented herein are based on the *in vitro* Bench performance of Progel™ PALS, and other commercially available products. Bench or Preclinical data may not correlate to performance in humans.

Preparation Time: Prep time is the time to prepare the sample product according to the manufacturer's IFU. Time is recorded from opening of the product packaging to spray readiness. (BioGlue®; n=1, Coseal®; n=3, DuraSeal™; n=3, Progel™ PALS; n=3 ,Tisseel™; n=2).

Gel Time: Gel time is the time duration required for a two-part self-curing sealant to change from a viscous solution to a non-flowing elastic material under ambient laboratory conditions.

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Sealants were prepared per each manufacturer's IFU; 200 µl of first pre-cursor was micro pipetted into a test tube with a micro stir bar. Stirring was initiated using Stirring Hotplate (Fisher Scientific Isotemp, catalog #11-100-49SH, Dubuque, IA) and the second pre-cursor added to make a 400 µl aliquot. Time to gel formation started when the second pre-cursor was added and stopped when the magnetic stirring bar settled in the hydrogel. Time to gel formation was measured using a Panasonic Video Camera frame capture system, 60 frames / second, (Panasonic SDR – S50, Indonesia). QuickTime Player version 7.6.9 was used to capture images and time. Three samples of each sealant were tested to determine average gel time (n=3).

Swelling and Diameter Measurement: Test samples were prepared by following the manufacturer's IFU and dispensing material into a PTFE mold 15mm diameter x 2mm deep. Samples cured in the mold geometry for a minimum time period recommended in the IFU or 5 minute maximum and removed carefully with a micro spatula. Samples were weighed (Mettler Toledo Compact Scale, MS105DU, Switzerland) and a high resolution camera (Canon T2i, 28-135 mm lens 3.5-5.6 IS USM, New York, USA) and calibrated scale and Mitutoyo 8" calipers (Mitutoyo, 0095, California, USA) used for dimensional measurements at time 0. Individual samples were placed in 20ml glass scintillation vials filled with PBS and .02% sodium azide. The vials were placed in a 37°C water bath (Grant Instruments LTD, OLS 200, England) with gentle agitation. At 24 hours, samples were removed from vials, excess moisture was blotted and weight and dimensional measurements repeated. Fresh PBS and sodium azide was used to fill the vials and the samples returned until the 48 hour duration was achieved and measurements were then repeated (n=5). Percent swelling was calculated as follows:

$$\frac{\text{Measurement (initial)} - \text{Measurement (Time x)} \times 100}{\text{Measurement (initial)}}$$

where "Measurement" equals weight or diameter. The mean and standard deviation of the individual swelling percentages is calculated at each time interval.

Degradation Time: Degradation time was determined by placing 15mm diameter x 2mm thick samples into scintillation vials covered with PBS solution. Vials were stored in a 37°C agitated water bath and the time to complete dissolution recorded (American Standard of Test Methods (ASTM) designation F 1635-04a, Standard Test Method for *in vitro* Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms of Surgical Implants, 2004). If samples did not dissolve the test was stopped at 90 days.

Burst Strength (Pressure) over Time: Samples were prepared by dispensing product onto a natural tissue substrate (Collagen sausage casing, #302, Nippi Casing Company, Tokyo, JP) with a centered 3mm hole forming a 15mm diameter x 2mm thick disc. Time zero samples are burst tested while interval test samples were covered in a PBS and sodium azide solution and stored in a 37°C water bath until tested. Five burst pressure (over time) measurements, per sealant, per time interval, were performed using a calibrated pneumatic insufflator (Sprint LC™ Leak Tester, Uson, L.P., Houston, TX, USA) under ambient room conditions on Day(s) 0, 1, 2, 4, and 7 post-application (n=5). Pressure ramped at 100 mmHg per second. Burst failures were noted as adhesion or cohesion failures. An adhesion failure occurred when there was delamination of the sealant from the substrate. A cohesion failure occurred when pressure caused a leak in the sealant while it was adhered to the substrate. If samples could not be handled without disruption of the sealant, the test was considered failed and no measurements recorded. Following burst testing, the sample was sliced in half using a razor blade. The thickness of each sample slice was measured and recorded using a Panasonic video camera and video inspection system software (i-Solution Lite, version 8.1, Vancouver, Canada).

Elasticity: Samples were made by injecting the test product into a steel dogbone mold (5 mm wide x 15 mm long x 2 mm deep test region) (n=5). The test region of each sample was demarcated using a fine Sharpie marker. The sample was mounted with test region visible on the Instron tester (Instron, Model 5943, Norwood, MA, USA) with a 10N load cell (Instron, Model S#103278, Norwood, MA, USA) using pneumatic grips (Instron, Model #2752-005, Norwood, MA, USA) and tensioned at 25mm per minute. A webcam (Logitech Webcam, Model C615 Newark, CA, USA) was set up to focus on the sample test region, the load balanced, gauge length reset on BlueHill software (Instron, version 3, Norwood, MA, USA) and recording started. The Instron was started and tension applied to the test sample until yield or failure was observed. The elongation and modulus of the sealant was captured using a combination of an image based measurement tool and software. Camera software (Frame-shots, version 3.1) captured the change in distance of the test region every 100 ms. Correlation was made between displacement and load. Elongation measurements followed ASTM D638, Standard Test Method for Tensile Properties of Plastics, 2003, where % Strain = % Elongation to break = (Lfinal - Linitial)/Linitial * 100 and L is length.

Young's modulus, a standard biomaterial metric, was calculated from images captured as a function of the distance between two points measured and the corresponding load of those points.

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Statistics: All variable data are presented as means ± standard deviations among samples. Samples deemed not testable were not included within the statistical evaluation. Statistical analyses were performed using ANOVA (GraphPad Prism 6.01, LaJolla, CA). A P value < 0.05 was considered statistically significant.

Results

Results for each test are described and data presented for the 6 sealants tested in Tables 1 through 5.

Preparation Time: Preparation time was the fastest for BioGlue® (2m:05s), followed by Coseal® (2m:48s), PROGEL™ PALS (3m:35s), DuraSeal™ (4m:21s) and Tisseel™ (18m:00s) (Table 1). (BioGlue®; n=1, Coseal®; n=3, DuraSeal™; n=3, PROGEL™ PALS; n=3 ,Tisseel™; n=2)

Gel Time. Gel formation time (gelation time) was 0.91s for DuraSeal™, 0.91s Coseal®, 14.26s for PROGEL™ PALS, 14.42s for Tisseel™, and 16.83s for BioGlue®. PROGEL™ PALS gelation time was comparable to Tisseel™, significantly less than BioGlue® ($P < 0.05$), and significantly greater than DuraSeal™ ($P < 0.05$) (Table 2). (BioGlue®; n=3, Coseal®; n=3, DuraSeal™; n=3, PROGEL™ PALS; n=3, Tisseel™; n=3)

Weight Measurements: The following sealants increased in weight at 24 hours: BioGlue® by 17.8%, PROGEL™ PALS by 82.6%, DuraSeal™ by 162.9%, and Coseal® by 295.6%, as compared to Tisseel™ that did not increase (Table 3). PROGEL™ PALS weight change at 24 hours was significantly less than Coseal® and DuraSeal™, and significantly greater than BioGlue®. The following sealants increased in weight at 48 hours: BioGlue® by 21.3%, PROGEL™ PALS by 97.1%, DuraSeal™ by 189.8%, and Coseal® by 446.0%, as compared to Tisseel™ that did not increase. PROGEL™ PALS weight change at 48 hours was significantly less than Coseal® and DuraSeal™ ($P < 0.05$), and comparable to BioGlue® (Table 3). (BioGlue®; n=5, Coseal®; n=5, DuraSeal™; n=5, PROGEL™ PALS; n=5, Tisseel™; n=5).

Swelling Diameter Measurements: The following sealants increased in diameter at 24 hours: BioGlue® by 6.1%, PROGEL™ PALS by 22.7%, DuraSeal™ by 36.0%, and Coseal® by 67.0%, as compared to Tisseel™ that did not increase in diameter. PROGEL™ PALS swelling diameter change at 24 hours was significantly less than Coseal® and DuraSeal™ ($P < 0.05$), and significantly greater than BioGlue® ($P < 0.05$). The following sealants increased in diameter at 48 hours: BioGlue® by 8.1%, PROGEL™ PALS by 28.4%, DuraSeal™ by 41.2%, and Coseal® by 81.3%, as compared to Tisseel™ that did not increase (Table 4). PROGEL™ PALS swelling diameter change at 48 hours was significantly less than Coseal® and DuraSeal™ ($P < 0.05$), and significantly greater than BioGlue® ($P < 0.05$) (Table 4). (BioGlue®; n=5, Coseal®; n=5, DuraSeal™; n=5, PROGEL™ PALS; n=5, Tisseel™; n=5).

Degradation Time: Mean time to degradation was 10 days for Coseal®, 15 days for PROGEL™ PALS, 28 days for Tisseel™, and 46 days for DuraSeal™. BioGlue® did not degrade over three months, and the test was halted (Table 3). (BioGlue®; n=5, Coseal®; n=5, DuraSeal™; n=5, PROGEL™ PALS; n=5, Tisseel™; n=5).

Table 1. Prep Time

Sealant	Mean Prep Time (Minutes: Seconds ± SD)
BioGlue®	2:05
Coseal®	2:48 ± :10
DuraSeal™	4:21 ± :34
PROGEL™ PALS	3:35 ± :13
Tisseel™	18:00 ± 4:15

Table 2. Mean Time to Gel Formation (Gelation Time)

Sealant	Mean Gelation Time (Seconds ± SD)
BioGlue®	16.83 ± 0.20
Coseal®	1.21 ± 0.05
DuraSeal™	0.91 ± 0.05
PROGEL™ PALS	14.26 ± 0.94
Tisseel™	14.42 ± 1.55

Table 3. Mean Swelling Weight Change and Degradation Time

Sealant	Mean Weight Change, %		Mean Degradation Time (Days)
	24 hr ± SD	48 hr ± SD	
BioGlue®	17.8 ± 23.7	21.3 ± 24.0	did not degrade
Coseal®	295.6 ± 16.1	446.0 ± 84.2	10
DuraSeal™	162.9 ± 18.4	189.8 ± 16.5	46
PROGEL™ PALS	82.6 ± 5.4	97.1 ± 5.4	15
Tisseel™	-6.1 ± 12.2	-3.1 ± 22.8	28

Table 4. Mean Swelling Diameter Change

Sealant	Mean Weight Change, %	
	24 hr ± SD	48 hr ± SD
BioGlue®	6.1 ± 3.6	8.1 ± 2.9
Coseal®	67.0 ± 0.8	81.3 ± 11.7
DuraSeal™	36.0 ± 4.8	41.2 ± 4.3
PROGEL™ PALS	22.7 ± 1.6	28.4 ± 1.0
Tisseel™	-1.6 ± 1.3	-2.1 ± 2.6

Burst Strength (Pressure) over Time: Mean burst strength was normalized for sample thickness. Mean burst strength at time zero per mm thickness post-application was 160.60 mmHg for PROGEL™ PALS, 115.47 mmHg for BioGlue®, 71.25 mmHg for Coseal®, 70.16 mmHg for DuraSeal™ and 13.98 mmHg for Tisseel™

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(n=5). Mean burst strength at Day 1 per mm thickness post-application was 131.77 mmHg for PROGEL™ PALS, 33.81 mmHg for Coseal®, 33.62 mmHg for DuraSeal™ and 17.42 mmHg for Tisseel™ (n=5). BioGlue® was not evaluated at this timepoint. Mean burst strength at Day 2 per mm thickness post-application was 114.55 mmHg for PROGEL™ PALS, 39.25 mmHg for DuraSeal™, 17.68 mmHg for Coseal® (n=5). Tisseel™ was not able to be evaluated as this timepoint. BioGlue® was not evaluated at this timepoint. Mean burst strength at Day 4 per mm thickness post-application was 64.95 mmHg for PROGEL™ PALS, 34.63 mmHg for DuraSeal™, 8.66 mmHg for Coseal® (n=5). Tisseel™ was not able to be evaluated as this timepoint. Also, BioGlue® was not evaluated at this timepoint. Mean burst strength at Day 7 per mm thickness post-application was 95.94 mmHg for BioGlue®, 17.11 mmHg for DuraSeal™, 4.78 mmHg for PROGEL™ PALS, 2.20 mmHg for Coseal® (BioGlue®; n=5, Coseal®; n=4, DuraSeal™; n=5, PROGEL™ PALS; n=2.) Tisseel™ was not able to be evaluated as this timepoint. In summary, at t=0 PROGEL™ PALS burst strength was significantly greater than Coseal®, Tisseel™, BioGlue® and DuraSeal™. At Day 1, PROGEL™ PALS burst strength was significantly greater than Coseal®, Tisseel™, and DuraSeal™ ($P < 0.05$). At Day 2 and 4, PROGEL™ PALS burst strength was significantly greater than Coseal® and DuraSeal™ ($P < 0.05$). At Day 7, PROGEL™ PALS burst strength was significantly less than BioGlue® ($P < 0.05$), and comparable to Coseal® and DuraSeal™ (Table 5; Figure 1).

Table 5. Burst Pressure over Time (±SD) Normalized for Gel Thickness

Timepoint	PROGEL™ PALS	DuraSeal™	Coseal®	Tisseel®	BioGlue®
0	160.60 ± 15.87	70.16 ± 17.83	71.25 ± 17.33	13.98 ± 5.50	115.47 ± 21.21
1	131.77 ± 3.92	33.62 ± 25.36	33.81 ± 17.47	17.42 ± 11.89	n/a
2	114.55 ± 6.24	39.25 ± 18.71	17.68 ± 4.38	n/a	n/a
4	64.95 ± 5.56	34.63 ± 17.41	8.66 ± 5.57	n/a	n/a
7	4.78 ± 2.24	17.11 ± 9.01	2.20 ± 0.53	n/a	95.94 ± 36.70

Cohesion vs. Adhesion: Burst failures led to evaluation of failure mode previously defined as adhesive or cohesive (n=5). At t=0 all failures occurred adhesively except for Tisseel™. PROGEL™ PALS and BioGlue® had consistent cohesive failures though PROGEL™ PALS failures were adhesive at Day 7. DuraSeal™ had an adhesive failure at Day 1 and Coseal® had an adhesive failure at Day 2 (Table 6).

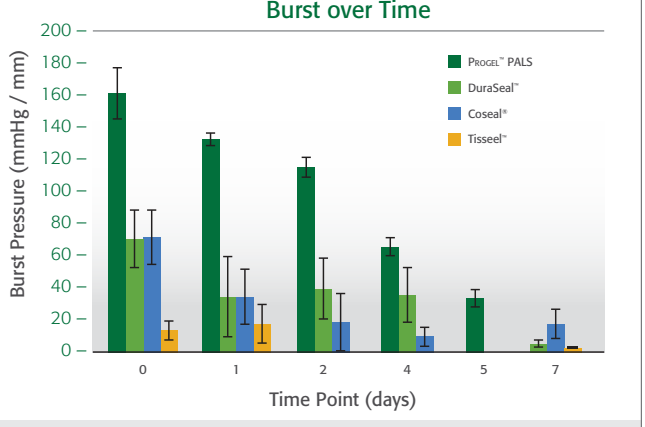

Figure 1: Burst Pressure over Time

Elasticity: The following sealants demonstrated varying degrees of elongation to break during evaluation. DuraSeal™ demonstrated the highest elongation of 121.86%, followed by PROGEL™ PALS at 77.24%, BioGlue® at 63.76% and Coseal at 62.35% (BioGlue®; n=5, Coseal®; n=4, DuraSeal™; n=5, PROGEL™ PALS; n=5). Tisseel™ could not be tested as it sheared during clamping in the test fixture. PROGEL™ PALS elongation was not significantly different than Coseal®, DuraSeal™ and BioGlue®. Furthermore, the following sealants demonstrated varying degrees of modulus (relative stiffness) during evaluation. BioGlue® demonstrated the highest modulus of 11.91 kPa, followed by Coseal® at 0.082 kPa, DuraSeal™ at 0.029 kPa and PROGEL™ PALS at 0.026 kPa (BioGlue®; n=4, Coseal®; n=4, DuraSeal™; n=5, PROGEL™ PALS; n=5). Tisseel™ could not be tested as it sheared during clamping in the test fixture. PROGEL™ PALS modulus was significantly less than BioGlue® ($P < 0.05$), and comparable to Coseal® and DuraSeal™ (Table 7).

Table 6. Burst Pressure over Time Failure Mode (Cohesive:Adhesive)

Timepoint	PROGEL™ PALS	DuraSeal™	Coseal®	Tisseel®	BioGlue®
0	5:0	5:0	5:0	2:3	5:0
1	5:0	4:1	5:0	1:4	n/a
2	5:0	5:0	4:1	n/a	n/a
4	5:0	4:1	4:1	n/a	n/a
7	0:5	3:2	3:1[1]	n/a	4:0[2]

[1] One sample was degraded and gel not adherent to substrate during handling
[2] One sample had fractured prior to testing and failed at the defect line

Table 7. Elongation Modulus (Elasticity)

Sealant	Mean % Elongation ± SD	Young's Modulus (kPa) ± SD
BioGlue®	63.76±25.19	11.91±5.68
Coseal®	62.35±4.34	0.082±0.05
DuraSeal™	121.86±35.69	0.029±0.013
PROGEL™ PALS	77.24±18.90	0.026±0.02
Tisseel™	n/a[1]	n/a[1]

[1] Tisseel could not be loaded in test fixture due to softness of material

In vitro evaluation of physical characteristics for lung applications, as compared to other surgical sealants.

Discussion

Sealant biomaterials for preventing postoperative air leaks must be sufficiently strong and flexible on the surface of the lung or repair edges during recovery to ensure normal lung function. The strength and elasticity of a sealant on the lung surface can result in an ineffective repair if there is poor tissue adhesion to the pleura, ineffective air pathway sealing and reduction in lung expansion. Device effectiveness is further challenged when attempting to reduce the risk of postoperative air leaks that are subjected to cyclic loading. Various surgical sealants were evaluated to determine their biophysical properties, which may play a role in their effectiveness to modulate air leak outcomes.

This study demonstrates varying degrees of gelation, swelling, degradation rates, burst strength, adhesiveness, and elasticity of the surgical sealants evaluated. Too rapid gelation can potentially lead to low or non-uniform tissue coverage and/or limited working time during application. PROGEL™ PALS gelation time was comparable to Tisseel™, significantly less than BioGlue®, and significantly greater than DuraSeal™.

Device swelling and weight increase is indicative of how much a given sealant can further hydrate post-application or if dynamic sealant efficacy changes may occur over time. This can be indicative of the potential for limited use in confined spaces. PROGEL™ PALS swelling diameter change at 24 and 48 hours was significantly less than Coseal® and DuraSeal™, and significantly greater than BioGlue®. Furthermore, PROGEL™ PALS weight change at 24 hours was significantly less than Coseal® and DuraSeal™, and significantly greater than BioGlue®. At 48 hours PROGEL™ PALS weight change was significantly less than Coseal® and DuraSeal™, and comparable to BioGlue®.

Degradation rate can potentially be a positive or negative attribute for a surgical sealant. A sealant that degrades too rapidly may have limited function *in vivo*, while a sealant that degrades too slowly may represent a nidus for infection and/or limit normal tissue function. Coseal® demonstrated the most rapid degradation rate following application (10 days), followed by PROGEL™ PALS (15 days), Tisseel™ (28 days), DuraSeal™ (46 days) and BioGlue® (which did not degrade).

Burst strength is indicative of how effectively a sealant would perform when placed under physiologic conditions. We used a standard collagen substrate with uniform thickness to develop a test that was not dependent upon *ex vivo* or *in vivo* tissue variability, user application techniques, or delivery mechanisms. The test represents a high pressure ramp rate presenting a clinically relevant challenge to model post-surgery air leak outcomes. Samples were subjected to simulated thoracic cavity conditions using 1 x PBS at body temperature. Immediately following application, PROGEL™ PALS burst strength was significantly greater than Coseal®, Tisseel™, BioGlue® and DuraSeal™. At Day 1, PROGEL™ PALS was significantly greater than Coseal®, Tisseel™, and DuraSeal™. At Day 2 and 4, PROGEL™ PALS was significantly greater than Coseal® and DuraSeal™. At Day 7, PROGEL™ PALS was significantly less than BioGlue® and comparable to Coseal® and DuraSeal™. PROGEL™ PALS demonstrated clear burst strength superiority through Day 4 post-application.

The adhesiveness of a sealant is also critically important, as it can have a direct effect upon sealant efficacy. Post-application sealants can fail in one of two manners when exposed to physiologic and/or supraphysiologic burst pressures. Failure can be characterized as "cohesive" in which a sealant fails at an interface within the material, or "adhesive" in which a sealant fails at the tissue interface. Early adhesive failures can be indicative of poor adhesion to the tissue surface post-application. Upon burst evaluation, PROGEL™ PALS demonstrated cohesive failures only throughout Day 4 post-application, as compared to Coseal®, DuraSeal™ and Tisseel™, which demonstrated a degree of adhesive failures indicative of non-uniformity between t=0 and Day 4.

The elasticity/compliance of a sealant is critically important, particularly on dynamic structures or organs within the body, such as the lung. PROGEL™ PALS elongation at break was not significantly different than Coseal®, DuraSeal™ and BioGlue®. However, PROGEL™ PALS modulus was significantly less than BioGlue®, and comparable to Coseal® and DuraSeal™. A lower modulus is indicative of a sealant that is less stiff and more compliant, as compared to a sealant with high modulus which may limit lung expansion.

Using an *ex vivo* porcine lung model, findings by Pedersen *et al.* corroborate our baseline burst results and model, in which BioGlue® demonstrated the greatest burst pressure, followed by Tachosil, PleuraSeal, Tissuepatch Dural, Evicel and Tisseel™ (Pedersen *et al.* 2012). BioGlue® maintained pressure in 7 of 9 samples, with failures identified with tissue tearing. In a canine model, Araki *et al.* also reported that intentional pleura-parenchymal defects demonstrated a mean air leakage pressure of 8.1 cm H_2O (6.0 mmHg), and burst pressure of 32.1 cm H_2O (23.6 mmHg) after application of fibrin glue (Araki *et al.* 2007). In a canine model, Kawamura *et al.* reported that intentional right lobe defects (with an approximate positive airway pressure of 20 cm H_2O) treated with a fibrin sealant demonstrated unstable burst pressures within the first 12 hours of application (Kawamura *et al.* 2005). Taken together, these animal study results validate our *in vitro* observations and furthermore suggest that coughing or positive pressure ventilation should be minimized during recovery, particularly for some unstable sealants or those with low burst pressure thresholds.

In vitro evaluation of physical characteristics for lung applications, as compared to other surgical sealants.

Selection of an optimal sealant for lung surgery and the prevention of air leaks necessitate a thorough consideration of multiple physical characteristics. Considering high ventilator peak pressure is a known risk factor for the development of post operative air leaks (Cho *et al.* 2006) and surging differential pressures observed during coughing (Byrd *et al.* 1975), sealant burst pressure is one of the most critical selection factors. Within this study, PROGEL™ PALS demonstrated high burst pressure between t=0 through Day 4 post-application. PROGEL™ PALS also demonstrated several other physical characteristics (gelation, swelling, degradation rate, adhesiveness, and elasticity) within the optimal range in relation to other commercially available products evaluated. Data suggest that PROGEL™ PALS possesses a unique combination of strength, compliance and tissue adherence that may contribute to the superior efficacy of this FDA-approved lung sealant.

References

1. Allen MS, Wood DE, Hawkinson RW, *et al.* Prospective randomized study evaluating a biodegradable polymeric sealant for sealing intraoperative air leaks that occur during pulmonary resection. Ann Thorac Surg. 2004;77:1792-1801.
2. Araki M, Tao J, Nakajima N, Sugai H, Sato T, Hyon SH, Nagayasu T, Nakamura T. Development of a new biodegradable hydrogel glue for preventing alveolar air leakage. J Thorac Cardiovasc Surg. 2007;134(5):1241-1248.
3. Artzi N, Zeiger A, Boehning F, bon Ramos A, Van Vliet K, Edelman ER. Tuning adhesion failure strength for tissue-specific applications. Acta Biomater. 2011;7:67-74.
4. Azadani AN, Matthews PB, Ge L, Shen Y, Jhun CS, Guy TS, Tseng EE. Mechanical Properties of Surgical Glues Used in Aortic Root Replacement. Ann Thorac Surg. 2009;87(4):1154-1160.Rajagopal P, Hakim N: The use of a powdered polysaccharide hemostat in live donor nephrectomies controls bleeding and reduces postoperative complications. *Transplant Proc* 2011, 43:424–426.
5. Baxter Healthcare Corporation, Hayward, CA, USA. Coseal® Instructions for Use. 2009.
6. Baxter Healthcare Corporation, Westlake Village, CA, USA. Tisseel™ Instructions for Use. 2012.
7. Bennet SL, Melanson DA, Torchiana DF, Wiseman DM, Sawhney AS. Next-generation hydrogel films as tissue sealants and adhesion barriers. J Card Surg. 2003;18:494-499.
8. Berchane NS, Andrews MJ, Kerr S, Slater NK, Jebrail FF. On the mechanical properties of bovine serum albumin (BSA) adhesives. J Mater Sci Mater Med 2008;19:1831– 1838.
9. Brunelli A, Monteverde, Borri A, Salati M, Marasco RD, Fianchini A. Predictors of prolonged air leak after pulmonary lobectomy. Ann Thorac Surg. 2004;77(4):1205-1210.
10. Byrd RB, Burns JR. Cough dynamics in the post-thoracotomy state. Chest. 1975;67(6):654-657.
11. Campbell PK, Bennett SL, Driscoll A, Sawhney AS. Evaluation of absorbable surgical sealants: *In vitro* testing. Confluent Surgical, Inc., Waltham, MA. 2005.
12. Cho MH, Malhotra A, Donahue DM, Wain JC, Harris RS, Karmpaliotis D, Patel SR. Mechanical ventilation and air leaks after lung biopsy for acute respiratory distress syndrome. Ann Thorac Surg. 2006;82(1):261-266.
13. Confluent Surgical, Inc. Waltham, MA, USA. DuraSeal™ Instructions for Use. 2005
14. Cryolife, Inc., Kennesaw, GA, USA. BioGlue® (Cryolife) Instructions for Use. 2010.
15. García Páez JM, Jorge Herrero E, Rocha A, *et al.* Comparative study of the mechanical behaviour of a cyanoacrylate and a bioadhesive. J Mater Sci Mater Med. 2004;15:109-115.
16. Handy JR, Denniston K, Grunkemeier GL, Wu YX. What is the inpatient cost of hospital complications or death after lobectomy or pneumonectomy? Ann Thorac Surg. 2011;91:234-238.
17. Kawamura M, Gika M, Izumi Y, Horinouchi J, Shinya N, Mukai M, Kobayashi K. 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(1):39-42.
18. Kjaergard HK, Velada JL, Pedersen JH, Fleron H, Hollingsbee Da. Comparative kinetics of polymerization of three fibrin sealants and influence on timing of tissue adhesion. Thrombosis Res. 2000;98:221-228.
19. 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;58:658-665.
20. McDermott MK, Chen T, Williams CM, Markley KM, Payne GF. Mechanical properties of biomimetic tissue adhesive based on the microbial transglutaminase-catalyzed crosslinking of gelatin. Biomacromolecules. 2004;5:1270-1279.
21. Neomend, Inc., Irvine, CA, USA. PROGEL™ Pleural Air Leak Sealant (PALS) Instructions for Use. 2011.
22. Okereke I, Murthy SC, Alster JM, Blackstone EH, Rice TW. Characterization and Importance of Air Leak After Lobectomy Ann Thorac Surg. 2005;79(4):1167-1173.

23. Pedersen TB, Honge JL, Pilegaard HK, Hasenkam JM. Comparative study of lung sealants in a porcine *ex vivo* model. Ann Thorac Surg. 2012;94(1):234-240.

24. Preul MC, Bichard WD, Muench TR, Spetzler RF. Toward optimal tissue sealants for neurosurgery: Use of a novel hydrogel sealant in a canine durotomy repair model. Neurosurgery. 2003;53:1189-1199.

25. Shazly TM, Artzi N, Boehning F, Edelman ER. Viscoelastic adhesive mechanics of aldehyde-mediated soft tissue sealants. Biomaterials. 2008;29:4584-4591.

26. Spotnitz WD, Burks S. Hemostats, sealants, and adhesives III: a new update as well as cost and regulatory considerations for components of the surgical toolbox. Transfusion. 2012; doi: 10.1111/j.1537-2995.2012.03707.x. [Epub ahead of print].

Disclaimer

Data presented herein are based on the *in vitro* Bench performance of PROGEL™ PALS, and other commercially available products. Bench or Preclinical data may not correlate to performance in humans. This study was funded by C. R. Bard, Inc. (Davol). Pierre Theodore, M.D. is a paid consultant for, and Pete Davis is a paid employee of C. R. Bard, Inc. (Davol-Neomend).

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