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Structural heteropolysaccharides as air-tight sealants of the human pleura

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Abstract

Pulmonary “air leaks,” typically the result of pleural injury caused by lung surgery or chest trauma, result in the accumulation of air in the pleural space (pneumothorax). Air leaks are a major source of morbidity and prolonged hospitalization after pulmonary surgery. Previous work has demonstrated structural heteropolysaccharide (pectin) binding to the mouse pleural glycocalyx. The similar lectin-binding characteristics and ultrastructural features of the human and mouse pleural glycocalyx suggested the potential application of these polymers in humans. To investigate the utility of pectin-based, we developed a simulacrum using freshly obtained human pleura. Pressure-decay leak testing was performed with an inflation maneuver that involved a 3 second ramp to a 3 second plateau pressure; the inflation was completely abrogated after needle perforation of the pleura. Using non-biologic materials, pressure-decay leak testing demonstrated an exponential decay with a plateau phase in materials with a Young's modulus less than 5. In human pleural testing, the simulacrum was used to test the sealant function of four mixtures of pectin-based polymers. A 50% high-methoxyl pectin and 50% carboxymethylcellulose mixture demonstrated no sealant failures at transpleural pressures of 60 cm H₂O. In contrast, pectin mixtures containing 50% low-methoxyl pectin, 50% amidated low-methoxyl pectins, or 100% carboxymethylcellulose demonstrated frequent sealant failures at transpleural pressures of 40–50 cm H₂O ($p < .001$). Inhibition of sealant adhesion with enzyme treatment, dessication and 4°C cooling suggested an adhesion mechanism dependent upon polysaccharide interpenetration. We conclude that pectin-based heteropolysaccharides are a promising air-tight sealant of human pleural injuries.

Keywords

air leak; sealant; lung; pectin

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Introduction

Pulmonary “air leaks” are the accumulation of air in the pleural space; typically, air leaks are the result of pleural injury caused by lung surgery or trauma.¹ Air leaks are the most common reason for increased hospital length of stay (LOS) after pulmonary surgery.^{2,3} The impact of air leaks on patient recovery and hospital resources is significant; air leaks result in prolonged hospitalization⁴ and increased healthcare costs.^{5,6} Pleural air leaks increase LOS by 5 to 13 days; more than doubling of the cost of hospitalization.⁵ Moreover, air leaks after pulmonary surgery can lead to atelectasis, pneumonia or empyema.⁷ Because of these late complications, post-surgical prolonged air leaks increase the rate of readmission within 30 days by 20.4%.⁶

In previous work, we have demonstrated the adhesion of pectin-based polymers to the mesothelial surface glycocalyx of the murine lung, liver, bowel, and heart.⁸ Pectins are structural heteropolysaccharides that comprise 30% of the primary cell walls of plants.⁹ Pectins differ from typical pressure sensitive adhesives as they do not bind to most nonbiologic compounds.¹⁰ The selective and strong binding of pectins to the mesothelial glycocalyx⁸ is likely the result of a mechanism of interdiffusion or interpenetration;¹¹ that is, adhesivity results from the entanglement of branched chain polysaccharides¹² based on chemical bonds and weak chemical interactions.^{13–15} Pectin-based mixtures have been shown to effectively bind mouse pleural mesothelium;^{8,10} the potential utility of these pectin adhesives in human applications is unknown.

In this report, we tested the effectiveness of pectin-based bioadhesives to function as “air-tight” sealants of human pleural injuries. The function of the sealants was quantitatively assessed using a sensitive pressure-decay leak test in a custom-designed pleural simulacrum.

Materials and Methods

Human and mouse pleura

Male mice, eight to ten week old wild type C57BL/6 (Jackson Laboratory, Bar Harbor, ME, USA) were anesthetized prior to euthanasia,¹⁶ The care of the animals was consistent with guidelines of the American Association for Accreditation of Laboratory Animal Care (Bethesda, MD, USA) and approved by the Brigham and Women’s Hospital Institutional Animal Care and Use Committee. Human pleural samples were obtained from surgical specimens. The collection of specimens was performed by the Brigham & Women’s Hospital (BWH) Tissue Bank under the direction of the BWH Institutional Review Board. The specimens were obtained from a lung region remote to the surgical pathology. The pleura was transported in normal saline prior to mounting in the simulacrum; no additional treatment of the pleura was performed.

Lectins

The biotinylated lectins were obtained from commercial sources. Concavalina ensiformis (ConA)¹⁷ was obtained from Dako (Carpinteria, CA, USA). The lectins obtained from Vector Laboratories (Burlingame, CA, USA) were as follows: Maackia amurensis (MAL-1),¹⁸ Sambucus nigra (SNL),¹⁹ Solanum tuberosum (STA),²⁰ wheat germ agglutinin (WGA),²¹

Lectin histochemistry

Cryostat sections were obtained from lung specimens perfused with O.C.T. compound and snap frozen. After warming the slide to 27°C, the sections were fixed for 10 minutes (2% paraformaldehyde and PBS at pH 7.43). The slides were washed with buffer (PBS, 5% sheep serum, 0.1% azide, 1mM MgCl₂, 1mM CaCl₂) and blocked with 20% sheep serum in PBS. The slides were treated with the lectin followed by avidin-fluorescein ((Southern Biotech, Birmingham, AL, USA) or avidin-fluorescein alone control. The slides were incubated for one hour at 27°C, washed 3 times and mounted with DAPI-containing medium (Vector Labs. Burlingame, CA, USA).

Enzyme treatment

The tissues were treated with a combination of commercially obtained enzymes previously used to treat mesothelium²² (Sigma-Aldrich, St. Louis, MO, USA): hyaluronidase cleaving the 1–4 linkages between N-acetyl-D-glucosamine and D-glucuronate; neuraminidase, also called sialidase, cleaving the glycosidic linkages of neuraminic acids; pronase is a mixture of proteases derived from *Streptomyces griseus*. Enzyme solutions and tissues were maintained at 37°C during a 60-minute incubation. Following enzyme treatment, the tissues were washed with PBS 3 times.

Pectin-based mixtures

The pectins and the carboxymethylcellulose (CMC) used in this study were obtained from a commercial source (Cargill, Minneapolis, MN, USA) as previously described.⁸ The proportion of galacturonic acid residues in the methyl ester form is determined by the degree of methoxylation. High-methoxyl pectins (HMP) were defined as those pectin polymers with a greater than 50% degree of methoxylation (range 70–75%). In contrast, low-methoxyl pectins were defined as the degree of methoxylation less than 50% (range 30–35%). The low-methoxyl pectins included non-amidated (LMC) and amidated (LMA) forms. The percentage of galacturonic acid converted to carboxylic acid amide was 10–15%.

Simulacrum

The simulacrum used for testing the pleural sealants was custom-designed adapter for the FlexiVent ventilator. The mounting chamber, 1.1ml in gas volume, was a polypropylene cylinder containing two Mediprene thermoplastic elastomer mounting gaskets (Hexpol TPE, Sandusky, OH, USA). The mounting gaskets provide a pressure resistant seal for pressure-decay testing. The surface area exposed to the test pressure was 0.785 cm². The pectin sheet applied to the pleura was 5x5 mm (25 mm²).

Pressure-decay testing

Prior to all measurements, the pressure transducers and ventilator tubing of the FlexiVent (SciReq, Montreal, QC) were calibrated as described.²³ Similar to previously described commercial²⁴ and biological²⁵ leak testing, the chamber was inflated to a target pressure (30 cm H₂O), then isolated from the supply pressure. In the standard inflation maneuver, a 3 second ramp to 30 cm H₂O was followed by a 3 second detection phase. The monitored pressure drop reflected the air leak. The rate of pressure decrease with time was used as an

index of the leak rate. To extend the pressure limits of the FlexiVent, the simulacrum was placed in a negative pressure chamber. The 30 cm H₂O positive pressure and the -40 cm H₂O chamber pressure produced a maximal -70 cm H₂O transpleural pressure gradient.

Non-biologic testing

The polymers used in non-biologic testing were obtained from commercial sources with physical properties obtained from Matbase (www.matbase.com) and the product manufacturer: vulcanized natural latex rubber (latex), Young's modulus 1–2 MPa; acrylonitrile butadiene rubber (nitrile), Young's modulus 2–4 MPa; polyvinyl chloride (vinyl), Young's modulus 10–15 MPa; polyvinylidene chloride (PVCD), Tensile modulus 300–400mPa. The plastic paraffin film (Parafilm) was obtained from Bemis NA (Neenah, WI, USA).

Pleural injury

The standard pleural injuries were created with either a 16g, 19g, 21g, 25g or 30g needle (Becton Dickinson, Mountain View, CA, USA), with an outer diameter of 1.651mm, 1.067mm, 0.8192, 0.5144mm, and 0.3112mm, respectively. The needle was used to penetrate the mounted pleural membrane.

Adhesion strength testing

The bioadhesion test was similar to a previously described method.⁸ Briefly, the load/displacement measurements were made using an apparatus customized for tissue application. Loads were applied at comparable rates; tissue displacement was digitally recorded and analyzed by MetaMorph 7.8 morphometry software (Molecular Devices, Downingtown, PA, USA). The pectin mixtures provided a rigid interface upon which the tissue adherend was applied. The tests were performed with uniaxial load application; all loads were applied to uniform cross-sectional areas of bioadhesion. The tissues were warmed to near-37°C. Adhesion development time was comparable for all specimens.

Statistical analysis

The statistical analysis was based on measurements in at least three different samples. The unpaired Student's t test for samples of unequal variances was used to calculate statistical significance. The data was expressed as mean $\pm$ one standard deviation. A long-rank (Mantel-Cox) testing was used to determine the significance of the product-limit estimator. The significance level for the sample distribution was typically defined as $p < .05$.

Results

Human pleural mesothelium

Previous work has demonstrated pectin binding to the murine mesothelial glycocalyx.⁸ To compare the glycocalyx of human and murine pleural mesothelium, we stained the pleural mesothelium of both species with a panel of fluorescent-labeled lectins. The pattern and intensity of the lectin binding was similar (Figure 1A). To compare the adhesivity of the pectin-based polymer to mouse and human mesothelium, adhesion strength testing (load-

distance) was performed.⁸ Adhesion of a mixture of 50:50 high-methoxyl pectin (HMP) and carboxymethylcellulose (CMC) to the skin, buccal mucosa and pleura of the two species was indistinguishable (Figure 2A). Notably, the adhesion of the pectin mixture to both pleural adherends was significantly greater than the adhesion of the HMP pectin mixture to the skin or mucosa ($p < .001$). In contrast to most pressure-sensitive adhesives (not shown), the pectin mixture did not bind to non-biologic commercial materials (Figure 2B).

Pressure-decay sealant testing

To test the effectiveness of pectin-based bioadhesives as “air-tight” sealants of human pleural injuries, we developed a pleural simulacrum that permitted sensitive pressure-decay testing of both human pleura and non-biologic materials. The system was pressurized with a standard inflation maneuver involving a 3 second ramp to a 3 second plateau at 30 cm H₂O (Figure 3A). Perforation of the pleura with various sized needles resulted in a reproducible pressure-decay; increasingly more perturbed inflation maneuvers were observed with larger holes (Figure 3C). In non-biologic testing, the peak pressures reached by the system were related to the size of the pleural injury (Figure 4A–D). As expected, elastomeric polymers, such as latex (Figure 4A) and nitrile (Figure 4B), were more leak resistant than polymers with minimal elastic properties such as vinyl (Figure 4C) and PVDC (Figure 4D).

Pectin-based sealants

In human pleural testing, freshly obtained human pleural samples were placed in the simulacrum and a baseline inflation maneuver was performed. The pleura was then perforated with a 19g or 21g needle (depending upon tissue thickness) so that a repeat inflation maneuver demonstrated a failure to pressurize the system (e.g. Figure 3C). After perforation, the sealant was applied and the inflation maneuver was repeated. Four different mixtures of pectin-based sealants were tested (Figure 5); all 4 mixtures demonstrated sealant efficacy at 30 cm H₂O transpleural pressure. To obtain a greater transpleural pressure gradient and simulate the negative pressure of a therapeutic tube thoracostomy²⁶, vacuum was applied to the negative pressure chamber (see Figure 2A). CMC and LMA demonstrated sealant failures (pressure-decay) at 30 to 40 cm H₂O of transpleural pressure (gray shading shows the level of transpleural pressure). LMC demonstrated no failures less than 50 cm H₂O. HMP demonstrated no sealant failures below transpleural pressure of 60 cm H₂O (Figure 5D). Although there was some variability with individual pleural tissue samples, sealant strength was reproducibly HMP>LMC>LMA>CMC. HMP mean peak pressures were higher than the other pectins ($p < .05$)(Figure 5E). Similarly, a product-limit estimator²⁷ was used to determine the transpleural pressure time-to-failure of the pectin-based polymer (Figure 5F). HMP sealant strength was significantly greater than the other three mixtures ($p < .01$).

Adhesion and interpenetration

The mechanism of adhesion was also investigated using the simulacrum and pressure-decay testing (Figure 6). A mechanism of interpenetration requires wetting and swelling of the polymer to permit intimate contact between the sealant and the pleura.¹³ Consistent with interpenetration, dehydration of the pleural samples resulted in markedly decreased HMP adhesion ($p < .01$)(Figure 6). Low temperatures (4°C), designed to minimize water flux and

polymer expansion, also significantly decreased HMP adhesion ($p < .01$) (Figure 6). Finally, the dependence of HMP adhesion on the glycocalyx was suggested by the effect of the enzyme treatment of the pleura; a mixture of neuraminidase, pronase and hyaluronidase markedly diminished binding of the pectin-based sealant ($p < .01$) (Figure 6).

Discussion

In previous work, we showed that structural heteropolysaccharides function as pleural sealants in mice.¹⁰ The similarity between the human and murine glycocalyx suggested the possibility of a comparable sealant function in humans. Here, we show the efficacy of structural heteropolysaccharides in sealing human pleura. The striking finding was the adhesion strength between the HMP pectin mixture and the human pleural glycocalyx. In normal human tidal ventilation, pleural pressures vary over a range of 30 cm H₂O.²⁶ Typically, peripheral airway pressures of 15–20 cm H₂O are required to recruit lung volumes from surgery-related or low ventilation-associated atelectasis.²⁸ A person with a strong chest wall can generate pleural pressure gradients over 50 cm H₂O.²⁹ In this work, we consistently measured HMP adhesion strength of 60–70 cm H₂O. We conclude that structural heteropolysaccharides in general, and high-methoxyl pectins in particular, are promising air-tight sealants of human pleural injuries.

Pectin-based sealants have several additional features that suggest the utility of their clinical application. In contrast to pressure-sensitive adhesives, pectin-based sealants are not “tacky” to the touch, but selectively bind to the pleural mesothelium.⁸ The sealant preparation requirements are negligible and binding occurs over a 2–3 minute development time. Mouse studies have demonstrated an effectively sealed pleural injury, with no pleural adhesions or evidence of toxicity, when studied over 7 days.¹⁰ Finally, the presence of pectin-based mixtures in human food and oral medication capsules^{30–32} suggests that the pectin-based sealant will be similarly nontoxic in humans.

The simulacrum was developed to permit the quantitative leak testing of human pleural tissue samples. In addition to sealant testing, the use of the simulacrum provided physiologic insights into pressure-decay observations in the intact lung. After pleural injury in an intact mouse lung, Servais and co-workers have previously demonstrated an initial pressure decay to a reproducible plateau pressure of 16–18 cm H₂O.²⁵ The observed pressure plateau suggested inspiratory flow limitation in the peripheral airways.²⁵ Consistent with this proposed mechanism, injury of the isolated pleura (without airways) did not result in flow limitation.

A variety of polysaccharide polymers have been used in tissue engineering applications. In addition to pectin, polysaccharide biopolymers used in medical applications including alginate,³³ agarose,³⁴ cellulose,³⁵ and chitin.³⁶ Pectin is a particularly intriguing polysaccharide because of its structural features.³⁷ Pectin is a structural heteropolysaccharide that comprises approximately 30% of the primary cell walls of plants⁹. Chemically, pectin consists predominantly of partially esterified linear chains of (1,4)-alpha-D-galacturonic acid residues.^{38,39} A proposed mechanism of bioadhesion is a wetting phase involving swelling of the polymer to permit intimate contact with the adherend.

followed by polymer chain interpenetration and entanglement.⁴⁰ Consistent with this mechanism, we found that pectin-based adhesion was limited by enzymatic treatment, drying and 4°C temperatures. Notably, the striking adhesion strength of the pectin-based polymer provides an insight into the binding energy of this proposed mechanism.

Finally, the pectin-based polymers have an additional structural feature with therapeutic implications. Upon gelation, the polymers form a corrugated “egg-box” structure.⁴¹ The structure of the pectin-based polymers has led to their use in the controlled release of oral medications formulated as tablets, gel beads and coated pellets.⁴² Pectins have also been incorporated in wound dressing.⁴³ In future studies, the ability to incorporate drugs or growth factors into the pleural sealant offers an intriguing opportunity to accelerate the healing of pleural injuries.

Acknowledgments

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Abbreviations

CMC	carboxymethylcellulose
Con A	concanavalin A
HMP	high-methoxyl pectin
LMA	low-methoxyl amidated pectin
LMC	low-methoxyl pectin
LOS	length of stay
MAL-1	<i>Maackia amurensis</i>
SNL	<i>Sambucus nigra</i> , STA, <i>Solanum tuberosum</i>
WGA	wheat germ agglutinin

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Figure 1.

Comparison of human and mouse pleural glycocalyx. Binding of 5 fluorescently-labeled lectins to human and mouse pleural glycocalyx and counterstained with a blue nuclear dye (DAPI) demonstrated similar pattern and intensity; Bars=50um. Con A, concanavalin A; MAL-1, *Maackia amurensis*; SNL, *Sambucus nigra*; STA, *Solanum tuberosum*; WGA, wheat germ agglutinin.

Figure 2.

Bioadhesion of the pectin-based polymer to biologic and non-biologic adherends. Adhesion was measured using a load/displacement assay⁸; uniaxial loads were applied at 90° to the surface area-adjusted adhesive interface. A representative load/displacement assay is shown in the inset; the arrow shows the yield strength. A) In biologic testing, the pectin-based polymer HMP demonstrated progressive adhesion strength in binding to human and mouse skin, buccal mucosa and pleura (N=5). Binding strength of the HMP to the pleura was greater than the binding to skin or mucosa ($p < .01$). There was no statistical difference between human and mouse pleura. B) In non-biologic testing, the HMP demonstrated minimal binding to the moist interface of the adherend; dry surfaces demonstrated no detectable binding. Human pleural binding is shown for comparison and is significantly greater ($p < .0001$). Quintuplicate samples are shown; error bars are ± 1 std dev.

Figure 3.

Pressure-decay leak testing of pleura using the simulacrum. A) The FlexiVent ventilator (SciReq, Montreal, CA) was used to monitor pressures during the computer-controlled 3 second ramp to 30 cm H₂O followed by a 3 second pressure plateau. In testing biologic samples, the pleural sample (shown in gray inset) was placed in a negative pressure chamber with a range of 0 to -40 cm H₂O to extend the transpleural pressure range of the system²⁵. B) In baseline testing of pleural samples, the inflation maneuver resulted in a stable pressure plateau. C) In the test conditions, the mounted pleura was perforated by a needle of various caliber: 30g, 25g, 21g, 19g and 16g. Representative pressure decays are shown. In most cases, perforation of a 21g or 19g needle resulted in inflation failure (arrow). All pleural samples demonstrated lower pressure-decay curves with increasing needle caliber. Because of the variable thickness of the pleural tissue, complete decompression was empirically obtained prior to application of the pectin-based sealant.

Figure 4.

Pressure-decay leak testing of non-biologic materials using the simulacrum. In non-biologic testing, the material was penetrated by needle of various caliber: 30g, 25g, 21g, 19g and 16g. Representative pressure decays are shown for latex (A), nitrile (B), vinyl (C), and PVDC (D). All materials demonstrated lower pressure decay curves with increasing needle caliber. Materials with a Young's modulus less than 5 MPa (A and B) demonstrated a pressure plateau with small caliber needles. Representative curves are shown.

Figure 5.

Pressure-decay leak testing of human pleura using the simulacrum. A–D) Perforated human pleura was sealed with four different pectin mixtures. Solid line shows baseline inflation maneuver to 30 cm H₂O; dotted lines reflect the pressure curves of 4 representative samples. The gray shading reflects the transpleural pressure level (ventilator pressure ± negative pressure chamber): A) –10 cm H₂O, B) –10 cm H₂O, C) –20 cm H₂O, and D) –30 cm H₂O. E) Mean peak transpleural pressures prior to sealant failure based on N=5 samples demonstrated a significant difference between HMP and CMC and LMA ($p < .001$). Error bars reflect ± 1 std dev. F) Similar data presented as a product-limit estimator shows the superior sealant performance of the 50% HMP mixture; N=12 samples, $p < .01$.

Figure 6.

Mechanism of interpenetration of the pectin-based sealant and human pleura. A) Median peak pressures show a markedly decrease in adhesive strength in the treatment conditions. Pretreatment of the pleura with dessication, 4°C temperatures, or enzymes (hyaluronidase, pronase, and neuraminidase) inhibited sealant binding. B) Composite data presented as a product-limit estimator shows the sensitivity of glycocalyx-dependent adhesion to dessication, temperature and enzyme digestion (N=3–5 samples; $p < .01$).