SUPPORTING INFORMATION

Pressure-Sensitive Tissue Adhesion and Biodegradation of Viscoelastic Polymer Blends

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Materials and Methods

Polymer Solutions for Producing Pressure-Sensitive Tissue Adhesives

Polymer solutions were prepared at a 20% w/v concentration in acetone. All solutions consisted of a low molecular weight (LMW) component, poly(D,L-lactide-co-caprolactone) (70:30 L:CL, acid endcap, Mn 15,000-25,000 Da, Akina), and a high molecular weight (HMW) component of either poly(D,L-lactide-co-caprolactone) (70:30 L:CL, acid endcap, Mn 35,000-45,000 Da, Akina) or poly(D,L-lactide-co-glycolide) (L:G 50:50, 0.76-0.94 IV, Lactel). Fibrin glue (Tisseel, Baxter International Inc.) was used as a control. An airbrush (Master Airbrush, G222-SET, 0.2 mm nozzle diameter) was used to deposit the surgical sealants. The airbrush was connected to a compressed CO2 tank equipped with a pressure regulator set to 20 psig. Unless stated otherwise, polymer samples were produced by solution blow spinning (SBS) onto a 22 mm by 22 mm glass coverslip, with the distance between airbrush nozzle and cover slip at approximately 10 cm.

Gel Permeation Chromatography

Polymer samples were produced by spraying 500 µL of polymer solution. Segments from the resulting polymer samples were cut and dissolved at 3 mg ml^{-1} in THF. Two Agilent Easical GPC calibration standards, A and B, were dissolved at 2 mg ml^{-1} in THF and a blank THF control was also prepared. Samples were run on the Waters e2695 Separations Module (GPC). Upon run completion, the calibration standards were analyzed to develop the calibration curve. The molecular weight, molecular number and polydispersity of each sample were then obtained from the sample curves and recorded. Each sample type was replicated 3 times (n = 3).

Differential Scanning Calorimetry

Polymer samples were produced by spraying (SBS) 500 µL of polymer solution. The resulting polymer samples were removed from the coverslips and trimmed to yield 10 mg samples. The samples were then sealed in aluminum hermetic pans (TA Instruments) using a sample encapsulation press. DSC measurements were made on a TA Instruments DSC Q100. Samples

were held isothermal at -50°C for 5 min and then heated and cooled from -50 to 80 to -50°C, at a rate of 3°C min^{-1}, ±0.20°C amplitude, with a modulation period of 60 s for two continuous cycles. T_g onset was calculated using the tangent intersection method on the reversing heat flow. The inflection point of the reversing heat flow during the T_g was used to determine the midpoint.

Atomic Force Microscopy

Polymer samples were collected on a glass slide and transitioned at 37° C for 30 minutes. Atomic force microscopy (AFM) was performed on an MFP-3D (Asylum Research) in AC mode using a Tap 300 DLC silicon cantilever tip coated with diamond-like carbon (BudgetSensors). Images were acquired using a 2 µm x 2 µm scan area with a 256 x 256 resolution. Height and phase data visualized in three dimensions using the Gwyddion software package.[1]

Oscillatory Shear Rheology

Oscillatory shear rheology was collected using an AR2000 stress-controlled rheometer (TA Instruments). Polymer samples were collected on a glass slide, transitioned at 37° C for 30 minutes, and then transferred to the 8 mm parallel plate geometry of the AR2000. Frequency sweeps were run using a 1% strain at room temperature.

Compression Creep Testing

Polymer samples were produced by spraying 500 µL of polymer solution. The polymer samples were removed and formed into a circular disk with 5 mm diameter. Using the compression clamp of the TA Instruments DMA, the samples were compressed under 20 kPa of pressure for 1 minute and then relaxed for 5 minutes. Creep behavior is calculated by dividing the time-dependent strain by the constant force. The creep compliance vs. time curve during the compression phase was plotted. Each sample was replicated once (n = 1).

Stress-Relaxation Testing

Polymer samples were produced by spraying 500 µL of polymer solution onto a coverslip. The resulting polymer samples were removed from the coverslips and trimmed to a rectangular shape, approximately 10 mm by 5 mm in size. Exact sample dimensions were measured immediately prior to testing. Stress-relaxation testing was performed on the TA Instruments DMA. With a preload force of 0.001 N, the samples were strained to 10% for 10 minutes and relaxed for 10 minutes. The strain vs. time curve of each sample was analyzed and the final strain after the 20 minutes was recorded. To calculate the strain recovery, the final strain was subtracted from the initial 10% strain, and this value was taken as a percentage of the 10% strain. Each sample type was replicated 5 times (n = 5).

Pull-off Adhesion Testing

Pull-off testing was performed on the TA Instruments DMA Q800. Polymer samples were produced by spraying 1 mL of polymer solution directly onto square 10 cm segments of Gore-Tex Cardiovascular Patch (polytetrafluoroethylene, Gore Medical) and porcine aorta. The coated heart patch and aorta were allowed to set for 15 minutes in 37°C ambient air. Prior to testing, the cardiac patch and aorta were brought into contact and superglued to the clamps of the mechanical analyzer—the aorta to the fixed clamp and the cardiac patch to the movable clamp. For fibrin glue, samples with similar size and mass to the SBS polymer samples were created by depositing approximately 500 µL of fibrin glue onto the aorta. To test the dependence of the polymer sample's strength on the time of applied pressure, the samples were compressed at 1 N for either 10 seconds, 1 minute, or 5 minutes. After compressing, a controlled force ramp was used to increase pull-off force at a rate of 1 N min^{-1} until failure. The

adhesion strength of each sample for each timepoint was recorded, as well as the mode of failure for each sample. Each sample type was replicated five times (n = 5).

Burst Pressure Testing

Porcine small intestine purchased from a local butcher was cleaned with water and cut into 20 cm segments prior to use. For testing, the small intestine segments were rehydrated and heated to 37 degrees Celsius by soaking in 37°C phosphate buffered saline (PBS) for two minutes, followed by exposure to 37°C ambient air for four minutes, repeating this process twice, and finally drying with gauze (Fisherbrand). Once rehydrated, a half diameter incision was made to simulate a leaky anastomosis and the ends of the tissue were closed with zip ties. Approximately 1 mL of polymer solution was deposited directly onto the intestinal tissue at the site of the anastomosis using the SBS process. For cyanoacrylate and fibrin glue, 500 µL of adhesive was applied. After applying the adhesive, it was allowed to set for 15 minutes at 37°C in ambient air.

Once the adhesive was set, the intestine was connected to the burst pressure testing set up. A syringe was used to inject 1x PBS dyed with 0.05% methylene blue into the intestine at a constant rate using an 18-gauge needle. A digital pressure gauge was attached to the injection line using a three-way stopcock to measure the injection pressure. The maximum pressure prior to bursting or leakage was recorded as the burst pressure. The entire test was captured on video so that the failure mode could be determined. Each adhesive was tested five times (n = 5).

Mass Loss, Degradation Testing, and Contact Angle Measurements

Polymer samples were produced by spraying 2 mL of polymer solution onto a coverslip. A microbalance (Sartorius ME-5) was used to determine the net increase in mass after the spinning process was complete, which is the initial sample mass, m_i. Samples submerged in 4 mL of 1x PBS in wells of a 6-well plate, and stored in a shaker incubator at 37°C and 50 rpm. PBS was changed every 48 h until the end of the study to prevent pH change. Samples were removed at time points of 0, 1, 3, 7, 14, and 28 days. At these points, the PBS was removed, and the samples were stored in a vacuum desiccator for three days. The samples were weighed again to determine the final mass, m_f, and mass loss ($m_i - m_f$) was calculated as a percentage of m_i. Samples that swell with water may produce a negative mass loss because of incomplete water removal and salt that remains in the polymer matrix. Five samples were used for each time point and surgical sealant type (n = 5).

Surface wettability was characterized by water contact angle measurements at room temperature, with images captured on a Nikon D3400 (Nikon) and subsequent analysis performed in ImageJ (NIH). Samples were degraded as above, with removal at time points of 0, 3, and 10 days. At these points, the PBS was removed and the samples were dried in a vacuum desiccator for three days. Advancing contact angle of 20 µL droplets of deionized (DI) water was measured using the sessile drop technique. Five samples were used for each surgical sealant type (n = 5).

Tensile Testing

Tensile testing was performed to determine the mechanical properties of the adhesive polymer samples over time. For the 0-day test, polymer samples were produced by spraying 500 µL of polymer solution onto a glass coverslip. For 3-day and 7-day testing, polymer samples were degraded according to the procedure used in the previous section. The SBS polymer samples were removed from the coverslips and trimmed to a rectangular shape, approximately 10 mm by 5 mm in size. Exact sample dimensions were measured immediately prior to testing. Tensile

testing was performed on a TA Instruments DMA Q800 equipped with a film tension clamp. Samples were stretched under a controlled force ramp from 0 N to 5 N at a rate of 0.001 N min^{-1}. Measurements were made either at room temperature or at 37°C after a 10 min isothermal period. Elastic modulus was calculated from the linear region of the resulting stress/strain curve. Failure strain was recorded as the strain at break. Each sample type was replicated 5 times (n = 5).

Cardiac Patch Adhesion Model

A porcine heart was purchased from a local butcher. The heart was warmed and rehydrated prior to use by wrapping in wet gauze and subsequently wrapping in aluminum foil before being exposed to 37°C ambient air for 20 minutes. Upon rehydration, a puncture was made using a punch biopsy and about 1 mL of polymer solution was deposited onto the biopsy site via SBS. A small cardiac patch was adhered to the wound under constant pressure for 1 minute and the cardiac patch was then slowly removed using tweezers.

Mouse Intraperitoneal Space Implantation Model

All animal procedures were approved by the Children's National Medical Center Institutional Animal Care And Use Committee (IACUC protocol #00030703), and the animals were treated in accordance with PHS Policy on Humane Care and Use of laboratory Animals, the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. Eighteen, 7-15 week-old C57BL/6 female mice were used (Jackson Laboratory). Mice were randomized into groups based on type of implant. Saline injection was used as a control. Experimental endpoints were 3 days and 10 days after initial surgery. A total of five mice were allocated to each treatment group per endpoint. Polymer adhesive implants were made under sterile conditions by solution blow spinning 2 mL of polymer solution onto a sterile cover slip then cutting the resulting fiber mat into disks weighing approximately 10 mg in a biosafety cabinet. After processing, the implants were sterilized by UV irradiation.

All mice were anesthetized with a solution of ketamine and xylazine. Buprenorphine was given for analgesic at the start of the surgery and then every 12 hours for 48 hours. After anesthesia, the mice were positioned supine, abdominal hair removed, and then skin prepped with betadine solution. In sterile fashion, a 1 cm laparotomy incision was made at the midline. After dissection into the peritoneal cavity, the polymer samples were implanted into the right lower quadrant. For the saline injection control, 0.2 mL of sterile saline was dripped into the right lower quadrant. Animals were sacrificed 3 or 10 days after initial surgery. After euthanasia, midline laparotomy was performed, and images of the peritoneal cavity were taken with a 15-megapixel digital camera (Canon). The intraperitoneal space was then examined by a surgeon for signs of inflammation, adhesions formation, and degradation (in the form of fragmentation) of the polymer sample.

After euthanasia, cardiac puncture was also performed for serum cytokine assessment. Whole blood was collected via sterile syringe and 25-gauge needle to determine cytokine levels in the serum. In sterile, uncoated vials, blood was allowed to clot over 15 minutes, then serum extracted from supernatant after centrifugation for 15 minutes at 4oC and 2000 RPM. Serum was stored at –80°C until ELISA analysis for INFγ and TNFα. Analysis was performed using ELISA kits (Mouse TNFα High Sensitivity ELISA and Mouse INFγ Platinum ELISA, Invitrogen, NY) on serum samples in duplicate. Serum concentrations were interpolated from standard curves.

Statistical Analysis

Statistical analysis was performed on Origin (OriginLab). Typically, one-way ANOVA was used to compare group variation, followed by post-hoc pairwise Tukey comparison to determine significant differences between the groups. Typically, averages were plotted with error bars representing standard error. Asterisks are used to indicate statistically significant differences: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. If no asterisks are shown, there are no significant differences amongst the groups.

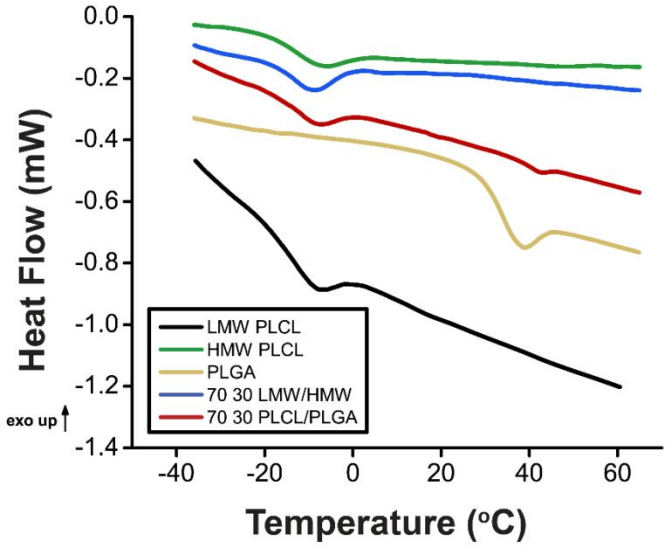

Figure S1. Differential scanning calorimetry (DSC) of pressure sensitive tissue adhesive polymer blends. Pure LMW and HMW PLCL (as well as their blends) have a T_g of approximately –7°C. PLGA has a T_g of approximately 40°C. 70:30 PLCL:PLGA displays two T_gs, one for PLCL, and one for PLGA.

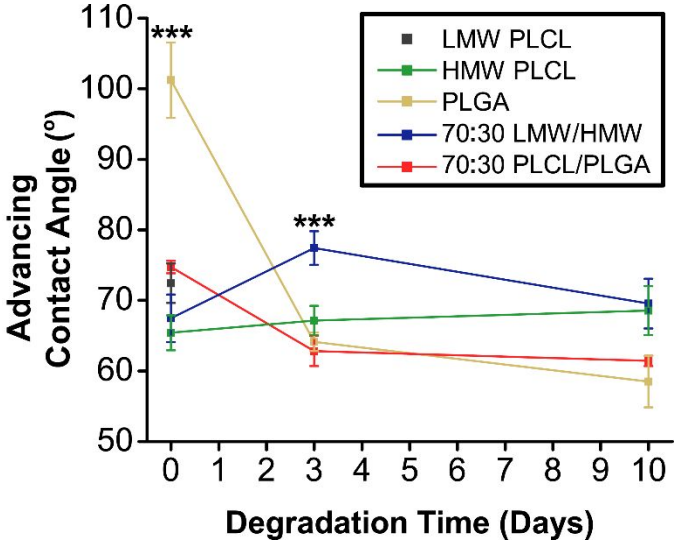

Figure S2. Advancing contact angle measurements on various pressure sensitive tissue adhesive polymer blends and pure polymers. LMW PLCL degrades completely within 3 days, and so it could only be tested at day 0.

Three-dimensional Regression Model Relating Adhesion Strength, Viscoelasticity, and Degradation Time

A second order logarithmic model was used to fit two data sets: pull-off adhesion strength vs. time, tan(δ) vs time:

$$I(t) = a + b\log t + c^2\log t$$

Here, "time" in the time vs. viscoelasticity plot can be thought of as the relevant time scale for adhesion:

$$t = \frac{100}{f}$$

where *f* is the frequency of oscillation used for oscillatory shear rheology. This transformation was applied to the x-axis to yield a plot of tan(δ) vs. time, which was then fit to the above logarithmic function.

A three-dimensional plot of the regression equations for adhesion strength and tan(δ) vs. time was created in MATLAB. The curves were colored according to the degradation rate of that polymer blend, which was calculated by:

$$degradation\ rate = \frac{\%\ degradation\ over\ 10\ days}{10\ days}$$

The fit of the resulting regression equation *I*(*t*) for each type of adhesive tested is shown in Figure 2.

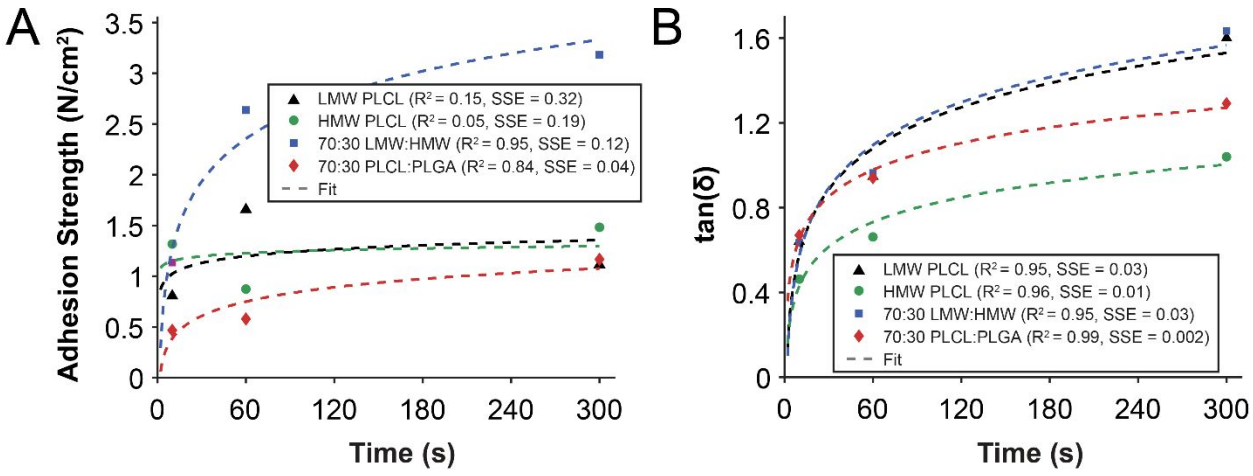

Figure S3. Plots showing a logarithmic model fit to the data sets for (A) adhesion strength versus time and (B) tan(δ) versus time. The goodness-of-fit statistics coefficient of variation (R^2) and sum of squares estimate of error (SSE) are listed alongside the respective curves in the key for each plot.

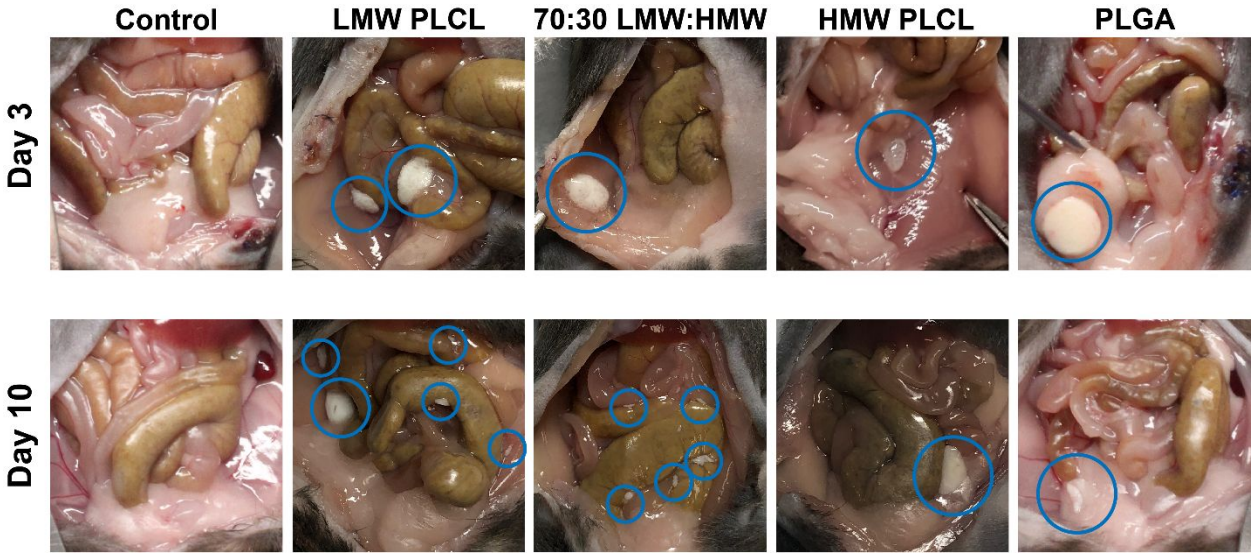

Figure S4. Array of images showing various polymers implanted into the intraperitoneal space of mice, at 3 and 10 days post-implantation. Polymer is highlighted with a blue circle.