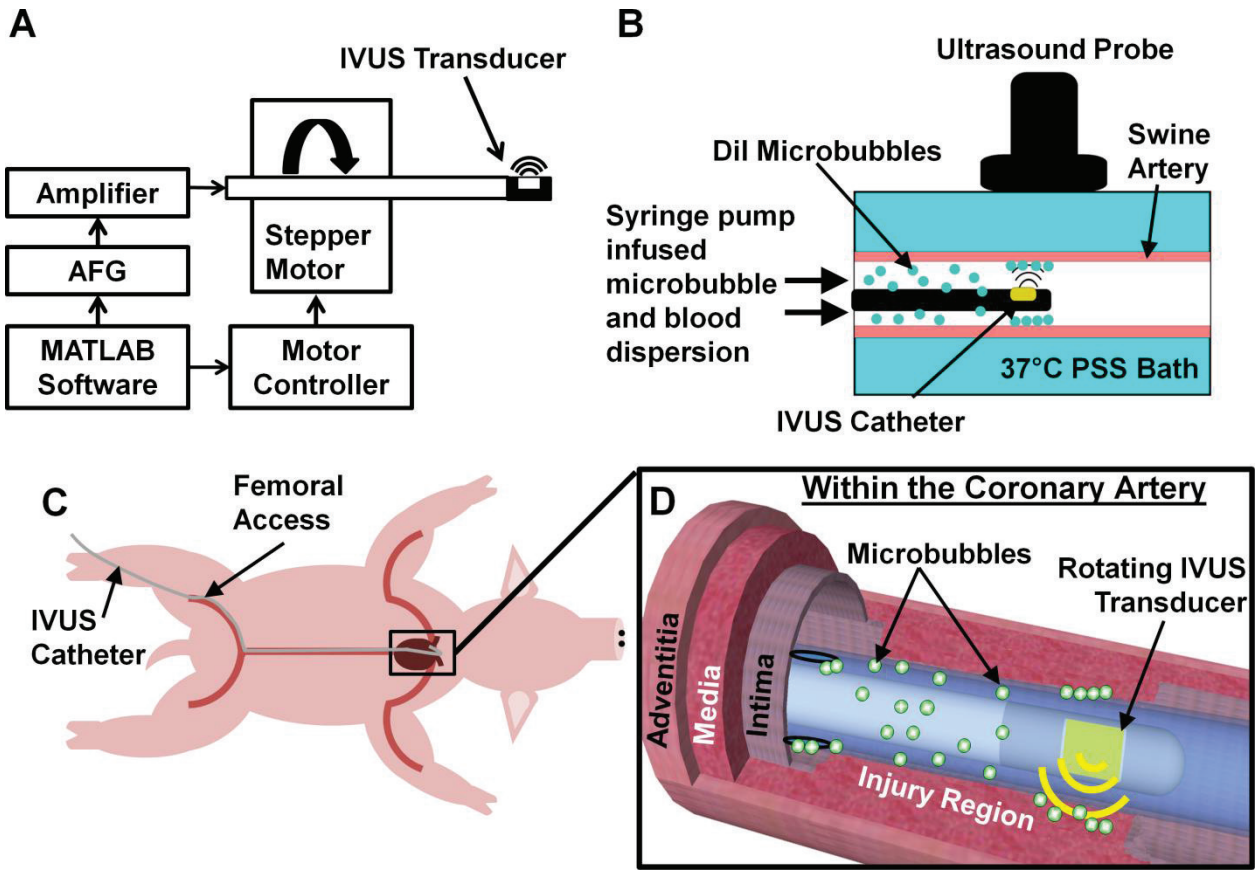
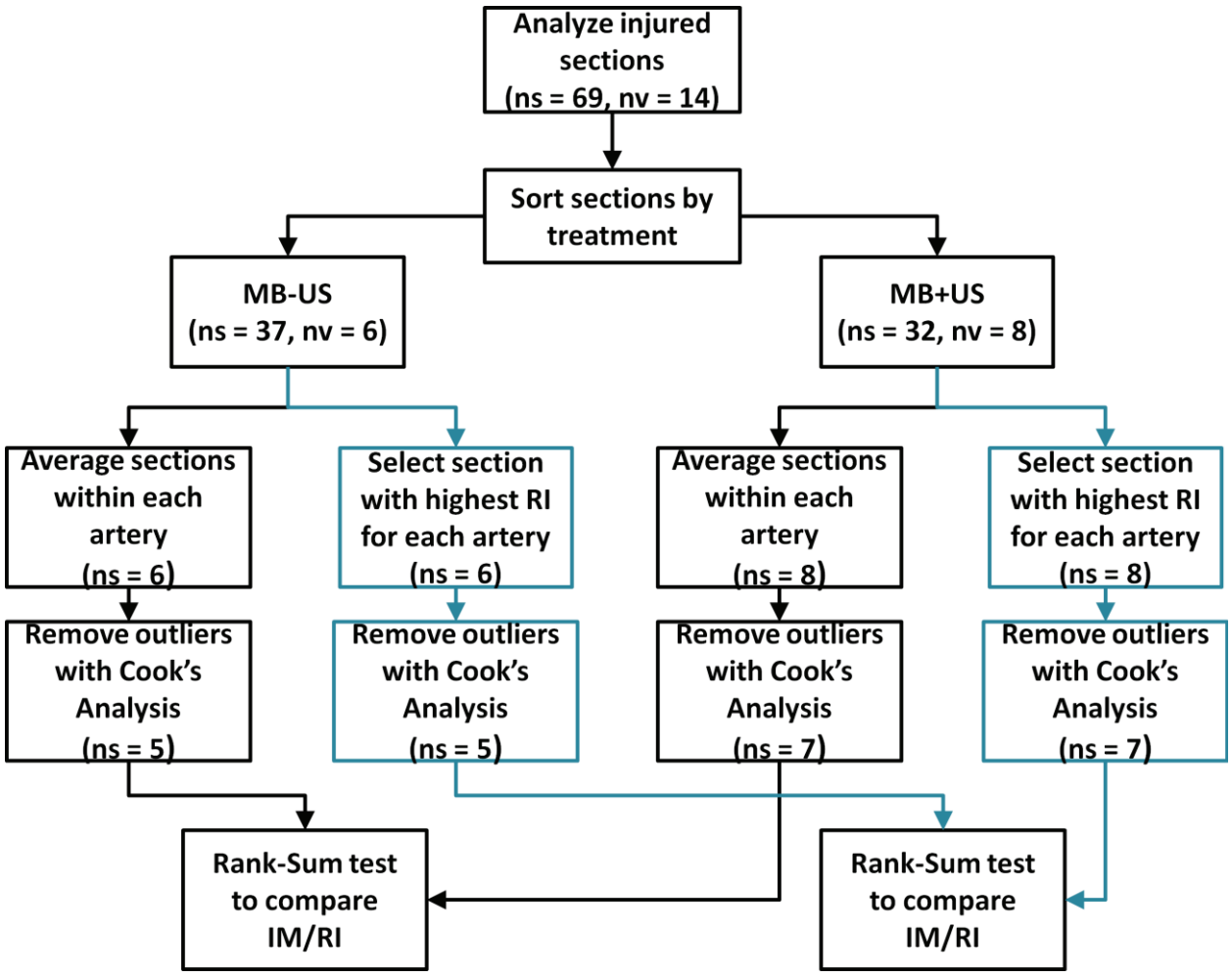
Supplemental Table I: 1x PSS solution components.

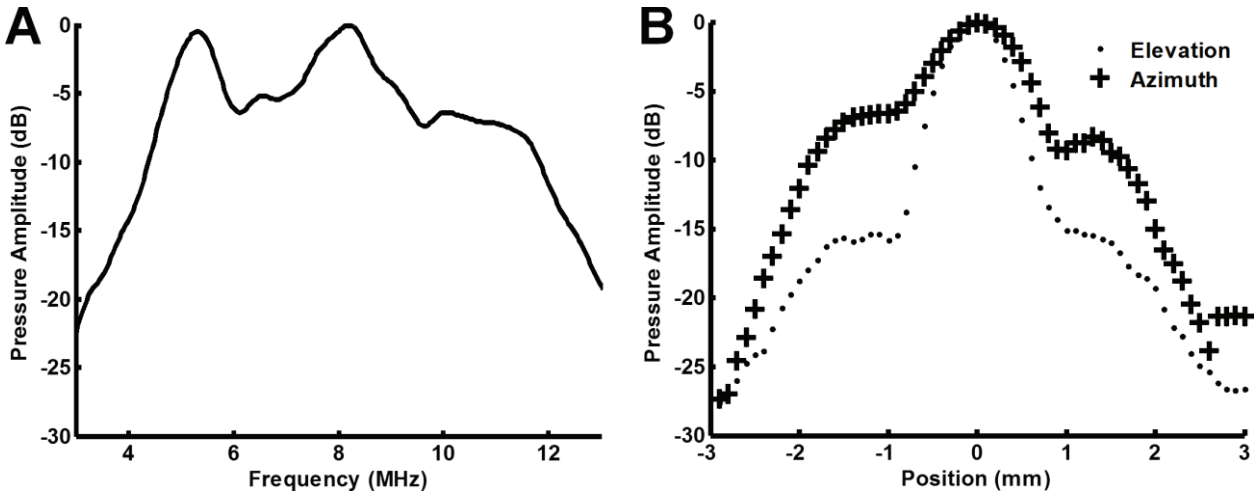
Ingredient	Molar Concentration	Source
NaCl	139 mM	Sigma-Aldrich, St. Louis, MO, USA
KCl	4.7 mM	Sigma-Aldrich, St. Louis, MO, USA
Na_2HPO_4	2.3 mM	Sigma-Aldrich, St. Louis, MO, USA
MOPS	2.0 mM	Sigma-Aldrich, St. Louis, MO, USA
EDTA	25.3 μM	Sigma-Aldrich, St. Louis, MO, USA
1M $MgSO_4$	1.2 mM	Sigma-Aldrich, St. Louis, MO, USA
1M $CaCl_2$	1.6 mM	Sigma-Aldrich, St. Louis, MO, USA
Glucose	5.55 mM	Sigma-Aldrich, St. Louis, MO, USA



Supplemental Figure 1: A) Intravascular ultrasound pulsing and rotation schematic. B) *Ex vivo* DiI delivery schematic. C) Schematic of the *in vivo* ultrasound and microbubble treatment. D) Schematic of ultrasound and microbubble enhanced delivery within the coronary artery. Sirolimus-loaded microbubbles are infused through the catheter while the IVUS transducer rotates and displaces the microbubbles to the vessel wall with ultrasound.



Supplemental Figure 2: Data processing work flow. ns = total number of samples across all vessels at each step. nv = number of vessels at each step.



Supplemental Figure 3: IVUS acoustic characterization results. A) Transfer function of a completed therapeutic IVUS transducer with output frequencies of 5.3 MHz and 8.2 MHz. The 5.3 MHz width mode frequency can be used to insonate microbubbles and the 8.2 MHz center frequency can be used for image guidance. B) The experimental beam profiles for the therapeutic IVUS transducer in azimuth (longitudinal in the artery) and elevation (circumferential in the artery) measured 2 mm from the transducer face. The -6 dB beamwidths was 1.5 mm and 0.9 mm for azimuth and elevation, respectively.

Materials and Methods

Microbubble Preparation

In a previous study[14], it was experimentally determined that 3 washes removed the majority of excess DiI/sirolimus and lipids from the microbubbles. For *ex vivo* experiments, microbubbles were washed by centrifuging in a 3 mL syringe containing 0.8 mL of microbubble stock in 2.2 mL of DFB saturated phosphate buffered saline (PBS) at 1000 RPM (225 xg), for periods of 10, 6, and 6 minutes[10]. An additional 6 minute centrifugation was performed for *in vivo* experiments. After each centrifugation, the infranatant was drained and additional DFB saturated PBS added. Microbubbles were used within 6 days of washing for *ex vivo* experiments and within 24 hours of washing for *in vivo* experiments. The average diameters of DiI and sirolimus microbubbles were 2.2 µm and 2.5 µm, respectively.

Microbubble Infusion Losses

To measure microbubble losses due to catheter infusion, microbubbles ($296x10^6$ MB/mL) were infused at a rate of 1 mL/min through the catheter as the IVUS transducer rotated within the catheter (250 rotations per minute), collected from the catheter's ejection port, and then counted using a Coulter Multisizer (Multisizer 3, Beckman Coulter Inc., Brea, CA, USA). A mean microbubble loss of 60 +/- 8% (n=3) was measured. To compensate for this loss, the concentration of microbubbles was increased 3-fold for the *ex vivo* DiI delivery experiments.

Intravascular Ultrasound Catheter and System

In order to provide the high duty cycles required for displacing microbubbles in blood[1], a custom 5.3 MHz mechanically rotated single element IVUS transducer was designed

and fabricated. Transducer dimensions were selected using finite element analysis (PZFlex, Weidlinger Associates Inc., Mountain View, CA, USA) to design a 5 MHz center frequency transducer that fit within a 500 μm x 700 μm area. A center frequency of 5 MHz was selected to match the resonance frequency of the microbubbles and to provide a frequency capable of sonoporation[2–4]. A hard, PZT-4 type ceramic (EBL#1, EBL Products Inc., East Hartford, CT, USA) was diced into 250 μm x 700 μm elements and backfilled with non-conductive epoxy (RE2039/HD3561, Henkel Corporation, City of Industry, CA, USA). The original ceramic was then removed from a commercial IVUS catheter (Volcano Revolution, Volcano Corp., San Diego, CA, USA) and replaced with the aforementioned transducer. The final catheter diameter when enclosed in the sheath was 3.2 Fr.

An arbitrary function generator (AFG3022B, Tektronix, Inc., Beaverton, OR, USA) controlled using MATLAB software (The Mathworks, Inc., Natick, MA, USA) was used to drive the ultrasound transducer. The function generator output was amplified by a 55 dB RF amplifier (A150, ENI, Rochester, NY, USA), and coupled to the IVUS transducer through a custom slip ring and rotation assembly (Supplemental Figure 1A). The transducer was rotated at a rate of 250 RPM to apply therapeutic ultrasound to the full vessel circumference. This ultrasound system was calibrated prior to use by measuring transducer output pressures with a calibrated hydrophone (HGL-0085, Onda Corporation, Sunnyvale, CA, USA) in a degassed water tank. A -6 dB beam width of 1 mm in elevation and 1.5 mm in azimuth was measured 2 mm from the transducer.

Ex Vivo Ultrasound Parameter Selection

This experimental apparatus was distinct from other *ex vivo* ultrasound drug delivery configurations because the experiment was performed using flowing blood instead of static saline and an IVUS transducer was deployed within the *ex vivo* artery.

The effect of acoustic radiation force and bursting pulses on drug delivery was evaluated using an *ex vivo* swine carotid artery model. Physiological saline solution was prepared in advance (Supplemental Table I). Common carotid arteries were harvested from swine at a local abattoir, immediately immersed into physiological saline solution (PSS), and stored on ice. Bovine blood was collected from recently slaughtered farm cows and 195.75 mg of EDTA was added per L blood. Arteries were cut to 4 cm in length and barbed luer lock connectors were secured to each end with sutures. Tubing was connected to the artery through the luer lock connections and the artery was placed in a PSS bath with an acoustically transparent window. 37°C PSS was constantly pumped through the bath in which the artery was submerged. An ultrasound probe was coupled to the acoustically transparent window using ultrasound coupling gel and the lumen of the artery was imaged using a research ultrasound scanner (Verasonics, Inc., Redmond, WA, USA) (Supplemental Figure 1B). Ultrasound imaging was performed at 9 MHz center frequency, MI=0.04, and PRF=30 Hz. PBS was infused into the artery using a syringe pump (PHD-2000, Harvard Apparatus, Holliston, MA, USA). The IVUS catheter was then positioned at four different longitudinal locations within the artery using the ultrasound images for guidance.

A dispersion of DiI-loaded microbubbles was prepared in 40-45% hematocrit bovine blood (2.25×10^6 MB/mL) based on previously published microbubble concentrations [5–7]. Previous research has demonstrated that microbubble displacement in blood is different

from water or saline, requiring a higher duty cycle ultrasound pulse[1,7]. Flow in the human coronary artery was modeled by infusing blood (105 mL/min flow rate) into the arteries (average diameter 5.78±0.1 mm). This corresponds to a laminar flow velocity of 13.3 cm/s, similar to the averaged peak velocity in a human coronary artery [8]. PBS was infused for 1 minute after each DiI microbubble infusion to remove excess microbubbles from the artery.

Four different longitudinal locations, separated by approximately 8 mm (5x the transducer beam width) within each artery received different acoustic treatments (Table I, Supplemental Figure 1B). Each acoustic treatment was a combination of up to two pulses, displacement and/or a burst pulse (1 MPa or 2 MPa). The displacement pulse was a long (500 cycle), high duty cycle (50%), low peak negative pressure (PNP = 600 kPa) pulse to displace microbubbles from flow to the vessel wall. The delivery pulse was a short (50 cycle), low duty cycle (1%), high peak negative pressure (PNP = 1 or 2 MPa) pulse designed to disrupt microbubbles and induce DiI release and uptake by cells. Ultrasound conditions were selected based on previous research by our group and others[5,6,9,10]. Each longitudinal location received four consecutive 15 second insonations divided between acoustic radiation force and delivery pulses. Every acoustic parameter set was applied to three arteries.

Following treatment, the arteries were cut longitudinally and laid flat on a glass cover slip. Arteries were imaged on a confocal microscope (LSM700, Carl Zeiss Microscopy LLC, Thornwood, NY, USA) with a 555 nm excitation. A mosaic of images along the circumference at each treatment region was collected using the ZEN software (Zeiss Microscopy LLC, Thornwood, NY, USA).

Custom software developed in MATLAB measured the increase in fluorescence intensity along the circumference in each treated region. A 250 μm wide region delimited the edges of a region of interest. The logarithm of the mean fluorescence intensity increase of the treated region from the surrounding untreated artery region was determined for each acoustic setting in order to compare different acoustic parameters for treatment.

In Vivo Treatment

Prior to catheter placement, animals received an intramusculuar injection of xylazine (2.25 mg/kg), telazol (5.0 mg/kg), and atropine (0.025 mg/kg). Animals received 325 mg aspirin 24 hours prior to treatment and both initial (0.03 ml/kg) and maintenance (0.01 ml/kg hourly) doses of heparin. In addition, each pig was treated with indomethacin (2x50 mg suppositories, Indocin, IROKO Pharmaceuticals, PA, USA) prior to treatment to prevent acute myocardial infarction due to a known allergic reaction in swine to microbubbles [42]. Balloon injury was performed in 2-3 coronary arteries per animal, the left circumflex (LCX), the left anterior descending (LAD), and the right coronary (RCA) arteries. In one case, the ramus was treated instead of the LAD due to anatomic limitations preventing access to the LAD. Vessel diameter was measured by angiography and inflation pressure was selected such that the balloon (Maverick 12-20 mm length, Boston Scientific, Natick, MA, USA) was 1.3x the artery diameter. The balloon was inflated three times for 30 s with a 1 minute rest between inflations[40,41]. Two microbubble ejection ports (Supplemental Figure 1C) were cut in the IVUS catheter sheath 2 cm proximal from the IVUS transducer tip with a 25 G needle. After injury, the IVUS transducer was positioned within the injured segment of the vessel under

angiographic guidance. The optimal acoustic parameters, as determined from the *ex vivo* experiments, were applied (5 MHz center frequency, 500 cycles, pulse repetition frequency (PRF) = 5 kHz, PNP = 0.6 MPa) as the IVUS transducer was pulled back within the catheter sheath using a modified syringe pump at a rate of 2 mm/min and the transducer rotated at a rate of 250 RPM. During DiI delivery, no rotation was performed in order to localize delivery along the vessel circumference.

During IVUS treatment, a dispersion of DiI or sirolimus-loaded microbubbles ($8.1x10^9$ MBs and $6.9x10^9$ MBs, respectively) was infused into the catheter lumen at a rate of 1 mL/min while agitating the syringe to prevent buoyancy-based separation of the microbubbles. The microbubbles then exited through the ejection port at the distal end of the catheter into the blood stream. IVUS treatment was applied to an average vessel length of 14.7±2.6 mm in sirolimus treated vessels and a 10 mm length in the DiI treated vessel.

Whole Blood Sirolimus Concentration

Whole blood samples were drawn from four pigs before treatment (T=0), after each vessel was treated (T1-3), and 24 hours after the initial treatment (T4). Sirolimus concentration of these samples was determined by HPLC analysis (iC42, Aurora, CO, USA).

Histology Analysis

Before analyzing the histology data, sections without rupture (RI=0) were discarded (Supplemental Figure 2). Then sections were sorted by treatment type (US+MB vs US-MB) and iteration. Following sorting, sections were selected for each artery using two methods: 1) the mean across all sections and 2) the section with the highest intima to

media ratio. Linear regression was performed with the intima to media ratio as the dependent variable and the rupture index as the independent variable. Cook's distance was measured for each artery and values with a Cook's distance greater than the mean + three standard deviations were labeled outliers and removed from the data set. Following Cook's distance outlier analysis, a Wilcoxon Rank-Sum test was performed to compare IM, RI, and IM/RI between the US+MB and US-MB cases (Table I).

Linear regression analysis of the IM exhibited a decrease in slope when ultrasound was applied to the sirolimus-loaded microbubbles (Figure 2C-D). The decreased slope indicates that the application of ultrasound and sirolimus-loaded microbubbles following balloon injury decreased neointimal formation across the RI range. This result was more prominent when the maximum IM was used to select the artery section for analysis (Figure 2D, slope = 9.06 vs. 4.41) than when mean section values were analyzed (Figure 2C, slope=6.17 vs. 4.13).

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