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ORIGINAL RESEARCH

Novel Guidewire Design and Coating for Continuous Delivery of Adenosine During Interventional Procedures

Mervyn B. Forman, MD, PhD; Erik C. Brewer, PhD; Zachary R. Brown, PhD; Elizabeth V. Menshikova, PhD; Anthony M. Lowman, PhD; Edwin K. Jackson 🆔, PhD

BACKGROUND: The "no-reflow phenomenon" compromises percutaneous coronary intervention outcomes. There is an unmet need for a device that prevents no-reflow phenomenon. Our goal was to develop a guidewire platform comprising a nondisruptive hydrophilic coating that allows continuous delivery of adenosine throughout a percutaneous coronary intervention.

METHODS AND RESULTS: We developed a guidewire with spaced coils to increase surface area for drug loading. Guidewires were plasma treated to attach hydroxyl groups to metal surfaces, and a methoxy–polyethylene glycol–silanol primer layer was covalently linked to hydroxyl groups. Using polyvinyl alcohol, polyvinyl pyrrolidone, and polyvinyl acetate, a drug layer containing jet-milled adenosine was hydrogen-bonded to the polyethylene glycol–silanol layer and coated with an outer diffusive barrier layer. Coatings were processed with a freeze/thaw curing method. In vitro release studies were conducted followed by in vivo evaluation in pigs. Coating quality, performance, and stability with sterilization were also evaluated. Antiplatelet properties of the guidewire were also determined. Elution studies with adenosine-containing guidewires showed curvilinear and complete release of adenosine over 60 minutes. Porcine studies demonstrated that upon insertion into a coronary artery, adenosine-releasing guidewires induced immediate and robust increases (2.6-fold) in coronary blood flow velocity, which were sustained for ≈30 minutes without systemic hemodynamic effects or arrhythmias. Adenosine-loaded wires prevented and reversed coronary vasoconstriction induced by acetylcholine. The wires significantly inhibited platelet aggregation by >80% in vitro. Guidewires passed bench testing for lubricity, adherence, integrity, and tracking.

CONCLUSIONS: Our novel drug-releasing guidewire platform represents a unique approach to prevent/treat no-reflow phenomenon during percutaneous coronary intervention.

Key Words: adenosine ■ cardiac guidewire ■ no-reflow phenomenon ■ percutaneous coronary intervention

In spite of numerous advances in pharmacologic modalities to modify associated risk factors, coronary artery disease remains a major cause of morbidity and mortality.[1] Because of the advent of drug-eluting stents and technical advances in equipment, percutaneous coronary intervention (PCI) has rapidly evolved as the most common modality to treat coronary artery disease.[2] Unfortunately, however, microvascular obstruction (MVO) and the "no-reflow phenomenon" (NRF) remain important obstacles to achieving optimal tissue perfusion after PCI. MVO and NRF are particularly problematic in anterior ST-segment–elevation myocardial infarction (STEMI) and saphenous vein grafts.[3–5] Magnetic resonance imaging studies emphasize the role of MVO as an independent risk factor for major cardiovascular events with a greater predictive power than ejection fraction or infarct size.[6–8]

The mechanisms responsible for MVO are complex and multifactorial and include embolization by atheromatous and thrombotic debris, release of potent vasoconstrictive substances, platelet aggregation, activation of immune cells resulting in leukocyte plugging

CLINICAL PERSPECTIVE

What Is New?

- Microvascular obstruction and the no-reflow phenomenon are major barriers that preclude optimal outcomes in patients undergoing percutaneous coronary interventions.
- Drawing upon numerous technologies, we have developed an adenosine-releasing guidewire that has the potential to prevent and treat microvascular obstruction and no-reflow phenomenon, thus greatly improving patient outcomes.

What Are the Clinical Implications?

- Our novel adenosine-releasing guidewire provides for an autonomous adenosine-releasing system that performs without thought or planning.
- Therefore, by using Adenowires, the interventionalist can protect patients from microvascular obstruction and the no-reflow phenomenon without altering their workflow.
- Adenowires have considerable translational potential.

Nonstandard Abbreviations and Acronyms

CFV	coronary flow velocity
MVO	microvascular obstruction
NRF	no-reflow phenomenon
PEG	polyethylene glycol
PRP	platelet-rich plasma
PVA	polyvinyl alcohol
PVAc	polyvinyl acetate
PVP	polyvinyl pyrrolidone
SS	sum of squares

and endothelial cell disruption of microvessels.[3–5] Since current vascular devices are only partially effective in preventing vascular and organ damage and do not reduce mortality, there is a dire need to develop new technologies to improve outcomes after PCI.[9–12]

Adenosine is an endogenous nucleoside that activates 4 well-characterized receptors. Adenosine preserves microcirculatory flow by (1) preventing and reversing constriction of the coronary microcirculation induced by numerous vasoconstrictors including thromboxane A_2, platelet-activating factor, angiotensin II, norepinephrine, and endothelin-1; (2) inhibiting activation of leukocytes; (3) inhibiting platelet activation; (4) blocking release of myeloperoxidase; (5) preventing calcium overload; and (6) reducing production of oxygen free radicals.[4,5] Indeed, case reports suggest that intracoronary adenosine does reverse MVO/NRF.[13] While the majority of experimental and clinical studies demonstrate beneficial effects of continuous infusions of adenosine on enhancing myocardial salvage and improving microvascular flow, it is likely that the full therapeutic potential of adenosine is compromised because of adenosine's ultra-short half-life (≈1 second) in blood and because of the dilution of the administered adenosine when delivered via a guiding or balloon catheter.[4,5] We therefore conceived the idea that since the guidewire is the first mandatory device placed for PCI, incorporation of adenosine on the guidewire would allow immediate and targeted continuous release of adenosine throughout the procedure and improve post-PCI perfusion.

In our initial attempt, we developed a nontoxic pentameric form of adenosine using polyurethane technology.[14] While cumulative adenosine release and modest in vivo coronary vasodilatation was demonstrated on micro-machined guidewires, it became apparent that it would not be an acceptable solution for 2 reasons. First, the surface area of the guidewire was small, resulting in an insufficient drug load. Second, the coating was degradable and exhibited poor lubricity, durability, and adhesive properties compared with guidewires with hydrophilic coatings. We therefore changed directions to address the inadequacies of the first iteration. This paper describes in detail an innovative device platform to include a new guidewire design and novel hydrogel coating that allows sufficient quantities of adenosine to be released over a typical PCI procedure to produce robust vasodilatation and reversal of severe pharmacologically induced vasoconstriction in the porcine model. Confirmation of coating lubricity, adherence, and durability and guidewire performance of the "Adenowire" is also presented.

METHODS

For data and for additional information on analytic methods or study materials, contact E.K. Jackson at edj@pitt.edu.

Guidewire Design

In preliminary studies, we determined that although micro-machined guidewires could elute adenosine, use of traditional guidewire designs, because of limited surface area, could not provide an optimal drug load.[14] In collaboration with 2 leading wire manufacturers (Asahi-Intecc, Justin, CA; and Lake Region Medical, Chaska, MN), we successively modified their popular wire designs by increasing the coil spacing in the distal 15 cm of the wire to 0.005 inches while maintaining the wire performance and characteristics of the predicated

device. Detailed drawings of the 2 wires are shown in Figure 1. The wires are made of stainless steel and contain a coil spacing zone of 150 mm, with slight variation in the placement of the radiopaque tungsten-platinum marker. The coil-wound section was left uncoated, with the solid mandrel covered with proprietary Teflon. Mechanical testing requirements of both wires (tensile testing of all joints, torque strength, torqueability, tip flexion, and biocompatibility) met all the acceptance criteria and were similar to the predicate device.

Hydrogel-Drug Coating

The proposed chemical reactions involved in coating the wire are illustrated in Figures 2–5. The materials used for coating were polyvinyl alcohol (PVA; Mowiol 28-99, MW 145 kDa; EMO Millipore, Billerica, MA), polyvinyl acetate (PVAc; Kollicoat SR 30D; Sigma-Aldrich, St. Louis, MO), polyvinyl pyrrolidone (PVP; Providone K-30; Spectrum Chemical, New Brunswick, NJ), methoxy–polyethylene glycol (PEG)-silane (Jenkem Technology, Plano, TX), and adenosine (Sigma-Aldrich). Coronary guidewires were cleaned in the distal 20- to 30-cm coiled region with an ultrasonic probe for 5 minutes in ethanol followed by deionized water for 5 minutes and dried via a nitrogen gas line as previously described.[15–17] The wires were then subjected to oxygen plasma treatment for 2 minutes using a commercial device (PE25-JW; Plasma Etch, Carson City,

NV), which resulted in deposition of hydroxyl groups on the surface (Figure 2, reaction 1).[18] A primer layer was then deposited on the wire utilizing methoxy-PEG-silane (MW, 20 kDa). In this regard, a solution of methoxy-PEG-silane (40 mg/mL) was prepared in an ethanol/water solution (95/5 w/w) that was titrated to a pH of 5.0 with glacial acetic acid. After 30 minutes to allow the methoxy-PEG-silane to hydrolyze (Figure 3, reaction 2), the wires were dipped in the hydrolyzed methoxy-PEG-silane mixture (now methoxy-PEG-silanol solution) for 1 hour and cured at 70°C for 90 minutes in a vacuum oven set at <0.1 bar. As shown in Figure 4 (reaction 3), the silanol groups covalently bond to the hydroxyl groups on the stainless steel and crosslink with neighboring silanol moieties to form a robust primer layer.

Next, the inner drug layer was prepared. To accomplish this, a 20% w/w solution of PVA/PVP (99:1 mass ratio) in deionized water was prepared and autoclaved for 1 hour while stirring. This yielded a supersaturated solution that was homogeneous and free of bubbles. Using a water bath, the temperature of the PVA/PVP solution was maintained at 65°C. A 25% concentration of adenosine was required in the drug layer to provide a sufficient drug load on the wire. However, adenosine has poor solubility in water (80 mg/mL), making it difficult to achieve this concentration of adenosine in the drug layer. Moreover, commercial adenosine has a mean particle size of 55 μm which would have compromised
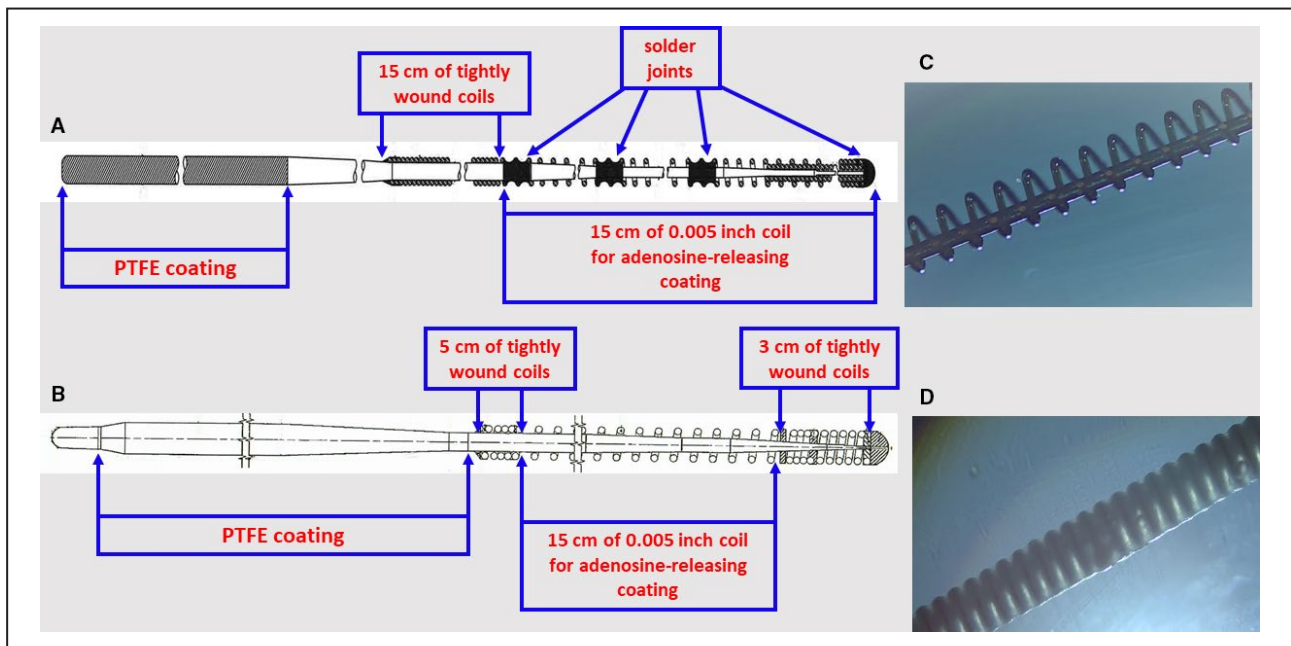

Figure 1. **The diagrams illustrate 2 types of 0.014-inch stainless steel guidewires used in the present study.**
Adenosine-releasing guidewires are redesigns of 2 popular commercially approved wires (0.014-inch stainless steel), which have been modified by increasing the coil spacing to 0.005 inches in the distal 15 cm to accommodate the hydrophilic-drug polymer. In (**A**) the radiopaque platinum-tungsten portion is incorporated in the mandrel and in (**B**) is in the 3 cm of tightly wound coil at the tip. **C**, A micrograph of the redesigned 0.005-inch coils before coating. **D**, Micrograph of the 0.005-inch coils after coating with the polyethylene glycol–silane primer layer, adenosine-loaded hydrogel layer and diffusion-barrier layer. PTFE indicates polytetrafluoroethylene.
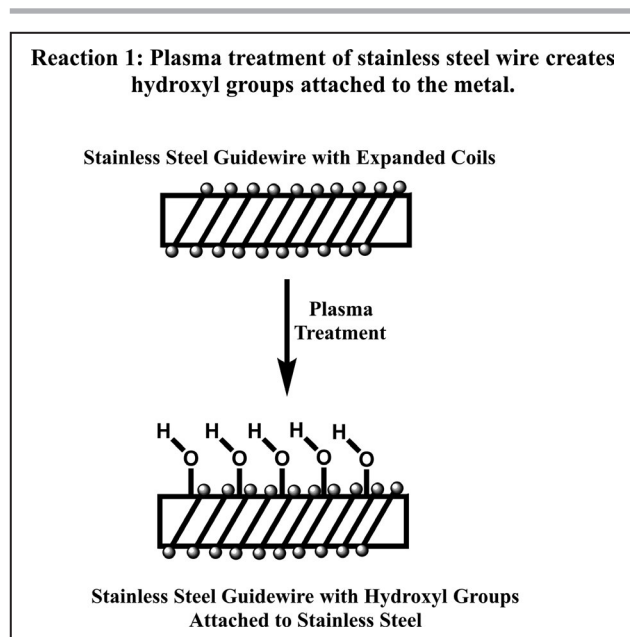
Figure 2. Reaction 1: guidewires were initially exposed to plasma treatment to create OH groups on the stainless steel surface.

Figure 3. Reaction 2: methoxy–polyethylene glycol (PEG)-silane was treated with acid to hydrolyze the ester linkages, thus producing PEG-silanols with exposed OH groups.

the lubricity of the wire. To overcome these barriers, we utilized a 2-pass jet-milling process to reduce the solid-phase adenosine particles to <5 µm. Micronized adenosine was mixed in deionized water, sonicated for 15 minutes, and then added to the PVA/PVP mixture, and the PVA/PVP/adenosine mixture was stirred until a homogeneous mixture was obtained. This resulted in a mixture that contained 7.5% PVA, 67.5% water, and 25% adenosine, which was kept at 65°C for the duration of the coating process. Next, the guidewire coated with the PEG-silanol primer was coated with the adenosine-containing hydrogel. Multiple hydroxyl groups in the hydrogel form hydrogen bonds with multiple ether, carbonyl and amine groups in the primer, thus creating an extensive network of hydrogen bonds between the drug-containing hydrogel layer and the primer layer (Figure 5, reaction 4). This results in a stable attachment of the drug layer with the wire surface. Laser diffraction and optical microscopy demonstrated stable particle sizes of the jet-milled adenosine even after 1 week in the hydrogel.

The outer barrier layer was produced using PVA with PVAc in a ratio of 1:3 (w/w). A 20% solution of PVA without PVP was prepared as described above. Commercially available PVAc packaged as a 30% dispersion with stabilizers and excipients was added to the PVA solution and mechanically mixed at 65°C to achieve a homogeneous solution. The final solution, consisting of 5% PVA, 80% deionized water and 15% PVAc, was left at room temperature.

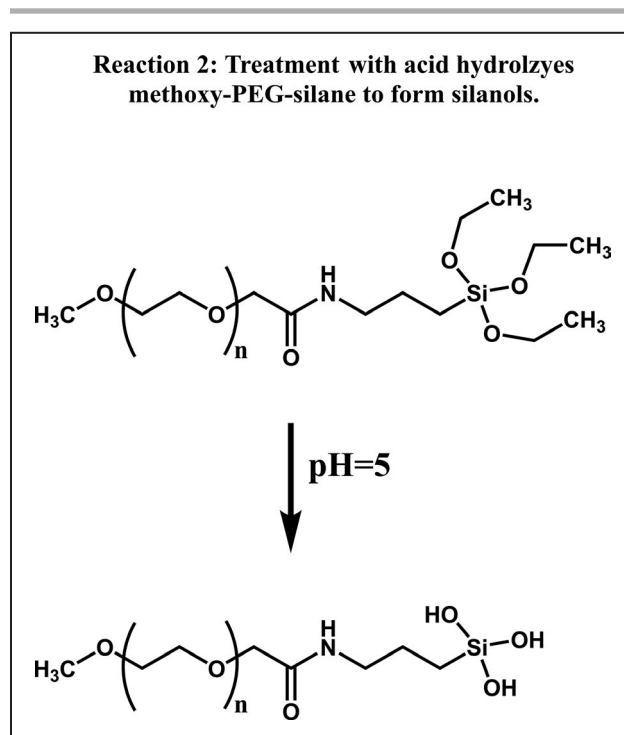The wires were coated using a mechanical dipper (Precision Dip Coater QPI-168; Qualtech, Denver, CO), with insertion and withdrawal parameters set to obtain a total polymer weight of ≈45 mg per 10-cm length of wire. For the drug layer, an insertion rate of 200 mm/min, dwell time of 10 seconds, and withdrawal rate of 40 mm/min was employed. After applying the drug layer, the wire was immediately flash frozen in liquid nitrogen and subjected to a 60-minute freeze/thaw cycle: 45 minutes at −20°C, followed by thawing at room temperature. This freeze/thaw cycle was then repeated to harden the gel. The wire was then coated with the barrier layer using a withdrawal rate of 500 mm/s, after which the wire was subjected to a further 2 freeze/thaw cycles. The final product contained a total polymer weight per 10-cm length of wire of ≈40 to 45 mg with a drug load of 2.7±0.2 mg of adenosine per 10-cm length of wire.

In Vitro Release Studies

These studies (n=6) were performed in 0.9% saline rather than in plasma or blood because adenosine has a short half-life (seconds) in the latter 2 media, making detection difficult. Tubes containing 15 mL of saline were maintained at 37°C, and 10 cm of the spaced coiled section of the guidewire was placed serially in tubes for 0 to 5, 5 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, and 50 to 60 minutes. One-milliliter samples were then taken from the tubes to measure the concentration of adenosine by spectrophotometry.[19]
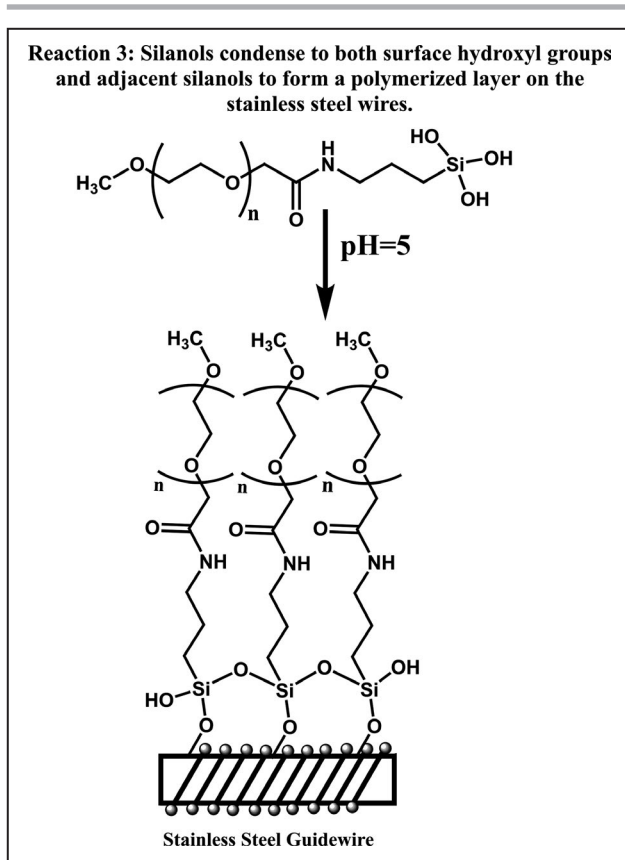
Figure 4. Reaction 3: PEG-silanols condensed with OH groups both on the wire and on neighboring silanols to form a polymerized primer layer.

Figure 5. Reaction 4: grafting of the polyvinyl alcohol/ polyvinyl pyrrolidone/polyvinyl acetate hydrogel to the primer layer is achieved via hydrogen bonding interactions between the carbonyl, ether and amine groups of polyethylene glycol and the OH groups of polyvinyl alcohol.

Animal Studies

All porcine studies were approved by Institutional Animal Care and the Committee of Synchrony Labs LLC (Durham, NC). Yucatán mini pigs (male and female, 25–30 kg, 6 months of age) were premedicated with Telazol (5 mg/kg). Anesthesia was maintained with 1% isoflurane and supplemental oxygen to maintain a pH of 7.4±0.6. Animals received aspirin 300 mg orally for 3 days before the study and were anticoagulated throughout the procedure with heparin (150 units/kg) to maintain activated clotting time >300. Heart rate, ECG changes, and blood pressure were monitored continuously using leads I, aVF, and aVL with a 6 F sheath in the left femoral artery. Another sheath was placed in the right femoral artery for coronary intubation of the left main vessel using a 6 F HS guide catheter. Coronary blood flow velocity (CFV) was measured using a 0.014-inch Doppler flow wire (Combowire; Volcano Corporation, Rancho Cordova, CA) and dedicated software. CFV was measured as a continuous average pulse wave velocity over 4 seconds, and mean values were recorded every 60 seconds.

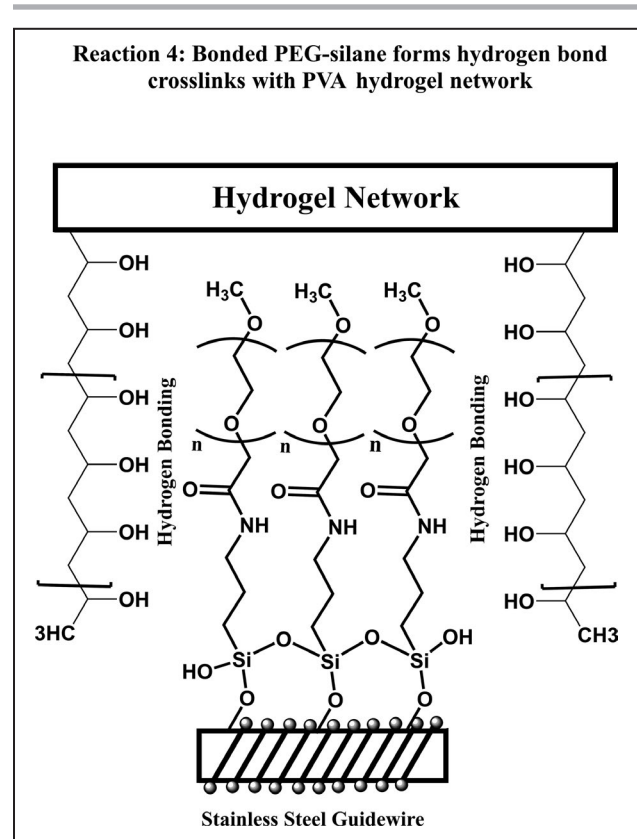Two experimental protocols were employed. In the first protocol (n=8), we assessed the efficacy and duration of vasodilatation induced by the guidewire under basal conditions. The Combowire was positioned in either the proximal circumflex or left anterior descending coronary artery (LAD), and baseline CFV was measured after obtaining an optimal velocity tracing. Care was taken to ensure that the Doppler wire remained in the same position throughout the experiment. An adenosine-loaded guidewire was positioned in the distal circumflex coronary artery or LAD, and heart rate, blood pressure, and CFV was measured serially every minute for 30 minutes. The drug-coated wire was removed, and CFV was measured until it had returned to baseline. Serial coronary angiography was performed at baseline, after Combowire placement and after removal of the medicated guidewire.

In the second protocol, we evaluated the ability of the wire to prevent and reverse intense vasoconstriction induced by intracoronary administration of acetylcholine. Release of numerous potent vasoconstrictors plays a key role in the pathogenesis of NRF. Acetylcholine is a powerful vasoconstrictor in the porcine model via direct muscarinic activation of smooth muscle cells in small coronary

arteries resulting in NRF.[20] A Transit infusion catheter (Codman, Roynham, MA) was placed in the proximal LAD for the drug infusion. Acetylcholine doses of 0.3 to 1.0 mg/min were infused into the LAD for 20 to 30 minutes. Preliminary studies showed that this results in an ≈80% reduction in CFV. In some studies, acetylcholine was commenced initially and after a >50% decrease in CFV was observed, an adenosine-loaded guidewire was rapidly inserted into the distal vessel for 15 to 20 minutes. These studies were designed to determine if the wire could reverse the NRF. In other experiments, we evaluated whether an adenosine-releasing wire could prevent the NRF by placing the adenosine-loaded wire initially into the LAD. Once an increase in CFV had occurred, an acetylcholine infusion was begun for 20 to 30 minutes. In some studies, the wire was removed at 20 minutes and the acetylcholine infusion continued, while in others the acetylcholine infusion was terminated and the wire was left in place for a further 10 minutes in the vessel.

Evaluation of Wire Performance, Coating Quality, and Integrity

Because guidewires are used to place a device into coronary vessels, it is essential that they easily negotiate a tortuous vasculature to facilitate placement of devices without inducing vascular damage. To assess guidewire lubricity, a 2-dimensional coronary model system (ASTM 2394) and a MSI IDTE 3000 device with dedicated software were utilized (Machine Solutions, Flagstaff, AZ). A 6 F coronary guide catheter was introduced 3 cm into the mock vessel. The wire was inserted through a hemostatic valve into the catheter and tracked for 3 cycles in the model for a distance of 38 cm. A proximal load cell with an encoder was used to measure the force applied to deliver and retract the guidewire and the distance in and out of the model. Microscopic images were obtained at baseline, after the track test and after hydration for 1 hour. Wire performance was determined in vitro and in vivo and included the ability to navigate the model or vessel, torqueability, and tip flexibility.

Platelet Aggregation Studies

Human venous blood was obtained from 4 healthy donors who had not taken any medications known to influence platelet function for at least 2 weeks before the study and as described by Gurbel and coworkers.[21] Procedures were performed according to institutional research committee standards, and informed consent was obtained. Venous blood samples were collected into 3.2% trisodium citrate Vacutainer tubes. After 15 minutes, the tubes were centrifuged at 200g for 10 minutes at room temperature to recover platelet-rich plasma (PRP). Platelet-poor plasma was then obtained by centrifuging PRP at 1500g for 10 minutes. Two hundred microliters of PRP were transferred to aggregation cuvettes with stir bars. One-centimeter pieces of guidewires with (Adenowire) and without adenosine (Control) were added to the cuvette and incubated in a multiple-channel optical aggregometer (Chrono-Log Corp, Hovertown, PA) and stirred for 20 minutes. Two commercially available guidewires, BMW (Abbott Vascular, Santa Clara, CA) and Runthrough NS (Terumo, Tokyo, Japan), were also evaluated. Following incubation, PRP was transferred to fresh cuvettes with stir bars and aggregation was initiated by adding 2 μmol/L ADP or 1 μg/mL collagen and monitored for 6 minutes. Aggregation, using platelet-poor plasma as a control reference, was assessed by the aggregometer software. Area under the aggregation curve, maximum aggregation, slope of the aggregation curve, and the lag time between adding the agonist and onset of aggregation were the variables included in the analysis.

Statistical Analysis

Data are expressed as mean and SEM. Statistical analyses of the effects of Adenowires on heart rate; systolic, diastolic, and mean blood pressures; and CFV in porcine experiments (n=8) were conducted on data that were transformed by the Box-Cox method so as to meet the assumptions of ANOVA. In this regard, assumptions of normality of groups and equality of variances among groups were tested using NCSS 2019 Statistical Software (Kaysville, UT;, ncss.com/software/ncss). Box-Cox transformed porcine data were subjected to ANOVA for repeated measures (RM 1-Factor ANOVA) followed by the Bonferroni test (versus control) using NCSS 2019 Statistical Software. This multiple comparison test adjusts the comparison-wise error rate, (c), in such a way that the experiment-wise error rate, alpha(f), is kept at a predetermined level of $P<0.05$. With k means, there are k–1 comparisons with a control. Hence alpha(c)=alpha(f)/(2(k–1)). Sample size was based on our previous study in which we infused adenosine into the coronary artery of closed-chest dogs and obtained significant results with a sample size of 8.[22] R^2 values were calculated by Prism version 8.4.3 for Windows (GraphPad Software, San Diego, CA; www.graphpad.com). R^2 is computed from the sum of the squares (SS) of the distances of the points from the best-fit curve determined by nonlinear regression (SS_{reg}). SS_{reg} is normalized to SS of the distances of the points from a horizontal line through the mean of all Y values (SS_{tot}). R^2 is calculated using the following equation: $R^2=1.0–(SS_{residuals}/SS_{total})$.

RESULTS

In Vitro Adenosine-Elution Study

The cumulative elution of adenosine from 6 guidewires over 60 minutes is illustrated for each individual guidewire in Figure 6. Figure 7 depicts the cumulative average release from the composite of all 6 guidewires, with results expressed as either absolute release (top panel) or relative release (percentage of 60-minute time point; bottom panel). As shown, the release of adenosine was curvilinear with ≈60% released in the first 20 minutes

and most of the remainder by 40 minutes. This release profile is optimal for a typical coronary interventional procedure, with the initial burst treating and the sustained release preventing NRF. The total amount of released adenosine was 2.7±0.2 mg/10-cm length of wire.

Effect of Adenosine-Releasing Guidewire on Basal Coronary Flow in the Pig

The effect of 8 guidewires on CFV in the circumflex coronary artery and LAD is shown in Figure 8A. CFV
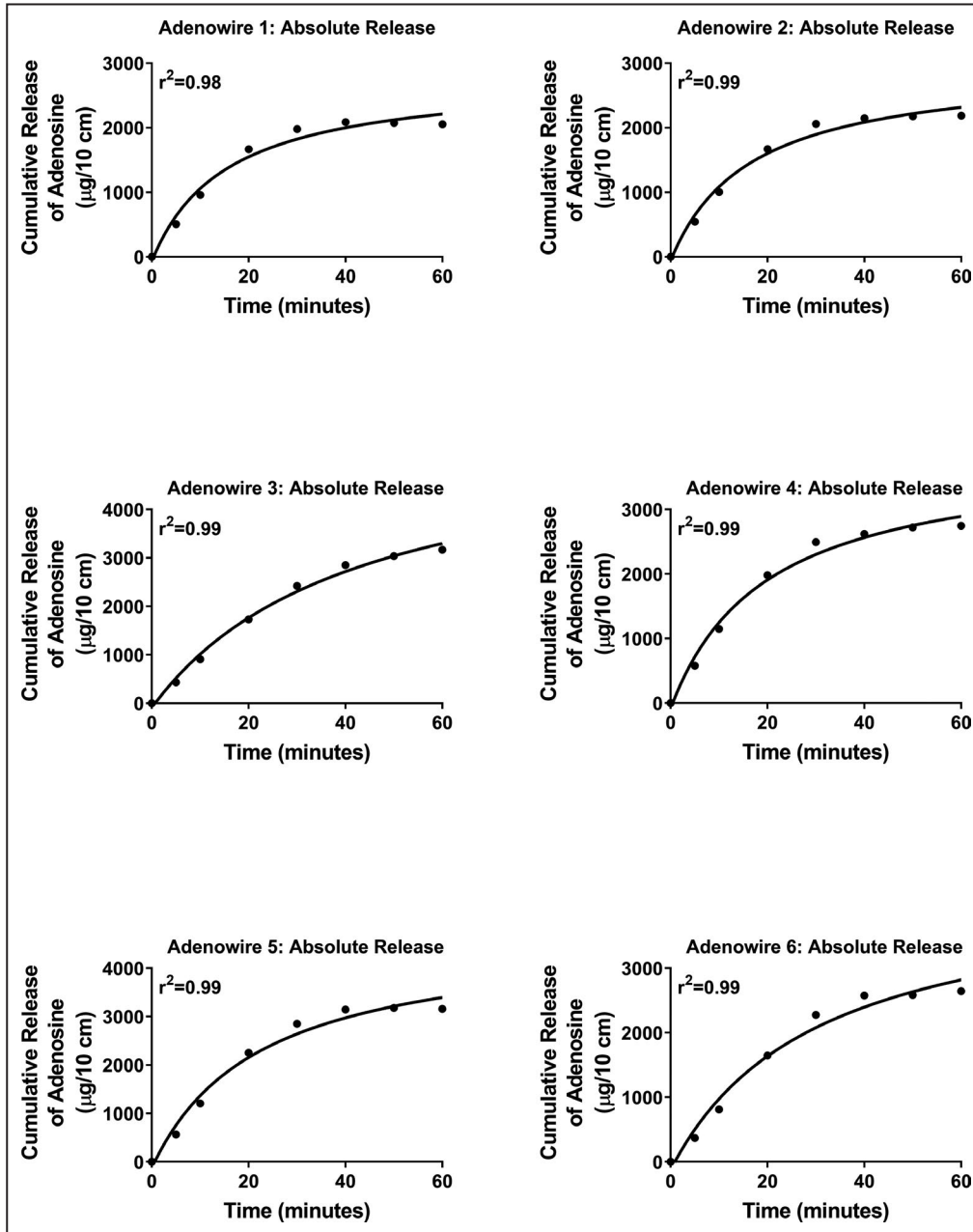

Figure 6. Line graphs demonstrate the elution profile of adenosine from 6 adenosine-loaded guidewires (Adenowires) in vitro.
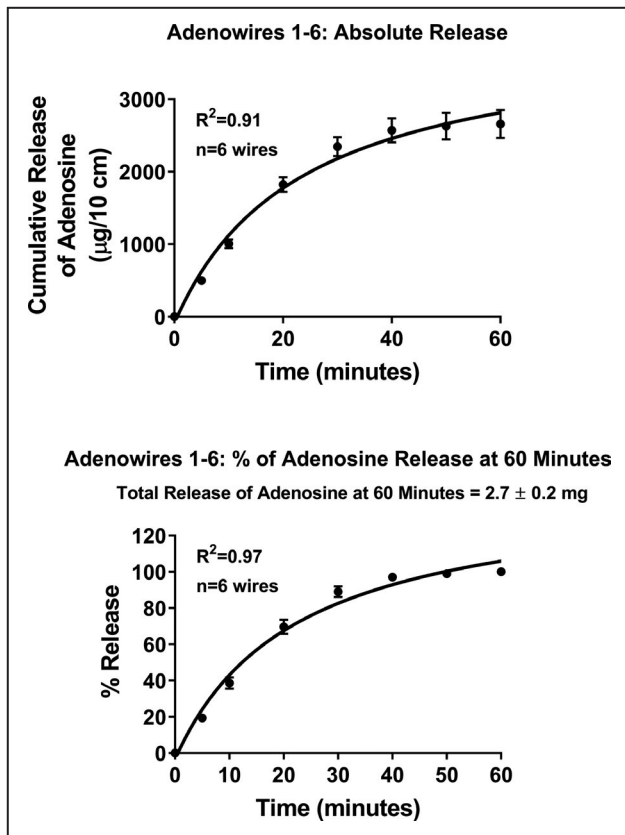The release was curvilinear over ≈60 minutes, a time frame which is ideal for an interventional procedure.

Figure 7. Line graphs depict the average elution profile from the 6 adenosine-loaded guidewires shown in Figure 6. The results are plotted either as cumulative release of absolute amounts of adenosine (top panel) or as % release relative to the 60-minute time point (bottom panel). The average release at 60 minutes was 2.7±0.2 mg per 10 cm of length. Values are means±SEM.

increased within 1 minute of guidewire insertion and remained significantly elevated above baseline for 27 minutes. This increase in CFV closely paralleled the adenosine-release profile in vitro. The fold increase in CFV (Figure 8B), a measure of coronary flow reserve, peaked at 2.5 and remained >2.0 for 15 minutes after wire insertion. Previous studies in our laboratory showed that a continuous infusion of adenosine into the LAD at 50 µg/kg per minute increased CFV ≈250%. Insertion of adenosine-releasing guidewires did not affect either mean arterial blood pressure (Figure 8C) or heart rate (Figure 8D), and no animal developed heart block. Coronary angiography after each guidewire removal showed normal flow and no filling defects (Figure 9).

Effect of Adenosine-Releasing Guidewire on Acetylcholine-Induced Coronary Vasoconstriction in the Pig

Figure 10 summarizes the results of 3 experiments in which acetylcholine was infused into the coronary artery to induce coronary vasoconstriction, and then an adenosine-releasing guidewire was inserted. In the first

experiment of this type (Figure 10A and 10B), coronary vasoconstriction was so intense that coronary flow ceased and the animal rapidly developed ventricular fibrillation. The heart was immediately defibrillated and an adenosine-releasing wire was inserted into the coronary artery. Despite continuing the acetylcholine, immediately upon insertion of the adenosine-releasing wire, CFV not only was restored but more than doubled from pre-acetylcholine baseline. CFV gradually returned to baseline and the acetylcholine infusion was stopped, and then the guidewire was removed. In 2 similar experiments (Figure 10C through 10F), insertion of the adenosine-releasing guidewire immediately reversed acetylcholine-induced coronary vasoconstriction and maintained CFV either above or near baseline during the acetylcholine infusion. Together, Figures 11 and 12 summarize the results of 5 experiments in which an adenosine-releasing guidewire was inserted before infusing acetylcholine into the coronary artery. As shown, in all experiments, insertion of the guidewire increased CFV and maintained CFV above baseline even when acetylcholine was infused. The experiment in Figure 12C was particularly striking. Here, the adenosine-releasing guidewire elevated CFV above baseline even while acetylcholine was infused. While maintaining the acetylcholine infusion, the guidewire was removed, and CFV rapidly began to fall nearly to zero. Coronary angiography in 2 animals showed acute NRF, with restoration of coronary patency after insertion of an adenosine-releasing guidewire (Figure 13).

Evaluation of Guidewire and Coating Performance

Light microscopy of unused wires (Figure 1) demonstrated that the hydrogel-drug coating filled the coil spaces and provided a smooth and thin coating on the outer coil surface. Track forces were obtained on 4 Adenowires and 3 predicated hydrophilic wires (Prowater; Asahi-Intecc, Justin, CA). Note that the frictional forces to deliver and retract the Adenowires were similar to the commercial wires indicative of similar lubricity properties (Figure 14). Light microscopy after track tests and hydration revealed that the coating integrity remained unchanged, with no evidence of swelling or coating loss.

Platelet Aggregation

Because a 1-cm length of an Adenowire contains ≈0.27 mg of adenosine, the concentration of adenosine present in the 0.2 mL of PRP would be estimated to be 5 mmol/L assuming total release of adenosine (0.27 mg/0.2 mL=1350 mg/L=5 mmol/L). Thus, there should be a sufficient concentration of adenosine to attenuate platelet activation. Figure 15 illustrates the
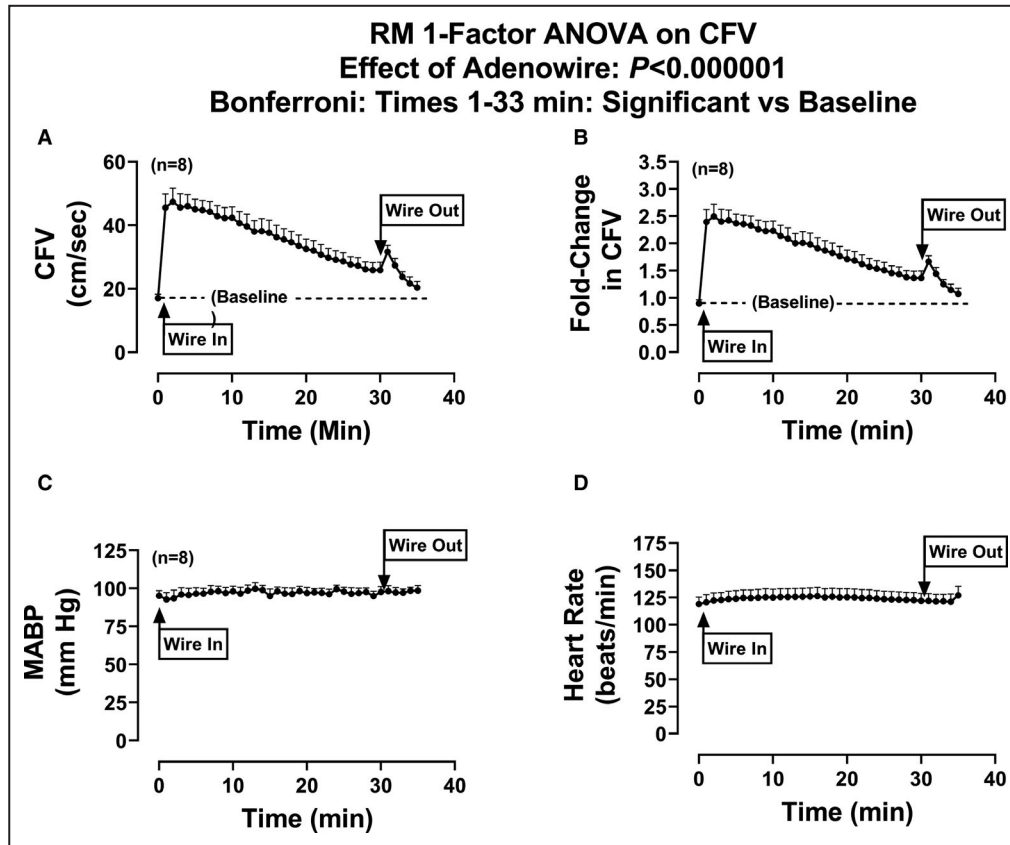
Figure 8. Line graphs summarize the effects on coronary flow velocity (CFV) of adenosine-releasing guidewires (n=8) inserted into the LAD or circumflex coronary artery of Yucatan minipigs.

Coronary flow velocity (**A**) was markedly increased immediately upon wire insertion and remained significantly elevated for 33 minutes. **B**, The fold increase in CFV, which equates to coronary flow reserve. The coronary flow reserve was >2.0 for the first 15 minutes. The small spike in CFV when the guidewire was removed was likely attributable to an incremental surge in adenosine release caused by wire manipulation. The adenosine-releasing wire did not affect mean blood pressure (MABP) or heart rate (**C** and **D**, respectively). Values are means and SEMs. RM 1-Factor ANOVA indicates repeated measures 1-factor analysis of variance. Bonferroni indicates Bonferroni test for comparisons to baseline (control) of subsequent time points after insertion of the Adenowire.

effects of Adenowires and Control wires (wires coated with the novel coating formulation but without adenosine) on area under the aggregation curve, maximum aggregation, rate of aggregation (slope), and lag time between addition of agonist and the onset of aggregation. Note that coated wires containing adenosine (Adenowires) resulted in a striking inhibition of ADP-induced and collagen-induced platelet aggregation as assessed by the reduction in area under the aggregation curve, maximum aggregation, and rate of aggregation. In addition, Adenowires delayed aggregation in response to collagen. Unexpectedly, Control wires exerted antiplatelet effects that were similar to, but not quite as striking as, Adenowires. Moreover, Control wires exerted more efficacious platelet inhibition compared with 2 commercially available hydrophilic wires (area under the aggregation curve: Control wires, 116±30 versus commercial wires, 258±32, $P<0.05$;

maximum aggregation: Control wires, 28±6 versus commercial wires, 62±8; $P<0.05$).

DISCUSSION

Guidewires are the first mandatory device placed in the culprit vessel before any interventional procedure. Current guidewires are coated with inert hydrophilic compounds such as PVP, polyacrylamides, and hyaluronic acid to enhance the lubricity and trackability. Here, we developed a platform guidewire to deliver adenosine without dissolution or disruption of the coating.

Our design employed a unique guidewire microstructure and a novel combination of coating technologies, as well as jet-milled adenosine. Since no data exist regarding formulations for drug-eluting guidewires, we developed a matrix (reservoir) system employing an
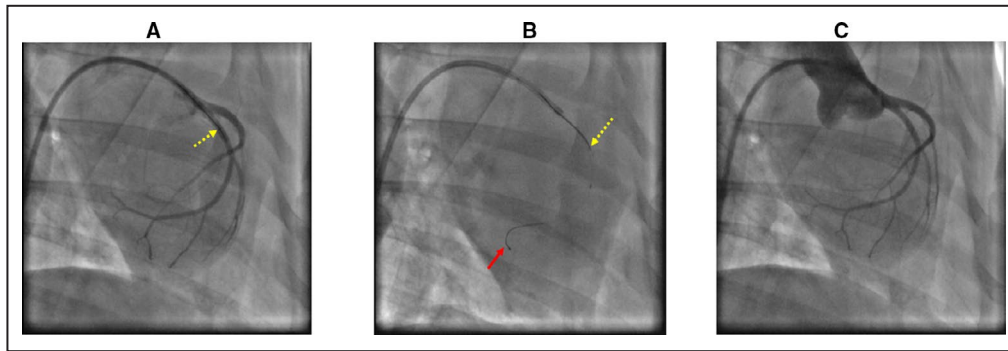
Figure 9. Coronary angiogram of circumflex in left anterior oblique projection.
A, Baseline; (**B**) adenosine guidewire positioned in distal vessel with radio-opaque tip (solid red arrow) clearly visible; (**C**) post wire removal after 30 minutes showing normal flow. Also shown (dashed yellow arrow) is the doppler Combowire used to measure coronary flow velocity.

inner drug-containing layer and an outer barrier layer. PVA has been used in other applications because it is nontoxic, noncarcinogenic, bioadhesive, and elastic.[23,24] The crystalline nature of PVA allows physical cross-linking by repeated cycles of freezing and thawing with pore size altered by the number of cycles and molecular weight.[23] As cross-linking is not stable, our group developed the concept that the addition of PVP would stabilize the hydrogel network through hydrogen bonding between carbonyl groups of PVP and hydroxyl groups of PVA.[25] In preliminary studies, we established that a PVA:PVP ratio of 99:1 provides both optimal mechanical stability and the best elution profile and was therefore used as the formulation for the inner drug layer of the coating.

Jet milling of adenosine was required to reduce the solid-phase particle size to obtain a smooth, lubricious coating. To optimize drug release for a typical PCI procedure, we determined that the addition of PVAc, a more hydrophobic polymer, was required for the barrier layer. PVAc is commercially available as a 30% dispersion that includes sodium lauryl sulfate as a wetting solution (Kollicoat; BASF, Florham, NJ, USA).[26] Following numerous trials, we determined that a ratio of PVAc:PVA of 3:1 in the outer barrier layer resulted in an optimal release profile. Light and scanning electron microscopy showed a smooth surface with a thin outer coating diameter of a few microns. Bench testing examined lubricity, durability, and integrity and determined that these important parameters met Food and Drug Administration standards. Also, in preliminary tests we found that electron-beam sterilization of the adenosine-releasing wires did not affect release kinetics.

In vitro studies demonstrated a curvilinear elution of adenosine over 60 minutes with a drug load of 2.7±0.2 mg per 10 cm of wire length. Porcine studies confirmed a robust pharmacologic effect with an immediate and significant increase in CFV for 30 minutes. The percentage increase in velocity, an estimate of coronary flow reserve, was 2.5 during the first 10 minutes of insertion, which is comparable to a 50 µg/kg per minute intracoronary infusion of adenosine in this model (M.B. Forman, unpublished data, 2018). Further studies using intracoronary infusions of acetylcholine to induce severe vasoconstriction of the microvasculature demonstrated that insertion of the adenosine-releasing guidewire both prevented and reversed the acetylcholine-induced decreases in CFV. The efficacy of the adenosine-releasing guidewire to immediately restore normal coronary perfusion was illustrated in 1 animal that developed acute NRF and severe ischemia, which was immediately reversed after wire insertion.

Since only a 5- to 6-cm length of guidewire could be placed in the pig coronary arteries, it is probable that the coronary vasodilatory response would be even greater in humans because human coronary arteries would accommodate at least 9 cm of the guidewire. Adenowire insertion into pig coronary arteries did not cause systemic hemodynamic effects, arrhythmias, or heart block. Nonetheless, because Adenowires likely would deliver more adenosine to patients because of the greater length of wire in the coronary arteries, going forward it will be important to carefully examine the effects of Adenowires on hemodynamics and arrhythmias in patients before manipulations, when contrast medium is injected, and when a stent or balloon is used in an actual PCI.

Platelets play a key role in MVO through release of potent vasoconstrictor substances such as serotonin, thromboxane A_2, and platelet-activating factor. Adenosine, through activation of high-affinity A_{2A} receptors, markedly inhibits platelet aggregation. Not surprisingly, in vitro studies demonstrated that Adenowires significantly inhibited platelet aggregation. However, we did not expect that the naïve coating alone, without adenosine, would also exert antiplatelet effects
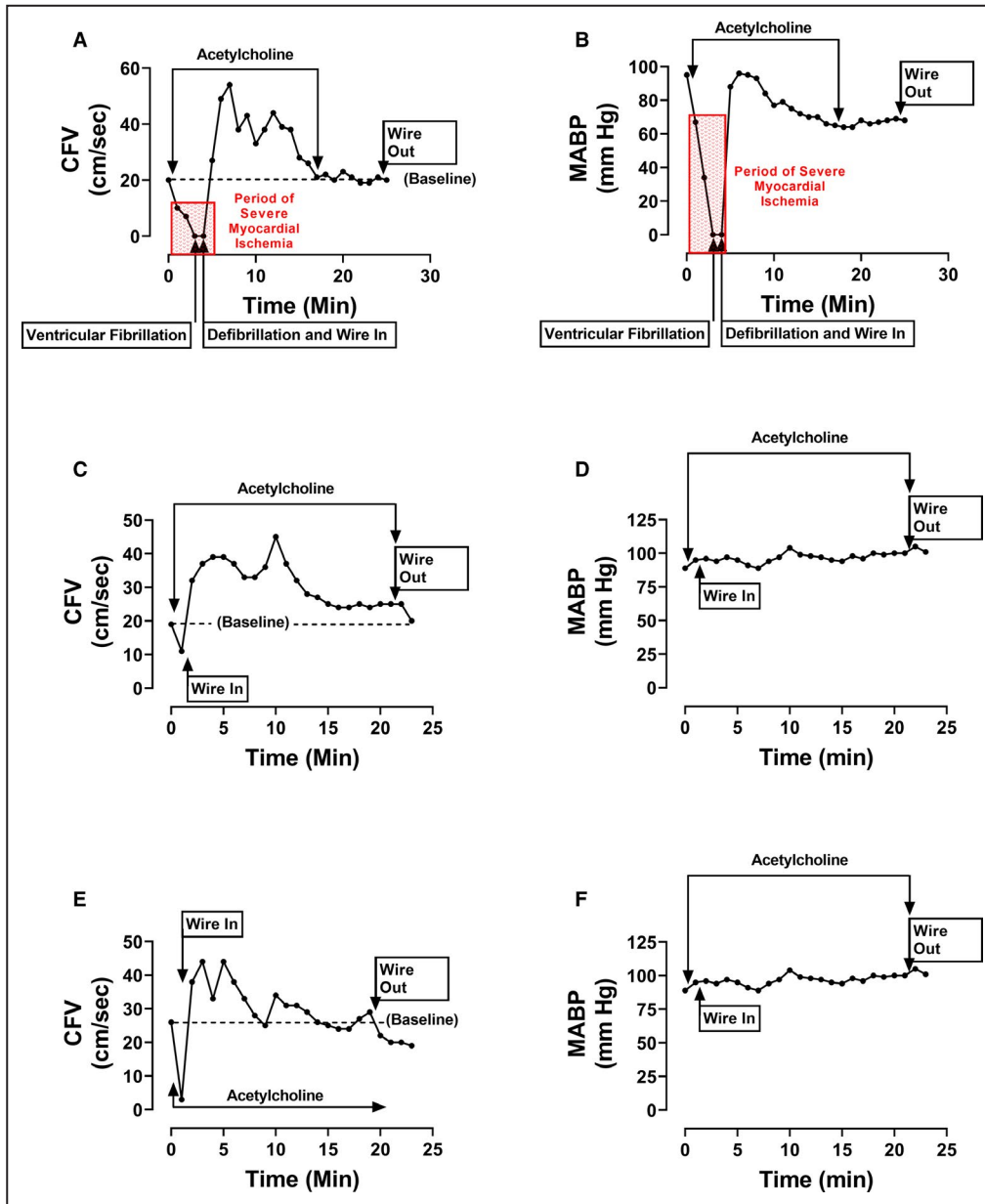
Figure 10. Line graphs summarize 3 experiments in which acetylcholine was infused into the coronary artery before the adenosine-loaded guidewire was inserted into the artery.
Experiment 1 is summarized in (**A** and **B**); experiment 2 in (**C** and **D**); and experiment 3 in (**E** and **F**). Coronary flow velocities (CFV) are illustrated in (**A**, **C**, and **E**) and associated mean arterial blood pressures (MABPs) are shown in (**B**, **D**, and **F**). In experiment 1, the animal developed severe ischemia that induced ventricular fibrillation requiring cardioversion. In all 3 experiments, acetylcholine caused coronary vasoconstriction that was reversed by insertion of the adenosine-releasing guidewire.

when compared with commercial hydrophilic wires. Since coadministration of A_{2A} agonists enhances the antiplatelet effects of $P2Y_{12}$ antagonists, Adenowires could provide additional benefits to reduce thrombus formation and embolization during PCI in clinical settings with large thrombus formation.[27]

MVO and NRF remain important obstacles that frustrate achievement of optimal tissue perfusion after PCI. All patients, irrespective of clinical presentation, are at risk for NRF, which occurs in 4% to 8% of elective procedures.[4,5] However, NRF is most prevalent in the setting of STEMI. In this regard, NRF manifests in >50% of patients with STEMI with anterior infarctions.[4,5,28] MVO is associated with larger infarct size, abnormal ventricular remodeling, arrhythmias, and CHF.[4,5,28] Moreover, MVO is an independent risk factor for major cardiovascular events with greater predictive power than
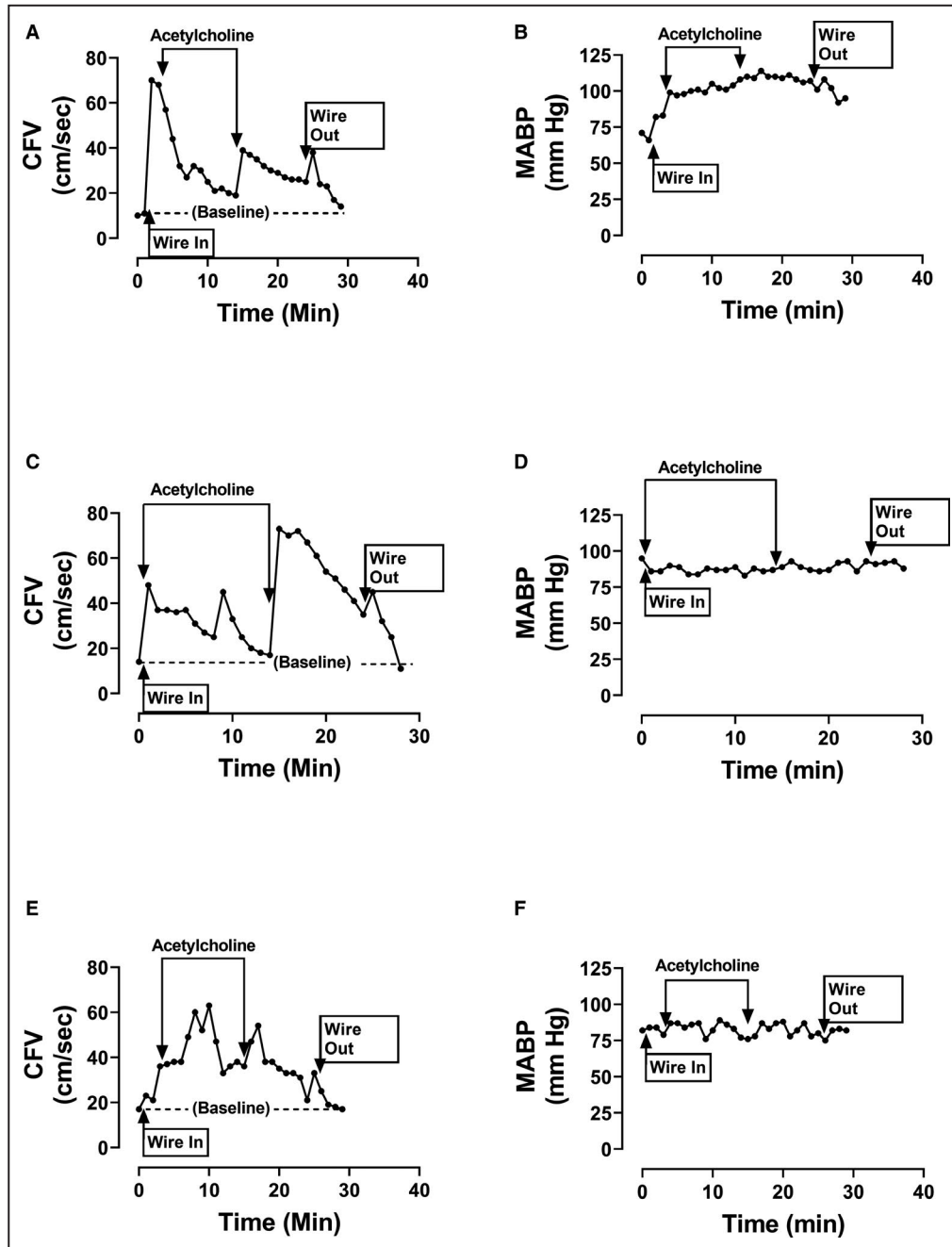
Figure 11. Line graphs summarize 3 experiments in which acetylcholine was infused into the coronary artery after the adenosine-loaded guidewire was inserted into the artery.
Experiment 1 is summarized in (**A** and **B**); experiment 2 in (**C** and **D**); and experiment 3 in (**E** and **F**). Coronary flow velocities (CFVs) are illustrated in (**A**, **C**, and **E**) and associated mean arterial blood pressures (MABPs) are shown in (**B**, **D**, and **F**). In all 3 experiments, insertion of the adenosine-releasing guidewire immediately increased CFV and prevented acetylcholine from reducing CFV below baseline.

ejection fraction or infarct size.[6,7,29] The mechanisms responsible for MVO are complex and include embolization by atheromatous and thrombotic debris, release of potent vasoconstrictors, and activation of the immune system resulting in leukocyte plugging and endothelial disruption of microvessels.[3–5] The realization that prevention and prompt reversal

of MVO is paramount to improve outcomes in high-risk PCI procedures has motivated the evaluation of numerous devices to ameliorate MVO. Various mechanical devices that induce thrombectomy, aspirate clots, or protect against emboli have failed to consistently reduce MVO, infarct size, or mortality, suggesting that they are not protective throughout
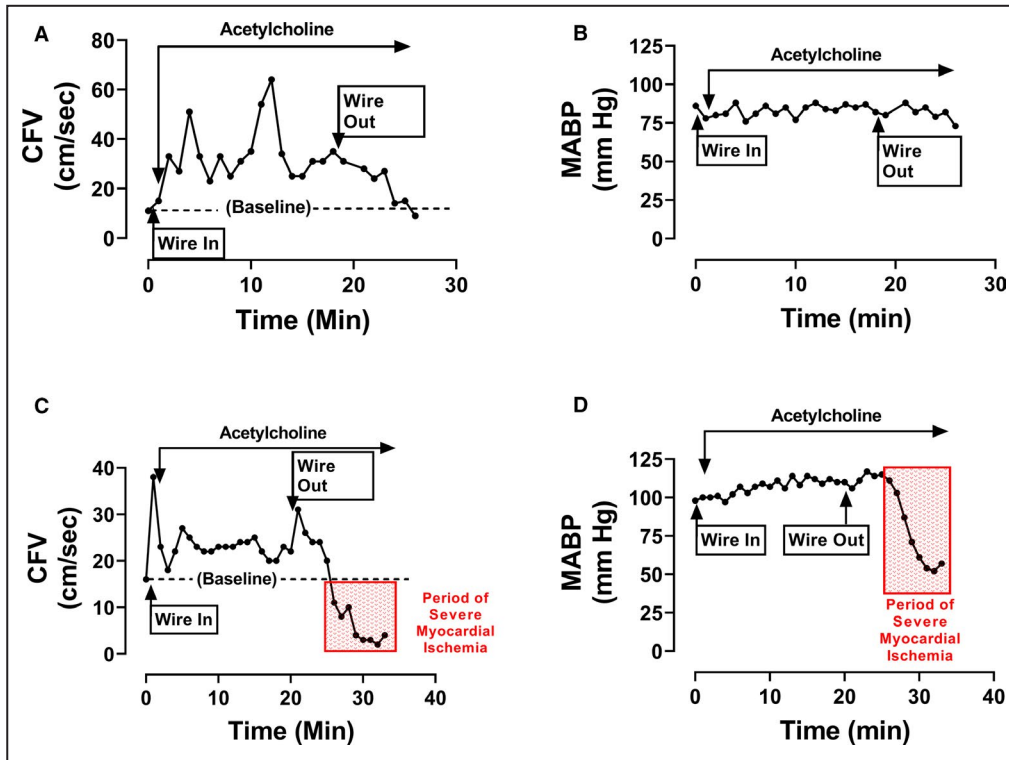
Figure 12. Line graphs summarize 2 additional experiments in which acetylcholine was infused into the coronary artery after the adenosine-loaded guidewire was inserted into the artery.
Experiment 1 is summarized in (**A** and **B**); and experiment 2 in (**C** and **D**). Coronary flow velocities (CFVs) are illustrated in (**A** and **C**) and associated mean arterial blood pressures (MABPs) are shown in (**B** and **D**). In both experiments, insertion of the adenosine-releasing guidewire immediately increased CFV and prevented acetylcholine from reducing CFV below baseline. Note that in (**C**) removal of the adenosine-releasing guidewire while maintaining the intracoronary infusion of acetylcholine resulted in a rapid and profound decline in CFV.

the whole procedure and do not affect humoral factors and cytotoxic compounds responsible for MVO.[9–12]

Given the negative impact of inadequate tissue perfusion with PCI, there is increased awareness of the need for new pharmacologic approaches to prevent
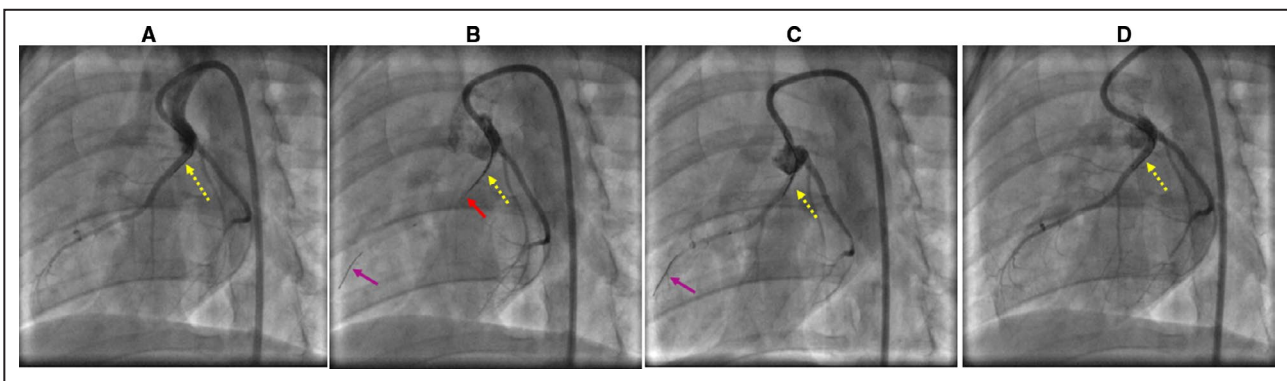

Figure 13. Coronary angiography showing an example of the no-reflow phenomenon (NRF) reversed by an adenosine-releasing guidewire.
A, A baseline angiogram was obtained with the Combowire (dashed yellow arrow) in the proximal LAD. **B**, Soon after administration of acetylcholine, severe NRF occurred with total cessation of blood flow in the proximal LAD (solid red arrow). **C**, Upon placement of an adenosine-releasing guidewire (solid purple arrow) in the LAD, coronary flow was rapidly restored and maintained even while infusing acetylcholine for an additional 20 minutes. **D**, After stopping acetylcholine and removing the guidewire, a final angiogram showed that normal coronary flow was maintained.
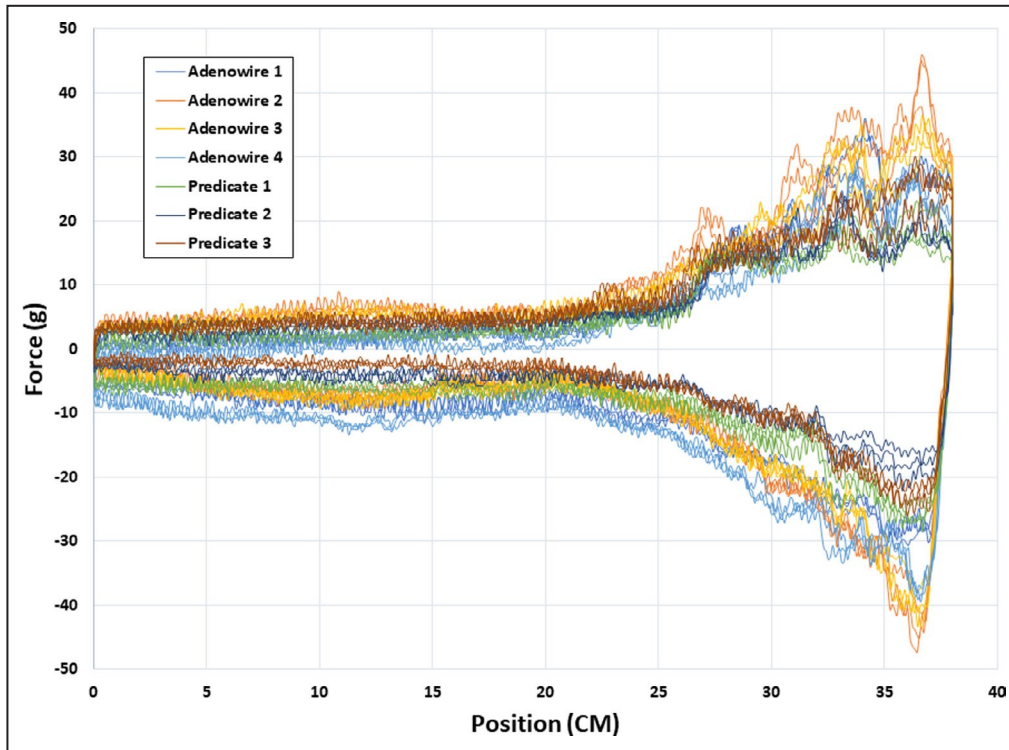
Figure 14. Track forces (g) of 4 Adenowires and 3 commercial wires with a proprietary hydrophilic coating is illustrated.

Note that the insertion and withdrawal forces were similar, which was indicative of comparable lubricity properties.

and reverse MVO. Adenosine is an endogenous nucleoside that activates 4 well-characterized receptors, producing a myriad of physiological effects that maintain microcirculatory flow.[4,5,30] These include potent vasodilatory, antiplatelet, and anti-inflammatory actions, restoration of profibrinolytic activity of endothelial cells, acceleration of vascular healing (vasculogenesis), and stimulation of new vessel formation (angiogenesis).[4,5,31] Adenosine also functions as an endogenous cardioprotective agent as established by the fact that adenosine is the mediator of pre- and postconditioning.[5] Activation of adenosine receptors stimulates survival kinases, which prevent opening of mitochondrial permeability transition pores.[5] These actions, in association with adenosine's antiapoptotic effects, prevent lethal myocyte injury. Indeed, in the acute myocardial infarction study of adenosine trial, a 3-hour intravenous infusion of adenosine significantly reduced infarct size in patients with anterior STEMI, and intracoronary or repeated boli of adenosine reduced MVO in STEMI and saphenous vein grafts.[5,27,32–35] The multiple mechanisms whereby adenosine attenuates MVO and cellular injury, which have been verified in numerous clinical studies, indicate that adenosine is superior to other pharmacologic agents for preventing and treating MVO.

In humans, adenosine has a plasma half-life of only ≈1 second. Despite the potential therapeutic benefits of adenosine in STEMI, the short half-life of adenosine is problematic because it mandates that adenosine be delivered by continuous administration to optimize its beneficial effects on MVO and infarct size. Moreover, intravenously administered adenosine induces unpleasant, and in some cases serious, side effects. Thus, there is a dire need to develop a convenient system that allows continuous delivery of adenosine in pharmacologic amounts directly into the coronary circulation while avoiding adverse effects. Since guidewires are the first mandatory device placed in the culprit vessel before PCI, an adenosine-releasing guidewire provides for an autonomous system that performs without thought or planning. In this regard, our adenosine-releasing guidewire is a platform guidewire product coated with a novel hydrophilic coating that allows delivery of a drug, without disruption of the coating, throughout the interventional procedure. This guidewire achieves high concentrations of adenosine at the target site, thereby optimizing pharmacologic efficacy yet avoiding side effects that would be induced with high-dose systemic administration. Moreover, since adenosine release is immediate, our adenosine-releasing
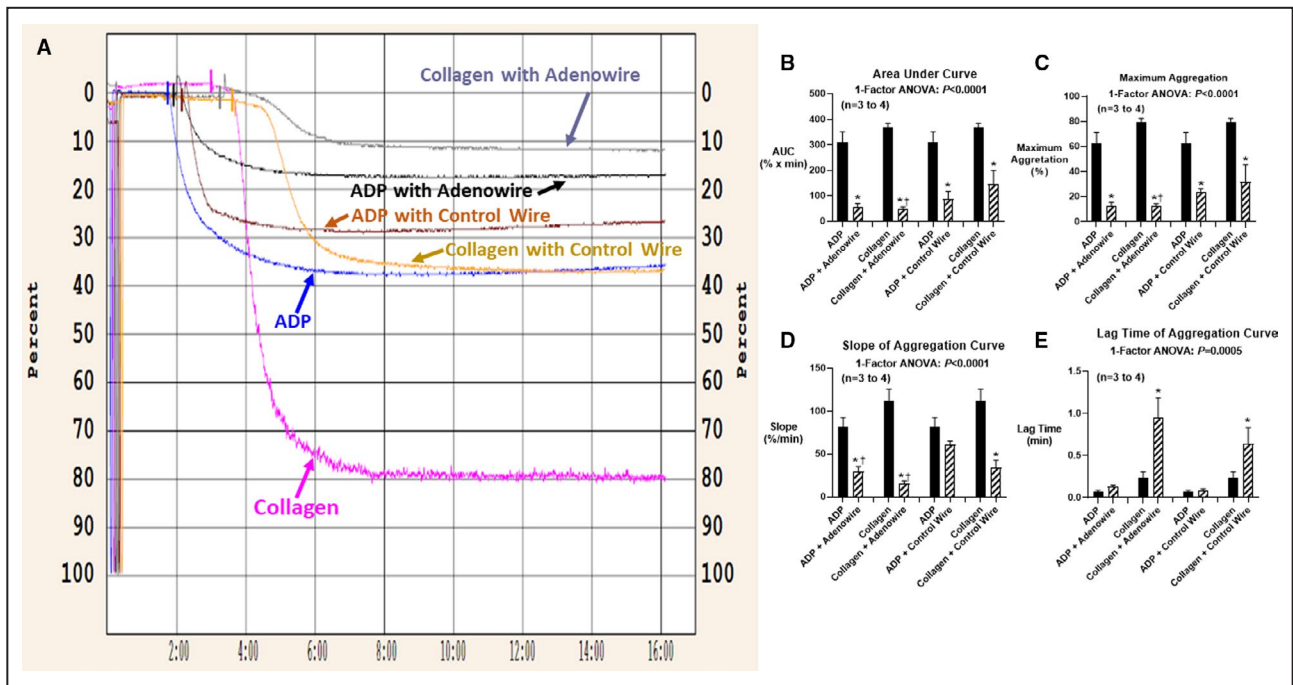
Figure 15.   Effects of Adenowires and Control wires (wires coated with the novel coating formulation but without adenosine) on area under the aggregation curve (AUC), maximum aggregation, rate of aggregation (slope) and lag time between addition of agonist and the onset of aggregation.

A, Representative platelet aggregation tracings showing aggregation responses to ADP (2 µmol/L), collagen (1 µg/mL), ADP+Adenowire, collagen+Adenowire, ADP+Control wire and collagen+Control wire. Adenowires were coated with our novel hydrophilic coating that contained adenosine; Control wires were similarly coated but did not contain adenosine. Bar graphs summarize the effects of treatments on (**B**) area under the aggregation curve, (**C**) maximum aggregation, (**D**) slope of the aggregation curve and (**E**) the lag time between adding the agonist and onset of aggregation. Note that Adenowires markedly inhibited platelet aggregation. The inert coating also manifested antiplatelet properties that was similar to, but not quite as efficacious as, Adenowires. Data were analyzed by 1-factor ANOVA after Box-Cox transformation. *Significantly different vs agonist without wire; †Significantly different vs corresponding Control wire.

guidewire would promote preconditioning in patients with STEMI before reperfusion of the myocardium following balloon or stent placement.

In conclusion, here we describe a therapeutic agent–releasing guidewire platform that allows for continuous release of pharmacologic amounts of adenosine directly into the microvasculature during a PCI procedure. The average time for the total PCI procedure is ≈60 minutes from start to finish. However, once the guide catheter has been positioned in the coronary ostium, guidewire insertion into the culprit vessel for placement of balloon and stent catheters rarely exceeds 30 minutes, even with complex lesions and acute coronary occlusions; thus, the current design of the Adenowire allows for the appropriate time frame for release of pharmacologic amounts of adenosine. In the present study, there was a small spike in CFV when the guidewire was removed; this was likely attributable to an incremental surge in adenosine release caused by wire manipulation. If this occurs in the human coronary circulation during balloon and stent insertion, it would be beneficial since a surge of adenosine during these critical moments would be

advantageous. The portion of the Adenowire in the guide catheter would also elute adenosine, and thus extra adenosine would be delivered with injection of contrast material; this design is also a plus. The development of this guidewire necessitated optimization of the surface area of the guidewire, use of novel hydrophilic and hydrophobic polymers, and creation of a reservoir (matrix) system comprising an inner drug layer and outer barrier layer. In vitro studies confirmed an ideal elution profile for adenosine that was verified in a large animal model where robust coronary vasodilatation and rapid reversal of vasoconstriction was demonstrated. Bench studies revealed that wire performance and coating quality (lubricity, durability, adhesion, and integrity) were comparable to inert hydrophilic guidewires. The novel inert coating also provides antiplatelet effects that were amplified with the addition of adenosine in the coating. Although we have not yet performed biocompatibility testing, the safety of PVA and PVAc is well established, as they are used as tissue replacement material and in drug and food products.[23–26] This technology could be used on a pressure wire to measure fractional flow

reserve, thereby avoiding the side effects of large intravenous infusions of adenosine. The coating formulation would also be applicable for controlled release of other drugs for interventional procedures such as anti-thrombotic and anti-platelet agents, anticoagulants and antimitotic agents. Furthermore, the process could be employed to coat other devices such as vascular sheaths, catheters, balloons, and stents.

While the overall incidence of NRF is low (4%–8%), NRF remains prevalent in high-risk patients undergoing procedures such as anterior STEMI and saphenous vein graft interventions. Furthermore, all patients, irrespective of a clinical presentation, are at risk. Since an Adenowire would add little to the overall cost of a procedure and since NRF is unpredictable, likely the cost-benefit ratio is favorable for use of the Adenowire as insurance against NRF.

ARTICLE INFORMATION

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Disclosures

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