
Environmental influences on endovascular stent platelet reactivity: An *in vitro* comparison of stainless steel and gold surfaces

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Abstract: Thrombosis is an initiating reaction to vascular injury that follows the placement of vascular devices. Platelets play a crucial role in this response. Their interaction with endovascular devices is not solely a function of device properties, but a multifaceted response dependent on several biological factors that interact in the context of a hemodynamic environment. We sought to investigate the role of local environmental variations on determining indices of biocompatibility. Using a recently described *in vitro* flow apparatus, we separately studied the platelet and coagulative component responses to stainless steel endovascular stents with and without gold coating. When allowed to interact, these biological

mediators of thrombosis enabled varied biocompatibility outcomes in a manner that was dependent on flow. Using platelet reactivity as an index, the stainless steel fared better under some conditions, while under other conditions, gold was superior. Considering such impacts of local environment on biocompatibility is important, both in the interpretation of experimental findings, as well as the continued use and optimized development of vascular devices. © 2004 Wiley Periodicals, Inc. *J Biomed Mater Res* 70A: 186–193, 2004

Key words: stent; thrombosis; biocompatibility; flow; environment

INTRODUCTION

Thrombosis is a complex process that initiates the body's response to vascular injury following placement of an endovascular stent. In early clinical examination, thrombotic vessel occlusion occurred in nearly 20% of stent implantation procedures. While advances in anti-thrombotic agents, stent design, and expansion technique have reduced occlusive thrombosis rates to less than 2%, there still remains a significant risk of mortality associated with thrombotic vascular occlusion.^{1–5} Moreover, silent mural thrombosis is an apparently ubiquitous process that promotes subsequent repair and remodeling events, which may lead to pathological restenosis in 20–50% of cases.^{6–8} Accordingly, elucidation and control of the local thrombotic process is important for the continued use and optimized development of vascular implants.

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Platelets play a central role in the thrombotic reaction to endovascular devices.^{3,9} Their activation, adhesion, and aggregation to sites of vascular injury serve both as a mechanism of primary hemostasis as well as a method of localizing and supporting the coagulation reactions in flow.^{10–15} Constituents of the enzymatic coagulation cascade further modulate the growing platelet plug through feedback signaling and the generation of a stabilizing fibrin meshwork.¹⁵ Given the host of biological and physical interactions that take place in the vicinity of the implant, we hypothesized that variations in local environmental factors may play a critical role in determining measures of stent biocompatibility. Specifically, we considered the influence of flow and coagulation on determining the relative platelet reactivity of stainless steel endovascular stents with and without gold coating.

METHODS

System overview

A recently described flow system was utilized whose basic components include fluid loops, rotor-stages, a driving

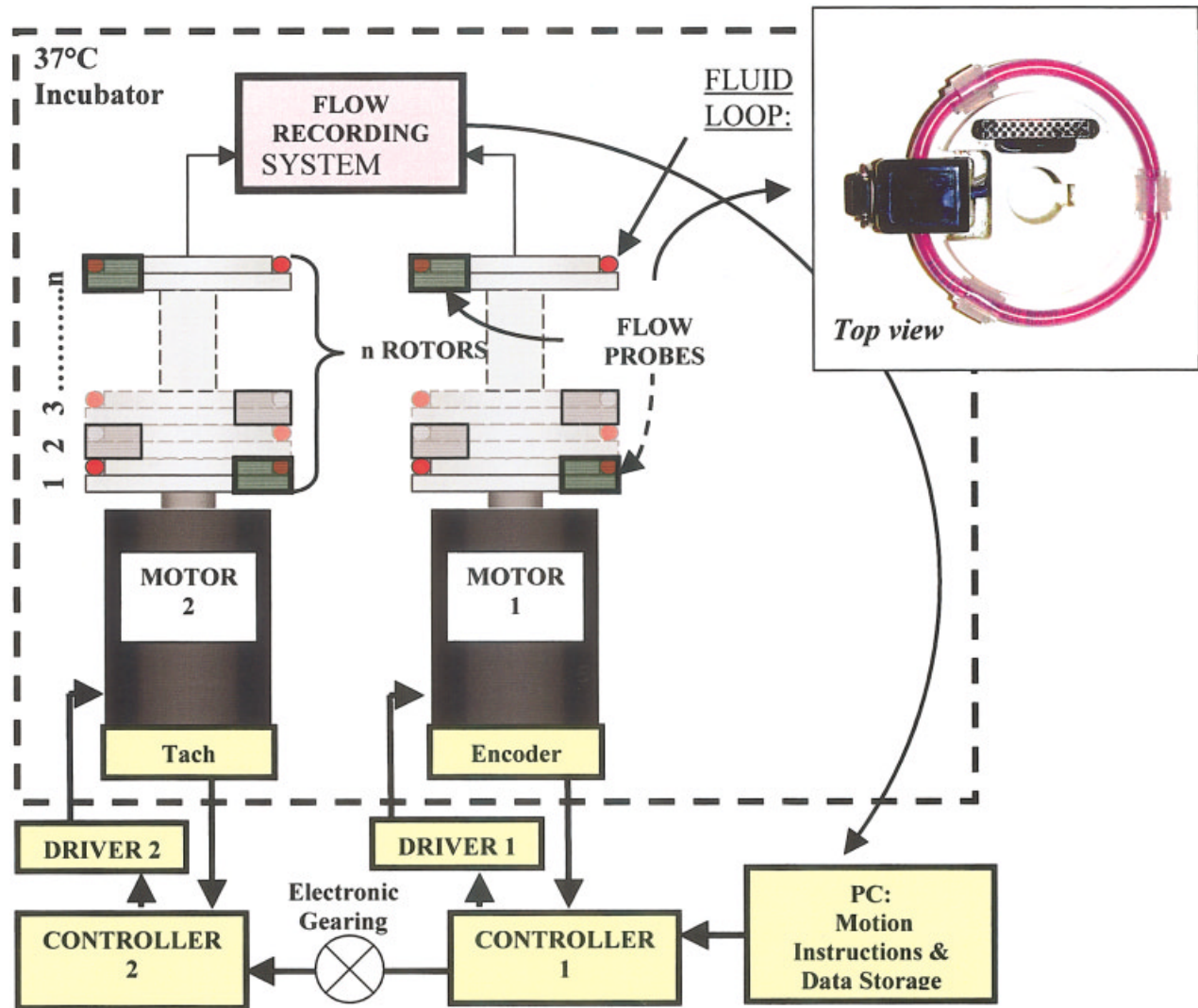


Figure 1. General two-axis system schematic and fluid loop design. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

motor, a motion controller, a measurement system, and an incubator.¹⁶ The fluid loops were made from a 24.0-cm-long length of silicone tubing (3.2 mm ID/4.8 mm OD). By controlling the angular loop accelerations, a wide variety of pulsatile flows can be maintained in a geometrically relevant setting while minimizing background disturbances (i.e., circuit platelet activation, flow disruptions).¹⁶ For the current work, two rotating axes were used to accommodate two simultaneous flow profiles (Fig. 1). A high flow, coronary-like pattern was approximated with a peak flow of 200 mL/min along with a low flow pattern with a peak of 40 mL/min (100 and 20 mL/min mean flow rates, respectively; Fig. 2). Although the flows produced were bidirectional in nature, physiological shear rates could be matched on a beat-to-beat basis as discussed elsewhere.¹⁶

Blood components were derived from whole blood obtained from healthy volunteers drawn via a 20-gauge needle into a 1/10 dilution of acid-citrate-dextrose anticoagulant (ACD, a calcium chelating agent; 85 mM trisodium citrate, 69 mM citric acid, 111 mM glucose, pH 4.6). The whole blood

was spun at 200g for 15 min and the top platelet-rich plasma (PRP; $1.5\text{--}2 \times 10^8$ platelets/mL) was collected. For trials requiring platelet-poor plasma (PPP), the platelets were removed by additional centrifugation at 1500g for 15 min. The platelet-depleted supernatant was re-centrifuged at 2500g for 20 min to ensure PPP (as verified through loss of CD61 detection on flow cytometry).

Stainless steel stents (7-cell NIR[®], Medinol, Jerusalem, Israel) left intact or coated with a $7 \pm 2 \mu\text{m}$ layer of gold [surface roughness, Ra, of 48.4 ± 2.2 and 154.6 ± 5.4 nm, respectively, as determined through atomic force microscopy (Topometrix Explorer)] were examined as a relevant clinical comparison.^{17–21} These geometrically identical stents¹⁸ were all 9 mm in length and were expanded within the tube circuit to a 3.5-mm post-inflation diameter under 12 atm of pressure via a balloon catheter (Maxxum[™] 3.5 Scimed[®] balloon catheter, Boston Scientific Corporation). After stent expansion, the tubes were closed into their loop format (Fig. 1), filled with the specified blood components, and rotated through the desired motion profile at 37°C.¹⁶ The flow was continuously monitored and motion of the

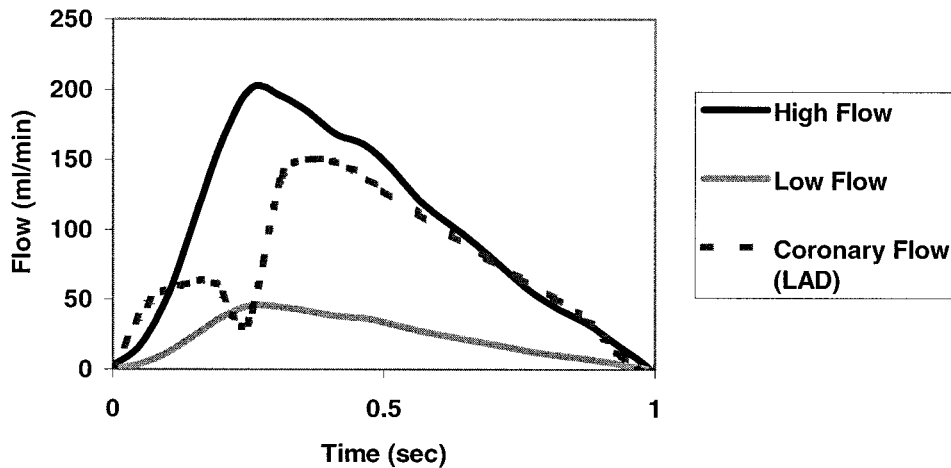


Figure 2. Generated flow profiles showing a representative pulse at high and low flow, superimposed on a typical, left, anterior, descending branch coronary profile.³⁷

loops could be stopped at any time to assess specific biological markers.

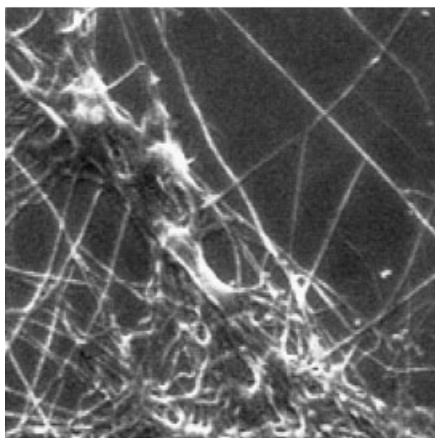
Measuring the endovascular stent platelet response

Platelet response was measured independently of coagulation using anticoagulated PRP. To suppress the coagulation reactions, we did not rely on the use of ACD given possible effects of calcium chelation on platelet interactions.²² Rather, 80 $\mu\text{g}/\text{mL}$ Corn Trypsin Inhibitor (CTI; FXIIa inhibitor) and 5 U/mL hirudin (thrombin inhibitor) were added. These agents blocked the initiation of the intrinsic coagulation pathway and the positive feedback effects of thrombin, respectively.²² Activated partial thromboplastin times (APTT; data not shown) and electron microscopy studies of post-run stent surfaces (Fig. 3) indicated the effectiveness of this method in eliminating coagulative function.

The CTI/hirudin anticoagulated PRP was recalcified prior to testing (13 mM; thus overwhelming ACD chelation) and injected into the pre-stented fluid loops. Twelve stents with and without gold coating and 4 nonstented controls were run in total under the high flow conditions. Four groups of 3 gold, 3 stainless steel, and 1 nonstented loop were considered in turn, thus allowing inter-stent comparisons despite inter-run variability. Each run was stopped after 1 h and the loops were quickly emptied and flushed with Hank's Balanced Salt Solution (HBSS) supplemented with 2 mmol CaCl_2 (pH 7.4). The tube surfaces and emptied volumes were then assessed for indices of platelet reactivity.

To measure volumetric platelet activation, 100 μL of each post-run PRP sample was suspended in 1 mL of 1% paraformaldehyde/PBS fixing solution and stored at 4°C for 24 hours. Fifty microliters of the fixed sample was incubated for 20 min with 20 μL of CD61-FITC (anti-gpIIIa) and CD62p-PE (Beckton Dickinson) (anti-p-Selectin) as constitutive and activation dependent platelet markers, respectively.

w/ out Anticoagulant



w/ Anticoagulant

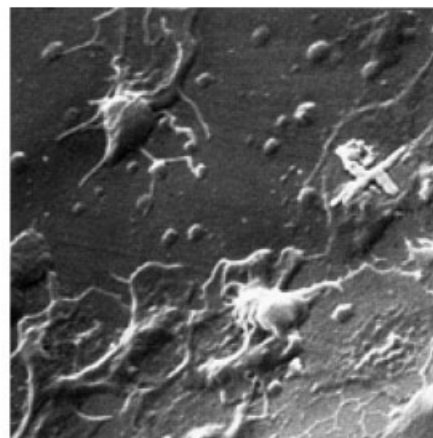


Figure 3. Electron microscopy of post-run stent surface with and without anticoagulant (80 mg/mL CTI, 5 U/mL hirudin). Elimination of fibrin strand formation in the anticoagulated case indicates the effectiveness of this technique.

The reaction mix was quenched with 1 mL 1% paraformaldehyde and assessed via flow cytometry (FAC scan™, Becton Dickinson) (FAC) by determining percentage of CD62p+ counts in the total CD61+ population (% activation).

For an index of surface activation, a platelet extraction protocol was developed. Briefly, the flushed tubes were filled with 1% paraformaldehyde fixative and stored at 4°C for 24 h. After fixing, the tubes were again flushed with HBSS and closed. Each loop was filled with 0.05% trypsin w/0.02% EDTA and spun in the flow setup under high shear conditions (500 mL/min peak flow rate) for 2.5 h (25°C) to extract adherent platelets. The loops were emptied into 200 µL trypsin inhibitor (60 mg/mL; Sigma). The platelets were pelleted (2500g for 15 min; 4°C) and concentrated in 100 µL PBS (pH 7.2). Following the antibody staining protocol, total CD61+ counts and counts expressing both CD61 and CD62p were determined through FACs analysis as measures of surface adherent and activated platelets, respectively. Fixation and trypsinization procedures were found not to alter CD61 or CD62p labeling.

Measuring the endovascular stent coagulation response

To assess the coagulation response independently of platelet function, 320 µg/mL phosphatidylethanolamine was added to the PPP. This served as an activated phospholipid source essential in catalyzing the coagulative reactions. Without this added source, typically provided by activated platelet membrane,²³ stented loops remained unoccluded for +2.5-h trial runs, whereas in its presence (320 µg/mL), occlusion occurred within 90 minutes (unpublished data, 2001).

The plasma was recalcified (13 mM) and injected into the respective fluid loops. Six stents of each type and 4 stentless controls were run in total under the high flow conditions (2 runs of 3 gold-coated, 3 stainless steel, and 2 non-stented loops). After one hour (pre-occlusion), the tubes were stopped and the plasma was assessed for PT1 + 2 fragment levels by ELISA (Bering-Dade) as an index of the coagulation response.

Influences of coagulation and flow on the endovascular stent platelet response

To evaluate the effects of coagulation on platelet reactivity, varying levels of CTI anticoagulant (40, 20, 4, 0 µg/mL) were added to recalcified PRP. Stainless steel stents were run under the high flow conditions until a reduction in the flow recordings could be detected under any of the CTI conditions. All loops were subsequently stopped and analyzed for surface and volumetric platelet response as described above. This procedure was repeated for a total of four runs.

The interactive influence of coagulation and flow on determining the relative platelet reactivity of the biomaterial surfaces was considered by performing four 1-h runs of 4

steel and 4 gold stents using recalcified PRP supplemented with 20 µg/mL CTI anticoagulant. In each run, 2 of each stent type were run under the high-flow and 2 under the low-flow conditions. Platelet response was measured as described above. Four similar runs were performed at a lower, 4 µg/mL CTI condition. In each of these trials, an additional 20 µg/mL CTI, high flow, stainless steel case was tested to allow relative scaling between the 4 and 20 µg/mL anticoagulant conditions.

Statistical analysis

To account for inter-run variability, all values are given after normalization with the most highly anticoagulated, stainless steel values for the given run. The results are expressed as normalized mean \pm SEM. A one- or two-way ANOVA was performed to evaluate global statistical significance, followed by post hoc, two-tailed (unless otherwise indicated) Student's *t*-tests for comparison between specific groups. Statistical significance was accepted at a value of $p < 0.05$.

RESULTS

We have previously considered the applicability of the *in vitro* flow system in studying stent thrombosis and found that while stentless control loops remained unoccluded for a 2.5-h trial duration, stainless steel stented loops occluded after approximately 40 min.¹⁶ In the current investigation, we more closely assessed such acute thrombotic events by considering individual components of the thrombotic process and their impact on inter-stent comparisons.

The use of highly anticoagulated PRP (CTI + hirudin) allowed us to consider a platelet response relatively independent of coagulative function. We found significantly more normalized surface-bound, activated platelets (CD61+, CD62p+) in the gold stented loops as compared to the stainless steel stented configurations (Fig. 4; 1.45 ± 0.10 vs. 1.00 ± 0.08 , $p < 0.05$). Of note, all surface-bound, CD61+ cells co-expressed the CD62p activation marker. The increase in platelet response was not well represented in the normalized volumetric platelet activation measure (gold 1.21 ± 0.16 , steel 1.00 ± 0.14 , control 1.19 ± 0.24). Phosphatidylethanolamine-doped PPP allowed us to consider the coagulation response independent of platelet function. In contrast to the surface platelet responses, we found that PPP exposed to the gold-coated stents exhibited lower normalized PT1 + 2 levels than plasma exposed to uncoated, stainless steel stents (Fig. 4; 0.50 ± 0.07 vs. 1.00 ± 0.13 , $p < 0.001$).

We then tested the interactive effect of coagulation on platelet reactivity by considering PRP with varying levels of anticoagulant (0, 4, 20, 40 µg/mL CTI). Each

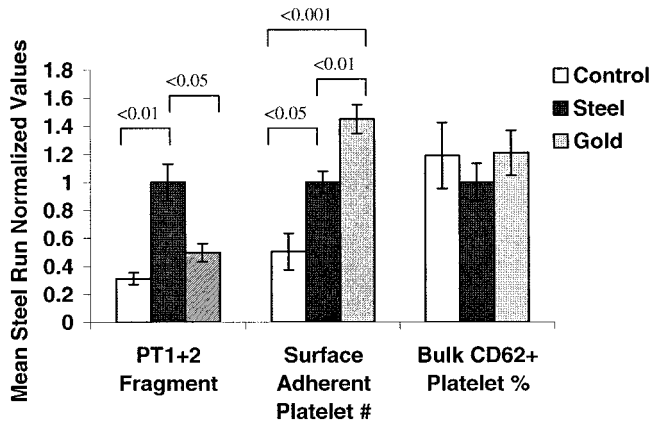


Figure 4. Mean steel normalized discrete coagulation (PT1 + 2) and platelet responses (activated surface bound number, bulk percent activation) of control, stainless steel, and gold stented loops. Significant comparison *p* values indicated on chart.

run was immediately stopped when a drop in flow was detected in any of the anticoagulant conditions. This decrease was the result of a reduction in luminal diameter that accompanied overt thrombus formation. In each trial, the initial drop occurred in the 0 µg/mL CTI case (33 ± 3 min). We found that the surface-bound, activated platelet number (CD61+, CD62p+ counts) rose significantly with decreasing levels of anticoagulation (Fig. 5). A significant rise in volumetric platelet activation only occurred under the actively clotting 0 µg/mL CTI conditions.

To determine whether flow could influence measures of stent biocompatibility, gold and stainless steel stents were run at high and low levels of CTI anticoagulant (20, 4 µg/mL) under both high and low flow conditions (200, 40 mL/min peak flow; Fig. 6). Platelet reactivity indices were normalized within each run to

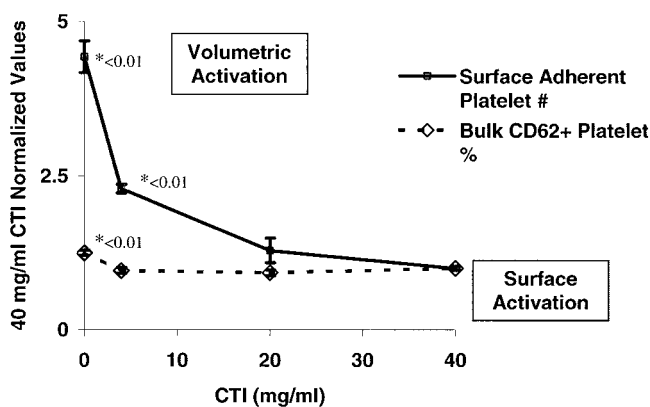


Figure 5. 40 mg/mL normalized platelet reactivity (activated surface bound number, bulk percent activation) of stainless steel stents with increasing coagulative function (decreasing CTI anticoagulant levels) showing regions of relatively low (surface) and high (volumetric) platelet activation. *Denoted points are significant as compared to higher CTI values.

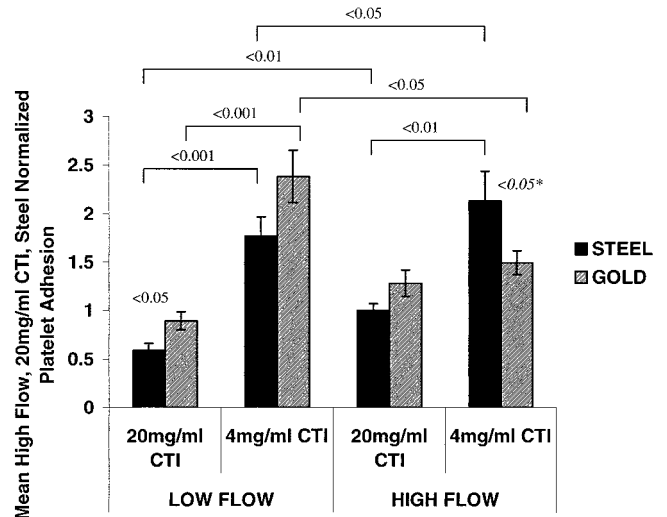


Figure 6. Parametric study of mean, high-flow, 20 mg/mL CTI steel normalized platelet reactivity (activated surface bound number) of stainless steel and gold-coated stents. Significant comparison *p* values indicated on chart. **p* value evaluated using a one-sided test hypothesis to test for directional differences.

the 20 µg/mL CTI, 200 mL/min, stainless steel condition. Two-way ANOVA analysis indicated significant effects on surface-bound, activated platelet measures (CD61+, CD62p+ counts) given changes in either flow or CTI levels. Significant interaction effects between flow and CTI levels occurred in the gold-coated stent group. In all conditions, volumetric platelet activation levels did not significantly vary between groups (data not shown). Table I summarizes specific findings made at a particular flow rate or coagulation strength given an increase in the other variable (coagulation or flow, respectively).

Increasing coagulation tended to increase platelet reactivity, while increasing flow only uniformly increased platelet reactivity under conditions of low coagulation. Considering the impact of these interactive effects on inter-stent comparisons, we found that under three test conditions (40 mL/min, 20 µg/mL CTI; 40 mL/min, 4 µg/mL CTI; 200 mL/min, 20 µg/mL CTI; Fig. 6), gold tended to react more strongly with platelets (first condition significant: 0.89 ± 0.09 vs. 0.59 ± 0.08; *p* < 0.05), while at low levels of CTI and high flow, the stainless steel stent reacted more strongly (2.13 ± 0.03 vs. 1.44 ± 0.12; *p* < 0.05 using a one-tailed Student *t* test to assess this directional change).

DISCUSSION

Implanted devices are increasingly used to combat diseases in a range of physiological environments.

TABLE I
Summary of Endovascular Stent Flow and Coagulation Interactions^a

Condition	Altered Variable	Finding
A. Low flow (40 mL/min peak flow)	Increasing coagulation (20 → 4 μg/mL CTI)	Increased platelet reactivity: (SS: 0.59 ± 0.08 vs. 1.77 ± 0.20; <i>p</i> < 0.001) (G: 0.89 ± 0.09 vs. 2.38 ± 0.27; <i>p</i> < 0.001)
B. High flow (200 mL/min peak flow)	Increasing coagulation (20 → 4 μg/mL CTI)	Increased platelet reactivity: (SS: 1.00 ± 0.07 vs. 2.13 ± 0.30; <i>p</i> < 0.01) (G: 1.28 ± 0.14 vs. 1.44 ± 0.12; NS)
C. Low coagulation (20 μg/mL CTI)	Increasing flow (40 → 200 mL/min peak flow)	Increased platelet reactivity: (SS: 0.59 ± 0.08 vs. 1.00 ± 0.07; <i>p</i> < 0.01) (G: 0.89 ± 0.09 vs. 1.28 ± 0.14; <i>p</i> < 0.05)
D. High coagulation (4 μg/mL CTI)	Increasing flow (40 → 200 mL/min peak flow)	No common trend: (SS: 1.77 ± 0.20 vs. 2.13 ± 0.30; NS) (G: 2.38 ± 0.27 vs. 1.44 ± 0.12; <i>p</i> < 0.05)

^aSS: stainless steel; G: gold.

Given the multiple interactions that take place at the body/device interface, issues of biocompatibility must encompass far more than a static surface reaction. In the setting of endovascular stenting, platelets serve as an important mediator of the biological response. The platelet reaction is not only a function of device surface composition, but a complex outcome dependent on several physical and biological factors. We show that varied independent responses (namely platelet and coagulative function) may exist *in vitro*, and further that it is a balance of these interactive components in the context of a local flow environment that determines measures of device biocompatibility such as stent-mediated platelet-reactivity.

Independent biological responses: platelets and coagulation

It is recognized that devices of different surface composition may stimulate different aspects of thrombosis to a variable degree.^{24–26} Currently, we considered stainless steel stents that were either polished in standard fashion or coated with a radio-dense layer of gold. We found that in the virtual absence of coagulation (obtained through high levels of anticoagulation), stainless steel stents exposed to PRP had reduced platelet reactivity as compared to gold stents. However, in the absence of a platelet component (as modeled with phosphatidylethanolamine-doped plasma), the stainless steel surface exhibited a higher coagulation response.

Such variability in material response can be rationalized by considering the unique quality of each surface. While stainless steel forms a protective oxide layer on its surface, gold does not.¹⁸ Materials that do not support such oxidation have been shown to enable strong surface protein interactions to highly abundant plasma proteins such as fibrinogen, thus promoting platelet reactivity.^{18,25,27} On less tightly binding, oxi-

dized surfaces, scarce proteins such as high molecular weight kininogen (HMWK) and FXII may accumulate over time, potentiating coagulative function.^{25–28} Surface oxidation is one mechanism that may account for the observed device differences. However, other aspects of surface quality may play a role as well. Indeed, we found surface roughness to be greater on the gold-coated as compared to the bare stainless steel stents. While further investigations are required to isolate and more directly analyze the impact of specific surface features on individual aspects of stent thrombosis, the observation of varied, independent device responses sets the stage for more complex, integrative, biocompatibility outcomes.

Interactive biological and physical environments: Coagulation and flow

As coagulation and platelets were allowed to interact through decreasing levels of anticoagulant (cases A and B, Table I; further evidence in Fig. 4), we found a generalized increase in endovascular stent platelet reactivity (trend for gold, case B). Additionally, as flow was increased under high anticoagulant conditions (case C), platelet reactivity also increased. These rises can be anticipated given the positive role of coagulative products and flow in facilitating platelet activation, adhesion, and aggregation.^{12,15,29–31}

With the role of coagulation increased (case D), the flow-dependent rise in platelet reactivity was no longer uniformly evident (nonsignificant increase with stainless steel; significant decrease with gold). These contrasting findings may reflect the flow-dependent balance of thrombotic products within the stent vicinity (a phenomenon evidenced by the nature of platelet-rich, high-flow arterial and fibrin-rich, low-flow venous thrombi.³² Whereas the higher coagulative potential of stainless steel may partly compensate

for high-flow clearance mechanisms, the lower potential of gold may not.

Variations in local environment and biocompatibility: Stainless steel vs. gold

Computational models of thrombotic reactions have predicted that flow and surface are key physical parameters that together may modulate the thrombotic response to biomaterials.^{32,33} Here, we provide experimental evidence for such interdependencies, showing that relative device biocompatibility is not solely a function of device properties. Under some conditions (decreased flow, decreased coagulation), gold coating supported more platelet adhesion than stainless steel while under other circumstances (increased flow, increased coagulation), stainless steel was more reactive.

There are several limitations to these observations that must be addressed. Firstly, the findings were made using an *in vitro* model of endovascular stent thrombosis. Factors such as direct wall injury or the added complexities of erythrocyte and leukocyte components were not incorporated. Furthermore, although the absolute flow profiles approximated typical coronary flow tracings on a beat-to-beat basis (Fig. 2), the oscillatory nature was a departure from the predominately unidirectional physiological condition.¹⁶ Thus, while the phenomenological importance of flow, surface, and coagulation levels in influencing device platelet reactivity is shown, it is not certain how these findings may quantitatively translate to physiological settings.

Additionally, our assessment of surface platelet reactivity as a measure of device biocompatibility is limited. While platelet response is a critical aspect of the body/device interaction, it is but a single component of the composite response. Moreover, our surface extraction assay yielded an indirect marker of platelet reactivity. This measure was chosen given that more standard volumetric activation measures (which also are indirect indices of a surface response^{34–36} did not appreciably change in our relatively short, pre-occlusive trials (illustrated in Fig. 4). What remains important for the scope of the current study was to have a sensitive index on which the hypothesis of environment-dependent device response could be tested.

In this report, we show that the benefit of one stent implant over another is not solely a function of the device itself, but a multifaceted phenomenon dependent on external conditions. The impact of external influences on vascular device biocompatibility is important to recognize given the varied vascular environments (*in vivo* and *in vitro*) that are regularly encountered.^{18–21,24} Devices suited for high-flow conditions (arterial settings) may not be best suited for

low-flow conditions (impeded arterial/venous settings). Alternatively, particular patient populations or the use of specific anti-thrombotic agents could significantly affect biological factors and, hence, the relative behavior of a given endovascular device. While we did not specifically study the effects of population variations on implant biocompatibility, the need to normalize data within each run is suggestive that such differences may exist.

Appreciating and understanding the role that local environment plays on the body/device interaction is important for the continued use and development of interventional technologies. Such recognition may lead to the tailored use of implants, materials, or concurrently administered drug regimes to a given individual or situation. These issues of local interactions are further highlighted given the trends toward local drug delivery and targeted therapies that offer methods of directly and specifically modulating the implant environment.

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References

1. De Servi S, Repetto S, Klugmann S, Bossi I, Colombo A, Piva R, Giommi L, Bartorelli A, Fontanelli A, Mariani G, et al. Stent thrombosis: incidence and related factors in the R.I.S.E. Registry (Registro Impianto Stent Endocoronarico). *Cathet Cardiovasc Interv* 1999;46:13–8.
2. Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. *Circulation*. 1995;91:2995–3001.
3. Cutlip DE, Baim DS, Ho KK, Popma JJ, Lansky AJ, Cohen DJ, Carrozza JP, Jr., Chauhan MS, Rodriguez O, Kuntz RE. Stent thrombosis in the modern era: a pooled analysis of multicenter coronary stent clinical trials. *Circulation*. 2001;103:1967–1971.
4. Baim DS, Carrozza JP, Jr. Stent thrombosis. Closing in on the best preventive treatment. *Circulation*. 1997;95:1098–1100.
5. Schomig A, Neumann FJ, Kastrati A, Schuhlen H, Blasini R, Hadamitzky M, Walter H, Zitzmann-Roth EM, Richardt G, Alt E, et al. A randomized comparison of antiplatelet and anticoagulant therapy after the placement of coronary-artery stents. *N Engl J Med*. 1996;334:1084–1089.
6. Kastrati A, Mehilli J, Dirschinger J, Pache J, Ulm K, Schuhlen H, Seyfarth M, Schmitt C, Blasini R, Neumann FJ, et al. Restenosis after coronary placement of various stent types. *Am J Cardiol*. 2001;87:34–39.
7. Fuster V, Falk E, Fallon JT, Badimon L, Chesebro JH, Badimon JJ. The three processes leading to post PTCA restenosis: dependence on the lesion substrate. *Thromb Haemost*. 1995;74:552–559.
8. Schwartz SM, Reidy MA, O'Brien ER. Assessment of factors important in atherosclerotic occlusion and restenosis. *Thromb Haemost*. 1995;74:541–551.

9. Badimon L, Chesebro JH, Badimon JJ. Thrombus formation on ruptured atherosclerotic plaques and rethrombosis on evolving thrombi. *Circulation*. 1992;86(Suppl 6):III74–85.
10. Basmadjian D. The effect of flow and mass transport in thrombogenesis. *Ann Biomed Eng*. 1990;18:685–709.
11. Savage B, Almus-Jacobs F, Ruggeri ZM. Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. *Cell*. 1998;94:657–666.
12. Turitto VT, Baumgartner HR. Platelet deposition on subendothelium exposed to flowing blood: mathematical analysis of physical parameters. *Trans Am Soc Artif Intern Organs*. 1975;21:593–601.
13. Savage B, Saldivar E, Ruggeri ZM. Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell*. 1996;84:289–297.
14. Rosenberg RD. Hemorrhagic disorders II. Platelets. In: Beck WS, editor. *Hematology*. Cambridge, MA: The MIT Press; 1994. p 507–542.
15. Kuter DJ. Hemorrhagic disorders I. Protein interactions in the clotting mechanism. In: Beck WS, editor. *Hematology*. Cambridge, MA: The MIT Press; 1994. p 443–576.
16. Kolandaivelu K, Edelman ER. Low background, pulsatile, *in vitro* flow circuit for modeling coronary implant thrombosis. *J Biomech Eng* 2002;124:662–668.
17. Herrmann R, Schmidmaier G, Markl B, Resch A, Hahnel I, Stemberger A, Alt E. Antithrombogenic coating of stents using a biodegradable drug delivery technology. *Thromb Haemost*. 1999;82:51–57.
18. Edelman ER, Seifert P, Groothuis A, Morss A, Bornstein D, Rogers C. Gold-coated NIR stents in porcine coronary arteries. *Circulation*. 2001;103:429–434.
19. Kastrati A, Schomig A, Dirschinger J, Mehilli J, von Welser N, Pache J, Schuhlen H, Schilling T, Schmitt C, Neumann FJ. Increased risk of restenosis after placement of gold-coated stents: results of a randomized trial comparing gold-coated with uncoated steel stents in patients with coronary artery disease. *Circulation*. 2000;101:2478–2483.
20. Tanigawa N, Sawada S, Kobayashi M. Reaction of the aortic wall to six metallic stent materials. *Acad Radiol*. 1995;2:379–384.
21. Alt E, Schomig A. The InFlow coronary stent. In: Serruys PW, Kutryk MJB, editors. *Handbook of coronary stenting*. London: Martin Dunitz; 2000. p 99–106.
22. Schneider DJ, Tracy PB, Mann KG, Sobel BE. Differential effects of anticoagulants on the activation of platelets *ex vivo*. *Circulation*. 1997;96:2877–2883.
23. Rollason G, Sefton MV. Measurement of the rate of thrombin production in human plasma in contact with different materials. *J Biomed Mater Res*. 1992;26:675–693.
24. Courtney JM, Lamba NM, Sundaram S, Forbes CD. Biomaterials for blood-contacting applications. *Biomaterials*. 1994;15:737–744.
25. Eriksson C, Nygren H. The initial reactions of graphite and gold with blood. *J Biomed Mater Res*. 1997;37:130–136.
26. Nygren H, Elam JH, Stenberg M. Adsorption of coagulation proteins and adhesion and activation of platelets at the blood-solid interface. An experimental study of human whole blood. *Acta Physiol Scand*. 1988;133:573–577.
27. Kanagaraja S, Lundstrom I, Nygren H, Tengvall P. Platelet binding and protein adsorption to titanium and gold after short time exposure to heparinized plasma and whole blood. *Biomaterials*. 1996;17:2225–2232.
28. Vroman L, Adams AL, Fischer GC, Munoz PC. Interaction of high molecular weight kininogen, factor XII, and fibrinogen in plasma at interfaces. *Blood*. 1980;55:156–159.
29. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature*. 2000;407:258–264.
30. Maalej N, Folts JD. Increased shear stress overcomes the anti-thrombotic platelet inhibitory effect of aspirin in stenosed dog coronary arteries. *Circulation*. 1996;93:1201–1205.
31. Makkar RR, Kaul S, Nakamura M, Dev V, Litvack FI, Park K, Mcpherson T, Badimon JJ, Sheth SS, Eigler NL. Modulation of acute stent thrombosis by metal surface characteristics and shear rate. *Circulation*. 1995;92:I–86.
32. Basmadjian D, Sefton MV, Baldwin SA. Coagulation on biomaterials in flowing blood: some theoretical considerations. *Biomaterials*. 1997;18:1511–1522.
33. Gregory K, Basmadjian D. An analysis of the contact phase of blood coagulation: effects of shear rate and surface are intertwined. *Ann Biomed Eng*. 1994;22:184–193.
34. Beythien C, Gutensohn K, Bau J, Hamm CW, Kuehnl P, Meinertz T, Terres W. Influence of stent length and heparin coating on platelet activation: a flow cytometric analysis in a pulsed floating model. *Thromb Res*. 1999;94:79–86.
35. Gutensohn K, Beythien C, Bau J, Meinertz T, Kuehnl P. Flow cytometric analysis of coronary stent-induced alterations of platelet antigens in an *in vitro* model. *Thromb Res*. 1997;86:49–56.
36. Shattil SJ, Cunningham M, Hoxie JA. Detection of activated platelets in whole blood using activation-dependent monoclonal antibodies and flow cytometry. *Blood*. 1987;70:307–315.
37. Berne RM, Levy MN. *Cardiovascular physiology*, 6th ed. Mosby Year Book: 1992.