Figure 3A.

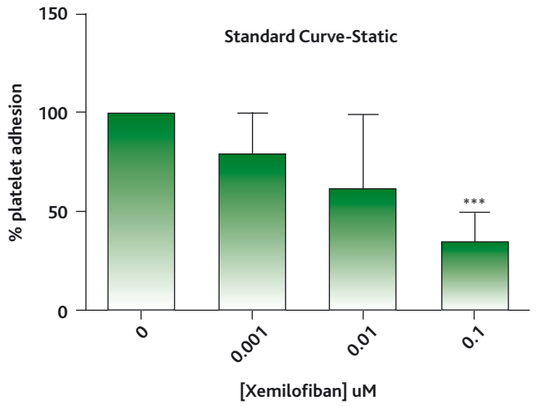

Figure 3A: Standard curve generated under static conditions to detect the effectiveness of a range of concentrations of xemilofiban on platelet adhesion to fibrinogen.

Figure 3B.

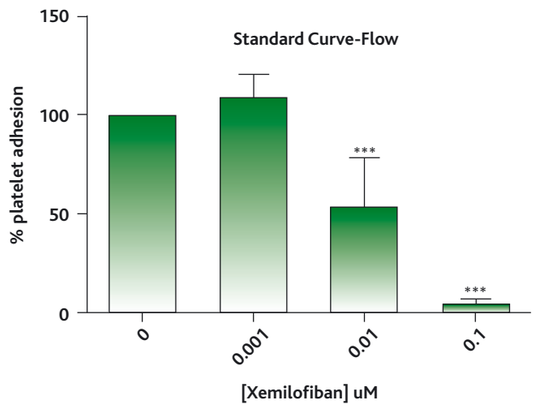

Figure 3B: Standard curve generated under physiologically relevant shear stress, detecting more statistically significant inhibitory concentrations of xemilofiban.

DISCUSSION

- System A and System B illustrate the adhesion profile of platelets treated with drug eluates over the course of 14 days. Day 1 suggests that there is a significant release of drug resulting in inhibition of platelet adhesion to fibrinogen. Day 3 shows a similar pattern (as shown by the median). It is noted that the drug is released at a slower rate after Day 9 and Day 14 resulting in more platelets adhering to fibrinogen (as shown by the median).
- System A and System B result in a more rapid release of drug during the first 3-day period compared to System C.
- System C results in a steady release of drug from the microgel/matrix composition from Day 3.
- Cellix's VenaFlux™ platform allows researchers to mimic physiological shear stresses. In comparison to static experiments, the VenaFlux™ platform allows for a more dynamic and sensitive detection method for platelet adhesion.
- Under flow conditions 0.1 µM xemilofiban results in an almost complete inhibition of platelet adhesion, whereas static experiments show a decrease by approximately 50%.
- Interestingly, under flow conditions platelet adhesion to fibrinogen is inhibited at a lower concentration of xemilofiban. Data from flow experiments show that 0.01 µM xemilofiban results in a statistically significant inhibition of platelet adhesion compared to static experiments where the 0.01 µM concentration shows no significant inhibitory effects.
- Statins are known to have pleiotrophic effects on the body. In this study, 0.1 µM fluvastatin showed a 70% inhibitory effect on the adhesion of platelets to fibrinogen (data not shown).
- Each drug-eluting co-polymer used in this study displayed effectiveness in lowering platelet adhesion, thus contributing to the prevention of in-stent thrombosis. This is achieved by the synergistic effects of both xemilofiban and fluvastatin.

ACKNOWLEDGEMENTS

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1. McGillicuddy FC, Lynch I, Rochev YA, Burke M, Dawson KA, Gallagher WM, Keenan AK: Novel "plum pudding" gels as potential drug-eluting stent coatings: Controlled release of fluvastatin. Journal of Biomedical Materials Research Part A, 2006; 79A: 923-933.

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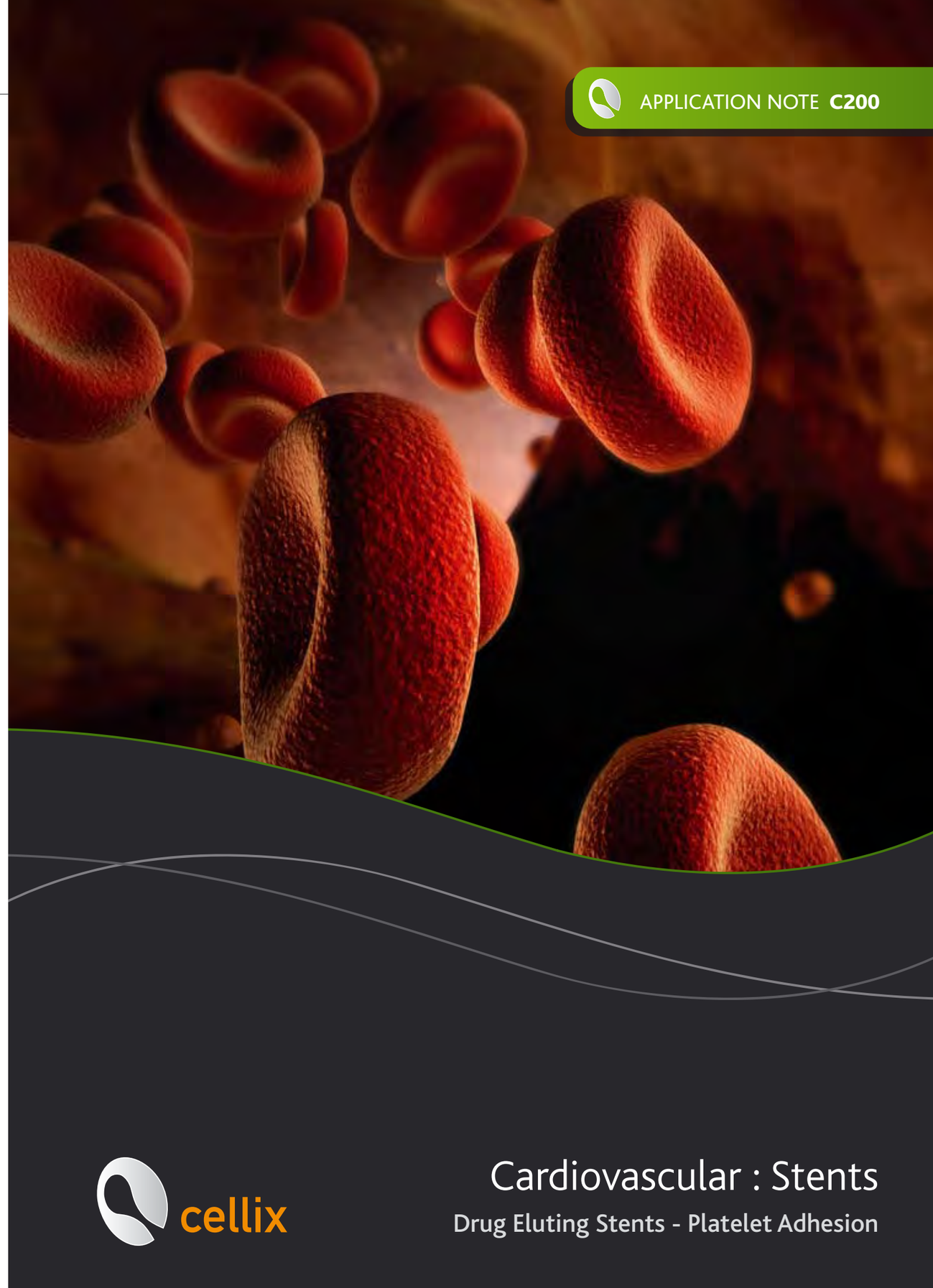


OBJECTIVES

To screen a range of **novel thermoresponsive polymers** designed to be used as dual drug-eluting systems in coating stents. Specifically, to assess the ability of xemilofiban released in conjunction with fluvastatin, to prevent **thrombus formation / platelet adhesion** to fibrinogen using **Cellix's VenaFlux**™ platform.

Figure 1.

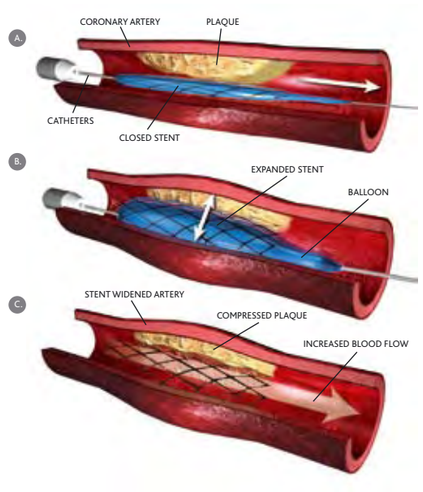


Figure 1: Schematic diagram illustrating the three stages of stent implantation.

INTRODUCTION

Stents are designed to line the inner walls of the blood vessel, holding the artery open and maintaining normal blood flow. Problems arise when scar tissue grows rapidly inside the stent, eventually narrowing the artery and causing reblockage, **or restenosis**, in as many as 25 % of patients. In addition to restenosis, in-stent thrombosis can occur following stent deployment. Until now, physicians have had few options for preventing restenosis and have used oral anti-platelet therapy to prevent in-stent thrombosis. New devices called **drug-eluting stents** have made a major contribution to reducing the incidence of restenosis. These devices consist of a metallic stent covered with a polymer coating, slowly releasing a medication that blocks inflammatory and proliferative processes in the vessel wall, thereby limiting the overgrowth of vascular tissue during the healing process.

Cellix have optimized a microfluidic set-up to study the interaction of whole blood incubated with fractions eluted over time from different co-polymer combinations. The co-polymers used in this study are **Thermoresponsive polymers** which are attractive materials for both biomedical engineering and cardiovascular applications. Treated whole blood was subjected to flow over microfluidic biochips coated with fibrinogen to provide varying platelet adhesion profiles for each co-polymer.

KEY WORDS

Platelet adhesion, thermoresponsive, drug elution, xemilofiban, fluvastatin, Vena8™

MATERIALS

- **Drug: xemilofiban** (an antiplatelet agent that blocks the binding of fibrinogen to specific membrane GPIIb/IIIa integrin receptors and thus prevents platelet aggregation induced by any known platelet agonist)
- **Drug: fluvastatin** (a cholesterol-lowering drug that reduces low-density lipoprotein (LDL) cholesterol and total cholesterol in the blood, helping to prevent heart disease and hardening of the arteries, conditions that can lead to heart attack, stroke, and vascular disease. Fluvastatin also possesses potent anti-proliferative activity, which prevents the overgrowth of vascular smooth muscle characteristic of restenosis)

Co-Polymers:

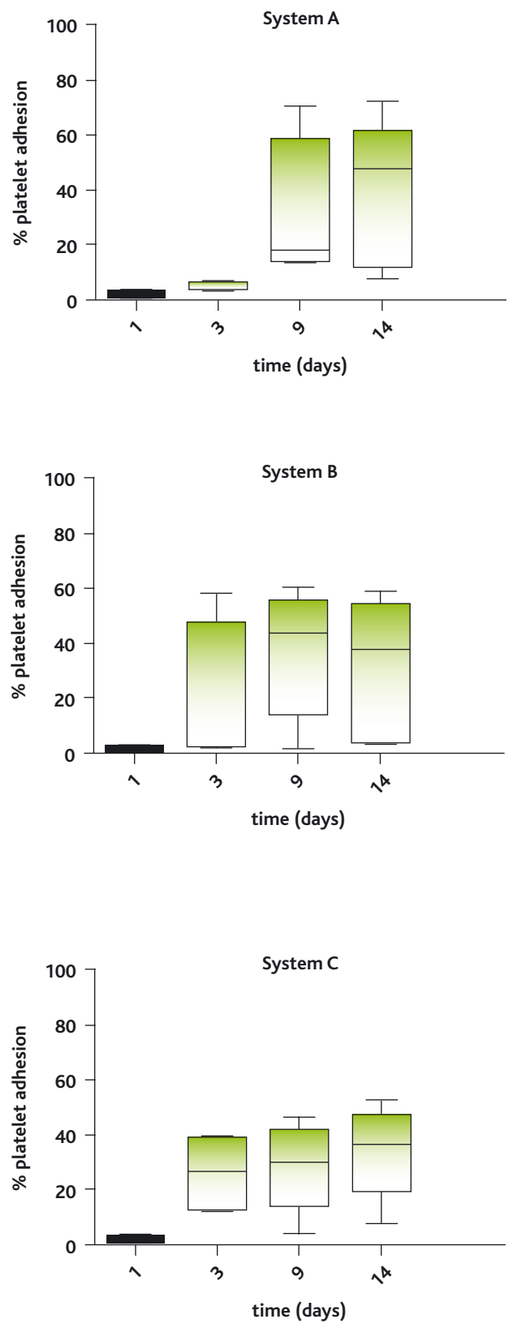
The copolymers contain different w/w ratios of the hydrophilic, temperature-sensitive poly(N-isopropylacrylamide, pNiPAAm) and the more hydrophobic, non-temperature-sensitive poly(N-tButylacrylamide, pNtBAAm). In each mixture, a condensed form (microgels) is dispersed throughout a matrix. The composition of each microgel or matrix system is indicated in terms of ratios, e.g. 85/15 denotes a co-polymer of composition 85 % pNiPAAm, 15 % pNtBAAm. The 85/15 matrix shows the most hydrophilic properties while the 50/50 matrix shows the most hydrophobic properties. The 65/35 microgel composition shows intermediate properties.

System A:
65/35 microgels containing fluvastatin embedded in a hydrophilic 85/15 matrix containing xemilofiban

System B:
65/35 microgels containing fluvastatin embedded in a hydrophobic 50/50 matrix containing xemilofiban

System C:
65/35 microgels containing xemilofiban embedded in a hydrophilic 85/15 matrix containing fluvastatin

Figure 2.



METHODS

1. Biochip coating procedures

Vena8™ biochips were coated with fibrinogen (20 µg/ml) and placed at 4 °C, overnight. Prior to experimentation, the coated channels were further coated with BSA (10 µg/ml) for 30 mins at room temperature and washed with JNL buffer. JNL buffer was also used to wash and prime the Mirus™ nanopump.

2. Adhesion profiles

Blood was collected from healthy volunteers who were not taking any medication and were free from aspirin and other anti-platelet agents within the preceding 2 weeks. The blood was drawn by venepuncture into light-blue Vacuette™ tubes containing a 1:10 volume of 3.8% (wt/vol) tri-sodium citrate, which is a reversible anticoagulant, and gently mixed. Blood was incubated with drug eluates for 5 minutes at room temperature and subjected to a constant shear stress of 32 dyne cm^{-2} for 3 min, followed by JNL buffer for a further 3 min at similar shear stress.

3. Image analysis

Platelet adhesion profiles were recorded using DucoCell software. Cell images were captured from three microscopic fields from each channel. Data were exported into Excel to allow further analysis.

4. Statistics

Data obtained were normalized against whole blood. Statistical analysis was conducted using 1-way ANOVA from Graphpad Prism® 5 software.

Figure 2: Adhesion of platelets to fibrinogen treated with drug eluates from day 1 to day 14. Whole blood was incubated for 5 min at room temperature with drug eluates from system A (Figure 2A), system B (Figure 2B) and system C (Figure 2C). These drug samples were eluted on day 1, day 3, day 9 and day 14. Statistically significant results were obtained with all three systems when expressed as a percentage of whole blood platelet adhesion. The percentage of adherent platelets was measured under constant shear stress of 32 dyne cm-2 for 3 mins, followed by a JNL wash for a further 3 mins. Box and whisker plot shows the median, interquartile and full range data obtained from 5 healthy individuals. $p<0.005$, $n=5$.