



Preclinical research

Intravascular thermography: Immediate functional and morphological vascular findings

Stefan Verheye^{a,b*}, Guido R. Y. De Meyer^{a,c}, Rob Krams^d, Mark M. Kockx^{a,e}, Luc C. A. Van Damme^d, Babak Mousavi Gourabi^d, Michiel W. M. Knaapen^e, Glenn Van Langenhove^b, Patrick W. Serruys^d

^aCardiovascular Translational Research Institute, Antwerp, Belgium

^bDepartment of Interventional Cardiology, Middelheim Hospital, Antwerp, Belgium

^cDivision of Pharmacology, University of Antwerp, Antwerp, Belgium

^dDepartment of Cardiology, Thoraxcenter, University of Rotterdam, Rotterdam, The Netherlands

^eDepartment of Pathology, Middelheim Hospital, Antwerp, Belgium

Received 14 August 2003; received in revised form 6 October 2003; accepted 16 October 2003

KEYWORDS

Atherosclerosis;
Catheters;
Imaging;
Plaque;
IVUS

Aim To investigate safety, feasibility, and injurious effect on endothelial cells of a thermography catheter as well as effect of flow on measured temperature in non-obstructive arteries.

Methods and results Safety and feasibility were tested in both rabbit aortas and pig coronary arteries. Evaluation of endothelial damage by the catheter (acute, 7 and 14 days) was performed in pig coronaries using Evans Blue, scanning electron microscopy (SEM) and Factor-VIII antibody and compared with normal arteries and arteries that underwent intravascular ultrasound (IVUS). The effect of flow on temperature heterogeneity was analysed both in vitro and in vivo conditions. All procedures were successful without any adverse events; intra- and inter-operator variability was low. Intracoronary use of the catheter was associated with acute but reversible de-endothelialization, paralleling the findings associated with IVUS use. Changes in flow velocities under physiologic flow conditions did not significantly influence the temperature differences measured both in vitro and in vivo; temperature heterogeneity was more pronounced in absence of flow.

Conclusion Intracoronary thermography using a dedicated catheter is safe and feasible with a similar degree of de-endothelialization as IVUS. Temperature heterogeneity remained unchanged under normal physiologic flow conditions allowing clinical use of thermography.

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Introduction

There is widespread agreement that early detection and treatment of rupture-prone coronary plaques is required. However, current techniques such as intravascular ultra-

sound (IVUS) and/or coronary angiography are incapable of detecting such 'vulnerable' plaques. Therefore, newer imaging strategies are being developed trying to identify those plaques.^{1,2}

Atherosclerosis is an inflammatory process characterised by presence of macrophages and lymphocytes.^{3,4} Cascells *et al.* reported that there is increased temperature heterogeneity in ex vivo atherosclerotic specimens of human carotid arteries.⁵ In vivo temperature

* Address for correspondence: Stefan Verheye, M.D., Department of Interventional Cardiology, Middelheim Hospital, Lindendreef 1, 2020 Antwerp, Belgium. Tel +32 3 280 32 55; Fax +32 3 230 65 11
E-mail address: stefan.verheye@pandora.be (S. Verheye).

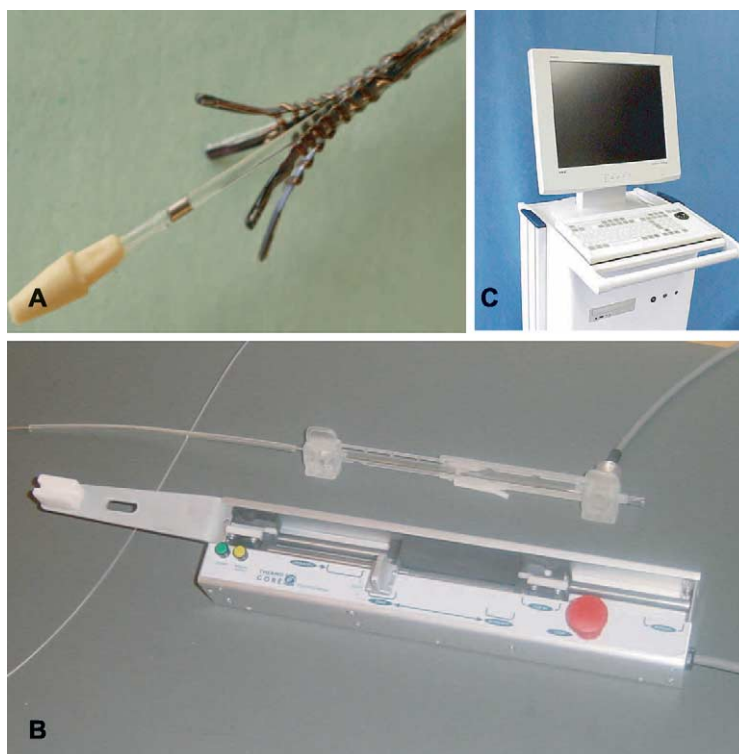


Fig. 1 (A) Close up photograph of the Thermosense™ catheter illustrating the four expanded thermistors, (B) Photograph of the catheter and pullback system, (C) Photograph of the console, which is connected to both the catheter and the pullback system.

heterogeneity was markedly increased in patients presenting with an acute coronary syndrome as opposed to patients having stable angina.⁶ We have recently shown that by using a dedicated temperature catheter in an animal model of atherosclerosis, in vivo temperature heterogeneity is determined by plaque composition and more specifically by the total macrophage mass.⁷ Furthermore, temperature heterogeneity in presence of flow appeared to be underestimated in patients with effort angina due to coronary stenosis which was related to cooling of the vessel wall by the blood flow.⁸ Since there are some unknown practical aspects of this technique such as safety, feasibility, effect of the catheter on endothelial cells and the effect of blood flow on wall temperature in non-obstructive lesions, we sought to determine these features associated with the use of a dedicated catheter in order to understand and incorporate its use in a clinical environment.

Methods

Thermography catheter and pullback-system specifications

The thermography catheter (Thermocore UK Ltd, Guildford, UK) is a 7F-compatible over the wire system that consists of a functional end that can be engaged by retracting a covering sheath.⁷ Briefly, the distal part has four dedicated thermistors at the distal end of four flexible nitinol strips. After engagement, the strips have an expansion width of 9 mm ensuring

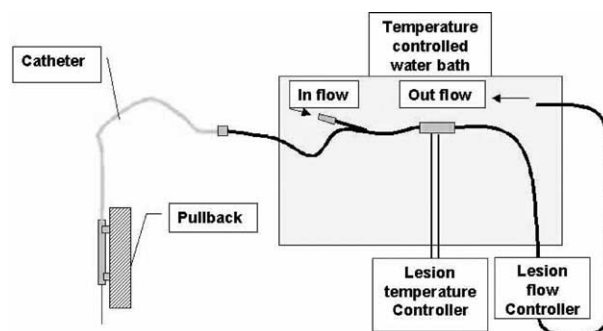


Fig. 2 Experimental set up evaluating the in vitro effects of flow on temperature measurement. Inside the bath, a 5 mm diameter aluminium tubular section wound with a heater coil, potted in resin was inserted to create an artificial change ('lesion'). The lesion temperature was controlled to maintain a surface temperature difference (measured for zero flow) of 1 °C. The flow rate was controlled through the lesion to achieve flow velocities of between 0 and 40 cm/s.

endoluminal surface contact of the vascular wall (Fig. 1A). The thermistors are made of 5k7 bare chips (5 kOhm resistance at 25 °C), which are gold metallized with 40 awg wires soldered onto it. The thermistor branches demonstrate an angle of $60 \pm 5^\circ$ when the sheath is retracted and the thermistors can perform up to 25 measurements per second; they are delivered with a certified accuracy of 0.006 °C. The response time of the thermistors to a small temperature change (1–5 °C) was found to be less than 100 ms. Once the catheter is inserted in the vascular system, the thermistors are normalized to a randomly chosen thermistor, and the proximal part of the catheter is then locked

Table 1 Measurements in temperature controlled water bath with and without conditions of sensor movement and/or flow past sensors ($n=6$)

	Average ($^{\circ}\text{C}$)	Max ($^{\circ}\text{C}$)
Average in-vitro deviation with no flow and stationary probe	0.00	0.01
Average in vitro deviation with no flow and pullback (0.5 mm/s)	-0.02	-0.02
Average in-vitro deviation with flow and pullback (0.5 mm/s)	0.01	0.03

onto a dedicated pullback system (Thermosense™; Fig. 1B), which by itself is connected to a dedicated thermography console (Thermosense™). Pullback at a predefined speed can then be started and recorded.

Data acquisition and processing

The thermistors are measuring resistance changes induced by changes in temperature. The latter changes are transformed into voltage changes via a Wheatstone bridge and recorded by the Thermosense™ Console that allows the data to be displayed in real-time (Fig. 1C).

In-vitro determination of system stability

To assess the stability of the measurement system a heated water bath was used to provide a stable temperature environment. The water bath was set to 37.0°C for 3 h so that a stable temperature was reached. Water in the bath was circulated by a pump to create an even heat distribution throughout the tank. A standard 7-Fr guiding catheter fitted with a standard Y-piece was used to introduce the catheter into the heated bath. The thermography catheter was introduced into the guiding catheter and into the water tank. A length of silicon tubing of 5 mm diameter was used to simulate an artery. The tubing was Y shaped, the catheter was introduced down one branch of the Y until the tubing could be attached to the distal end of the guiding catheter. The other branch was open to allow water to enter. A pump was connected to the distal end of the silicon tubing so that heated water from the tank was pumped down past the catheter. The flow rate was controlled to achieve flow velocities of 40 cm/s. The console and pullback were interfaced to the catheter and three sets of temperature measurements performed: (1) measurement of the temperature with no sensor movement and no water flow past the sensors, (2) measurement performed with a pullback of 60 mm at a velocity of 0.5 mm/s with no flow past the catheter temperature sensors and (3) measurement performed with flow past the sensors and with a pullback of 60 mm at a velocity of 0.5 mm/s.

Safety and feasibility

All animal experiments were approved by the local ethics committee and were conducted in compliance with Good Laboratory Practice. We performed experiments in both rabbits and pigs to test safety and feasibility of the catheter.

In order to assess the in vivo validity of the catheter measurements, intra- and inter-operator variability was tested in normocholesterolaemic male New-Zealand White rabbits ($n=6$; 4.0 ± 0.4 kg). Briefly, the marginal ear vein was cannulated and the rabbit was anesthetized with sodium pentobarbital (30 mg/kg i.v.). After shaving the groin, the femoral artery was dissected and a 6F sheath was introduced. Under fluoroscopy, a 0.014 inch guide wire (Boston Scientific) was positioned in the aortic arch prior to thermography of the descending aorta. A 30 mm seg-

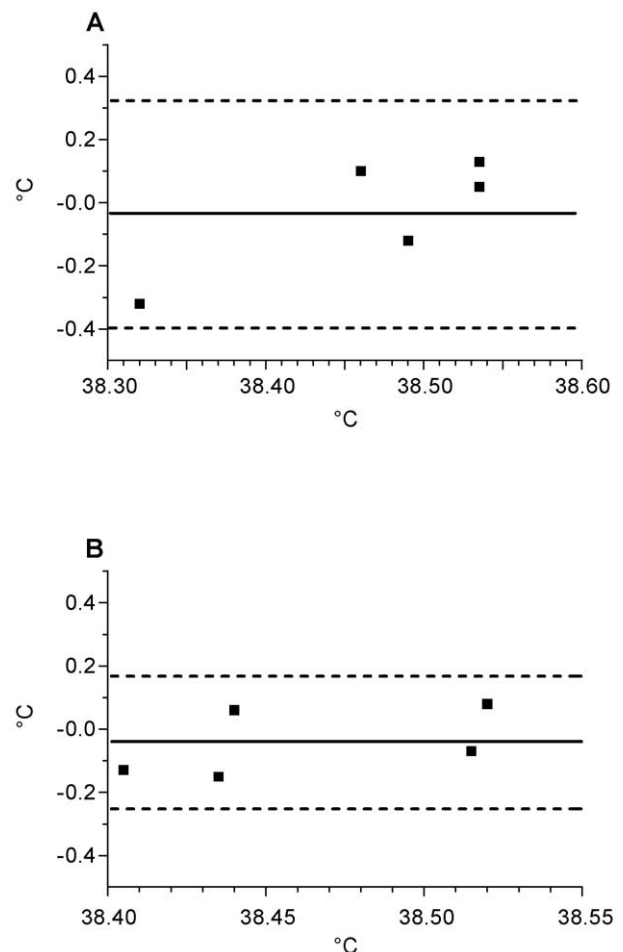


Fig. 3 Intra-operator (Panel A) and inter-operator (Panel B) variability in the descending thoracic aorta of normocholesterolaemic rabbits as assessed by Bland and Altman plots of the difference (second – first pullback, panel A; operator B – operator A, panel B) against their temperature mean ($n=5$ segments). The central line denotes the mean difference, the dotted lines represent $+1.96\times\text{SD}$ and $-1.96\times\text{SD}$ respectively.

ment in the descending thoracic aorta (between 4th and 7th rib) was chosen and operator A performed two consecutive pullback measurements, blinded to the temperature data output. Then, operator B performed the same measurement on the same aortic segment blinded to the previously obtained temperature data.

Safety and feasibility studies were further expanded in normal coronary arteries ($n=24$) of 12 juvenile domestic pigs (*Sus scrofa*). Animals received ketamine (20 mg/kg) and midazolam (3 mg/kg) prior to intubation. The animals were then mechanically ventilated after sedation with Thiopental (10–15 mg/kg);

Isoflurane (1–2.5% volume) given as needed for anaesthetic maintenance. A similar approach, this time using a 7F sheath via the carotid artery was used. After positioning the guiding catheter in the ostium of the left coronary artery, intravenous heparin (5000 IU) and aspirin (300 mg) were administered prior to placement of the intracoronary guide wire. Afterwards, scanning of the proximal segments (6 cm) of each coronary artery (both left anterior descending and left circumflex artery) using the thermography catheter was performed.

Effect on endothelium

To evaluate the acute and sub-acute effects of the catheter on the coronary endothelium, we performed thermography in four coronary arterial segments of four pigs and compared the effects to a similar pullback by IVUS (CVIS, Boston Scientific) in four other coronary arterial segments of the same four pigs; the non-analysed arteries served as controls. Immediately after angiography, the catheters were advanced into the coronary artery, their position was recorded, and a motorised pullback was performed. In two animals, 150 to 200 ml Evans Blue (0.3% in saline) were infused immediately after the procedure, directly into the coronary circulation following a saline flush. After completion of the Evans Blue-infusions, the coronary arteries were flushed with approximately 300 ml saline before pressure fixation in situ (approximately 100 mmHg) with 500 ml electron microscope-fixative (4% buffered paraformaldehyde and 2% glutaraldehyde).⁹ The heart was excised and vessels were prepared for further analysis.

The remaining animals were allowed to recover from anaesthesia and returned to the animal care facilities for 7 days during which 300 mg of aspirin was administered daily until restudy including Evans Blue and scanning electron microscopy (SEM).

The excised vessels were opened longitudinally and evaluated under a dissection microscope for penetration of the blue dye. Macroscopic images of the arteries were documented in digital format. All arteries were processed for, and analysed using SEM using routine techniques.⁹

Endothelial assessment using immunohistochemical techniques was performed in six animals at three different time points ($n=2$ acute, 7 and 14 days). These animals underwent intravascular thermography in a segment of a coronary artery. At each time point, animals were euthanized and vessels were prepared for immunohistochemical staining by using the indirect peroxidase antibody conjugate technique (Factor-VIII antibody, dilution 1/250, Binding Site). Sections were incubated with a goat anti-mouse peroxidase antibody (Jackson Laboratory) for 45 min. The polyclonal sheep anti-Factor-VIII antibody was visualized by a pig anti-sheep peroxidase. Two experienced operators blinded to the invasive strategy performed histologic assessment of the endothelium. Four sections of each scanned arterial segment were analysed for circumferential lack of endothelial cells and presence of thrombus.

Effect of flow

To investigate the potential influence of blood flow on temperature changes, we performed additional in vitro and in vivo tests in an experimental set up. For the in vitro experiment, we used a water bath in which the water was maintained at a constant temperature of 37 °C (Fig. 2). Inside the bath, a 5 mm diameter aluminium tubular section wound with a heater coil, potted in resin was inserted to create an artificial change ('lesion'). The lesion temperature was controlled to maintain a surface temperature difference (measured for zero flow) of 1 °C. The flow rate was controlled through the lesion to achieve flow velocities

of between 0 and 40 cm/s. The upper values resemble velocities within the human coronary artery system. Pullbacks were performed to determine the measured temperature profile of the lesion.

To evaluate the effects of flow in vivo, we changed the flow in the infrarenal denuded aorta of rabbits that were fed a high cholesterol (2%) diet for 2 months, by inflating a balloon (5.0 Maxxum, Boston Scientific, USA) upstream of the region of interest. Velocity was measured by an intravascular Doppler wire (Flowmap®, Cardiometrics, USA) located downstream of the inflated balloon. In each animal, zero flow was induced and a pull back was performed. The highest, median and lowest temperature difference were then identified. Afterwards five flow steps (0–9, 10–19, 20–29, 30–39 and 40–49 cm/s) in each of the animals were induced and changes in temperature difference at the previously defined locations were analysed.

Analysis and statistics

Data are given as mean±standard deviation (SD). Intra- and inter-observer variability was evaluated using Bland and Altman plots. To investigate the effect of flow, a regression analysis using an exponential fit was applied. The SPSS 10.0 software package was used for all analyses. A P -value <0.05 was considered significant.

Results

System stability

Measurements in a temperature controlled water bath with and without flow are shown in Table 1. The measurements performed with the catheter position stationary and with no flow past the sensors showed ± 0.01 °C variation. With flow and movement, the maximum temperature variation was 0.03 °C.

In vivo and ex vivo experiments

All procedures were completed successfully, i.e. at no point during or after the procedure any adverse event (death, stroke, infection, allergic reaction or misbehaviour) occurred in any of the rabbits or pigs. Intracoronary spasm of pig coronary arteries was seen in the first two measurements, which were reversible with intracoronary administration of nitrates and did not occur anymore in any of the following animals after prior intracoronary administration of nitrates.

Intra-operator and inter-operator variability was assessed in the descending thoracic aorta of normocholesterolaemic rabbits by using Bland and Altman plots, i.e. of the difference (second – first pullback, Fig. 3A and operator B – operator A, Fig. 3B) against their temperature mean. The coefficients of repeatability ($1.96 \times \text{SD}$ of the differences between the measurements) were 0.37 and 0.21 respectively.

Effect on endothelium

In acute experiments, control arteries showed a normal endothelium or only minor changes with small spots

of Evans Blue coloration (Fig. 4A). These minor changes were characterised on SEM by raised nuclei and some surface folds (Fig. 4B). Evans Blue staining of arteries that underwent intracoronary thermography revealed intense coloration of the artery wall in clear lines that seemed to demarcate the pullback line of the thermistors (Fig. 4C). SEM showed endothelial denudation at the site of Evans Blue staining with occasionally adherence of leucocytes and platelets to the denuded surface. In areas of marked blue staining, correlating with the catheter tip, SEM showed the presence of islands of endothelium (Fig. 4D). In another coronary artery of the same pig, the IVUS catheter induced a similar degree of acute de-endothelialization (Fig. 4E). The areas stained following IVUS catheter injury also showed extensive denudation with patches of endothelium remaining (Fig. 4F).

At 7 days following intervention, Evans Blue staining showed coloration throughout the scanned segment (Fig. 4G). SEM analysis of some parts of dark staining showed a denuded surface on which smooth muscle-like cells with occasional adherent leucocytes and platelets can be seen. Less intense stained areas showed isles of re-endothelialization, but with dysfunctional cell-cell contact (Fig. 4H).

Intravascular ultrasound catheter injury also showed deep Evans Blue staining of the arterial wall (Fig. 4I). The injury inflicted by both the guiding- (ostium of the vessel) and IVUS-catheter, as visualized by the Evans Blue coloration, was characterized by denuded patches. Areas with less intense Evans Blue staining showed re-grown endothelium with dysfunctional cell-cell contact (Fig. 4J).

Immunohistochemical staining of the endothelial cells for Factor-VIII showed partial de-endothelialization in the thermography-interrogated segments immediately after the procedure (Fig. 5A–B); small mural thrombi were found in a small number of sections (Fig. 5C) and were absent in control arteries (Fig. 5D). At 7 and 14 days, endothelial cells were visualized circumferentially in both control un-scanned and thermography-scanned pig coronary arteries (Fig. 5E and F) and mural thrombi were absent.

Effect of flow

The results of the in vitro experiments are shown in Fig. 6A. The relationship between flow velocity and temperature was logarithmic with a large percentage of the measurable temperature variation being dissipated at low flow velocities of less than 10 cm/s. For velocities above 10 cm/s, the measured temperature remained relatively constant. For flow velocities in the normal physiological range between 20 and 40 cm/s, the measured temperature amounted between 8 and 13% of the actual no-flow surface temperature. The results of the in vivo experiments in the atherosclerotic rabbit aortas are shown in Fig. 6B. The results parallel the in vitro findings with greater temperature heterogeneity during no-

or slow-flow conditions as compared to physiologic conditions.

Discussion

We demonstrated that intracoronary thermography using a dedicated catheter in an experimental setting is safe and feasible. Intracoronary thermography was associated with an initial and partial de-endothelialization, which normalised within 2 weeks and paralleled the findings observed with an intravascular ultrasound catheter. Finally, temperature heterogeneity was minimally influenced under normal flow conditions but was more pronounced in absence of flow.

Detection of vulnerable plaque is becoming increasingly important since the majority of acute coronary syndromes with subsequent and often unpredicted adverse events arises from rupture of such plaques. Since these plaques or associated with an increase in inflammatory cells, especially at sites of ruptures,¹⁰ thermography may therefore help in determining these plaques. It is suspected that inflammatory cells may be associated with increased temperature perhaps due to increased cellular metabolism.^{5,7,11} Intracoronary thermography may therefore serve as a tool to determine these patients who are or might be at increased risk by characterizing these arteries showing increased temperature heterogeneity by means of a dedicated intravascular thermography catheter.

We demonstrated that intravascular thermography is at least as safe and feasible as IVUS, a widely applied technique of which an increase in the incidence of adverse events related to the technique has not been reported.^{12,13} In a multicentre survey of 2207 examinations, IVUS was associated with a minor acute clinical risk.¹³ Vessel coronary spasm was the most frequent event occurring during IVUS. We have seen some spasm during intracoronary thermography in pigs, which was completely reversible after administration of nitrates. However, by administering nitrates prior to thermography, spasms remained absent. There is no published data on endothelial damage caused by or associated with IVUS; however, we have shown that there is de-endothelialization after passage of an IVUS catheter. Thermography did also cause some initial and partial de-endothelialization but only to a same degree as IVUS did. Our histology findings at 14 days strongly suggest that repair of the endothelium occurred within 7–14 days after intracoronary thermography and ultrasound since staining for endothelial cells was complete on all samples. In addition, early and late events were absent and all vessels analysed were patent and free of intracoronary or mural thrombi at 7 and 14 days.

Today, there is only sparse information on the amount of temperature heterogeneity. In the landmark report of Cascells *et al.*, temperature variations in ex vivo atherectomy specimen up to 2.2 °C were documented.⁵ The degree of heterogeneity was less pronounced in in vivo settings.^{6,7} One might suspect that flow may play an important role since flow along the

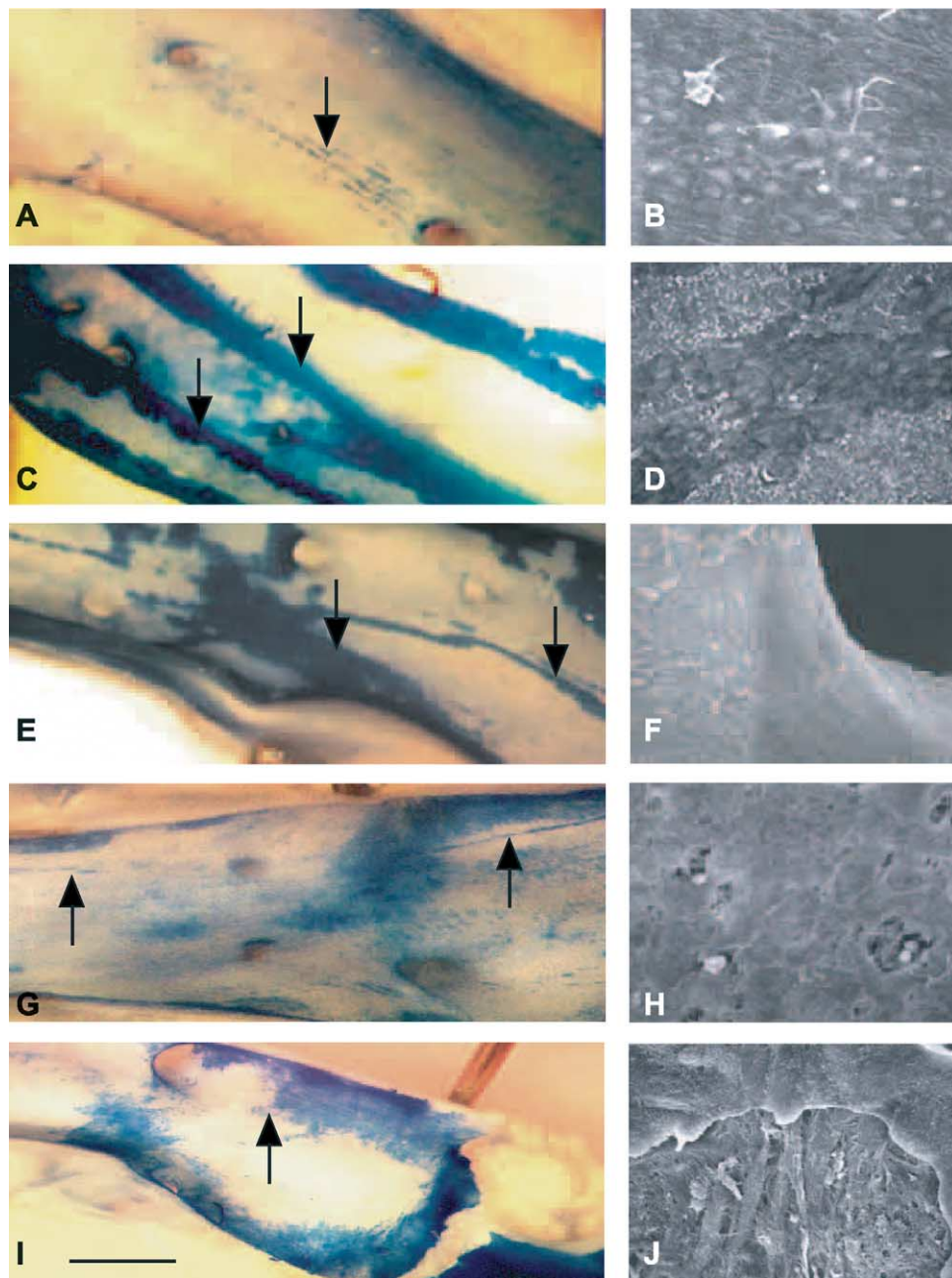


Fig. 4 Photograph of (A) Evans Blue staining of control pig coronary artery shows only mild staining (arrow), (B) SEM of control pig coronary artery with minor endothelial damage, (C) Evans Blue staining of pig coronary artery that underwent thermography (acute) illustrating the deep coloration corresponding to the tracing of the thermistor (arrow), (D) SEM of pig coronary artery that underwent thermography (acute) shows endothelial denudation at sites of Evans Blue staining, (E) Evans Blue staining of pig coronary artery that underwent IVUS (acute) also shows clear blue demarcation of the surface after passage of the IVUS catheter (arrow), (F) SEM of pig coronary artery that underwent IVUS (acute) illustrates endothelial denudation with patches of remaining endothelium, (G) Evans Blue staining of pig coronary artery that underwent thermography (7 days) shows still positive coloration along the traced path (arrow), (H) SEM of pig coronary artery that underwent thermography (7 days) shows re-endothelialization with dysfunctional cell-cell contact, (I) Evans Blue staining of pig coronary artery that underwent IVUS (7 days) parallels the thermographic findings with positive coloration (arrow), (J) SEM of pig coronary artery that underwent IVUS (7 days) shows areas of de- and re-endothelialization with dysfunctional cell-cell contact. (Scale bar is 5 mm).

vascular wall may cause a cooling effect due to dissipation at the inflamed site and therefore decrease the temperature. Recently, Stefanadis *et al.* have shown that there is indeed a cooling effect of the blood flow on temperature heterogeneity in human stable plaques.⁸ However, these findings were observed in

patients with stable angina having mean diameter stenoses of 73%. Even in lesions showing no baseline temperature heterogeneity, there was an increase of 76% by blocking flow, which may suggest that other energy sources (i.e. myocardium) may be responsible for increased temperature in circumstances of absence

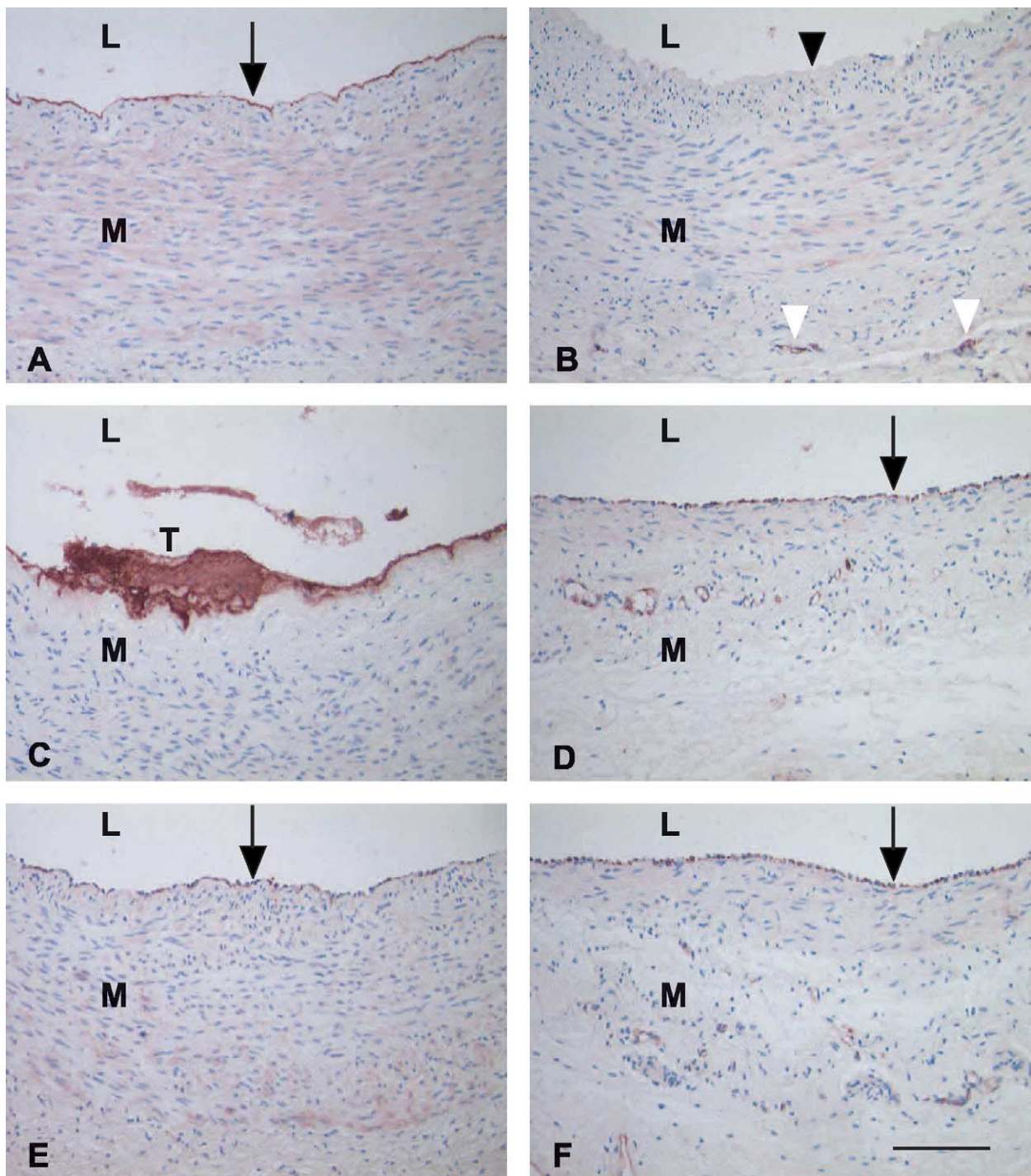


Fig. 5 Acute (A, B, C and D), 7 days (E), and 14 days (F) immunohistochemical endothelial staining (Factor VIII) of pig coronary arteries. Thermography-interrogated segments immediately after the procedure show areas of normal endothelium (A [arrow]), absence of endothelium (B [arrowhead]); note positive staining of endothelium in adventitial vessels [white arrowhead] and small mural thrombi (T) in a small number of sections (C). Control arteries show normal endothelium (D). At 7 and 14 days, endothelial cells were visualized circumferentially in both thermography-scanned (E: 7 days; F: 14 days) and control un-scanned (not shown) pig coronary arteries (L: lumen; M: media). Scale bar is 50 μ m.

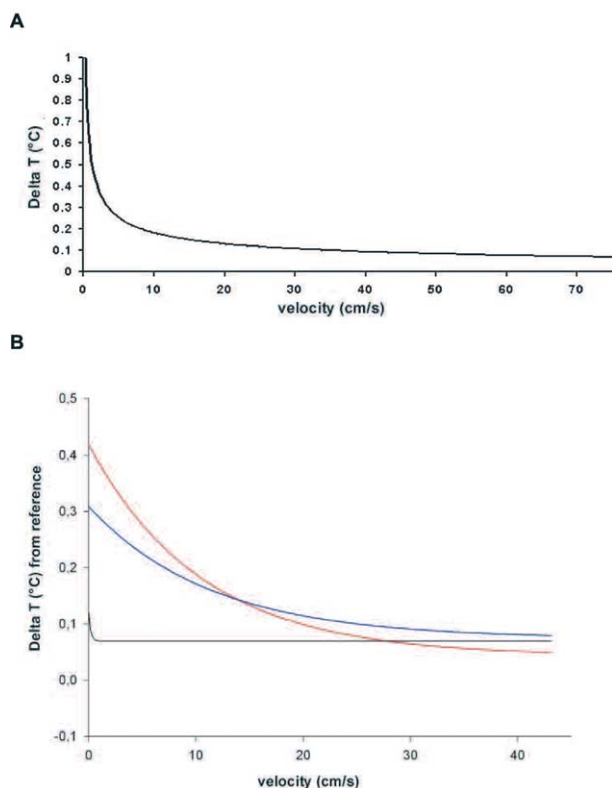


Fig. 6 (A) In vitro effect of flow on temperature performed as described in Fig. 2. The measured temperature for flow velocities between 20 and 40 cm/s (normal physiological range) amounted between 8 and 13% of the actual no-flow surface temperature, (B) In vivo effect of flow on temperature in denuded rabbit atherosclerotic aortas. In each animal, zero flow was induced by inflating a balloon and a pull back was performed. The highest, median and lowest temperature difference were identified. The three graphs (red line represents hot, blue line median and black cold spots) show the grouped results of the temperature differences obtained at each of the subsequent flow steps.

of flow. It remains to be seen whether these observations can be extrapolated to 'vulnerable' plaque lesions for which this technique has originally been designed for. Stable lesions are known to have less inflammatory cells and more smooth muscle cells with collagen fibres, which are less likely to show an increased temperature.⁷ Our in vitro and in vivo experiments illustrate that temperature heterogeneity is not influenced by flow variation in the physiologic range. Although temperature heterogeneity is more pronounced in absence of flow, it remains unclear what the value of reduction or stop in flow

means in a clinical setting of vulnerable plaque. Perhaps absence of flow in a clinical setting may lead to false positive temperature heterogeneity due to other energy expenditure unrelated to the vulnerable plaque.

In conclusion, intracoronary thermography using this dedicated catheter is a safe and feasible technique with a similar degree of de-endothelialization as the frequently used IVUS catheter. Temperature heterogeneity remained unchanged under normal flow conditions but was more pronounced in absence of flow. Intracoronary thermography may therefore be proposed as a valuable clinical tool for assessing the degree and meaning of temperature heterogeneity in human atherosclerotic arteries.

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