JACC Vol. 30, No. 3
September 1997:689–93



# Albumin Microbubble Adherence to Human Coronary Endothelium: Implications for Assessment of Endothelial Function Using Myocardial Contrast Echocardiography

FLORDELIZA S. VILLANUEVA, MD, FACC, RON J. JANKOWSKI, MS, CHRISTINE MANAUGH, BS, WILLIAM R. WAGNER, PHD

*Pittsburgh, Pennsylvania*

***Objectives.*** **We hypothesized that sonicated 5% human albumin microbubbles (Albunex) adhere to disrupted vascular endothelium and that this interaction is a marker of endothelial integrity. This study sought to identify sites and determinants of Albunex–endothelial cell (EC) attachment.**

***Background.*** **Under normal conditions, Albunex microbubbles used in myocardial contrast echocardiography (MCE) pass unimpeded through the coronary microcirculation. During pathophysiologic states associated with endothelial dysfunction, however, microbubbles linger in the myocardium despite normal flow. The sites and conditions regulating microbubble adhesion are unknown.**

***Methods.*** **Coverslips with cultured human coronary artery ECs were mounted in a parallel plate perfusion system and perfused with a suspension of fluorescein-labeled Albunex in culture medium, followed by a bubble-free wash at a wall shear rate of 100 $s^{-1}$. To create inflammatory ECs, phorbol myristate acetate was added 4.5 h before perfusion, and flow cytometry was used to confirm an inflammatory response. Perfusions were performed under normal and inflammatory conditions using surfaces of confluent and subconfluent ECs and isolated extracellular matrix. Bubble adherence was quantified in 20 random fields per coverslip using epifluorescent video microscopy.**

***Results.*** **No microbubbles adhered to normal confluent ECs, although small numbers adhered to inflamed ECs (0.03 ± 0.01 bubbles/cell, $p < 0.01$ vs. normal cells). Fewer microbubbles attached to normal versus inflamed matrix of both partially exposed (1,800 ± 520 vs. 4,100 ± 1,000 bubbles/$mm^2$, $p = 0.05$) and completely denuded (2,700 ± 1,300 vs. 7,200 ± 1,100 bubbles/$mm^2$, $p < 0.03$) endothelium.**

***Conclusions.*** **Albunex microbubbles preferentially adhere to inflammatory endothelial extracellular matrix. These data suggest that MCE can be used to noninvasively study endothelial integrity and may have implications for the assessment of preclinical atherosclerotic heart disease.**

**(J Am Coll Cardiol 1997;30:689–93)**



Myocardial contrast echocardiography (MCE) is a perfusion imaging technique utilizing two-dimensional echocardiography during transit of exogenously administered microbubbles through the myocardial microcirculation. Experimental studies performed in animals under normal physiologic conditions have confirmed that air-filled microbubbles made from sonicated 5% human albumin are kinetically similar to erythrocytes and pass unimpeded through the microcirculation (1,2), validating the use of this agent as a red blood cell tracer in vivo (3,4).

It has been noted, however, that under certain pathologic conditions, albumin microbubble transit through the myocardium is delayed despite normal flow, a phenomenon that manifests as persistent echo brightness in regions of myocardium (5,6). These observations were first made with MCE during infusion of cold crystalloid cardioplegia in beating canine hearts (5), but the mechanisms underlying this phenomenon are unknown. To date, studies to elucidate this observation have employed gross video intensity measurements in canine models (6), which have not permitted a more specific inquiry into the actual sites and determinants of microbubble persistence in myocardium.

Because microbubbles remain within the intravascular space, one possible explanation for bubble persistence may be adherence of the contrast agent to the endothelial surface. Furthermore, because the observations of delayed microbubble transit were made during conditions that are known to perturb endothelial function (7,8), it is conceivable that microbubble behavior within the microvasculature may be affected

From the Division of Cardiology, Division of Cardiothoracic Surgery and Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, Pennsylvania. Dr. Villanueva was supported in part by grants-in-aid from the National Center of the American Heart Association, Dallas, Texas and the Pennsylvania Affiliate of the American Heart Association, Camp Hill, Pennsylvania. Dr. Wagner was supported in part by the William and Sue McGowan Foundation, Pittsburgh, Pennsylvania. This study was presented in part at the 45th Annual Scientific Session of the American College of Cardiology, Orlando, Florida, March 1996. Molecular Biosystems, Inc., San Diego, California provided the Albunex used in this study.

Manuscript received December 11, 1996; revised manuscript received April 18, 1997, accepted May 16, 1997.

Address for correspondence: Dr. Flordeliza S. Villanueva, Division of Cardiology, University of Pittsburgh, 200 Lothrop Street, S568 Scaife Hall, Pittsburgh, Pennsylvania 15213. E-mail: villan@card2.cath.upmc.edu.



0735-1097/97/$17.00
PII S0735-1097(97)00197-6

**Abbreviations and Acronyms**

| | | |
|---|---|---|
| DTAF | = | dichlorotriazin amino fluorescein |
| ECs | = | endothelial cells |
| FITC | = | fluorescein isothiocyanate |
| HCAECs | = | human coronary artery endothelial cells |
| ICAM-1 | = | intercellular adhesion molecule 1 |
| IgG | = | immunoglobulin G |
| MCE | = | myocardial contrast echocardiography |
| PBS | = | phosphate buffered saline |
| PE | = | phycoerythrin |
| PMA | = | phorbol myristate acetate |

by the physiologic status of the endothelial surface. If such were the case, MCE could be a useful clinical tool to assess coronary endothelial cell (EC) dysfunction, a major early event in the pathogenesis of ischemic heart disease (9–11).

We hypothesized that microbubble–endothelial interactions reflect the structural and/or functional integrity of the endothelium. Accordingly, the goals of this study were to characterize the interactions between albumin microbubbles and vascular endothelium and to determine conditions that promote microbubble adherence to the endothelial surface. To more specifically interrogate vascular ECs, a perfused cell culture model was used to microscopically locate sites of bubble adhesion to vascular endothelium and to determine the effects of endothelial injury on bubble adherence.

## Methods

**Perfusion system.** Perfusions with nonpulsatile flow were performed using a rectangular, parallel plate system in which coverslips with cultured human coronary artery ECs (HCAECs) were mounted (12). A coverslip was mounted on the top of the perfusion chamber to permit contact between the endothelial surface and microbubbles. The perfusion chamber (perfusion space 200 $\mu$m in height) employed a silastic gasket and vacuum system to seal the compartment and secure the coverslip in place. The coverslip surface (2.65 $cm^2$) was exposed to culture medium flowing through the chamber at a preselected wall shear rate, which was regulated using a syringe pump in withdrawal mode (Harvard Apparatus) connected to a perfusate reservoir.

**Cell culture.** Commercially available HCAECs (Clonetics Corp.) were subcultured on glass coverslips and grown to confluence over 4 days in culture medium composed of endothelial basal media with 5% fetal bovine serum (Clonetics Corp.). To create inflammatory HCAECs, 4-beta-phorbol 12-myristate 13-acetate (PMA, 20 ng/ml) (Sigma) was added to the cultures 4.5 h before the experiment (13,14).

To isolate the endothelial extracellular matrix, coverslips of confluent HCAECs were exposed to 0.1 mol/liter of $NH_4OH$ for 15 min at room temperature and gently shaken. This technique results in cell lysis and exposure of the underlying extracellular matrix (14). Isolated matrices were washed with phosphate buffered saline (PBS) and used immediately thereafter.

**Flow cytometric confirmation of inflammation.** To confirm the presence of an inflammatory phenotype after exposure to PMA, flow cytometry was used to quantify cell surface expression of tissue factor and intercellular adhesion molecule 1 (ICAM-1) (15). Briefly, HCAEC monolayers were harvested with collagenase (0.2 mg/ml) and 2 mmol/liter of EDTA in PBS after incubation with or without PMA for 4.5 h. The harvested cells were washed and incubated for 20 min at 4°C with either immunoglobulin G (IgG) antihuman tissue factor murine monoclonal antibody (50 $\mu$g/ml, Biodesign International) or nonspecific murine IgG negative control antibody (50 $\mu$g/ml, Accurate Chemical and Scientific Corp.). After washing with PBS, the cells were incubated with a 1:50 dilution of fluorescein isothiocyanate (FITC)–conjugated sheep antimouse IgG (Sigma) for 20 min at 4°C, rewashed and incubated for 20 min with phycoerythrin (PE)-conjugated antihuman ICAM-1 murine monoclonal IgG (3.12 $\mu$g/ml, Immunotech) or nonspecific PE-conjugated murine IgG (5 $\mu$g/ml, Becton Dickinson). The cells were washed two more times and subsequently fixed in 1% paraformaldehyde in PBS. Samples were analyzed for fluorescence (5,000 events/sample) with a fluorescence-activated cell sorter (FacScan, Becton-Dickinson).

**Fluorescein labeling of microbubbles.** A suspension of air-filled microbubbles (mean bubble diameter 4.3 $\mu$m and concentration $0.5 \times 10^9$ bubbles/ml) produced by sonication of 5% human albumin (Albunex, Molecular Biosystems, Inc.) (2–4) was fluorescently labeled with 5-([4,6-dichlorotriazin-2-*yl*]amino)-fluorescein (DTAF, Sigma) using the following process: A 10-ml vial of Albunex was allowed to stand until the foamy bubble layer was separated from the clear albumin layer; 8 ml of albumin was removed from the vial and mixed with 6 mg of DTAF; the albumin–DTAF mixture was returned to the vial, mixed with the foamy layer for 15 min and allowed to stand and separate into two discrete layers; the clear albumin layer was removed and replaced with an equivalent volume of unlabeled 5% human albumin (Swiss Red Cross) to reduce background fluorescence.

**Cell perfusions.** Cell culture medium prewarmed to 37°C was used as the perfusate. A coverslip with confluent HCAECs or isolated matrix was mounted in the perfusion chamber, and the system was primed with culture medium and $10^{-5}$ mol/liter of mepacrine (Sigma) to fluorescently label the HCAECs. The chamber was perfused for 3 min at a wall shear rate of 100 $s^{-1}$ with a 1:10 suspension of fluorescein-labeled Albunex in culture medium, followed by a 3-min bubble-free perfusion. Experiments were performed during four conditions: intact endothelium was perfused at baseline (8 coverslips) and after stimulation (10 coverslips) with PMA. Similarly, completely exposed extracellular matrix was perfused under basal (6 coverslips) and inflammatory (6 coverslips) conditions.

After each perfusion, microbubble adherence was quantified in ~20 random microscopic fields (1,000×) per coverslip using epifluorescent video microscopy (Axiovert 35, Zeiss). Occasionally, after a coverslip with confluent ECs was perfused, isolated areas of extracellular matrix became exposed due to lifting of cells during the perfusion. Video images of

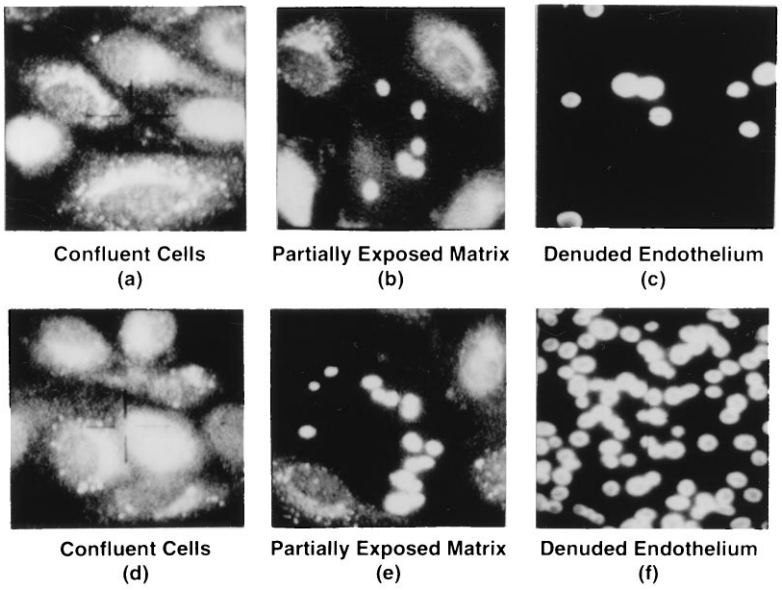


**Figure 1.** Photomicrographs (1,000×, reduced by 23%) demonstrating patterns of microbubble adherence to normal (**a to c**) and activated (**d to f**) endothelial surfaces. Microbubbles preferentially adhered to extracellular matrix, with more bubbles adhering to inflamed matrix. (See text for details.)

such fields were digitized (MIPRON, Kontron Electronics) and planimetered to determine the absolute area of denuded endothelium.

**Statistical analysis.** Results are expressed as mean values ± SEM. Comparisons between experimental conditions with respect to the numbers of adherent microbubbles were made using the *t* test. Statistical significance was defined as $p < 0.05$ (two-tailed).

## Results

**Patterns of microbubble adherence.** Figure 1 illustrates microbubble adherence patterns for normal (panels a to c) and PMA-exposed (panels d to f) endothelial surfaces. Microbubbles did not adhere to normal confluent cells (panel a). When normal surfaces were partially denuded during the perfusion, small numbers of bubbles adhered to the exposed matrix (panel b). Similarly, bubbles adhered to the completely exposed matrix (panel c).

There was greater microbubble adherence in the presence of EC activation (Fig. 1, d to f). Microbubbles occasionally adhered to a PMA-stimulated cell (Fig. 2), although in most instances bubbles did not attach to confluent inflamed cells (Fig. 1d). Partially denuded surfaces previously activated with PMA (Fig. 1e) had a greater number of bubbles adherent to the extracellular matrix than to the corresponding nonactivated matrix (Fig. 1b). When the PMA-stimulated cells were completely removed, large numbers of bubbles adhered to the inflamed matrix (Fig. 1f).

**Microbubble interactions with endothelial cells.** The EC–bubble adherence data for the 18 perfusions performed on cellular surfaces indicate that bubble attachment to cells was uncommon. Bubbles did not stick to any of the eight coverslips perfused under normal conditions. In 7 of 10 coverslips exposed to PMA, a total of 25 bubbles were found to be adherent to cells. Although microbubble adherence to cells was sparse in the PMA-treated condition, the number of adherent microbubbles per total number of activated cells in each coverslip (0.03 ± 0.01) was greater than that for normal cells (0.00 ± 0.00, $p < 0.002$).

**Microbubble interactions with extracellular matrix.** Microbubbles adhered almost exclusively to exposed extracellular

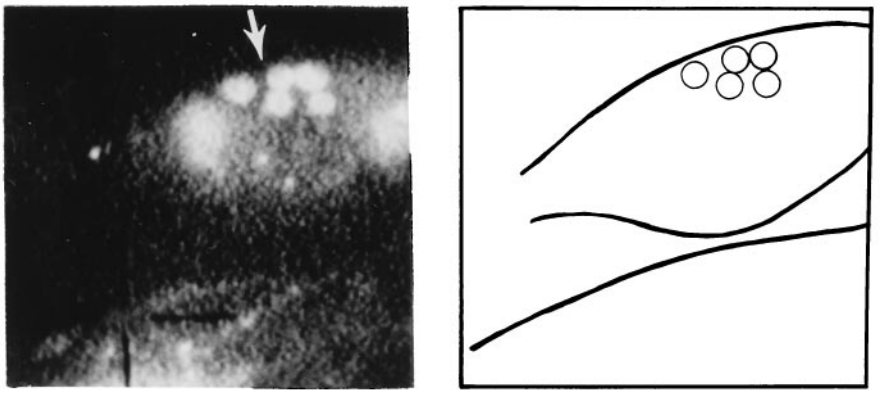

**Figure 2.** Photomicrograph (**left**) and diagram (**right**) demonstrating microbubble adherence to a single EC previously activated with PMA. (See text for details.)

matrix, with preferential adherence to activated matrix. Sixty three of the 165 microscopic fields studied under normal conditions had regions of exposed matrix, and 53 of the 214 inflammatory fields demonstrated patches of exposed matrix. There tended to be a greater number of adherent bubbles to the partially exposed matrix of PMA-exposed coverslips ($4,100 \pm 1,000$ bubbles/mm$^2$) than to that of control coverslips ($1,800 \pm 520$ bubbles/mm$^2$, $p = 0.05$).

Because bubbles appeared to selectively adhere to partially denuded surfaces, perfusions were performed after lysis of the HCAECs and complete exposure of the matrix. Similar to the findings with partially denuded endothelium, there was an approximate doubling in the number of microbubbles sticking to activated ($7,200 \pm 1,300$ bubbles/mm$^2$) compared with normal ($2,700 \pm 340$ bubbles/mm$^2$) matrix ($p < 0.03$).

**Confirmation of inflammatory response to PMA.** Phenotypic markers of inflammation, such as tissue factor and ICAM-1, were quantified in HCAEC cultures with respect to factor-specific IgG expression beyond background fluorescence. Under normal conditions, there was minimal expression of either tissue factor ($1.9 \pm 0.3\%$, $n = 4$) or ICAM-1 ($18 \pm 8\%$, $n = 5$). After PMA exposure, there was significant upregulation of tissue factor ($40 \pm 9\%$, $p < 0.03$) and ICAM-1 ($88 \pm 3\%$, $p < 0.001$), confirming that the phorbol ester generated an EC inflammatory response.

## Discussion

This study tested the hypothesis that characteristics of the endothelial surface affect albumin microbubble transit. Using an in vitro perfusion model to microscopically characterize interactions between albumin microbubbles and vascular endothelium, our three major findings were that 1) microbubbles do not adhere to normal cultured HCAECs; 2) during an experimentally induced inflammatory state, small numbers of microbubbles adhere to activated endothelial cells; and 3) microbubbles preferentially stick to exposed extracellular matrix, with significantly greater adhesion to inflamed matrix.

**Mechanisms of adherence.** The mechanisms mediating albumin microbubble adherence to endothelial surfaces remain incompletely understood. Microbubble "stickiness" could be a nonspecific mechanical phenomenon in which matrix exposed between cells may entrap the spherical bubbles. This explanation seems unlikely, however, because the microbubble diameter is greater than the EC height, and it would not completely account for the greater bubble adherence to activated matrix. Another possibility is that EC surface proteins that are upregulated during inflammation, such as leukocyte adhesion molecules or tissue factor (13,15–17), may have affinity for the microbubbles.

The preferential adhesion of bubbles to the matrix of PMA-exposed surfaces suggests that factors secreted by activated HCAECs and which alter the composition and organization of the matrix may potentiate bubble attachment (13,14,18). Similarly, as the inflammatory reaction involves not only synthesis of new matrix components but also degradation of preexisting ones (14), it is possible that matrix degradation products could be avid for albumin bubbles.

It is also conceivable that albumin within the bubble shell is involved in adherence, because albumin normally binds in vivo to endothelial glycocalyx through at least four putative albumin binding proteins (19,20). Nonetheless, the relative paucity of bubble adherence to cells in these experiments suggests that membrane-bound albumin receptors do not bind the albumin within the bubble shell.

**Study limitations.** As with all in vitro preparations, the cell–culture approach used in this study does not completely replicate the in situ human phenotype. For example, the lack of fluid shear during culture may alter EC secretion of vasoactive substances and proteins and the expression of surface proteins (21). Shear stress also modulates the synthesis of glycocalyx components (22). Although we did not confirm extracellular matrix synthesis by the ECs, the differential microbubble binding to denuded inflamed versus denuded normal endothelium suggests that the binding was not simply an epiphenomenon related to bubble adhesion to exposed glass.

Another limitation of this model is that cells were exposed to culture medium rather than blood, which would be a more clinically relevant perfusate. The absence of leukocytes or platelets could have affected the interaction of the microbubbles with the ECs and matrix. For example, activated ECs express P-selectin and ICAM-1 (16). If microbubbles adhere by means of these leukocyte-adhesive receptors, it is conceivable that a leukocyte-bearing environment might have caused a competitive decrease in microbubble binding. Similarly, platelets, which adhere to denuded endothelial surfaces (13,14), could have competitively affected microbubble binding to exposed extracellular matrix.

Despite the inherent limitations of this model, however, the physiologic and structural features of the cells and extracellular matrix of our preparation represent an approximation of the in vivo endothelial surface that has previously been used by other investigators to study endothelial biology and interaction with blood elements (12–14,18). Furthermore, flow cytometric confirmation of the expected increase in tissue factor and ICAM-1 expression in response to PMA tends to validate the physiologic integrity of our system.

The present study leaves unanswered which, if any, of the potential mechanisms discussed earlier participate in microbubble adherence. More extensive studies, both in vitro and in vivo, will need to be pursued to more precisely characterize the conditions promoting albumin microbubble adhesion to endothelium.

**Clinical implications.** The endothelium plays a critical regulatory role in vascular homeostasis. Normal endothelial surfaces are antithrombotic and fibrinolytic, inhibit migration of leukocytes and are vasoactive in response to physiologic stimuli (23). Endothelial injury due to hypertension, smoking, hyperlipidemia, diabetes mellitus, coronary angioplasty or postischemic reperfusion results in abnormal endothelial function, which may clinically manifest as inappropriate vasoconstriction, atherosclerosis, thrombosis or restenosis (10,23–26).

The experimental model of endothelial activation and injury used in this study could pertain to several clinical scenarios in which endothelial dysfunction is a prominent feature. Endothelial activation and expression of leukocyte adhesion molecules are associated with vascular injury during ischemia-reperfusion, such as occurs during postischemic reflow in acute myocardial infarction, coronary artery bypass graft surgery and cardioplegia delivery (7,27). In addition, the early lesions of atherosclerosis and the predisposition toward thrombosis are coincident with EC activation and upregulation of leukocyte adhesion molecules (9,10,28). Also, structural disruption of the endothelium and enhanced adhesion molecule expression occur after percutaneous transluminal coronary angioplasty (29).

As endothelial dysfunction plays a prominent role in the biology of atherosclerosis, its identification could have important implications for the early treatment of coronary artery disease. Because derangements in endothelial function may exist without overt manifestations of myocardial ischemia, however, they are not readily identified in vivo. Current techniques for assessing endothelial function are limited to invasive approaches in the cardiac catheterization laboratory (11,26) or to noninvasive peripheral measures of brachial artery reactivity, which are extrapolated to coronary endothelial function (30). There are currently no available noninvasive methods for directly studying coronary endothelial function at the microcirculatory level in vivo.

The development of a noninvasive tool to study coronary microvascular endothelial function would thus be a major advance in the diagnosis and treatment of patients at risk for ischemic heart disease. The preliminary data presented here suggest that measures of microbubble transit during MCE, an index of microbubble–endothelial interaction, may ultimately enable the noninvasive in vivo assessment of endothelial structure and function in the clinical setting. Furthermore, the concept of microbubble–endothelial adherence can ultimately be extended to the design of contrast agents targeted to specific markers of cell phenotype, thus opening possibilities for tissue-specific contrast ultrasound imaging.

## References

1. Keller MW, Segal SS, Kaul S, Duling B. The behavior of sonicated albumin microbubbles within the microcirculation: a basis for their use during myocardial contrast echocardiography. Circ Res 1989;65:458–67.
2. Jayaweera AR, Edwards N, Glasheen WP, Villanueva FS, Abbott RD, Kaul S. In-vivo myocardial kinetics of air-filled albumin microbubbles during myocardial contrast echocardiography: comparison with radiolabeled red blood cells. Circ Res 1994;74:1157–65.
3. Villanueva FS, Glasheen WP, Sklenar J, Kaul S. Characterization of spatial patterns of flow within the reperfused myocardium by myocardial contrast echocardiography: implications for determining extent of myocardial salvage. Circulation 1993;88:2596–2606.
4. Villanueva FS, Glasheen WP, Sklenar J, Kaul S. Assessment of risk area during coronary occlusion and infarct size after reperfusion with myocardial contrast echocardiography using left and right atrial injection of contrast. Circulation 1993;88:596–604.
5. Keller MW, Spotnitz WD, Metthew TL, Glasheen WP, Watson DD, Kaul S. Intraoperative assessment of regional myocardial perfusion using quantitative myocardial contrast echocardiography: an experimental evaluation. J Am Coll Cardiol 1990;16:1267–79.
6. Ismail S, Spotnitz W, Jayaweera A, Goodman N, Skyba D, Kaul S. Myocardial contrast echocardiography can be used to assess dynamic changes in microvascular function in vivo [abstract]. J Am Coll Cardiol 1995;25 Suppl:246A.
7. Aoki M, Kawata H, Mayer JE Jr. Coronary endothelial injury by cold crystalloid cardioplegic solution in neonatal lambs. Circulation 1992;86 Suppl II:II-346–51.
8. Milhoan KA, Lane TA, Bloor CM. Hypoxia induces endothelial cells to increase their adherence for neutrophils: role of PAF. Am J Physiol 1992;263:H956–62.
9. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991;251:788–91.
10. Gimbrone MA. Vascular endothelium: an integrator of pathophysiologic stimuli in atherosclerosis. Am J Cardiol 1995;75:67B–70B.
11. Reddy KG, Nair R, Sheehan HM, Hodgson JM. Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. J Am Coll Cardiol 1994;23:833–43.
12. Lawrence MB, McIntire LV, Eskin SG. Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. Blood 1987;70:1284–90.
13. de Groot PG, Reinders JH, Sixma JJ. Perturbation of human endothelial cells by thrombin or PMA changes the reactivity of their extracellular matrix towards platelets. J Cell Biol 1987;104:697–704.
14. Zwaginga JJ, Sixma JJ, de Groot PG. Activation of endothelial cells induces platelet thrombus formation on their matrix: studies of new in vitro thrombosis model with low molecular weight heparin as anticoagulant. Arteriosclerosis 1990;10:49–61.
15. Mulder AB, Hegge-Paping KSM, Magielse CPE, et al. Tumor necrosis factor-$\alpha$–induced endothelial tissue factor is located on the cell surface rather than in the subendothelial matrix. Blood 1994;84:1559–66.
16. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994;84:2068–2101.
17. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA. Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. J Clin Invest 1985;76:2003–11.
18. Houdijk WPM, de Groot PG, Nievelstein PFEM, Sakariassen KS, Sixma JJ. Subendothelial proteins and platelet adhesion: von Willebrand factor and fibronectin, not thrombospondin, are involved in platelet adhesion to extracellular matrix of human vascular endothelial cells. Arteriosclerosis 1986;6:24–33.
19. Schnitzer JE, Carley WW, Palade GE. Specific albumin binding to microvascular endothelium in culture. Am J Physiol 1988;254:H425–37.
20. Schnitzer JE, Oh P. Antibodies to SPARC inhibit albumin binding to SPARC, gp60, and microvascular endothelium. Am J Physiol 1992;263:H1872–9.
21. Mitsumata M, Fishel RS, Nerem RM, Alexander RW, Berk BC. Fluid shear stress stimulates platelet-derived growth factor expression in endothelial cells. Am J Physiol 1993;265:H3–8.
22. Arisaka T, Mitsumata M, Kawasumi M, Tohjima T, Hirose S, Yoshida J. Effects of shear stress on glycosaminoglycan synthesis in vascular endothelial cells. Ann N Y Acad Sci 1995;748:543–54.
23. Meredith IT, Anderson TJ, Uehata A, Yeung AC, Selwyn AP, Ganz P. Role of endothelium in ischemic coronary syndromes. Am J Cardiol 1993;72:27C–32C.
24. Anderson TJ, Gerhard MD, Meredith IT, et al. Systemic nature of endothelial dysfunction in atherosclerosis. Am J Cardiol 1995;75:71B–74B.
25. Nabel EG. Biology of the impaired endothelium. Am J Cardiol 1991;68:6C–8C.
26. Vita JA, Treasure CB, Nabel EG, et al. The coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1989;81:491–7.
27. Ma X, Lefer DJ, Lefer AM, Rothlein R. Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule-1 in myocardial ischemia and reperfusion. Circulation 1992;86:937–46.
28. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature 1993;362:801–9.
29. Naruko T, Ueda M, Becker AE, et al. Angiographic-pathologic correlations after elective percutaneous transluminal coronary angioplasty. Circulation 1993;88:1558–68.
30. Corretti MC, Plotnick GD, Vogel RA. Technical aspects of evaluating brachial artery vasodilatation using high-frequency ultrasound. Am J Physiol 1995;268:H1397–404.