In vitro construction of a human blood vessel from cultured vascular cells: A morphologic study

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Purpose: The purpose of this study was to create a tubular vascular model exclusively made of human cells and collagen.

Methods: The blood vessel equivalent was constructed with the three following human cell types: vascular smooth muscle cells, endothelial cells, and fibroblasts. A tissue-like structure was obtained from the contraction of a tubular collagen gel (human origin) by vascular smooth muscle cells, which created a media-like structure. An adventitia-like tissue was added around the media-like structure by embedding fibroblasts into a collagen gel. An endothelium was established within the tubular structure after intraluminal cell seeding.

Results: Cell orientation and gel contraction were followed up over time. Vascular smooth muscle cells developed a complex tridimensional network and were oriented in a circular fashion around the tube's axis. In contrast, fibroblasts were randomly oriented. A viable, homogeneous, and well-characterized endothelium was observed. These endothelial cells showed a slightly elongated structure and were oriented parallel to this vascular equivalent axis.

Conclusion: An in vitro tridimensional vascular model that exhibits some phenotypic characteristics of in vivo vascular cells could be useful in the study of events that lead to atherosclerotic plaque formations. (J VASC SURG 1993;17:499-509.)

Atherosclerosis and related cardiovascular diseases are the major cause of morbidity and mortality in the industrial world. Initial advances in understanding this disease were largely the result of histopathologic observations of resected sclerotic arteries. More recently, crucial insights have been obtained mostly through animal models such as rabbits, primates, and spontaneously hypertensive rats. Furthermore, advances in cell culture have permitted the use of pure vascular cell lines (endothelial and smooth muscle cells) to establish, in vitro, some of the basic principles on which vascular cell interactions are interpreted nowadays.

However, limitations of these different methods created the need for new approaches. Recently new coculture systems have proved to be such a novel and challenging approach to the study of vascular cell dynamics. Direct coculture of endothelial cells (EC) and smooth muscle cells (SMC), with or without contact, has been extensively used for short-term analysis of intercellular growth control, secretory activity, and responses to a variety of stimuli. However, the instability of some models and the failure to respect the basic tridimensional arrangement of respective cell populations led to the development of more complex models. Such models include EC grown on a microfilter at different proximities of an SMC culture, microcarrier beads covered with EC and deposited on an SMC culture, and EC grown on collagen gels containing SMC in various layouts.

We have developed a three cell type vascular model that contains human EC, SMC, fibroblasts, and human type I and III collagen (no animal products). This vascular equivalent respected the natural tridimensional arrangement of vascular cells and...
imitated the macroscopic aspect of the blood vessel, that is, a tubular form. Furthermore, this model provided vascular cells with a physiologic extracellular matrix. This model resembled the one presented by Weinberg and Bell in 1986. However, their model included a synthetic material (Dacron; E. I. du Pont de Nemours and Co. Inc.) and was produced from bovine collagen and cells. This new vascular equivalent could allow, in a single model, in vitro study of various human cell behaviors and interactions: changes induced by blood flow, intercellular growth inhibition, migration, SMC access to blood components, and others.

We have observed interesting cell behavior with respect to collagen contraction, cell orientation, and forces exerted by SMC in a tridimensional collagen lattice. The structure of EC was modified by the presence of SMC in the gel. Furthermore, the EC layer was found to have several characteristics of an in vivo endothelium.

**MATERIALS AND METHODS**

**Materials.** The HEPES buffer (N'-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid), Dulbecco’s modified Eagle medium (DME), Ham’s F12 modified medium, fetal bovine serum (FBS), the 0.4% trypan blue stain solution, and the 0.5% phenol red solution were purchased from Flow Laboratories (McLean, Va.). Collagenase (collagenase H, 0.153 U/ml) was obtained from Boehringer-Mannheim Canada (Dorval). The trypsin was from ICN Biochemical (Montreal, Canada). Heparin sodium (25,000 U/ml, from porcine intestinal mucosa) came from Leo Laboratories Canada Ltd. (Pickering). The pepsin extracted human placenta collagen was a mixture of type I and type III collagen (46:54) and was a gracious gift from Iméderex (Chaponost, France). Acetylated low density lipoprotein labeled with 1,1′-dioctadecyl-1-1-3,3,3′,3′-tetramethyl-indo-carbocyanine perchlorate (Ac-LDL-Dil) was purchased from Biomedical Technologies Inc. (Stoughton, Mass.). The mouse anti-human factor VIII-Von Willebrand’s factor monoclonal antibody (clone 1A4) was purchased from Chemicon International Inc. (El Segundo, Calif.). The mouse anti-smooth-muscle actin antibody (clone 1A4) was from Sigma Chemical Co. (St. Louis, Mo.).

**Solutions.** The HEPES buffer contained 10 mM HEPES, 119 mM NaCl, 6.7 mM KCl, and 11 mM glucose, at pH 7.35. For calcium, 5 mM CaCl2 was added. The collagenase solution was prepared in HEPES buffer with calcium and phenol red to a final concentration of 0.160 U/ml, at pH 7.35, or, dissolved in DME, to a final concentration of 0.200 U/ml. The trypsin solution used contained 0.05% trypsin and 0.01% ethylenediamine tetraacetic acid in phosphate buffered saline (PBS = 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, and 1.5 mM KH2PO4) with 2.8 mM glucose and phenol red, at pH 7.45. Antibiotics were used (100 U/ml penicillin G and 25 μg/ml gentamicin). The travel medium contained DME-Ham with a double dose of antibiotics and 1 μg/ml amphotericin B. FBS was heat-inactivated at 56°C for 30 minutes. A collagenase solution was obtained by planar agitation of collagen in distilled water at pH 4.0 (obtained by adding HCl) for 12 hours at 4°C.

**Cell isolation.** Human umbilical vein endothelial cells (HUVEC) were obtained by the method of Jaffe with some modifications. Briefly, umbilical cords were steriley collected from healthy newborns and kept in ice cold travel medium for 12 hours or less. Veins from 15 to 30 cm long unclamped cords were cannulated at both ends, washed with calcium-free HEPES solution, and placed in a 37°C bath of sterile water. A warm collagenase solution was injected to rinse and fill the vein. After a 15-minute incubation, the veins were gently massaged and vigorously perfused with two times 50 ml of M199 containing 10% FBS and antibiotics. The cell solution obtained was centrifuged and the cell pellet was resuspended in medium for endothelial cells (ME). HUVEC were then plated into gelatine-coated tissue culture flasks.

Human vascular smooth muscle cells (HVSMS) were isolated by the method of Ross. The human skin fibroblasts (HSF) were obtained from a normal adult skin specimen removed during reductive breast surgery of healthy human subjects (15 to 37 years old). The isolation was performed within 3 hours. The skin was trimmed by removing the fatty part of the dermis. The remaining skin fragments (5 mm × 5 mm) were then floated on a 500 μg/ml thermolysin solution in HEPES buffer for 2 hours at 37°C. The dermis was separated from the epidermis with forceps, cut into small pieces, and incubated for 20 hours at 37°C in a solution of collagenase (200 U/ml in DME). After centrifugation, fibroblasts were plated into tissue culture flasks.

**Tissue culture.** All cells were plated at an initial density of 1 × 10^4 viable cells/cm^2 in tissue culture flasks and were kept in a humidified atmosphere (92% air and 8% CO2). Cells were fed every 2 days. On passage, cells were counted and their viability was evaluated by the trypan blue dye exclusion test. Cell
viability always exceeded 95%. The MEC was made of medium M199 supplemented with 20% FBS, 2 mmol/L L-glutamine, 50 U/ml heparin, 25 μg/ml EC growth factor supplement, and antibiotics. EC were harvested when they approached confluence and used at passage 3 or 4. SMC and fibroblasts were cultured in the following fashion and used at passage 4 through 6 and 5 or 6, respectively. The medium for smooth muscle cells (MSMC) consisted of DMEM-Ham, 10% FBS, and antibiotics. These cells were harvested 2 to 4 days after having reached confluence. All centrifugations were made at 3000 × g, for 10 minutes. All cells were tested at different passages for mycoplasmal infection with Hoechst fluorescent staining for cytoplasmic deoxyribonucleic acid and were always found to be negative.

Construction of an artificial blood vessel. The media-equivalent was made with the use of a mold constituted of a 15 ml tube in which a glass cylinder was maintained in its center. A mixture of HVSMC were always found to be negative. Four times concentrated, and distilled water was made media-equivalent with the use of a mold constituted of a 15 ml tube in which a glass cylinder was laid on its side in an incubator and released it in a Petri dish. In experiments concerning SMC reorientation, the media-equivalent was kept off its mandrel and cannulated. A solution of HUVEC (1 × 10⁶ cells/ml) was then injected into the vessel. The glass mandrel, surrounded with the newly formed media-equivalent structure, was then carefully transferred into a larger tube filled with MSMC.

To obtain an adventitia-equivalent structure, the same protocol as in the case of the media-equivalent was used except that a mandrel surrounded with a contracted media-equivalent structure was covered by a collagen solution (3 mg/ml) containing HSF (5 × 10⁵ cells/ml).

EC seeding was performed in the following manner: The adventitia-media-equivalent structure, or a simple media-equivalent structure, was slipped off its mandrel and cannulated. A solution of HUVEC (1 × 10⁶ cells/ml in MEC) was then injected into the vessel. The cannulas were then closed and the vessel put in a tube filled with MEC, which was laid on its side in an incubator and frequently rotated. The next day, cannulas were taken off.

Media-equivalent contraction. The contraction process was followed by keeping the media-equivalent on the mandrel for 2 days and then releasing it in a Petri dish. In experiments concerning SMC reorientation, the media-equivalent was kept on the mandrel for the entire experiment.

Immunofluorescence staining. Frozen sections (4 μm) and cells grown on glass coverslips were fixed 10 minutes in acetone at -20°C and labeled by indirect immunostaining as previously described.

Metabolic labeling. MEC containing 10 μg/ml Ac-LDL-Dil was centrifuged at 10,000 × g, 10 minutes at 4°C. A small specimen of the model containing a living endothelium, or cells grown on glass coverslips, was then immersed in the supernatant at 37°C for 4h and washed. Frozen sections were fixed in formaldehyde (3% in PBS) for 20 minutes and processed as previously described herein.

Histology and electron microscopy. Specimens were processed according to standard methods previously described.

RESULTS

Cell characterization. The endothelial nature of the HUVEC was established by their characteristic cobblestone morphology in culture, their positive staining with an anti-human factor VIII-Von Willebrand's factor monoclonal antibody, and their uptake of Ac-LDL-Dil (Fig. 1, a, c, and e). Furthermore, the characteristic Weibel-Palade bodies were abundant in HUVEC as observed by transmission electron microscopy (TEM). HVSMC and HSF differed from HUVEC by their fusiform morphology in culture (Fig. 1, b). HVSMC could be discriminated from HSF and HUVEC by their positive staining with a mouse monoclonal anti-smooth-muscle α-actin antibody (Fig. 1, d, f).30,31 Moreover, isolated HVSMC observed by TEM displayed a discontinuous basal membranelike deposit and bundles of actin filaments interspersed with dense bodies (Fig. 1, f). HSP did not react with anti-α-actin or anti-factor VIII antibodies.

Genesis of a media-equivalent. The first step in the construction of this vascular model was the molding of the media-equivalent even though it is the middle layer. Just after casting and unmolding, the media-equivalent was a very flimsy "Jello-like" cylinder, which floated around the glass mandrel. However, within 24 hours, an obvious contraction of the gel had taken place. In this time, the HVSMC had changed from rounded cells to well-spread and ramified cells. Within 2 to 3 days, a complex tridimensional network of well-differentiated, star-like, and very elongated interconnecting cells was established (Fig. 2). Intercellular junctions could be seen by TEM (Fig. 1, f, open arrowhead). This firmer gel further contracted to a final state of being fairly manipulable in 3 to 4 days (at concentrations of...
Fig. 1. Characterization of HUVEC and HVSMC. Phase contrast micrographs of standard cultures: a, HUVEC (original magnification ×60); b, HVSMC (original magnification ×50). Immunofluorescence labeling: c, HUVEC stained with anti-human factor VIII-Von Willebrand's factor monoclonal antibody (original magnification ×240); e, HUVEC labeled with Ac-LDL-Dil (original magnification ×250); d, HVSMC stained with anti-smooth muscle α-actin monoclonal antibody (original magnification ×240). f, Electron micrograph of HVSMC embedded in collagen gel (original magnification ×11,000). Note discontinuous basement membranelike deposit (arrowheads), dark bodies surrounded by bundles of actin filaments (arrows), and cell junction (open arrowhead). N, Nucleus; RER, rough endoplasmic reticulum.

3 × 10⁵ cells/ml and more) and was then quite opaque. Observations by phase contrast microscopy became more and more difficult.

**Contraction of the media-equivalent.** The extent of the contraction after 48 hours was directly proportional to the cell concentration (Fig. 3) and was concomitant with cell spreading and ramification (Fig. 2). While contracting, the media-equivalent adhered to the glass mandrel. This adherence was repeatedly broken up at first by a gentle tapping of the tube on a hard surface, but as the contraction proceeded the detachment of the media-equivalent demanded much more vigorous shocks. This detachment was induced at least once a day and was necessary for proper HVSMC alignment. It was necessary to permit maximum longitudinal contraction on the mandrel to prevent a significant twisting contraction that would otherwise occur on the final release from the mandrel. Furthermore, massive HVSMC migration onto the glass tube was observed at both ends of undisturbed media.

Fig. 3 shows the time course of the contraction of media at different cell concentrations. Abrupt increases of contraction at 24 hours were due to the release of media. At 48 hours, media were slipped off their mandrels and put in a Petri dish (liberation). It was obvious that longitudinal tension was accumulating in the media before release or liberation occurred. On liberation, the diameter of the lumen rapidly diminished to roughly one third to one fourth of its original size (transverse contraction) at concentrations of 3 × 10⁶ cells/ml and more. This contraction stopped in 2 to 3 days after liberation and always left a visible lumen.

To observe the effects of long-term culture of HVSMC in MEC, which contains heparin, media-
Fig. 2. Morphologic appearance of HVSMC in media-equivalent structure at different times after gel formation (3 × 10⁵ cells/ml), as seen by phase contrast microscopy: (a) just after gel formation; (b) after 2 hours first cells start to spread, and (c) after 48 hours (original magnification ×40). d, A closer view of complex tridimensional network of interconnecting cells (original magnification ×95). Note that the three pictures were taken at slightly different focal levels to show extent of cell ramification.

equivels were kept in culture for as long as 60 days. The media-equivalent was then incubated with collagenase, and cells obtained from this digestion were plated in MSMC. HVSMC obtained were observed to be in a “growth arrested” phenotype, but were able to proliferate at a normal rate after a 5-day lag period (results not shown).

Reorientation of HVSMC in the media-equivalent. If media-equivalents were allowed to contract by periodic release from the mandrel, the incorporated HVSMC slowly reoriented themselves from a random orientation to a circular one around the tube’s axis. This phenomenon was visible at the end of the tubes as early as 24 to 48 hours after casting (Fig. 4, a). At a given time, HVSMC at the 4 level of the media-equivalent could be seen as being entirely circularly arranged cells, but as the center of the media-equivalent was approached the cells appeared more randomly oriented (Fig. 4, a and b). Actually, cells relatively far from the tubes extremities were longitudinally oriented (Fig. 4, b). Early in culture, cells toward the exterior of the media-equivalent were oriented mostly in a circular fashion, whereas the inner cells were more longitudinally oriented. After 2 weeks of culture on the mandrel, the vast majority of cells had become circularly oriented. The circular HVSMC reorientation can be seen on transverse (Fig. 4, c) and longitudinal (Fig. 4, d) histologic sections.

Addition of an adventitia. As in the case of HVSMC, HSF could be observed with phase contrast microscopy for the first few days. HSF spread in about 24 hours and displayed their usual fusiform appearance. However, they did not form a complex network, as did HVSMC, and appeared quite quiescent (Fig. 5). For the first 2 days, the physical link between the adventitia and the media-equivalent was quite weak as observed during medium change. However, after that time the adventitia was sufficiently attached to the media-equivalent to resist the downward pull of its own weight during medium changes. Furthermore, it withstood the vigorous shocks needed to release the media from the glass (Fig. 6, a). This attachment was stable when the adventitia-media-equivalent structure was liberated from the mandrel. On the other hand, the attachment did not always resist histologic preparation (Fig. 6, d).

Endothelialization. The endothelialization process of the adventitia-media-equivalent structure was
Fig. 3. Longitudinal contraction of media-equivalent structures as function of time. Detachment was induced by mechanical shocks at 24 hours, and release from mandrel occurred at 48 hours. One of three similar experiments.

done by cannulation and injection of HUVEC into the lumen (Fig. 6, b). After 1 to 2 weeks, an endothelium could be detected by incorporation of labeled Ac-LDL-Dil, which showed it to be a living and metabolically active confluent monolayer (Fig. 7, b). By TEM, Fig. 7, a, endothelial cells were seen as a continuous string of well-linked cells containing the characteristic Weibel-Palade bodies. A typical high nucleus to cytoplasm ratio was observed. Some small cell projections were seen penetrating the collagen gel and possibly contributing to cellular attachment. No continuous basement membrane-like deposits were detected. The endothelium could also be seen on histologic sections as a ruffled string of cells clinging to the collagen surface (Fig. 6, c). A lumen was observed at all times in all experiments.

Fig. 4. Reorientation of HVSMC in media-equivalent. Phase contrast micrographs of one end (a) and center (b) of tubular structure (original magnification ×60). Note that tube's axis is in horizontal direction. Obvious change of orientation can be observed from largely longitudinal orientation in middle (b) to exclusively circular one at tube's extremity (a). Transverse (c) and longitudinal (d) histologic sections of 3-week-old reorientated media in which cells are circularly arranged (original magnification ×330). Arrows indicate the longitudinal cell axis.

Conditions preventing contraction, and therefore opacity, were used to allow observations by phase contrast microscopy of an established endothelium on a media-equivalent (equivalent containing

1 × 10^5 cells/ml). HUVEC grew in a very dense fashion and had a typical high nucleus to cytoplasm ratio (Fig. 7, c). Interestingly, cells were slightly but unmistakably elongated and their longitudinal axis
was largely parallel to the one of the media-equivalent. This confluent endothelium was kept in culture for 50 days without any visible changes as observed by phase contrast microscopy.

Successful establishment of an endothelium depended on HVSMC concentration and the time the media-equivalents spent on the mandrel. With higher cell concentration (>3 × 10^5 cells/ml), HVSMC sometimes migrated and proliferated inside the lumen of the forming vessel and, in so doing, deterred the spreading of the endothelial cells to the entire inner surface.

**DISCUSSION**

A small caliber blood vessel equivalent was obtained through coculture of three different cell types. We used pure and well-characterized populations of nontransformed human cells. Furthermore, we provided an extracellular matrix (ECM) composed of human type I and III collagens (46% and 54%), which are present in normal vascular tissues in similar proportions, that is, more type III than I. 34,35 Many authors believe that cell differentiation and normal behavior may be more readily observed when cells are given a more physiologic ECM. 33,36-38

Different types of cells, particularly fibroblasts, can contract collagen lattices into a tissue-like structure and have been largely used for various research applications. 27,39-42 Paradoxically, very little is known about the mechanism by which cells contract hydrated collagen gels. This phenomenon may be caused by collagen fiber displacement on cell surfaces during cell locomotion and involve the cytoplasmic actin/myosin system. 43-45 This phenomenon is believed to be relevant to such pathologic situations as wound healing and intestinal chronic inflammatory fibrosis. 42,46 We have produced a media-like tissue with HVSMC embedded in a human type I and III collagen gel, which was contracted to produce a relatively dense collagen lattice populated by living HVSMC.

We have shown that lattice contraction by HVSMC was directly proportional to cell concentration for the first 72 hours for concentrations between 1 × 10^5 to 5 × 10^5 cells/ml. Moreover, we have determined that at concentrations of 3 × 10^5 cells/ml and higher, media will have contracted to the same extent as that after 4 days. Less concentrated gels contracted less when on the mandrel but contracted at a "catch up" rate when freed of the mandrel. This could be the result of a physical limitation of gel contraction and may be relative to the cylindrical shape of the collagen lattice.

Gel contraction by HVSMC resulted in collagen and cell concentration. However, cell concentrations obtained were still lower than those in human blood vessels (approximately one fourth). 20 On the other hand, the circular arrangement that cells adopted, if kept on a glass mandrel, resembled their physiologic disposition in vivo. To explain reorientation of HVSMC, we believe that cell orientation was directly influence by tensions opposing cell contraction. In regions where longitudinal resisting forces were minimal (at the media's ends), maximal circular orientation was observed, whereas in the center of the media, longitudinal orientation prevailed. Furthermore, as longitudinal contraction was allowed, only radial resisting forces remained, and this coincided with circular reorientation of cells in the center of the media.

Weinberg and Bell 29 observed a largely longitudinal orientation of the SMC embedded in a collagen gel in their model of an artificial bovine blood vessel. This model resembles ours in its coculture concept, but differs in many other respects, primarily by the use of a Dacron mesh to circumscribe different cell populations and to increase physical resistance. The low resistance of their model without a Dacron mesh was attributed partially to the longitudinal orientation of the bovine SMC. To overcome this weakness, we have developed a method to stimulate the reorientation of HVSMC into a circular network around the axis of the vessel-equivalent to thus mimic the cell organization found in vivo. According to the hypothesis stated herein, the lack of circular orientation of the cells is compatible with the absence of the periodic release of longitudinal tensions.

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*For review, see reference 16.*

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Fig. 5. Phase contrast micrograph of HSF in adventitia-equivalent structure (5 × 10^5 cells/ml). This adventitia was cast on media-equivalent and kept on mandrel for 5 days. HSF displayed stretched aspect but no complex network or particular alignment (original magnification × 65).
The adventitia-equivalent structure was a significant part of the model, as revealed by its distinct physical attachment to the media-equivalent. However, HSF appeared to be quite quiescent once embedded in the collagen gel. 47

The established EC layer showed numerous in vivo characteristics. A continuous monolayer was observed by TEM, phase contrast microscopy, and histologic sections and was shown to be metabolically active by incorporation of acetylated low-density lipoproteins. 26 In TEM, HUVEC looked like well-adapted endothelial cells with a characteristic high nucleus to cytoplasm ratio and contained the distinctive Weibel-Palade bodies that store the von Willebrand protein. 7,48 The HUVEC also appeared to anchor themselves on the collagen with small cell projections penetrating into the collagen gel. Furthermore, a very interesting feature of this endothelium was the slightly elongated form of the cells. Even more interesting was the fact that the longitudinal axis of the cells was largely parallel to the axis of the vessel, which is a characteristic of the vascular endothelium in vivo.

This elongated aspect of HUVEC in coculture with HVSMC has been observed once before in a two-dimensional coculture system involving an HVSMC multilayer in a collagen gel on which EC were seeded. 45 This phenomenon suggests that other factors than blood flow may be involved in the slightly elongated morphology of the EC in vivo. 49,80 ECM modifications, produced by HVSMC, have been suggested as a possible cause for this phenomenon. 17 Since HUVEC were not only elongated but also oriented parallel to the axis of the tubular collagen gel, we believe that ECM modifications by HVSMC is the most probable explanation of this phenomenon. ECM alterations could be caused by collagenolytic activity, secretion of new matrix com-
ponents, or alignment of collagen fibers as a result of HVSMC collagen contraction. Furthermore, it is known that collagen fiber orientation influences the behavior of other cell types.

To obtain massive HVSMC reorientation, media-equivalent must be kept on mandrels for at least 1 week. During such time, cells tend to migrate onto the tube and into the lumen, which would interfere with HUVEC adhesion and/or proliferation. The use of heparin in the MEC, which is known for its ability to inhibit SMC growth and migration (in vitro), was intended, in part, to prevent such problems, but with partial success. This failure to inhibit human SMC can be explained by the fact that these cells may be able to adapt to a heparin-containing medium in as little as 7 days. Modifications in the growth medium (lower FBS concentration) may help maintain HVSMC in a quiescent state, as in vivo, and heparin contained in MEC could keep them in that state, since heparin has been shown to be more inhibitory to growth arrested SMC than to proliferating ones. Furthermore, the use of a plastic mandrel to prevent cell attachment gave us promising preliminary results. It is then possible that substrata proximity may stimulate migration and proliferation of HVSMC.

This model can be used for a multitude of in vitro research applications, from immunologic cell migration through the endothelium to hemostasis and atherogenesis experiments. The structure of the model may permit interesting experiments in atherogenesis to address such phenomena as intraluminal SMC migration and proliferation. Furthermore, mechanisms of hypertension such as HVSMC contraction and response to pharmacologic treatment might be investigated.

One of the most interesting prospective applications of this model would be its use as an autologous vascular graft. However, before in vivo experiments could be initiated, three aspects of the model require further analysis. First, the physical resistance of the vascular-equivalent must be improved, since the model can withstand some intraluminal pressure, but not at a physiologic level. Second, a study of cell function such as endothelial secretion of antithrombogenic products, the ability of EC to act as a permeability barrier, HVSMC secretion of collagen, and HVSMC contraction is imperative. Finally, a long-term evaluation of stability and interactions of the cell populations in vitro is essential to justify in vivo experiments. These three issues are presently under intensive investigation in our laboratory.

Fig. 7. Characterization of endothelium. a, EC on media-equivalent structure as observed by TEM (original magnification ×3130). Note small cell projections into collagen gel (arrows) and typical high nucleus to cytoplasm ratio. Inset, Weibel-Palade bodies (original magnification ×42,000). Three bodies are visible, two in cross section and one cut at an angle. Note one-layer membrane and longitudinal inner small rods. b, Ac-LDL-Dil labeled endothelium as observed by fluorescence microscopy (original magnification ×275). Some part of endothelium may have been lost during preparation. c, Phase contrast micrograph of confluent monolayer of EC in lumen of media-equivalent structure (original magnification ×115). Note slightly but unmistakably elongated and parallel morphologic appearance. Cell orientation was parallel to axis of tube. Endothelium was stable for at least 50 days.
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