Surface Features of Human Aortic Atherosclerosis as Seen with Scanning Electron Microscopy

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Using SEM, we have observed surface structures of atherosclerotic lesions of human aortas obtained from autopsies ranging from 59 to 84 years of age (5 males and 4 females). We have found four major interesting features on the luminal surface of the aortas: 1) blood cells including leukocytes adhering to the endothelial surface, 2) a de-endothelialized surface showing both elastogenesis and elastolysis, 3) abundant cholesterol-ester crystals in extracellular spaces, and 4) cave-like structures possibly suggesting new capillarization in the thrombotic atherosclerotic plaques. We concluded that SEM has a great value in revealing more interesting surface structures if morphological studies are previously done in detail so that the characteristic shapes can be identified, and perhaps then meaningful interpretations can be made on the mechanism of human atherogenesis.

Key Words: Human aortas, atherosclerosis, elastolysis, elastogenesis, cholesterol crystals, angiogenesis

Atherosclerosis occurs in the aorta and large arteries of virtually all humans past middle-age. It manifests itself clinically by decreasing blood flow due to raised plaques, with aneurysm development, or with emboli from platelets or atheromatous gruel (Day and Wahlqvist, 1970; Lang and Insull, 1970; Katz et al. 1976 ). All of these phenomena occur at the intimal surface of the artery, and so they should be clearly visible with scanning electron microscopy (SEM). While there have been hundreds of studies of atherosclerosis with light microscopy and transmission electron microscopy, there have been very few with SEM (Kawamura et al. 1974; Weber et al. 1977; Jones and Wissler, 1978; Repin et al. 1984).

SEM is ideal for studying surface features as it provides three-dimensional images of relatively large regions. Since the tissue must be coated with gold (or other conductor) rather than stained, tissue components can be identified only if they have a characteristic shape or size.

Red cells with their biconcave shape are readily identifiable and distinct. White cells and platelets have characteristic sizes and can be identified, especially if seen in association with red cells. Foam cells, or lipid-containing cells, are large and can be separated from surface structures, and furthermore, circulating leukocytes can be identifiable at the surface of atherosclerotic lesions (Steinberg et al. 1997).
Crystals of various shapes and sizes are also readily identifiable. The ones of greatest interest here are cholesterol ones which are flat and rhomboidal in shape (Loomis et al. 1979). In histological preparations, they are often seen as clefts (Ross et al. 1984) because cholesterol is soluble in ethanol and acetone used to prepare the specimens. Elastin in arteries is in the form of fenestrated sheets which are clearly identified after hot alkali digestion in both animal (Roach and Song, 1988; Song and Roach, 1983, 1984, 1985) and human aortas (Song and Roach, in preparation). Endothelial cells are identifiable because of their location and flat shape (Kawamura et al. 1974; Ioris et al. 1983).

Atherosclerosis is a patchy disease associated with lipid deposits both intra-cellular (in foam cells) and extracellular (Duguid, 1926; Kruth, 1984), infiltration of monocytes through the endothelium (Kawamura et al. 1974), possible endothelial cell damage (Moore, 1979), fragmentation or reduplication of elastin (Hauert, 1979), with later fibrosis and calcification (Hass et al. 1961). Complications include ulceration which would be clearly visible with SEM. Platelets and monocytes adhere to the surface early (Ross, 1981; Scharf and Harker, 1987), and all blood cellular components may adhere if thrombus develops. Between 1981 (Ross) and 1997 (Ross) and his colleagues published a few research papers for examples (Ross et al. 1984; Gown et al. 1986), including review articles and suggested several hypotheses. Yet none of the unique theories had been established with clear ideas because the human lesion of atherosclerosis appears with pleomorphic features, but also implicates multiple etiological factors. There is also a proliferation of smooth muscle cells (Hauert, 1977; Thomas and Kim, 1983), although this probably cannot be identified with SEM alone.

The advantage of SEM is to allow identification of the spatial arrangement of objects on surfaces. Because so many of the constituents of atherosclerotic lesions have shapes and sizes which allow them to be identified, SEM should increase our understanding of how the plaque constituents are arranged. In this study, we have attempted to present surface features of human aortas with respect to atherosclerotic changes observed with SEM.

MATERIALS AND METHODS

Detailed SEM studies were done on nine human aortas obtained at autopsy, and ranging from 59 to 84 years of age. The aorta was removed and opened from the arch to the iliac bifurcation. They were x-rayed to identify the location of calcium deposits and then photographed in color to identify the location of fatty and fibrous plaques, ulcers, and thrombus. We have also tried to fix the tissues with 10% formaldehyde and 2% or 4% glutaraldehyde in isotonic phosphate buffer solutions, but we found that because of polymerizations in the tissues it was very difficult to dehydrate the tissues. The graded alcohol dehydration procedure was used once with defattenning results.

The aortas were washed with distilled water to remove loose debris, frozen, freeze-dried in a Virtis Freeze Drier (Model No. 11-030), coated with gold (less than 0.5 μm thick) in a cool sputter coater (Polaron Instruments Inc. SEM Coating Unit E5100, Series V) for 2.5 min, and scanned with a Philips SEM (model 501). This method is well documented in a previous paper (Roach and Song, 1988). Zones with different types of gross lesions were compared. Areas adjacent to those reported here were digested in 0.1 N NaOH at 75°C for 5 h to assess elastin which was in fenestrated sheets to show evidence of both elastolysis and elastogenesis (Song and Roach, in preparation). Specimens 2×1 cm² were studied here.

RESULTS

All of the aortas showed atherosclerotic lesions grossly, although the extent varied. As usual, there were more lesions in the abdominal than in the thoracic aorta.

Fig. 1 shows the surface features from regions of the aorta that appeared normal grossly. In all cases, the surface does not show the smooth surface with endothelial cell features seen in normal aortas. The changes get more severe from Fig. 1 to 3. All of them suggest some elastin at or near the surface. This is based on our previous study (Song and
Roach, in preparation) where all non-elastin was removed with hot alkali. There is a suggestion of new elastin seen as film-like sheets (Figs. 1, 2b, 5c) with or without holes in it. Fig. 1 (arrow) looks like 'naked' internal elastic lamina (IEL) and is very similar to the IEL seen after hot alkali. Fig. 1 shows the irregular edges typical of elastolysis. In most regions of elastolysis, cellular elements are common as shown in Fig. 1. Fig. 1 also shows crystals at the center of the picture, presumably cholesterol ones, which could have formed there either before or after the development of the ulcer.

**Fig. 1.** Surface view of the thoracic aorta of a 75-year-old man. The surface is much rougher, and many of the thin sheet-like regions on the surface (e.g. white arrow) are very similar to regions of new elastin seen in digested preparations. Crystals are seen at the arrow-head. The small bars are 10 μm.

**Fig. 2a.** A region which appeared thrombosed grossly from the abdominal aorta of a 59-year-old man. Note the large number of cells and the fine fibrils of fibrin. The adjacent surface has many small holes and resembles elastin. Bars are 10 μm. **2b.** A region which appeared grossly to have atheromatous plaque from the abdominal aorta of a 73-year-old man. A large cellular type structure (arrow) is shown in a deep hollow or cave. The size suggests a foam cell. Bars are 10 μm.
Fig. 3. Base of an atherosclerotic ulcer from the abdominal aorta of 59-year-old man. Note the large number of crystals and how they often appear in stacks. The surface is very rough. The arrow-head shows one which appears broken. The white bars are 100 μm.

Fig. 4. 'Caves' formed in the region of a fibrous plaque from the abdominal aorta of a 67-year-old man. The smaller ones appear to be blood vessels, and the lower one contains red cells. The white bars are 10 μm.
Fig. 2 shows SEM photographs from regions with gross lesions at higher magnification than in Fig. 1. The surface is irregular with various sizes of hole-like probably elastin fenestrations (Fig. 2a), others elastolysis (Fig. 2b), others neovascularization (Fig. 4, 5a). There are many adherent blood cells, probably foam cells (Fig. 2b, arrow), and occasional crystals (Fig. 3). The surface is much rougher than in regions where the aorta appeared grossly normal.

Fig. 3 shows the base of an atherosclerotic ulcer.

Cholesterol crystals are abundant and appear to be extracellular. They are often arranged in stacks (arrows), and some appear to line holes. They are both parallel and perpendicular to the surface. The surface itself is very rough and probably stimulates thrombus production. It is totally different from the surface in non-ulcerated areas. There is no evidence of normal endothelial cells or internal elastic lamina.

Fig. 4 shows one of the large cavernous holes or caves seen in or near fibrous plaques. There are two

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Fig. 5a. The lumenal surface of a plaque from the abdominal aorta of a 67-year-old man. Note the large number of holes which are roughly perpendicular to the surface. We believe these are vessels. The bars in all cases are 10 μm. 5b. The cut edge of the media of a thoracic aorta of an 84-year-old man. This is indistinguishable from the cut edge of an aorta after hot alkali digestion, so we believe the septa are elastin, and the holes indicate where muscles have been. The edges are rough. The IEL is shown at the arrow. Tilted angle was 15 degrees, and because of focus depth, the size may not be significantly augmented. 5c. The endothelial surface of a fibrous plaque from the thoracic aorta of a 68-year-old man. The surface is very rough. The cavities look to have the shape and size of cells, and we speculate that the cells have left the surface, although we do not know how. 5d. The center of the media of the thoracic aorta from a 68-year-old man. The picture is of all wall components. The globules are similar in shape and size to those seen on digested aortas and are probably lipid.
other holes within the large one and both appear to be the orifices of vessels. Red cells are seen within the lower one. Another hole, which may be another vessel, is seen in the upper right hand corner. We believe these are vessels forming within the plaques. Fig. 5a shows another area with the holes penetrating and branching deep into the surface.

Fig. 5 shows a number of types of hole, and illustrates the problem of interpreting SEM photographs in the absence of other knowledge. Fig. 5a and 5c are from the luminal surface and Fig. 5b and 5d from a cut edge to show the tunica media. Fig. 5a shows holes of decreasing diameter invading the tissue at an angle close 90° to the surface. These look very similar to vasa vasorum seen on the adventitial surface and can be identified positively by injecting casting material into them (Song et al. 1985). We believe they are new vessels penetrating into plaque.

Fig. 5b shows the cut edge of the media just under the internal elastic lamina (IEL). This is identical to tissue digested in 0.1 N NaOH at 75°C for 5 hours to remove all nonelastin. Thus, we conclude that these are elastin layers. The holes are assumed due to egress of smooth muscle cells (SMC), but the picture does not say where they went. This tissue was freeze-dried and this was one of the exposed surfaces.

Fig. 5c shows another part of the luminal surface. Here there are indentations which have the shape of cells. These could have been produced by cells being ‘shelled out’ of the surface, or by removal of cells from under a surface layer. These have the shape of SMC. The walls between are too thick to suggest that the two holes are produced by shelling out of endothelial cells. Foam cells are usually more spherical than these contours.

Fig. 5d shows the interior of the media which has been exposed after freeze drying. This picture is dramatically different from that in Fig. 2b. The holes here could probably be due to dehydration artefacts and are quite different in size and shape from those in Fig. 5b. This surface should have muscle, elastin, and a small amount of collagen. The globules may be fat as we have seen very similar ones on the elastin after hot alkali digestion (Song and Roach, in preparation). Guyton et al. have shown that lipid remains firmly bound to elastin even after hot alkali (Guyton et al. 1985).

DISCUSSION

Atherosclerosis is a complex disease which affects the intima of arteries initially, but in the later stages also invades the media. In this paper we have discussed how scanning electron microscopy (SEM) of human aortas can help our understanding of the disease.

The SEM shows that even regions which appear grossly normal have rough surfaces in aortas with moderate atherosclerosis. This is even truer with a severe disease. We have shown with digestion studies which removed all non-elastin and that there is evidence of elastogenesis with the new elastin appearing as very thin sheets, often in the shape of blebs or parachutes, with fenestrations. These same structures are occasionally seen even without digestion (Fig. 1 and 3). Our previous digestion studies also showed evidence of elastolysis with holes in the IEL with ragged edges. A similar picture is shown in Fig. 1 and suggests that elastolysis, at least in some regions, is associated with damage and probably absence of the overlying endothelium and basement membrane. We believe that elastolysis is probably produced by leukocyte elastase (Werb and Gordon, 1977), but it is not evident if the elastase comes from polymorphonuclear leukocytes or from the monocytes that create foam cells as suggested by Steinberg et al. (1997). If the former is true, then the elastolysis suggests that the body is treating the plaque as an inflammation or foreign body and attempting to remove it (Gown et al. 1986). If it is produced by monocytes which are precursors of foam cells, the elastase may be released as the cells imbibe enough fat that the membrane ruptures. The ragged edges seen in the base of ulcers (Fig. 3) also suggest elastolysis has occurred. There is some evidence from the work of Tomazic et al. that calcium is laid down in association with elastin, and our data suggests, but does not prove that it is more common on zones with elastolysis (Tomazic et al. 1988). This raises the question of whether ‘intact’ elastin can calcify or if the ‘ragged edges’ exposed by elastolysis are more apt to attract calcium with lipid
cells in patients with severe atherosclerosis.

Even though normal endothelial cells were absent in the lesion, the normal shaped biconcave red blood cells with small lymphocytes shown in Fig. 2a indicate that, rather than artificial techniques, the damages presented in these SEM pictures must be due to naturally occurring processes. We are very confident with freeze-dry procedures as we realize that sperms can be stored in dry ice at very low temperatures for a long period.

Some of the pictures, such as those in Figs. 2b and 4, are highly suggestive of vascularization. Normal vessels have vasa vasora, but these arise primarily from the adventitial side of the artery (Song et al. 1985). The vessels seen here appear to arise on the surface of the plaque and divide to form branches in the depths of it. Paterson described these new vessels many years ago with light microscopy and proposed that hemorrhage into plaques could lead to sudden vessel occlusion and myocardial infarction (Paterson, 1936, 1952). It is easy to speculate that some growth factor derived from components of the plaque stimulates this angiogenesis (Leu et al. 1987). This remains to be proved. Since SEM shows these vessels clearly, a more detailed study of their location and proximity to other plaque components seems needed.

Many of our pictures are similar to those of Tomazic et al. on deproteinated human aortas (Tomazic et al. 1988). They saw smooth spherical particles and layer structures. They agreed that the particles are probably lipid, but have not suggested the layers are elastin, although we are confident they are. Over the small age range studied here, we have not seen differences due to age. There may be minor ones masked by the more severe changes of atherosclerosis. If we could observe in human aortas that any temporal progressive or even regressive changes, this might be the ideal investigations. Unfortunately, at present, we all agree that human autopsies are very scarce from the younger patients. Our present observations must be the newest findings in terms of elastolysis-related atherosclerotic lesions in human aortas.
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