

Atherosclerotic alterations in human carotid observed by scanning electron microscopy

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Presented at a meeting in honour of Prof. G. Orlandini, Florence, February 15, 2010

Summary

Atherosclerosis involves all the layers of the artery wall, but the events involving the intimal portion are fundamental to understand the evolution and gravity of lesions. This study shows that scanning microscopy is instrumental for better understanding the physiopathology of this disease.

Key words

Atherosclerosis, Endothelium, Scanning Electron Microscopy

Introduction

The development of atherosclerotic lesions involves several factors that during different timescales lead to deep modifications of the arterial wall structure. Thickening and stiffening of arterial wall lead to stenosis, while atherosclerotic lesions may calcify and ulcerate leading to thrombus formation (Chen et al, 2006).

Recent studies supported by clinical and experimental evidences demonstrate that intima dysfunction and disruption are initiating processes of atherosclerotic plaque. The endothelium is subject to mechanical and chemical stress by the variations of blood stream, pressure and composition and is especially exposed to inflammatory processes. Intimal lesions may invest a wide area of the arterial wall and, at variance with the in vitro models, they can display a wide range of functional and structural alterations in the same anatomical location (Congiu et al, 2010)

Conventional section-based techniques are therefore far from optimal in studying the local progression and the different aspects of the disease. Scanning electron microscopy (SEM) offers three major advantages: large areas of exploration, a wide range of magnification and the possibility of unrestricted access to bulk specimens (Congiu et al, 2004; Schembri et al, 2008).

In the present study we demonstrate the morphological features of human carotid atherosclerotic lesions, an investigation topic where direct visualization is still an indispensable tool of knowledge.

Material and methods

A carotid specimen were obtained from a patient undergoing endarterectomy for stenosis. The vascular wall was distended and the atherosclerotic lesions were

exposed and transversally sectioned in correspondence of the plaque. Tissue slices were fixed in 2% paraformaldehyde and 2% glutaraldehyde for an hour, washed in phosphate buffered saline (PBS, pH 7.2) and postfixed in a solution of 1% osmium tetroxide and 1.25% potassium ferrocyanide for 2 hours. They were then immersed in 0.1% osmium tetroxide in PBS for 24 hours at room temperature and washed in PBS. The specimens were dehydrated in ascending grades of ethanol, critical point dried in CO₂, coated with 10 nm of pure gold in a vacuum sputter coater Emitech K550 and studied in secondary electrons imaging with a Philips XL 30 SEM-FEG scanning electron microscope.

Results

The most evident alteration revealed by SEM is the increased thickness of the carotid wall in correspondence of the plaque formation and the local loss of elastic sheets (Fig. 1). Wide areas of the intima are deprived of endothelial cells, which detach from the basal lamina leaving it exposed in many zones (Figs 2, 3). On this uncovered basal lamina initial fibrin deposition occurs, which entraps many erythrocytes (Fig. 2). Clusters of aggregated platelets are also present (Fig. 3).

In other parts of the intima, endothelial cells lack their normal junctional complexes and display abnormal, intertwined digitations that overlap on multiple levels (Figs 4,5). In the gaps that appear between cells, leucocytes are visible (Fig. 5).

The subendothelial layer shows thick delaminated sheets (Fig. 2). Groups of foam cells, easily recognizable by their lipid droplets, are pushing their way through the collagen sheets (Fig. 6). The lipid vacuoles are clearly visible causing protrusions of the plasmalemma (Fig. 7). In some cut cells it is possible to observe the inner foam cell architecture, where lipid droplets are clearly visible (Fig. 8).

Discussion

Although atherosclerotic alterations are known to involve all the layers that compose the arterial wall, their development is activated and started by alterations of endothelial cells. The functional role of endothelium is not limited to the simple lining of the inner surface of the artery, and all events taking place in proximity of the intima are fundamental to understand the evolution and the gravity of lesions.

It is therefore important that the technique used is able to offer a clear picture of the whole intimal surface and then to zoom on the details as they are found during the microscopic observation. The pictures shown demonstrate how the SEM approach can allow for a clear view of the different wall layers which is instrumental to a further comprehension of the physiopathology of the atherosclerotic lesion. Loss and disorganization of endothelial cells, uncovering of basal lamina, fibrin and platelet deposition, leukocytes crossing the wall and forming foam cells causing the delamination of subendothelium, are all well-known elements of this process (Bobryshev, 2006), but are not observable together and simultaneously with any other technique.

An interesting additional advantage of this methodology is the unique, three-dimensional imaging of the interplay of the different components of the atheroscle-

rotic arterial wall: the foam cells with the surrounding collagen matrix, the epithelial cells with each other and with their basal lamina, and so on.

SEM imaging is, in our opinion, the better and most flexible approach to the study of atherosclerotic plaque and should be adopted as the gold standard technique.

Aknowledgments

This work was performed with the technical instrumentations of the "Center of Major Instrumentation for Biomedical Research" of the University of Insubria, Varese, Italy.

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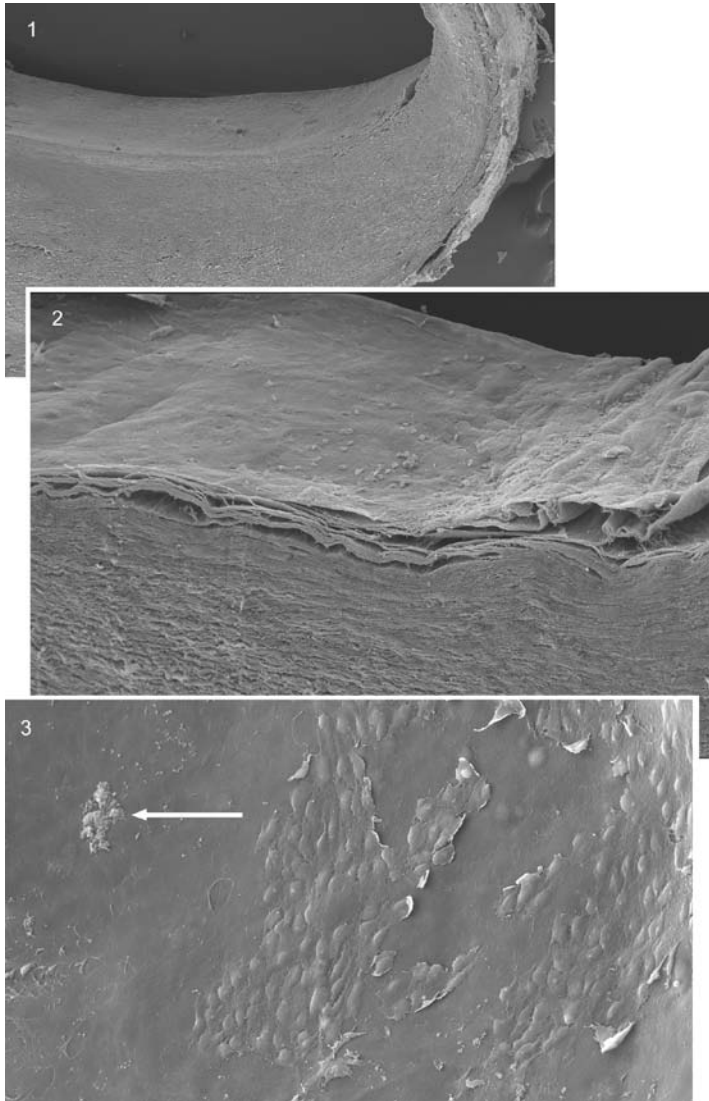
Figures

Fig 1 – Slice of human carotid. The transversal section clearly shows how the wall thickness varies in the same artery. Note the loss of endothelial cells, the delamination of subendothelial layer and the loss of elastic sheets. 65X

Fig 2 – The endothelial cells have disappeared from the intimal surface. The basal lamina is partially covered with fibrin and some entrapped erythrocytes. The subendothelial layer clearly shows how the elastic sheets delaminate. 200X

Fig 3 – The endothelial cells are leaving the basal lamina. There are wide uncovered areas of basal lamina with occasional platelets (arrow). 250X

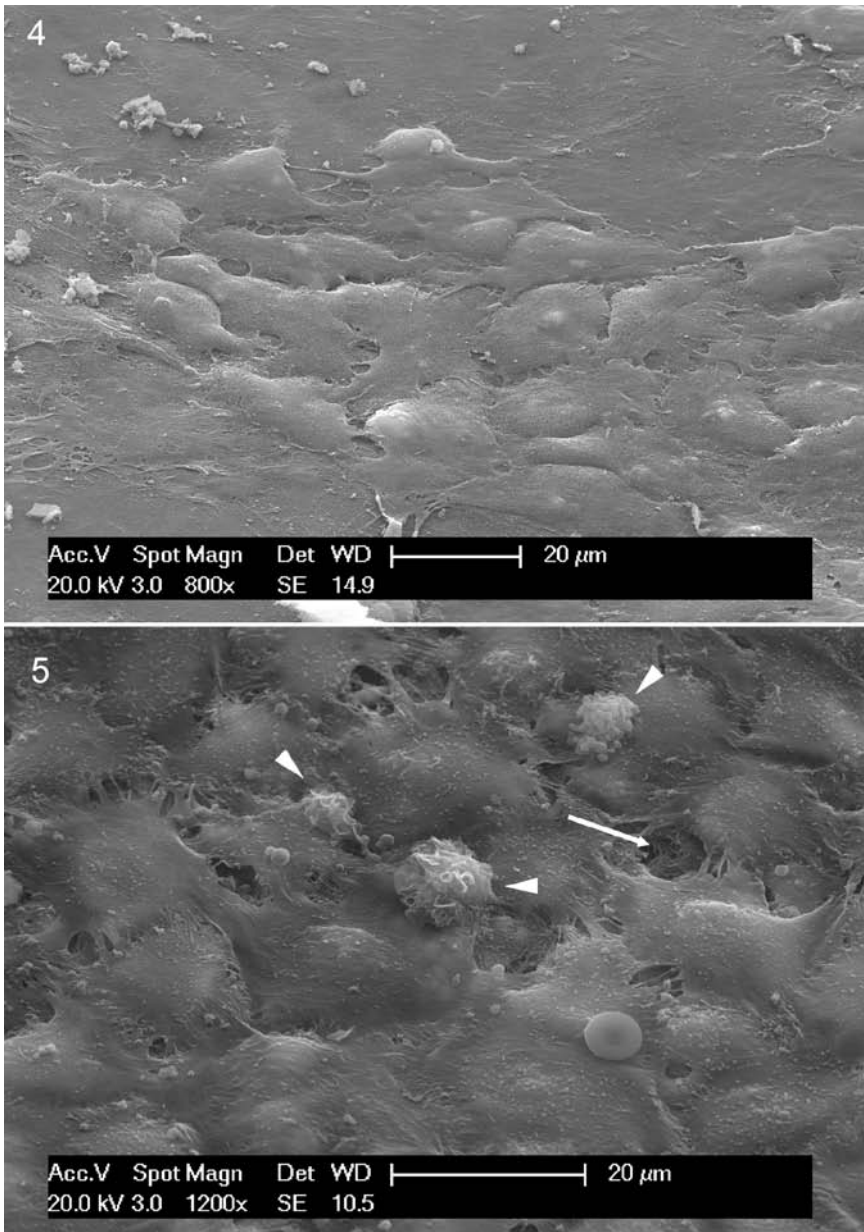


Fig 4 – Small groups of endothelial cells spread out in an extreme attempt to cover the basal lamina. Note the abnormal cell digitations and the lack of a normal junction complex between these extremely flattened cells. 800X

Fig 5 – Here the endothelial coating is more abundant. The cells, however, interdigitate and overlap to form multiple unorganized and intertwined layers and in some cases (arrow) the basal lamina is uncovered. Some leukocytes are shown entering the intima (arrowheads) are shown. 1200X

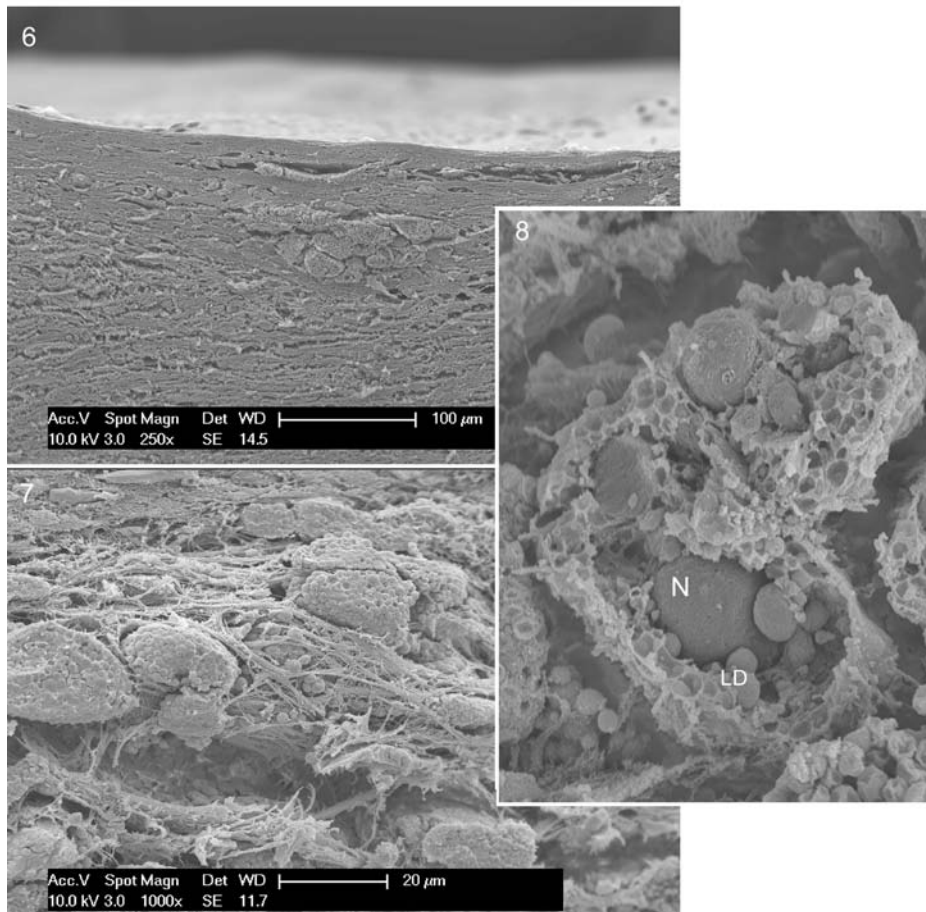


Fig 6 – Groups of foam cells are pushing their way through the collagen sheets of subendothelium. 250X

Fig 7 – external details of foam cells. The lipid droplets are clearly distinguishable through the plasmalemma. The subendothelial stroma is dissected. 1000X

Fig 8 – Two foam cells sectioned show the inner architecture. Nucleus (N) and lipid droplets (LD) are easily recognizable. 3500X