EFFECT OF QUARTZ DUST DQ 12 ON HUMAN MONOCYTES/MACROPHAGES *IN VITRO* —AN ELECTRON MICROSCOPICAL STUDY

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INTRODUCTION

Quartz particles are highly cytotoxic to rodent macrophages in vitro.[4,9,10] This cytotoxic effect is thought to be mediated by the lysis of phagolysosomal membranes through ingested quartz particles.[2] In comparison to cultured rodent macrophages human monocytes/macrophages are more resistant to quartz-induced cytotoxicity in vitro.[6] Exposure of human macrophages to quartz mainly leads to the formation of fibroblast-proliferating factors and to the release of mediators which activate neutrophils to generation of oxygen species.[3,8] This stimulatory action of quartz dust DQ 12 on human macrophages in vitro is morphologically paralleled by the development of a labyrinth of dilated vacuolar spaces within the cells.[1] Sequential analysis of quartz-exposed human macrophages by means of transmission electron microscopy further indicates the manifestation of specific autophagolysosomal processes which result in a vacuolar network filled with degradation products intrinsic to the cell in addition to quartz particles. From this study,[1] it has been concluded that human macrophages in vitro develop a protection mechanism against toxic quartz particles which is initiated by phagocytosis and which results in consecutive cell stimulation.

In the present study we report about surface alterations in human monocytes/macrophages in vitro which are induced by quartz DQ 12 and which further explain the considerably higher resistance of human macrophages to quartz particles in vitro.

MATERIALS AND METHODS

Cell Cultures

Isolation of human monocytes from peripheral blood in Ficoll-Hypaque gradient and cultivation of monocytes to maturation of cells with characteristics of macrophages has already been described in detail.[6,7]

For transmission electron microscopical studies 25×10^6 mononuclear cells were seeded in tissue culture flasks (Falcon 3013), for scanning electron microscopy 2.5×10^6 cells were distributed on tissue culture plate with 24 wells (Falcon 3047, Multiwell tissue culture plate) containing round glass coverslips with a diameter of 12 mm. After removing of non-adherent cells monocytes were cultivated for 7–14 days to differentiate into mature macrophages as already reported.[6,7] The cells were then incubated with 100 µg quartz DQ 12 (particle size $< 5\mu m$) per ml medium for 2, 24 and 48 hours. Cell viability has been tested by dye exclusion test.

Transmission Electron Microscopy

For transmission electron microscopy the cells were briefly fixed *in situ* by the addition of 2% buffered glutaraldehyde. After gently shaking to remove the cells from their substrate the cells were spinned down, pelleted and postfixed with osmium tetroxide. They were then dehydrated in a graded series of ethanol, and embedded in Araldite. Ultrathin sections were investigated in a Philips 400T electron microscope.

Scanning Electron Microscopy

For scanning electron microscopical investigations the cells were fixed *in situ* with buffered glutaraldehyde, post-fixed with osmium tetroxide and dehydrated in a graded series of ethanol. After short immersion in hexamethyldisilazane the cells were air dried according to the method of Nation,[5] coated with gold, mounted and analyzed in a Philips SEM 515 electron microscope.

RESULTS

At concentrations which are highly cytotoxic to guinea pig macrophages, human monocytes/macrophages react with a considerable higher survival rate (Figure 1). Macrophages which had been exposed to 100 µg/ml quartz DQ 12 for 48 hours display a vacuolar network filled with flocculent material and quartz particles (Figure 2) but no signs of cytotoxicity. No disruptions of lysosomal membranes were ever detected. The morphological picture rather results from processes of cell activation induced by quartz particles in vitro. The dilated vacuolar network is open to the extracellular space (Figure 2, Figure 3), so that the intravacuolar degradation products as well as the quartz particles are exposed to the extracellular micro-environment.

Connections between quartz-induced labyrinth formation and extracellular space—supposed to occur for the analysis of transmission electron micrographs[1]—are also evident in cell samples which have been exposed to 100 µg/ml quartz DQ 12 for 24 hours and subsequently investigated by scanning electron microscopy. As is indicated in Figure 6, deep in-

dentations or 'holes' can be seen on the surfaces of nearly all macrophages. One single foramen is usually characteristic for these cells. Since the formation of foramina can never be observed in cultured control macrophages (Figure 4) nor in cells being exposed to shorter times (2 hours, Figure 5) of the same quartz dust concentration, 'holes' are characteristically late phase alterations in otherwise vital cells. A comparison between Figure 3 and Figure 6 clearly indicates the similarity between sections through parts of dilated vacuoles containing fingerprint-like structures[1] and the formation of a foramen on their surface.

DISCUSSION AND CONCLUSIONS

Results obtained by scanning electron microscopy (presented in this paper) and by transmission electron microscopy[1] indicate that cultured human monocytes/macrophages display unique features upon contact with toxic quartz particles. The higher resistance of the cells to quartz concentrations which are toxic for animal cells is underlined by a special mechanism of phagocytosis in combination with autophagolysosomal processes[1] and cell secretion.[3,8] Similar observations have never been reported to occur in silica-exposed rodent macrophages. Therefore, the effects demonstrated by us are species specific characteristics of human cultured monocytes/macrophages which have been exposed to quartz particles.

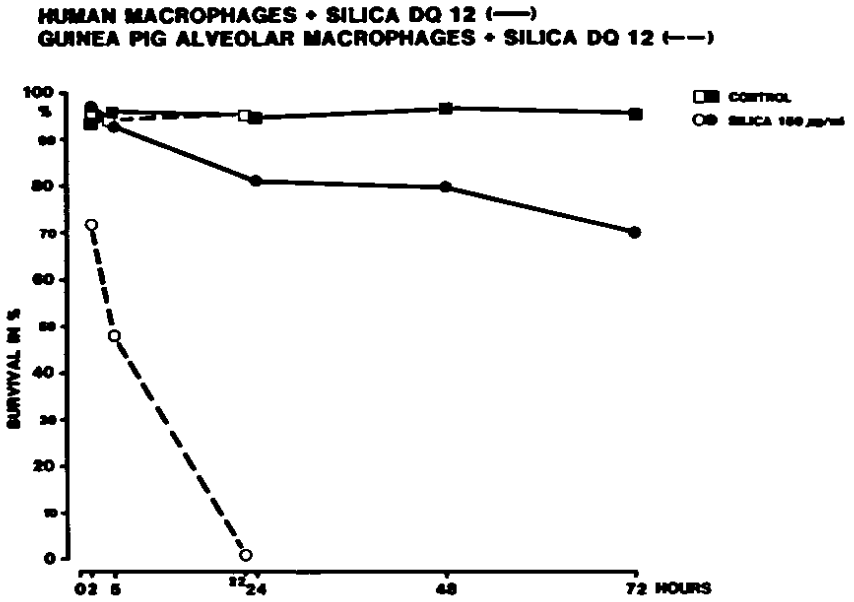

Figure 1. Effect of quartz DQ 12 on the survival rate of guinea pig alveolar macrophages and of human macrophages *in vitro*.

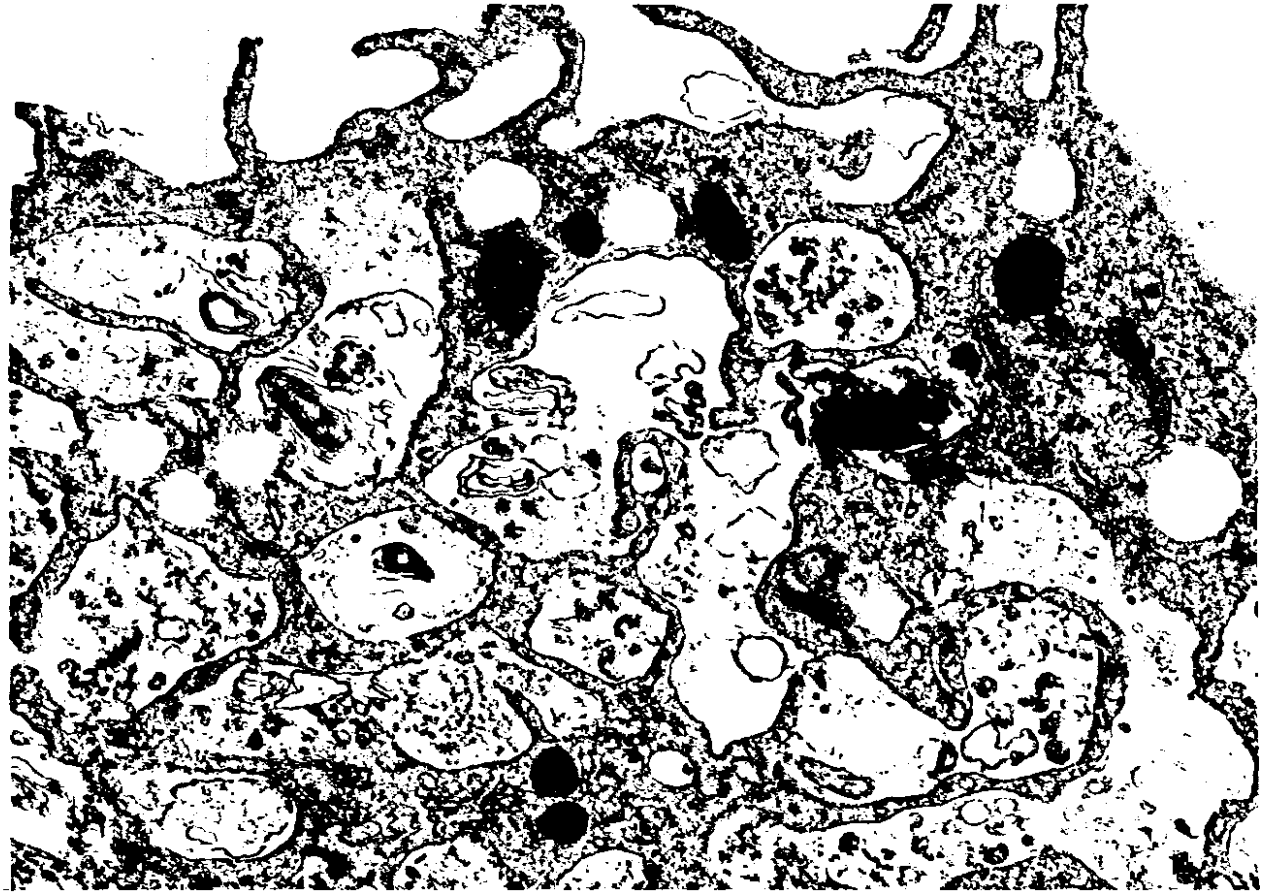

Figure 2. Part of a 12 days old human macrophage after incubation with 100 µg/ml quartz DQ 12 in vitro. Development of a vacuolar network containing quartz particules and flocculent material. Transmission electron micrograph. Magn. x25000.

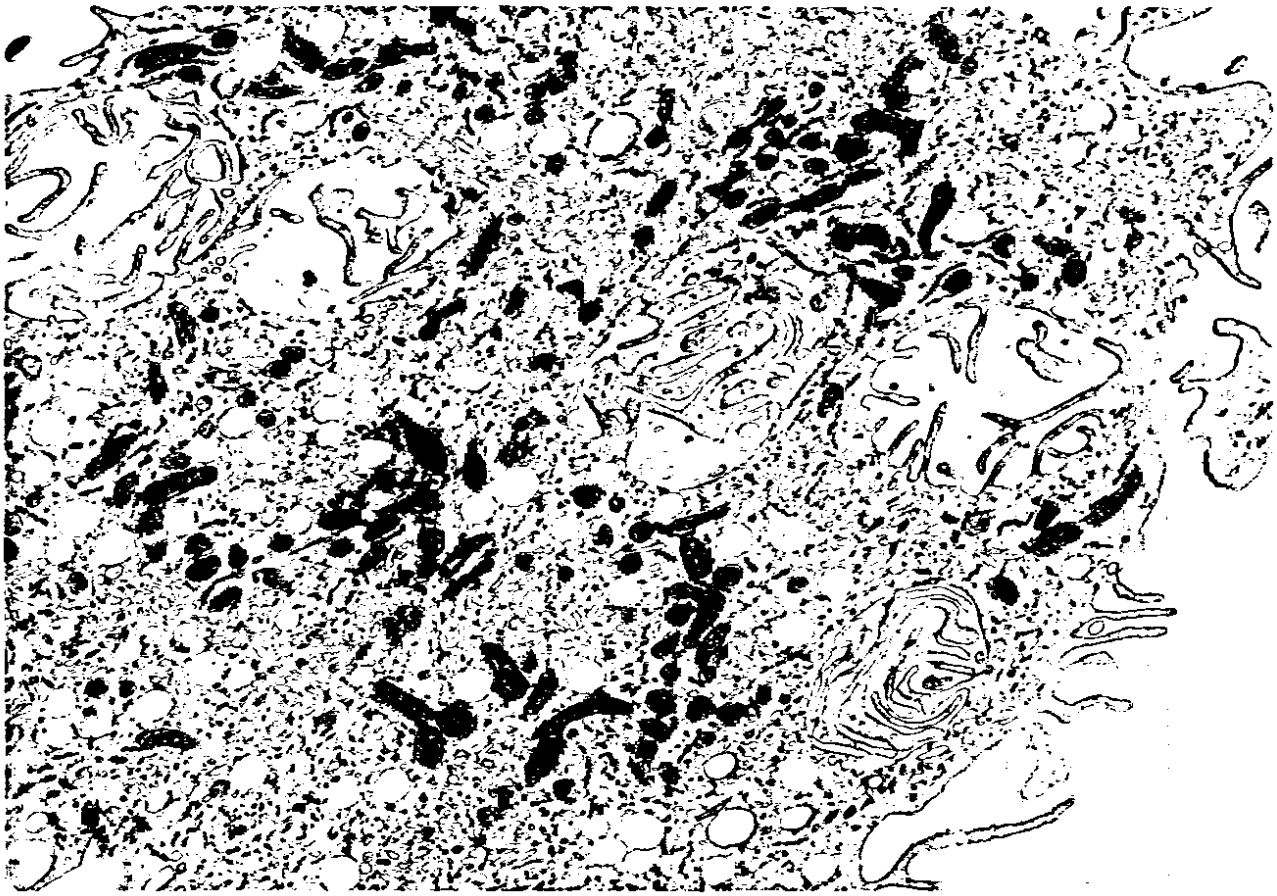

Figure 3. 12 days old human macrophage after incubation with 100 µg/ml quartz DQ 12 in vitro. Section through a 'hole.' Transmission electron micrograph. Magn. x9200.

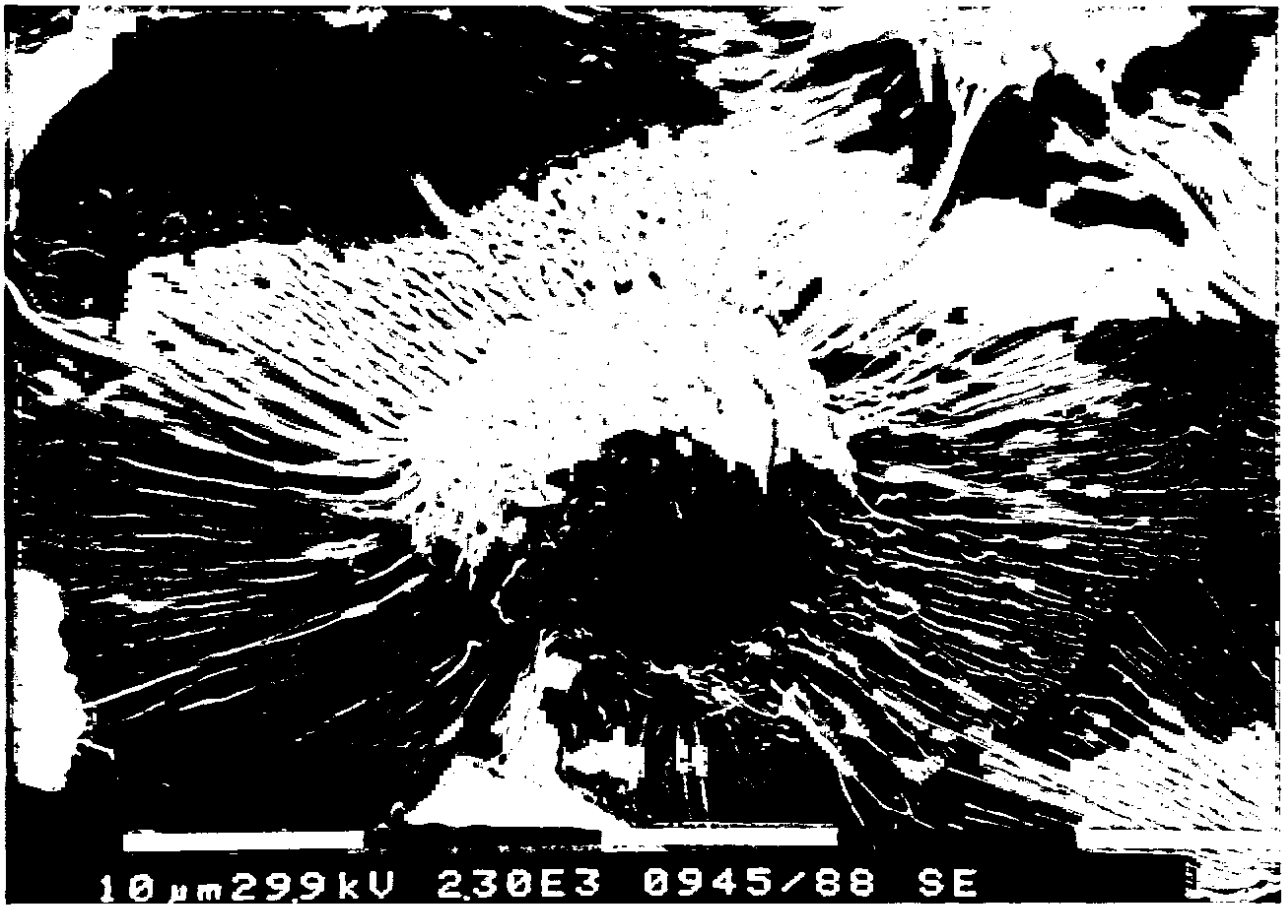

Figure 4. Human macrophages from a 12 days old culture. Scanning electron micrograph.

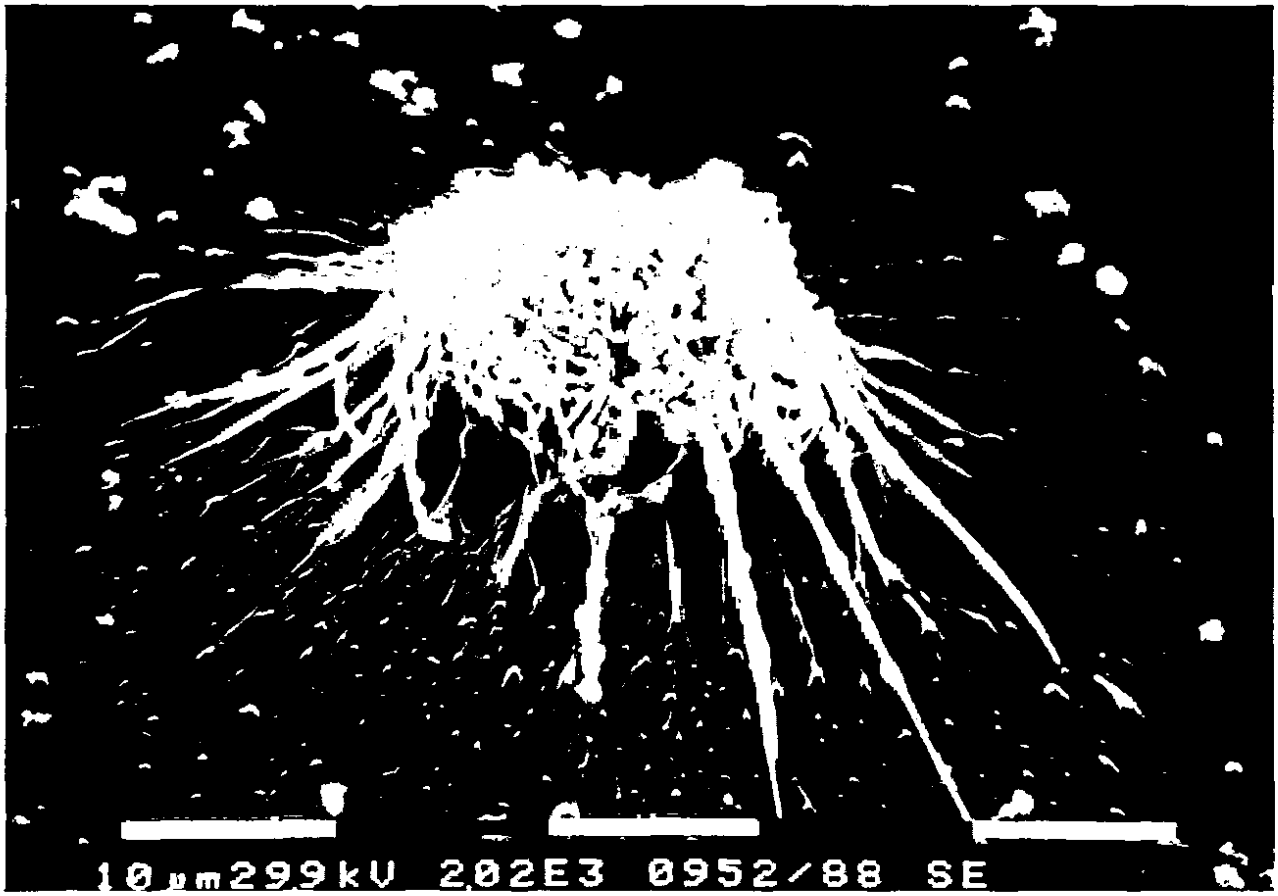

Figure 5. Scanning electron micrograph of a human macrophage after exposure to 100 µg/ml quartz DQ 12 for 2 hours in vitro.

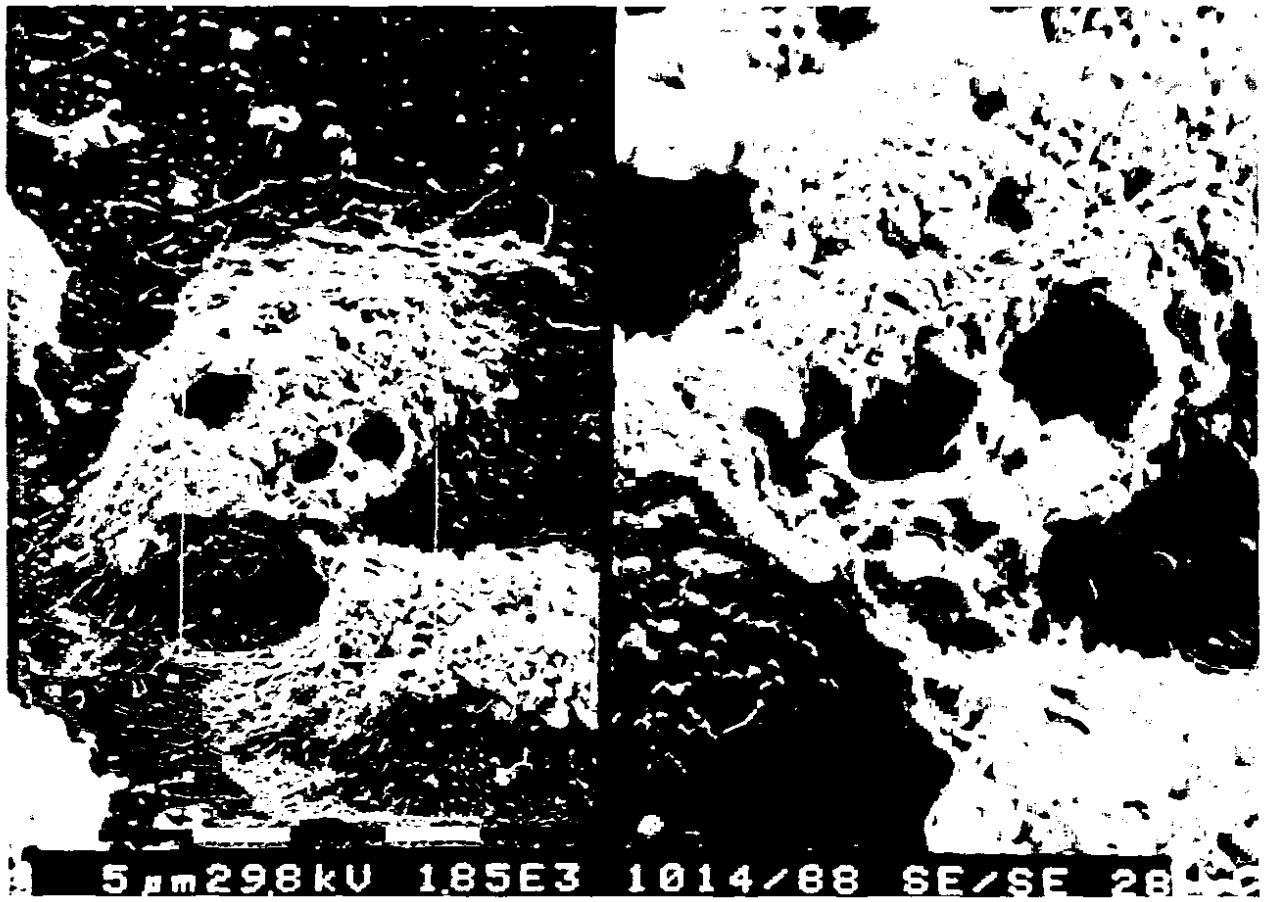

Figure 6. Scanning electron micrograph of a human macrophage after exposure to 100 µg/ml quartz DQ 12 for 24 hours, displaying characteristic 'holes.'

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