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On the topological complexity of human alveolar epithelial type 1 cells

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To the editor

Alveolar epithelial type 1 (AE1) cells and their thin cytoplasmic extensions cover ~95 % of the alveolar surface. These extensions keep the blood gas barrier thin and enable efficient gas exchange (1). Electron microscopic (EM) studies revealed the complex structure of these cells and suggested that AE1 cells form interconnected cytoplasmic plates protruding from the cell at different sites (perinuclear or peripheral), which reach the other side of the septal wall (1, 2). Much of the current conception is still schematic and based on inferences from images gained from conventional microscopic techniques (1, 2). Single images, however, can only contribute pieces to a larger puzzle and to see (and understand) a motive completely the puzzle has to be assembled, i.e. AE1 cells have to be reconstructed in three dimensions (3D). AE1 cell reconstructions from the cat lung based on tedious serial sectioning transmission EM have been described in the past (3), but to the authors' knowledge in the human lung such an attempt has not been reported yet.

With the new volume EM techniques like serial block face scanning electron microscopy (SBF-SEM) new devices for 3D reconstructions based on automated serial sectioning of tissue blocks and scanning their surfaces in between, became available (4).

The aim of this study was to extend current knowledge about human AE1 cell morphology by 3D reconstructions using SBF-SEM. This knowledge provides an essential basis for a better understanding of alveolar epithelial development, maintenance and repair.

Methods and results of this study have been reported previously in form of abstracts (5, 6)

Methods

A sample of a human lung fixed via endotracheal instillation of 2.5 % buffered glutaraldehyde in context of previous studies (7, 8) was used. The sample kept in fixative was prepared for SBF-SEM according to an adapted protocol from Deerinck et al. (9), including reduced osmium tetroxide (OsO_4), thiocarbohydrazide, OsO_4 (rOTO protocol), uranyl acetate and Walton's lead aspartate, and embedded in Durcupan. A trimmed specimen was scanned and sectioned with a Zeiss Merlin VP

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Compact (Zeiss, Jena, Germany) equipped with Gatan 3View® (Gatan Inc., Pleasanton, USA) to generate an image stack of 2046 images (6144 x 6144 pixels^2, pixel size 18.5 nm, section thickness 80 nm). A substack of 901 images (comprising 110x105x72 µm³) was converted to 8 bit, inverted, cropped, aligned and compressed in Fiji (10). 28 images were removed and replaced by preceding or following images because of artefacts that might interfere with alignment. The edited data set was imported into 3dmod (part of the iMOD package (11)) and AE1 cells, their nuclei and the lumen of the alveolar capillary network (ACN) were segmented manually by delineating their profiles on the EM images. In general, segmentation intervals were chosen according to the complexity of structures (Normally: ACN: every 16th or 8th image; blue AE1 cell: every 2nd image; gold AE1 cell: every 16th up to consecutive images; yellow AE1 cell: every 8th up to consecutive images. Nuclei: normally, every fourth (blue cell) or eighth image and poles). AE1 cells were segmented by tracing the electron-dense plasma membrane. Identification of relevant structures and their interpretation was supported by the uncompressed dataset incl. the application of filters, new alignments etc., and the 3D information from consecutive images. Based on 2323 contours 3D models were generated.

Results

The edited data set comprises 831600 µm³ of a human alveolar region with interalveolar septa and airspaces. Figure 1 shows the resulting 3D models. The ACN shows regions with a denser or coarser mesh. The alveolar surface formed by the cytoplasmic extensions of AE1 cells shows a relief caused by the ACN beneath indicating their intimate contact and the thin blood gas barrier.

Three entire AE1 cells were reconstructed. While the blue and gold cell form rather vast, almost quadrangular scales, the yellow cell forms several processes spreading out into different directions forming a propeller-like structure. The cell nuclei are located more or less eccentrically. The gold AE1 cell is primarily restricted to one side of the interalveolar septum but participates in the formation of an interalveolar pore. The yellow and blue cell also line pores but both clearly contribute to the lining of both sides of the septum. Their routes to the other side are totally different: The yellow cell reaches the other side via interalveolar pores, while the blue cell spans with the entire cell body

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containing the nucleus through the septum (figure 2). However, the majority of this cell is found on one side of the septum and only the cell body and a very small thin portion contribute to the lining of the other side. The dataset reveals that AE1 cells are able to make cell junctions with themselves: The gold cell encircles an AE2 cell and the yellow cell encircles a capillary segment and a pore completely (figure 2). The cell junctions help to seal the epithelial ring. According to Crapo et al. (8) the mean AE1 cell volume in this lung was 1996 µm³ and the mean basement membrane surface covered 4053 µm². The cells presented here are smaller.

Discussion

It was EM that proved the existence of a continuous epithelial lining on the alveolar septa, demonstrated the morphological complexity of AE1 cells and revealed the mystery of Kölliker's "non-nucleated plates" (1, 2). With the advent of SBF-SEM EM imaging of the alveolar epithelium has reached a new level, since SBF-SEM enables the automated image acquisition of rather large volumes with EM resolution (4), which is the prerequisite for reconstructing AE1 cells. Here we provide human AE1 cell reconstructions based on SBF-SEM. Reconstructions unveiled the great morphological diversity of AE1 cells, their ability to make cell junctions with themselves and, that entire AE1 cell bodies can span through the alveolar wall. The results demonstrate the value of SBF-SEM in lung research and indicate a spatial complexity of AE1 cells which is relevant for plasticity during alveolar epithelial development, maintenance and repair.

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Figure Legends

Figure 1

Top: Three AE1 cells (blue, gold, yellow) in the context of the alveolar capillary network which was modelled by segmenting its lumen (ACN, grey, transparent). Both images show the same models from different angles. The transparency of the ACN enables the visualization of cell parts on the other side of the septal wall like the major portion of the blue cell on the left image. Note the epithelial surface relief caused by the ACN beneath. The arrows indicate the positions of the nuclei. Scale bar: 5 µm.

Bottom: 3D models of the three AE1 cells (transparent) in a row. Transparency enables the visualization of their nuclei (pink in the blue cell and red in the gold and yellow cells). Note the morphological diversity of AE1 cells, ranging from cells with rather vast cytoplasmic plates (blue and gold cells) to a propeller-like shape (yellow cell), their eccentric nuclei and their relief surface caused by the ACN beneath. Scale bars: 5 µm.

Figure 2

Top: On the left side, the EM images 444, 396 and 348 of the data set including the segmentation of the blue cell and its nucleus (pink) are shown. The z-distance between the images is given in black text boxes (444 to 396: 3.84 µm; 396 to 348: 3.84 µm). The images 444 and 348 suggest two different AE1 cells with their own nucleus on either side of the septum. However, the 3D information gained from sequential images in between reveals that the nucleus and surrounding cell profiles on images 444 and 348 belong to one and the same AE1 cell that spans with its cell body through the entire alveolar wall (image 396); thus giving rise to two apical surfaces. On the right side, the transparent model of the cell is shown. The transparency enables the visualization of the nucleus (pink). The positions of the EM images 444, 396 and 348 are indicated by black section planes. The 3D information and the resulting model reveal that this cell serves both sides of the alveolar wall with

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two apical surfaces. However, the majority of the cell is found on one side of the septum, while it is only the nucleus containing cell body and a very small thin portion that contribute to the epithelial lining of the other side. Scale bars: 5 µm.

Bottom: On the left side, the EM images 676, 685 and 720 of the data set including the segmentation of the yellow cell, its nucleus (red) and the indications of lateral luminal cell borders (black/pink circles/arrowheads) are shown. The z-distance between the images is given in black text boxes (676 to 685: 0.72 µm; 685 to 720: 2.80 µm).

The 3D information gained from consecutive images reveals which of the different epithelial profiles on each single EM image belong to the yellow AE1 cell (yellow cell segmentations, nucleus segmented in red). The lateral luminal cell border of the cell was delineated in 3D by following and marking the lateral luminal edges of the profiles by black and pink circles on the EM images (arrowheads in the same color, see below).

The 3D information reveals that this AE1 cell is capable of making cell junctions with itself by different cell portions meeting and contacting each other. The sites of “self-contact” are indicated by pink circles (pink arrowheads) and cannot be identified as such by investigating the single images 676, 685 and 670 alone. However, following the cell in 3D reveals which profiles belong to the same cell (for example the two non-nucleated cell profiles in the interalveolar pore and lower side of the septum on image 676 and the largest cell profile on image 720). The lateral luminal cell borders of cell portions contacting other epithelial cells are indicated by black circles (black arrowheads).

The EM images show two Pores of Kohn, a larger Pore of Kohn in the right half of the images 685 and 720 (gap in the septal wall, #) and a smaller pore in the left half of the images 676, 685 and 720. The course of the smaller pore through the septum is indicated by arrows. At the edge of the larger pore the yellow AE1 cell crosses to the other side of the septum (center of image 676, *).

On the right side, the 3D model of the yellow cell is shown in the context of the alveolar capillary network, which was modelled by segmenting its lumen (grey, transparent). The delineation of the

lateral luminal cell borders in 3D resulted in one single closed contour demonstrating that despite the overall complexity of this cell, the cell has only one apical surface. The lateral luminal cell border contacting other epithelial cells is indicated by black contour segments and the lateral luminal cell border contacting other parts of the same cell is indicated by pink contour segments. The circles on the EM images can be regarded as transects through the lateral luminal cell border contour in 3D.

As demonstrated by the 3D model, these "self-contacts" between different portions of the same cell enable this cell to envelope a capillary segment and to encircle a Pore of Kohn completely. Scale bars: 5 µm.

Video: Human Alveolar epithelial type I cells in 3D

A video is available on the ATS YouTube page at the following: https://youtu.be/-iKowVFGKgY
The video shows a serial block face scanning electron microscopy (SBF-SEM) data set of a human lung sample, segmentations of three alveolar epithelial type 1 (AE1) cells (blue, gold and yellow), their nuclei (red) and the lumen of the alveolar capillary network (ACN, grey) as well as rotating 3D models.

The video shows one after another:

- the EM data set back and forth
- the EM data set including the segmentations back and forth
- the stack of contours in 3D
- rotating 3D models. Intermittent transparency of the models enables the visualization of the cell nuclei (red or pink) or focusing on certain objects.

The images for the video were acquired with 3dmod of the IMOD package (Kremer et al. 1996) and assembled in Fiji (Schindelin et al. 2012). For more details, see (Schneider et al. 2019).

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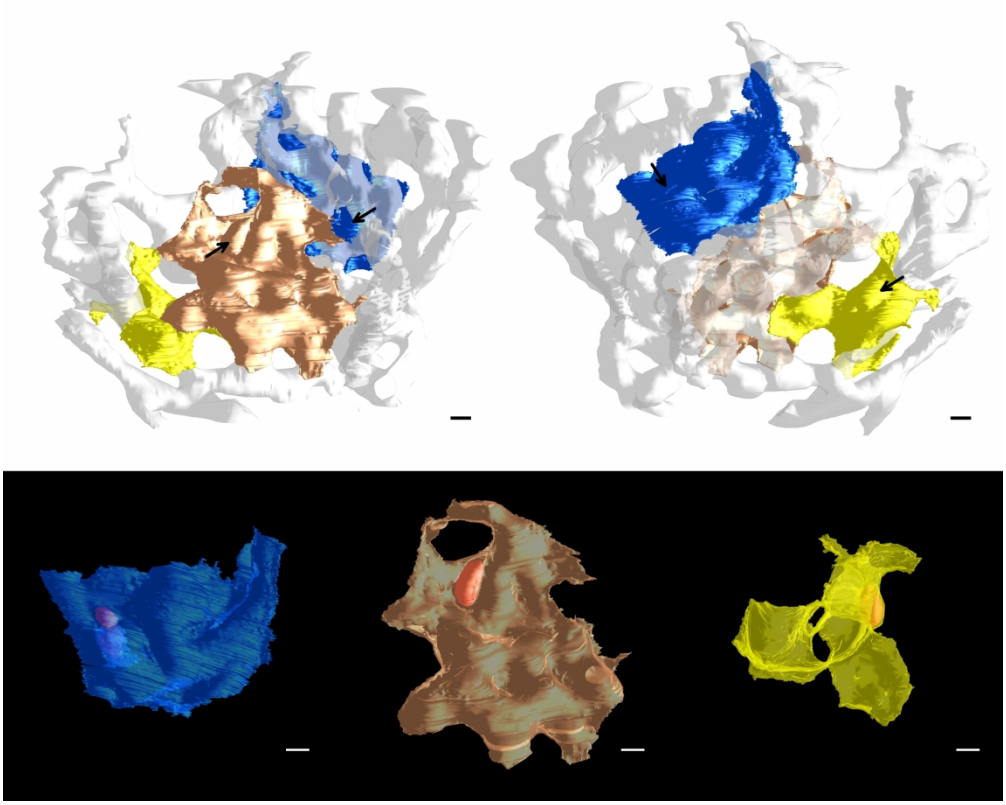

Figure 1

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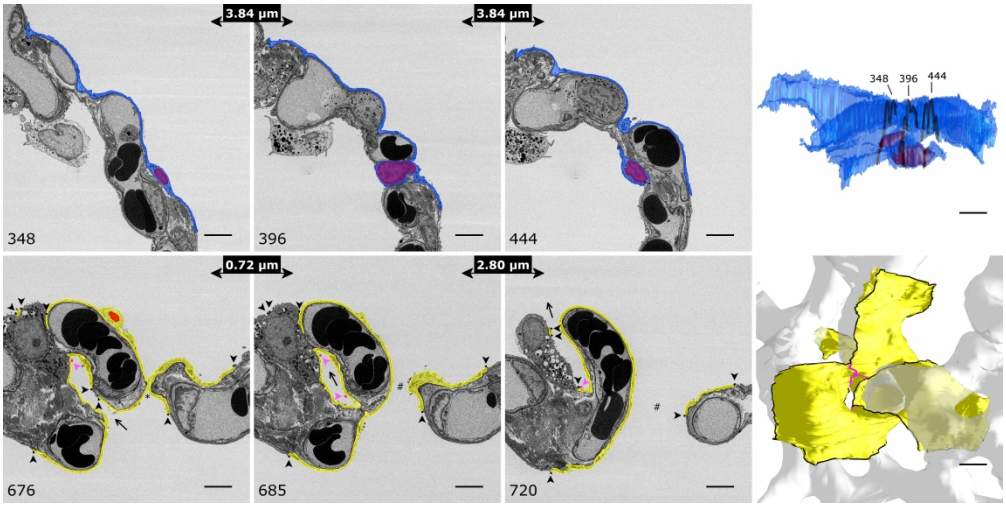

Figure 2

411x204mm (300 x 300 DPI)