Correlation between Optical and SEM Measurements of Wool Cortical Cell Size

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ABSTRACT

Wool fibres consist of micro to nano scale protein constituents that could be used for innovative applications. While techniques for extracting these constituents or making wool fibres into organic powders have been developed, effectively dispersing the particles and accurately determining their size has been difficult in practice. In this study, an ultrasonic method was employed to disperse cortical cells extracted from wool fibres into an immersion oil or ethanol. Specimens of the cortical cells were then observed under optical microscopy and scanning electron microscopy, respectively. Cell length and maximum cell diameter were measured to quantify the cell size. The results suggest significant discrepancies exist in the cortical cell size obtained from the two different measurement techniques. The maximum diameter of wool cortical cells obtained from the optical microscope was much larger than that from the scanning electron microscope, while the length was much shorter. A correction factor is given so that cortical cell size obtained from the two measurement techniques can be compared.

Keywords: Wool, Cortical Cell, Dispersion Medium, Image Analysis, Particle Size Analysis

1. Introduction

Fine wool fibres consist mainly of two types of cell, 10% of the external cuticle and 86.7% of the internal cortex comprised of ortho and para cells. The cortical cells are usually spindle shaped and surrounded by the cell membrane complex, overlapping each other and parallel to the fibre axis. The cortical cell size is believed to be approximately 100µm long and 3-6µm in diameter. These cortical cells can be extracted by treatment with enzymes, by ultrasonic disruption or by other techniques (Rippon, 1992; Brady and Wang, 2004). These cells may be used in different areas. For instance, particular keratinous materials have been used for cosmetics, bone grafts and functional surface coating (Lu et al., 1994; Tsubouchi, 1998; Pavlath et al., 1999; Joko and Yamazaki, 2000).

Various cortical cells from different animal hairs including wool were obtained by the enzyme digestion system. The size distribution has been reported (Brady and Wang, 2004). Meanwhile, mechanically milling, grounding or puffing wool fibres into fine powder has also been examined and the relationships between the treatment parameters and the powder properties have been investigated (Miyamoto et al., 1982; Joko and Yamazaki, 2000; Xu et al., 2003; Xu et al., 2004). However, effective dispersion and accurate size measurement of these organic particles remain a challenge that requires further investigation.

In this study, ultrasonic dispersion of wool cortical cells was used to prepare specimens for size measurement. Both optical microscope (OM) and scanning electron microscope (SEM) techniques have been used to quantify the diameter and length of the cortical cells.

2. Experimental

Wool cortical cells were extracted from Australian Merino wool via enzyme treatment. The treatment procedures are similar to those already reported (Brady and Wang, 2004).
2.1 Optical Microscope Analysis

To measure the cell size using an optical microscope (OM), the particles must be uniformly dispersed onto a glass slide, using an appropriate liquid medium. In addition, the refractive index of the medium should be about 1.500 at room temperature, which is nearly the same as that of the glass slide, in order to avoid light halo during measurement. In this research, the Olympus immersion oil (for microscopy) was used with its refractive index being 1.516 at 23 °C.

To prepare the specimen for measurement, 10ml immersion oil was firstly put into a 20ml sample bottle, then 0.2mg of the wool cortical cells was added. The cell particles were dispersed into the immersion oil with the help of a Unisonics FXP 8D ultrasonic device (Unisonics Australia Pty Ltd, Australia) for 20 min. Finally, a drop of the oil containing the cortical cells was spread in a thin layer, onto a microscope slide and covered with a glass slip of 1 ounce ready for use. An Olympus BX51 microscope fitted with Differential Interference Contrast (DIC) mode and an Optronics digital camera was used for imaging and measurement.

2.2 Scanning Electron Microscope Analysis

In the preparation of specimens for observation under the Scanning Electron Microscope (SEM), ethanol was used as the dispersion medium instead of the immersion oil as for the OM. The preparation procedure was also similar, except when spreading the ethanol drops containing the wool cortical cells onto a pin type SEM mount, a self designed device as shown in Figure 1 was employed. Before spreading, a pin type SEM mount B, with the face covered by a double sided conductive tape A, was fixed to a bar which was rotated by a small motor C. Ethanol containing the cortical cells was dropped onto the centre of the mount. This liquid was immediately heated by a fan heater D until the ethanol was entirely evaporated. Upon completion, the specimen was ready for coating with gold. A sputter coater SCD 050 (Balzers AG, Switzerland) using argon gas was employed for 30 seconds under 0.05 mbar working pressure and 40 mA current. Once this was prepared, a LEO 1530 SEM was used for imaging.

2.3 Image Processing and Data Analysis

The images captured by the OM and SEM were both used for measuring the particle size, using Image-Pro plus 4.5.1 software (Media Cybernetics, Inc., USA). The length and maximum diameter of the cortical cells were specified as shown in Figure 2. Meanwhile, the projected area as well as the ratios of the length to the maximum diameter of the cortical cells could be also measured, if necessary. The obtained data were then analysed with the SPSS 12.01 software.

3. Results and Discussion

3.1 Morphology of Wool Cortical Cells

As indicated in a great deal of literature, wool cortical cells are spindle-like and overlap each other parallel to the fibre axis (Rippon, 1992; Höcker, 2002). Figure 3 presents a cortical cell bundle which was treated by the enzyme treatment but did not separate completely. The cell membrane complex between the cells was removed and the cortical cells were freed from each other. As a result the unseparated cell bundles had an even larger diameter than that of intact wool.
With appropriate dispersion, the individual cortical cells were obtained as shown in Figure 4. Some had forked tips. The striated surface shown in Figure 5 represented the aggregation of macro fibrils as reported in literature, in which the macro fibrils are assumed to have a diameter of approximately 200 nanometres (Rippon, 1992).

3.2 Image Processing and Size Measurement

Images obtained from both OM and SEM were processed and measured using a coded macro program, which included functions such as image transformation, image processing, particle count and size measurement, data transfer and analysis.

3.2.1 Image Transformation

In order to gain a good contrast, the colour images were captured by the OM using the DIC mode (Rost, and Oldfield, 2000). Image segmentation usually works very well and quickly on greyscale images (Castleman, 1996). Therefore, the colour images from the OM were firstly converted to 16 bits greyscale, not requiring images from the SEM.

3.2.2 Image Processing

There are two frequently used image processing techniques. One is for enhancement and the other is for morphological modifications. Therefore, in the macro program, flatten and median filters were used to even out the background variations and impulse noises. The best-fit equalization was also applied to improve the enhancement of the filtered images. The resulting image is shown on the left in Figure 6, compared with the original on the right.

3.2.3 Image Segmentation

Image segmentation is a key step toward the quantitative interpretation of image data (Poille, 1998). In order to obtain a binary image with clear bright objects on a black background, thresholding was imposed on the previously processed greyscale image. Although thresholding can be an automatic process, a more accurate result can be achieved by manually
locating the threshold values in a zoomed in intensity histogram (Niblack 1986).

After thresholding, a binary image with the particles clearly identifiable from the background was created on the left in Figure 7, compared to the original image on the right. If the effect was not satisfactory, the same procedure could be repeated until a satisfactory one is obtained.

![Fig. 7. The thresholded image (left) and the original (right).](image)

### 3.2.4 Particle Size Measurement

The size of the processed image was automatically measured, according to the measurement criteria for the particles. During the course of each measurement, the program could be stopped to manually toggle off those particles which crossed each other or were believed not to be wool cortical cells, as marked in Figure 8. The resulting image with separated individual cortical cells was ready for final measurement, using the count / size function of the program.

![Fig. 8. The final processed image (left) for calculation against the original (right).](image)

The original data and the statistical results could be presented for each image, but they made no significant sense regarding the overall properties until enough samples were measured. Therefore, the data collected from several measurements together with further data analysis were required of the same batch sample.

### 3.2.5 Data Transfer and Collection

The raw data obtained from each measurement could be transferred into an Excel spread sheet or a database through the dynamic data exchange (DDE) function of the program. In every transfer, repeated measurements for the same batch sample needed to be identified, in order for the data to be exported to correct cells of the spread sheet. On the first sample measurement, the program would transfer all information about the data, including the captions (headings) for each column. If the measurement was from subsequent samples of the same batch, the program would transfer the data consecutively, without the captions. After all measurements for a batch of samples are complete, the spread sheet is ready for further data analysis.

### 3.2.6 Data Analysis and Result Presentation

The data processing and analysis were carried out using SPSS software. The detailed statistical results of the particle size could be obtained in numerical and graphical formats, including the data central tendency and variability. As an example, the results of a measurement from the OM are presented in Table 1 in numeral format and in Figures 9 & 10 by histogram, respectively.

![Table 1. Statistical results of a measurement with the optical microscope](image)

<table>
<thead>
<tr>
<th>Statistics Number</th>
<th>Area (µm²)</th>
<th>Length (µm)</th>
<th>Diameter (µm)</th>
<th>Ratio (L/D)</th>
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<tbody>
<tr>
<td>Valid</td>
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<td>2696</td>
<td>2696</td>
<td>2696</td>
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<tr>
<td>Missing</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Mean</td>
<td>275.708</td>
<td>46.575</td>
<td>7.801</td>
<td>6.954</td>
</tr>
<tr>
<td>Median</td>
<td>262.780</td>
<td>46.020</td>
<td>7.460</td>
<td>6.860</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>110.159</td>
<td>13.247</td>
<td>2.4989</td>
<td>1.945</td>
</tr>
<tr>
<td>Skewness</td>
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<td>0.137</td>
<td>1.216</td>
<td>0.151</td>
</tr>
<tr>
<td>Std. Error of Skewness</td>
<td>0.047</td>
<td>0.047</td>
<td>0.047</td>
<td>0.047</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>1.105</td>
<td>0.255</td>
<td>2.735</td>
<td>-0.575</td>
</tr>
<tr>
<td>Std. Error of Kurtosis</td>
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<td>0.094</td>
<td>0.094</td>
<td>0.094</td>
</tr>
<tr>
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<td>84.760</td>
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<td>9.305</td>
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</table>

Note: L means length and D maximum diameter of the cortical cells.
Fig. 9. A histogram of the measured length of wool cortical cells.

Fig. 10. A histogram of the measured maximum diameter of wool cortical cells.

3.3 Discrepancy between Results from OM and SEM

For organic particles such as spindle-like wool cortical cells, the dispersion media used in the sample preparation may affect the particle size in optical microscopy due to the light halo. Also, its lower resolution including the short depth of focus would account for the loss of some detail about the fine structures of particles, especially when a relatively low magnification is selected. On the other hand, the SEM that has a long depth of focus can clearly reveal the particle details and will give accurate results, but it is very time consuming and costly. It is not practical for measuring a large sample pool. Therefore, corrections to the results from the OM by the SEM are required.

Figure 11 shows that the maximum diameter values from the SEM are smaller than those from the OM. This phenomenon may be caused by two factors: (a) the existence of light halo during optical observation and (b) the dried state of cortical cells for the SEM observation (cortical cells will swell after absorbing moisture).

As shown in Figure 12, the cell length results do not follow the same trend as for the maximum cell diameter. In fact, the opposite is true for the statistical values of the cell length. The shorter length results obtained from the OM suggest some loss of information concerning the fine tips of the spindle shaped cortical cells, due to the short depth of focus of the OM.

The discrepancies for cell length and diameter results exist at different magnifications (e.g., X100, X200 or X400) for the optical microscope. The ratio of the maximum cell diameter values from the SEM to those from the OM is almost constant at around 65.3%, while the length at around 155.3%. Therefore, based on the present research, the following corrections can be made between the maximum diameter (D) and length (L) of wool cortical cells obtained from the OM and SEM:

\[
\frac{D_{SEM}}{D_{OM}} = 0.653, \quad \frac{L_{SEM}}{L_{OM}} = 1.553
\]
4. Conclusion

For size measurement, wool cortical cells can be dispersed properly in a liquid medium by using the ultrasonic method. The dispersion medium can be either ethanol for the scanning electron microscope or immersion oil for the optical microscope.

The optical microscope was more appropriate for measuring a large pool sample in a short time than the scanning electron microscope. However, the maximum diameter of the cortical cells from the optical microscope is larger than that from the scanning electron microscope, while the opposite is the case for cell length. The shorter cell length from the optical microscope indicates that the optical microscope is unable to ‘see’ the fine tips of the cortical cells owning to its low resolution. The larger value for the maximum cell diameter from the optical microscope is likely to be due the existence of light halo during observation and the dry state of the cells for SEM observation. The discrepancies between optical microscopy and scanning electron microscopy results for cortical cell diameter and length exist at different magnifications used for the optical microscope observation, and corrections can be made to account for the discrepancies.

REFERENCES


