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TEXTILES

Evaluating transmission electron microscopy as a method for assessing the condition of archaeological wool

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KEYWORDS: transmission electron microscopy, archaeological wool, degradation, pigment granules

ABSTRACT

Transmission electron microscopy (TEM) was investigated to clarify the applicability of the method for assessing the condition of archaeological textiles from waterlogged environments. A description and evaluation of the TEM protocol is presented and the method was tested on new and artificially soil-aged wool, as well as on archaeological samples. TEM can give more information on the ultrastructure of the wool cortex than other microscopic techniques. It is the only method that can give precise information on the amount, size, shape, and condition of natural pigment granules. TEM analyses were also used to investigate the presence of natural pigments in the yarns.

INTRODUCTION

Archaeological textiles can be a special challenge when it comes to identification of the fibers, documentation of the textiles and assessment of their condition. The most common methods used for these tasks are light microscopy (LM) and scanning electron microscopy (SEM) (Peacock 1996 and 2004). Both can give valuable information, but LM has its limitations concerning resolution and depth of field and SEM only permits observation of the surface of the fibers. These methods are therefore not sufficient for a full understanding of the internal structure of the fibers and how this structure may change due to degradation.

This issue became of interest during the work by one of the authors on a unique collection of wool textiles from a waterlogged Iron Age burial site in Denmark, Lønne Hede. The textiles originate from burials excavated in 1969 (one grave) and 1995 (11 graves). They are unique because they are clearly patterned in a variety of red, orange, brown, greenish blue, yellow, and cream colors (Munksgaard and Østergaard 1988, Demant 2007). Unfortunately, dye analysis has given limited results; although many fragments have been analyzed, only indigotin (mainly in the 1969 grave) and a few yellow dye components were detected, which leaves the majority of the colored textiles unidentified (Jørgensen and Walton 1986, Walton 1988, Walton Rogers 1997).

This raised several questions. First of all, the macroscopic observations of the textiles showed a great variation in the degree of deterioration of the yarns which seemed to be related to their color. Was it possible to verify this correlation by structural investigations of the wool cross sections? It is well known that the state of preservation of archaeological textiles does not solely depend on the fiber type and their burial environment, but is also greatly influenced by their prehistory, for example, dyestuff and dyeing procedures, bleaching, and wear and degradation due to use. Several studies have reported that dyestuffs can either reduce or enhance the fiber degradation, depending on the dyestuff and the degradation factors. These include the tapestry project MODHT (Quye et al. 2009, Odlyha et al. 2005 and 2007), and a variety of soil burial projects (Peacock 2004, Ringgaard 2010). The latter, for example, has shown that undyed wool was the first to degrade during waterlogged anaerobic soil burial, while alum-mordanted madder dyed wool was always the best preserved.

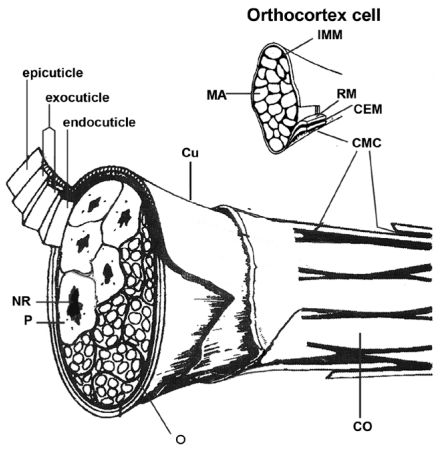

Figure 1. Schematic illustration of a wool fiber: the cross section (left), the scale surface (middle) and a longitudinal cut through cortex (right). This fiber is a composite structure consisting of two types of cortex cells (Co) (ortho- (O) and para- (P)), cuticle (Cu) cells, macrofibrils (MA), nuclear remnant (NR), intermacrofibrillar material (IMM) and a cell membrane complex (CMC) that is composed of two resistant membranes (RM) with intercellular cement in between (CEM). Drawing based on illustrations from Rippon 1992

The second question raised by these textiles was whether the coloring components of the red and brown yarns could be due to natural pigmentation. Frequently, observation of these melanin granules is done by transmitted light microscopy (TLM), but degradation might have changed their visibility. Could they have become invisible due to dissolution of the pigments in the soil, or had the melanin granules become colorless due to exposure of the wool to reducing conditions during burial (Ryder 1990, Wilson et al. 2007b)?

An obvious method for investigating the above questions was transmission electron microscopy (TEM). Rogers (1959) gave a thorough introduction to the TEM of wool fibers, but the method has rarely been used for archaeological textiles. Wilson et al. (2007b) has used the method for studying the head hair of the Danish bog body known as the Grauballe Man. Both high-resolution LM and TEM images of longitudinal sections of the hair showed how well preserved the morphology of the hair cells were, and furthermore how much the resolution was improved by TEM. With TEM it was possible to see a five-layer (at least) intact cuticle, individual cortex cells, and the shape of the natural pigment granules. Wilson et al. (2007a) also used TEM to study the impact of aerobic soil burial and keratinolytic microorganisms on hair ultrastructure. A sequence of degradation could be determined, which showed that pigment granules were the least vulnerable to degradation under these conditions.

THE MORPHOLOGICAL AND CHEMICAL COMPOSITION OF WOOL

The morphological structure of wool fiber (Figure 1) is very complex and can be divided into two or three different cell types (cortex, Co, cuticle, Cu), and medulla cells) that are linked together by the cell membrane complex (CMC). The CMC is a structure that runs through the entire fiber, both between the cuticle and the cortex cells. It is composed of two resistant membranes (RM) with intercellular cement in between (CEM). Each cell (cortex and cuticle) is thus covered by the resistant membrane, and the cement acts as a liquid and humidity transporter through the entire fiber.

The cortex cells, which can be divided into ortho- (O) and paracortex (P) cells, comprise the main part of the body. The cortex cells are surrounded by the cuticle cells, a multilayer structure that covers the entire fiber. The medulla cells are filled with air and only found in the center of coarser fibers, where they are visible as either continuous or fragmented canals (Rippon 1992).

The cuticle cells consist of three layers: the epicuticle, a thin membrane covering the surface of the cuticle; the exocuticle (ExCu), a highly cross-linked resistant layer; and the endocuticle (EnCu), which is less cross-linked and readily swells in liquids (Rippon 1992).

Wool belongs to a group of proteins known as keratins. These fibers are chemically very inhomogeneous and despite their classification as keratins, they can be divided into a keratinous part with more than 3% half-cystine and a non-keratinous part with a maximum of 3% half-cystine. The low concentration of disulfide crosslinks in the non-keratinous proteins makes them more labile and less resistant to chemical attack. The orthocortex is a more open structure where each macro fibril is surrounded by non-keratinous

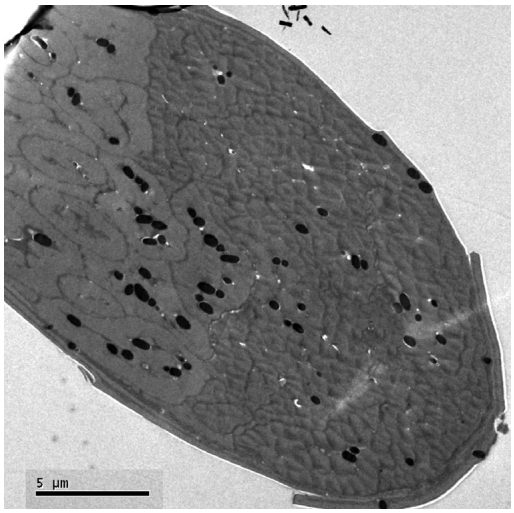

Figure 2. TEM of new brown wool , illustrating paracortex cells (left) and orthocortex cells (right)

material, while the macrofibrils in the paracortex are fused together, resulting in slower water absorbance. The macrofibrils are composed of rod-like crystalline microfibrils (composed of alpha-helix low-sulfur proteins) surrounded by an amorphous matrix (high-sulfur proteins) (Rippon 1992). The microfibrils in the paracortex are hexagonally arranged parallel to the fiber length, and fusion between the individual macrofibrils is therefore possible. In the orthocortex, the microfibrils are spun like a yarn, and therefore have a whorl-like appearance when observed in cross section. This difference between the macrofibrils of the ortho- and the paracortex cells is obvious when the fibers are analyzed in TEM (Figure 2).

MATERIALS AND METHODS

North Ronaldsay wool was chosen as the reference material for the analyses because the fleece, according to Wild (2003), is very similar to the one that existed in the Iron Age. New and soil-buried wool (24 months in waterlogged peat) was analyzed, i.e., eight samples altogether (undyed and indigo-dyed white and brown wool). The procedure for the indigo dyeing and the soil burial are described in Ringgaard (2010). Four archaeological fragments were sampled for analysis: the blue- and red-checked, tabby-woven shawl (Type 4 red); the blue- and red-striped waistband from the 1969 grave (Type 7 blue and Type 7 red); a cream- and brown-striped twill from grave 1 (Tx 1.6); and a diamond twill from grave 2 (Tx 2.3). All samples were analyzed by LM, SEM and TEM. As this paper focuses on the TEM analysis, results of the other methods are only discussed when appropriate. The microscope slides for LM were prepared as part of the TEM protocol, and analyzed by a Nikon 80i microscope. Samples for SEM were mounted on carbon adhesive tabs on SEM aluminum stubs, coated with gold and analyzed at 7.0 kV in a JEOL 5310LV scanning electron microscope. TEM grids were analyzed in a Jeol 1010 CX transmission electron microscope at 80 kV.

TEM PROTOCOL (JØRGENSEN AND FREDERIKSEN 2007)

Samples for TEM were prepared as follows:

- Samples (min 0.5 cm yarn) were tied with cotton sewing yarn to avoid separation during preparation.
- Fixation in 2.5% glutaraldehyde + 2% paraformaldehyde in 0.1 M phosphate buffer (monobasic and dibasic sodium phosphate), pH of 7.0 (15 hours at 5°C).
- Rinsing in the same buffer (3× for 20 min).
- Post-fixation in 1% osmium tetroxide in 0.1 M phosphate buffer, pH of 7.0 (2 hours at room temperature), or 2% osmium tetroxide in 0.1 M phosphate buffer, pH of 7.0 (6 days at room temperature).
- Rinsing in 0.1 M phosphate buffer, pH of 7.0 (2× for 15 min.), followed by distilled water (20 min.).
- Dehydration in 30, 50, 70, 80, 90, 95, 100 and 100% acetone (20 min in each solution).
- Infiltration and embedding in Spurr's epoxy medium (acetone-Spurr's, 3:1 (16 hours), 1:1 (8 hours), 1:3 (16 hours), 100% Spurr's (8 hours) and 100% Spurr's (16 hours).

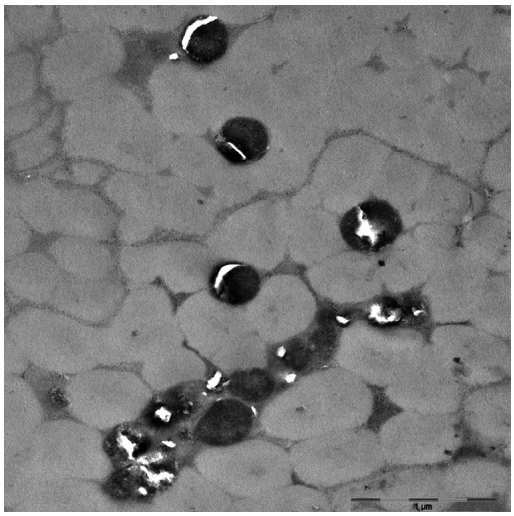

Figure 3. Indigo-dyed brown wool, degraded in waterlogged soil for 24 months

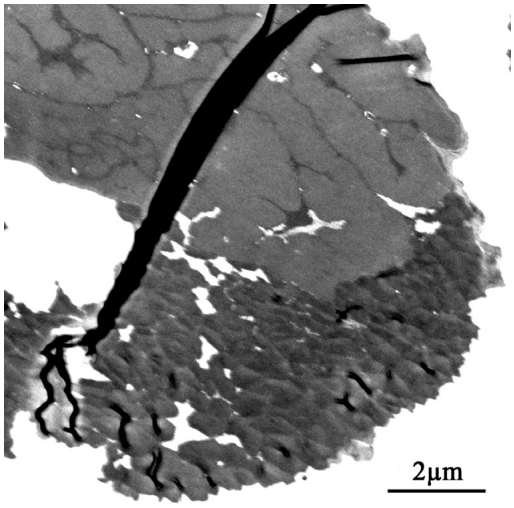

Figure 4. TEM of blue-green fiber from the waistband Type 7. Black lines are artifacts

- Samples and Spurr's transferred to molds and polymerized at 70°C for 12 hours.
- Block cut with embedded fibers using a dry 45° glass knife on an ultramicrotome. This was continued until a good overview of the fiber cross sections was obtained.
- Then two sections were cut with the glass knife (1–2-µm thick) to be used for LM. The sections were mounted on a slide. One section was stained with Toluidine Blue O (TBO) (1% TBO and 2% borax in distilled water), the other remained unstained. The mounting medium was DePeX (R.I. 1,529).
- The block was further cut: the area with characteristic fibers was selected for trimming of the mesa (an area about 300 × 300 µm). The block was trimmed and cut with a diamond knife.
- The first two mesa sections (about 1 µm thick) were mounted for LM (treated as above).
- Several ultrathin sections were cut with a diamond knife (50–100 nm thick) for TEM.
- The sections were transferred to formvar-coated copper slot grids.
- The sections were stretched by exposing the grids to chloroform vapor for a few seconds.
- Six grids were prepared. Three without post-treatment, three contrasted with uranyl (10% uranyl acetate in 50% ethanol for 10 min at 70°C) and lead citrate for 10 min at 70°C (Reynolds 1963).

RESULTS

It is only possible to see grayscale images in TEM. The gray color represents variations in the electron densities, with black as the most electron-dense material. TEM gives an excellent overview of the internal structure of wool fibers. It is possible to see details like microfibrils (diameter: 7–11 nm), cell membrane complex, nuclear remnants, natural pigment granules, intermacrofibrillar material, ortho- and paracortex and to determine the number of cuticle cells surrounding the fibers, including the individual layers within the cuticle. The TEM analyses showed that in order to observe structures such as microfibrils, CMC and intermacrofibrillar material (above 50.000×), the images were improved when the osmium fixation lasted six days in 2%, but two hours in 1% was often sufficient. The best TEM images were achieved when the ultrathin sections were contrasted with uranyl acetate and lead citrate.

The artificially aged wool (24 months in waterlogged peat) showed very few signs of degradation, and the fibers looked essentially unchanged except for the brown indigo dyed wool. In these samples, the pigment granules sometimes looked deteriorated with craquelure and in some cases the granules were partly lost (Figure 3).

The archaeological wool samples showed very different signs of degradation. The indigo-dyed fibers were the most degraded. The cuticle and the orthocortex were often totally or partly missing, and the paracortex had started to break up, but the paracortex cells were seldom detached. The

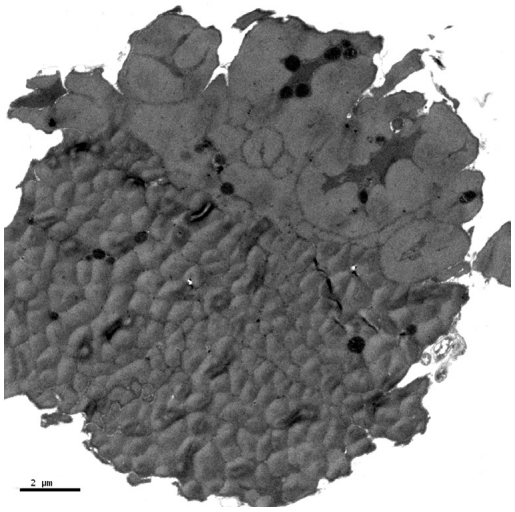

Figure 5. TEM of red fiber from red- and blue-checked shawl Type 4

indigo-dyed fibers had no natural pigmentation, only crystal-like structures (probably indigotin) that were evenly distributed in the nuclear remnants and the cell membrane complex (Figure 4).

The red and red-brown yarns had fairly intact ortho- and paracortex cells, and did not show the orthocortex-selective degradation pattern seen in the indigo-dyed fibers (Figure 5), except for fibers that had been degraded by microorganisms (Figures 6 and 7). When one disregards the latter degradation factor, then differences in the appearances of the cortex cell occur. Samples from graves 1 and 2 had cortex cells similar to new wool, while those of the two red yarns from the 1969 grave were clearly changed. Even though the cortex cells appeared intact in both samples, the fibers from the red-checked shawl showed destruction along the cell membrane complex, which had resulted in a slow unzipping of the cortex cells (Figure 5). All the red fibers from the waistband had cortex cells that seemed fused, and, in general, all the cross sections were obscured by artifacts (Figure 6). The pigment granules, when present, either looked degraded, partly or totally lost, and they often seemed to be one of the first structures to degrade, together with the cuticle cells. Concerning the color of the yarn then, TEM analyses showed that only in one sample (Tx 2.3) were the natural pigments present in sufficient amounts to justify the color of the yarn. The other samples had either no pigments (Tx 1.6), few (Type 7) or a moderate amount of granules (Type 4).

The results of the TEM analyses are listed in Table 1. Each structural component that could be recognized without any visible damage is marked

Table 1. The condition of the structural components of the wool samples when analyzed in TEM. An X indicates that the structure was recognizable without visible damage

Structural component	New Wool	24M Wool Brown Indigo dyed	Type 4 Red	Type 7 Red	Type 7 Blue	Tx 1.6	Tx 2.3
Cu – Cuticle	X	X Lifting observed	Mainly total loss	Total loss	Seldom observed fragmentary	X Occasional lifting. fragmentary or total loss	X Occasional lifting. fragmentary or total loss
EnCu – Endocuticle ExCu – Exocuticle	X	X	-	-	Eroded	Often eroded	Often eroded
CMC – Cell Membrane Complex Intercellular cement surrounded by 2 resistant membranes	X	X Crystalls observed probably indigotin	X Damage to cement along edge of fiber	X Obscured by artefacts	X Damage to cement along edge of fiber Crystalls observed	X	X
NP – Natural Pigentation	X (Brown fiber)	Damaged and occassional partly lost	X Moderate amount. Damaged and sometimes lost	Very seldom observed Damaged	No pigments	No pigments	X Many pigments Mostly damaged with partly or totally loss
O – Orthocortex	X	X	X Fibrillation occur along edge of fiber	X Obscured by artefacts Damaged by microorganisms	X Obscured by artefacts, partly of totally lost	X	X Damaged by Microorganisms
P – Paracortex	X	X	X Fibrillation occur along edge of fiber	X Obscured by artefacts Cells sometimes look fused	X Fibrillation occur along edge of fiber	X	X
IMM – Intermacrofibrillar material In Orthocortex	X	X	X	X	X Partly damaged	X	X
MA – Macrofibrills In Orthocortex	X	X	X	X	X Partly damaged or lost	X	X
MI – Microfibrills Ma – Matrix	X	X	X	X	Not observed	X	X
NR – Nuclear Remnant	X	X Crystalls observed probably indigotin	X	X	X Partly damaged	X	X

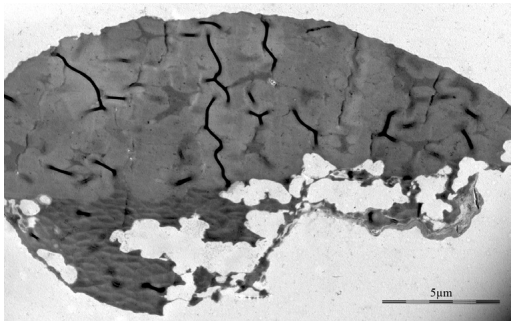

Figure 6. TEM of red fiber from blue- and red-striped waistband Type 7. Black lines are artifacts

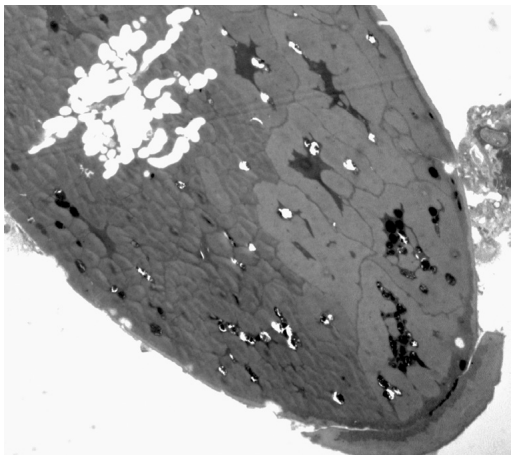

Figure 7. TEM of red-brown fiber from cream and red-brown diamond twill Tx 2.3

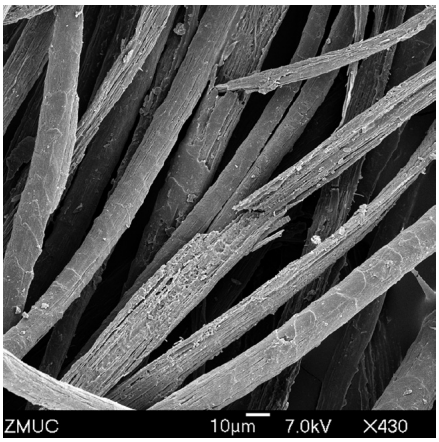

Figure 8. SEM of blue fibers from the blue and red waistband Type 7

with an X. Only new and soil-buried, indigo-dyed wool is listed in the table, because no visible changes appeared in the other samples.

DISCUSSION

Comparison of TEM and SEM analyses of the fibers shows that, when dealing with degraded fibers, the cuticle cells can be lost during the TEM protocol. The SEM image of the indigo-dyed fibers from the 1969 grave clearly shows cuticle cells on the fiber surface, even though they sometimes are very degraded (Figure 8). These cuticle cells were seldom seen in TEM. This cuticle loss was seen in several of the samples and is something that must be taken into consideration when analyzing fibers with TEM. Attempts to infiltrate and embed a sample in epoxy without any pretreatment did not work out. Soil particles within the yarn made it impossible to cut ultrathin sections without risk of damage to the diamond knife. Artifacts occur in nearly all the fiber sections, but they seldom obscure the information on the TEM image. However, artifacts (Figure 6) heavily damaged one sample. It was still possible to see details, but it was remarkable how consistent the problem was. Previous dyestuff analyses showed that this yarn had also dissolved during the acid dyestuff extraction procedure, which had not happened with any of the other samples. The large amount of artifacts could therefore be a signal of severe deterioration. The paracortex cells also seemed partly fused when the cells were observed in TEM (Figure 6). This was not seen when the other red yarn from the 1969 grave was analyzed (Figure 5). The TEM analyses have thus enhanced knowledge on the condition of the red yarns and indicated that they may not have been dyed with the same dyestuff or the same dyeing method.

TEM analysis showed that although the natural pigment granules were sometimes partly or totally dissolved, it was possible to estimate the amount of pigments that had originally colored the fibers. This pigmentation could not be documented by TLM on the whole amounts of the fibers. In the TLM of cross sections it was difficult to detect the pigment granules if they were degraded. Only TEM gave precise information on the condition and amount of pigment granules.

CONCLUSION

In comparison with other microscopic techniques, TEM gives valuable information on the ultrastructure of wool cortex cells. The information on cuticle cells is less reliable for degraded fibers. Because there seems to be a risk of losing the cuticle cells during the TEM protocol, it is crucial that samples are analyzed with SEM prior to the selection of samples for TEM. TEM is the only method that can give precise information on the condition, size, shape, and quantity of the pigment granules. The four red and red-brown archaeological yarns turned out to possess different characteristics when observed in TEM, which suggests that at least three of them had been dyed. Only one sample (Tx 2.3) contains pigments in sufficient quantities that can justify the color of the yarn. The TEM analysis of the two red yarns from the 1969 grave showed great differences in their appearance, and it is therefore likely that they had

not been exposed to the same pretreatment before burial; it is possible that two different dyestuffs were used for the yarns.

ACKNOWLEDGEMENTS

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