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Three-dimensional architecture of macrofibrils in the human scalp hair cortex

Duane P. Harland [a,*], Richard J. Walls [a], James A. Vernon [a], Jolon M. Dyer [a], Joy L. Woods [a], Fraser Bell [b]

[a] Food & Bio-Based Products, AgResearch Lincoln Research Centre, Lincoln, New Zealand
[b] Hair CTI, Unilever R&D, Port Sunlight, Bebington, UK

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ABSTRACT

Human scalp hairs are comprised of a central cortex enveloped by plate-like cuticle cells. The elongate cortex cells of mature fibres are composed primarily of macrofibrils-bundles of hard-keratin intermediate filaments (IFs) chemically cross-linked within a globular protein matrix. In wool, three cell types (ortho-, meso- and paracortex) contain macrofibrils with distinctly different filament arrangements and matrix fractions, but in human hair macrofibril-cell type relationships are less clear. Here we show that hair macrofibrils all have a similar matrix fraction (~0.4) and are typically composed of a double-twist architecture in which a central IF is surrounded by concentric rings of tangentially-angled IFs. The defining parameter is the incremental angle increase (IF-increment) between IFs of successive rings. Unlike the wool orthocortex, hair double-twist macrofibrils have considerable inter-macrofibril variation in IF increment (0.05–0.35°/nm), and macrofibril size and IF increment are negatively correlated. Correspondingly, angular difference between central and outer-most IFs is up to 40° in small macrofibrils, but only 5–10° in large macrofibrils. Single cells were observed containing mixtures of macrofibrils with different diameters. These new observations advance our understanding of the nano-level and cell-level organisation of human hair, with implications for interpretation of structure with respect the potential roles of cortex cell types in defining the mechanical properties of hair.

1. Introduction

Recent advances have improved our understanding of the proteomic basis of hair (Langbein and Schweizer, 2005; Langbein et al., 2007; Rogers et al., 2006), but the ability to match this information to hair at the tissue level has lagged because the links between cell-types and its underlying nano-structure in human hair is less well characterised. Of the mammalian fibres studied ultrastructurally, wool has received by far the most attention and, not surprisingly, forms the basis for discussing the internal structure of other fibres, including that of human hair. As observed using transmission electron microscopy (TEM), the keratin intermediate filament (IF) bundles (or macrofibrils) of wool orthocortex and paracortex cells have differing architectures by which IFs are arranged within the surrounding matrix material (Kaplin and Whiteley, 1978; Orwin et al., 1984; Rogers, 1959). Separated by intermacrofibrillar material (IMM), orthocortex macrofibrils are approximately cylindrical with IFs arranged in a double-twist arrangement as if wound around a central core (Caldwell et al., 2005; Harland et al., 2011b; McKinnon and Harland, 2011). Paracortex macrofibrils are laterally fused, making boundaries indistinct, and IFs are arranged along the macrofibril axis, but locally (over tens of nanometres) there is noticeable lateral disorder in IF packing, sometimes called 'pseudo-hexagonal' packing. Paracortex macrofibrils contain more matrix than those of the orthocortex (Dobb, 1970; Harland et al., 2011b; Rogers, 1959). Mesocortex cell macrofibrils can appear superficially similar to either ortho- or paracortex macrofibrils (Orwin et al., 1984), except that they contain patches within which IF packing is especially regular. This packing regularity over tens or hundreds of nanometres gives mesocortex macrofibrils a distinct hexagonal texture. In Merino wool the mesocortex matrix fraction is intermediate between ortho- and paracortex (Harland et al., 2011b). In human scalp hair there appears to be less certainty of what kinds of macrofibrils and what types

Abbreviations: IF, intermediate filament; IMM, intermacrofibrillar material; TEM, transmission electron microscopy.

* Corresponding author. Address: AgResearch Research Centre, Private Bag 4749, Christchurch, New Zealand. Fax: +64 3 321 8811.

E-mail addresses: duane.harland@agresearch.co.nz (D.P. Harland), james.vernon@agresearch.co.nz (J.A. Vernon), jolon.dyer@agresearch.co.nz (J.M. Dyer), joy.woods@agresearch.co.nz (J.L. Woods), fraser.bell@unilever.com (F. Bell).

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of cortical cells occur. The cortex of hair has been described as composed of the same three cell types as in wool (Popescu and Höcker, 2007; Thibaut et al., 2007), as just orthocortex (Robbins, 1994), as wool cell types but with reservations (Swift, 1997), or sometimes as 'ortho-like' and 'para-like' (Kajiura et al., 2006). Others describe hair cortex cells as having features of both the ortho- and paracortex of wool and use new terms (Bryson et al., 2009; Kaplin and Whiteley, 1985; Kassenbeck, 1981; Orfanos and Ruska, 1968; Randebrock, 1964) (e.g., hetero- or meta-cortex).

In an earlier study (Bryson et al., 2009) we identified that wool cortex cell type descriptions were incompatible with observations of Japanese hair cortex. We applied a tentative classification scheme based on the degree of fusion of macrofibrils within cells based on TEM. One key finding was that many cells appeared to contain mixtures of fused macrofibril aggregate (similar appearance to paracortex) and discrete macrofibrils with a circular cross section (similar appearance to orthocortex). Using electron tomography we established that narrow 'ortho-like' macrofibrils had double-twist architecture similar to that of wool orthocortex macrofibrils. However, our study did not extend to thoroughly investigating larger 'ortho-like' macrofibrils or macrofibrils within cells containing mixed macrofibril types (called Type C in that study). One of the few tomographic observations we made of Type C macrofibrils revealed an architecture that appeared to be intermediate between orthocortex and paracortex IF arrangements, something only rarely encountered in wool (Harland et al., 2011b). How TEM projections relate to the underlying structure revealed by tomography and the variability of macrofibril architecture in human scalp hair were unresolved issues. Characterisation of macrofibril structure is important if we are to uncover the mechanisms underlying their supramolecular assembly in the follicle, its transformation into a hard material, and subsequent performance modifications brought on by environment.

Here we examine relationships between macrofibril appearance in TEM, and its three-dimensional architecture using electron tomography, explain why this has led to challenges in interpreting macrofibril architecture in the past, and introduce new terms necessary to describe the relationship between macrofibril diameter and IF arrangement.

2. Methods

Scalp hair samples from a Caucasian female with naturally straight hair which had not been previously damaged by harsh chemical treatments were prepared for TEM and electron tomography by first cutting the hair transversely with a razor to allow reagents (mercapto-ethanol followed by osmium tetroxide) to penetrate all parts of the cortex directly. Following reduction–osmication, samples were treated with uranyl acetate, dehydrated under vacuum and embedded in epoxy resin. Full details are given elsewhere (Harland et al., 2011a). Additional samples of high-curl ethnic African hair (gender unknown) were prepared using the same methods for TEM only to allow us to confirm macrofibril observations in hair with different fibre morphology. Sections of 90 nm (standard TEM) or 150–300 nm (electron tomography) were cut with a Leica UCT ultramicrotome fitted with a 35° Diatome diamond knife. Section stains (uranyl acetate and lead citrate) were applied, and for electron tomography, 15 nm gold fiducials were applied to both sides before examination to assist with subsequent image alignment. Standard TEM micrographs were collected using an FEI Morgagni 268D operating at 80 kV with a side-mounted SIS/Olympus Megapixel III camera. Electron tomography tilt-series were composed of images (pixel size 0.6 nm) taken at 1° increments over 120° of specimen tilting on a FEI Tecnai G^2 T30 TEM operating at 300 kV and a via a Tietz camera (2048 × 2048 pixels, 12 bit). Tomograms were reconstructed, visualized, modelled and IF angles measured using the IMOD suite (Kremer et al., 1996) version 3.13.5, and fast Fourier transform analysis of filament spacing was carried out in *analySIS*® pro version 5. Methods for electron tomography, modelling and analysis of models were similar to earlier studies on wool (Caldwell et al., 2005; Harland et al., 2011b) and hair (Bryson et al., 2009). Statistical analyses were carried out using Graphpad Prism version 4.03. IF spacing was measured from the peak to centre distance of Fourier transformed electron tomography slices; further details of IF spacing methods and data for wool are given elsewhere (Harland et al., 2011b).

3. Results

3.1. Appearance of macrofibrils using TEM

In 90 nm thick TEM sections, macrofibril appearance was highly variable in all hair examined (Caucasian: 2153 macrofibrils from 102 cells from 3 hairs. African: 1305 macrofibrils, 113 cells, from 2 hairs). We grouped macrofibrils based on size, shape, internal texture and degree of fusion. Macrofibrils in both Caucasian and African hair cortices had an identical range of appearances, with no macrofibril type uniquely associated with either type of hair. At low magnification (~7,000x, IFs indiscernible), the macrofibrils of some cells were roughly circular, partly or entirely surrounded by IMM, and therefore reminiscent of those in the wool orthocortex. Other cells contained larger irregular-shaped complexes that sometimes filled most of the cell, thereby appearing paracortex-like. Many cells from all hairs contained a mixture of discrete and fused macrofibrils of various sizes (Fig. 1A), similar to previous observations of straight and curly ethnic Japanese hair (Bryson et al., 2009). Differences in the amount and distribution of macrofibrils between the Caucasian and African hair types are not reported here.

At high magnification (~70,000x), small discrete hair cortex macrofibrils resembled those from wool orthocortex cells because they had cores of transversely viewed IFs surrounded by rings of obliquely viewed IFs (so-called "whorl" pattern), and local packing (i.e., packing between an IF and its immediate 6 neighbours) was close to hexagonal. What appeared to be multiple cores within these macrofibrils were common and often the apparent core, or cores, occurred close to the macrofibril edge rather than at its centre. Electron tomography results (below) confirmed these features to be artefactual. Many larger fused regions that had, at low magnification resembled paracortex, were instead clearly composed of closely packed roughly circular macrofibrils without any visible IMM. Compared to the wool orthocortex, elongation and distortion of macrofibrils into non-circular profiles were especially noticeable (Fig. 1B). In addition, many macrofibrils were unlike those found in wool, having a large core of pseudo-hexagonally-packed IFs surrounded by a thick coat of obliquely viewed filaments, similar to the periphery of an orthocortex macrofibril (Fig. 1C). Macrofibrils with this appearance do not fit into the traditional wool cell type descriptions, but have been recently observed in the hairs of deer antlers (Woods et al., 2011) and in the body hairs of a range of species (Thomas et al., 2012). We also found large less-well organised macrofibrils that appeared to be composed of patches of pseudo-hexagonally packed IFs oriented in different directions that did not resemble the expected projected pattern consistent with the double-twist architecture seen in other macrofibrils (Fig. 1D).

3.2. Simulated TEM sections from electron tomograms

Electron tomography data from thicker sections (150–300 nm) allowed us to use thin slices (~0.6 nm) viewed from many angles