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Visibilia ex invisibilibus: seeing at the nanoscale for improved preservation of parchment

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This paper describes the application of atomic force microscopy (AFM) for the imaging of collagen denaturation as observed in parchment. Parchment is prepared from processed animal skin and collagen is the main component. Large collections in national archives, libraries and religious institutions contain numerous documents written on parchment. Their preservation presents an unsolved problem for conservators. The main challenge is to assess the state of collagen and to detect what conservators refer to as the pre-gelatinised state, which can cause surface cracking resulting in a loss of text and can increase the vulnerability of parchment to aqueous cleaning agents. Atomic force microscopy (AFM) was first used within the Improved Damage Assessment of Parchment (IDAP) project, enabling the characterisation of the collagen structure within parchment at the nanoscale. Damage categories were also established based on the extent of the ordered collagen structure that was observed in the AFM images. This paper describes the work following the IDAP project, where morphological changes in the fibres due to both artificial and natural ageing were observed and linked to observations made by AFM. It also explores the merits and drawbacks of different approaches used for sample preparation and the possibility of using a portable AFM for imaging directly on the surface of documents. A case study on a manuscript from the 18th century is presented.

Introduction

Parchment was widely used in Europe as a writing medium from the 2nd century BC until the 18th century AD[1]. Notable examples include the Dead Sea Scrolls, which are the oldest known parchment documents[2], the Magna Carta and the Faddan More Psalter[3]. Current practice in British Parliament is to still use parchment as a writing medium for all Acts of Law. Parchment is a complex biological material obtained from processed animal skins, usually from calf, sheep or goats. It consists mostly of collagen Type I (95%) and other constituents are inorganic minerals and lipids[4,5]. During the manufacturing process, the epidermis and subcutaneous layer of the skin are removed, leaving the dermis layer. The remaining skin is subsequently treated by liming, scraping and drying under tension[6]. Although parchment has demonstrated its longevity as a bearer of our written history, it may still be prone to conservation issues, such as surface damage with loss of text, wrinkling and damage from unsuitable conservation treatments. All of these occur as a result of the ageing process of the parchments and therefore it has become apparent for scientists and conservators alike that the impact of such ageing processes on parchment needs more attention.

The structural and physico-chemical properties of parchment are directly linked to those of collagen. In most connective tissues, such as the skin from which parchment is derived, collagen exists in the form of large fibres or sheets, composed of smaller fibrils, which are themselves made of collagen molecules. A collagen molecule is composed of three polypeptide chains, with a left-handed twist, which combine together to form a right-handed triple helix[12]. Collagen molecules are staggered axially relative to their nearest neighbours, in order to form fibrils via an entropic reaction *in vivo*[13,14]. The external appearance of the collagen fibrils presents an annular periodicity (D-banding), which is conserved across all tissue types and species. The D-banding periodicity in skin is approximately 65 nm and in tendons is about 67 nm. Collagen molecule fibrils are subjected to various post-translational modifications and glycation whilst present in the body. These various chemical modifications change both the intermolecular and interfibrillar degree of crosslinking in collagen, which results in greater thermal stability and physical properties.

However, the processes of parchment manufacture and subsequent ageing lead to irreversible changes in the collagen structure and degree of crosslinking[15-17]. This results in a change from an intact fibrillar arrangement of collagen molecules in a triple helix to a random conformation and a more disordered system. Detection of this change in collagen poses a challenge for conservators. Current practice involves the heating of fibres in water using hot-stage microscopy and then measuring the shrinkage temperature of the fibres (*Ts*); the lower the temperature, the more gelatinised the sample[18-22].

Two EU interdisciplinary projects, Microanalysis of Parchment (MAP)[4] and Improved Damage Assessment of Parchment (IDAP)[7], have focused on characterising a large number of both

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accelerated aged and historical parchment samples. The two projects used a range of advanced analytical techniques, but it was in the IDAP project that AFM was first used for the damage assessment of parchment. Skin collagen displays a characteristic banded fibril structure on the submicron scale when visualised by atomic force microscopy (AFM). Changes in the banded fibril structure were considered as useful damage markers[8,9]. Damage assessment by AFM has also been used in the medical area for nano-scale structural characterisation of: (a) natural heart valves and bioprosthetic heart valve material under slightly altered physiological conditions[10]; and (b) collagen-based tissues (including dentin, tendon, cartilage, skin, fascia, vocal cords and cornea). Some studies have also been undertaken on bone[11].

In the IDAP project, some correlation was observed between the shrinkage temperature and the extent of the ordered collagen structure as recorded by AFM[23]. Studies were initially performed on a model rat tail to study the effect of collagen denaturation. On heating the samples to 70°C for 15 min, partially intact banding and a fibril structure was observed. In other locations, the characteristic structure for native collagen was not present and the surface developed a 'glass-like appearance'[23,24]. A Fourier transform-based algorithm was used to quantify changes in the AFM images and this provided a measure of the extent of intact collagen remaining in the parchment[23,25].

Following the IDAP project[26,27], attention was focused on changes in the morphology of fibres and a damage assessment protocol was established based on shape differences[28-30]. One of the aims of this paper is to link these observations with AFM imaging performed on fibres with varying morphology[31]. Various methods of sample preparation are reviewed. A second aim is to present a case study where AFM imaging is performed directly on the surface of a historical document from the 18th century (National Archives, UK).

Materials and methods

Samples

The parchment samples analysed in this study included two new calfskin hides bought from different tanneries (Z H De Groot in The Netherlands and Pergamena in the USA), dehaired in $Ca(OH)_2$ with the addition of sodium sulphide to speed up the dehairing process, and a historical parchment document dating back to the reign of King George the Third (18th century) from The National Archives, UK (Kew, London) (see Figure 1). The document is folded in three parts and written on the recto with carbon ink. Viewing with the naked eye, it appears to be in a good state of conservation and the surface is covered by a 'patina' (layer of superficial dirt).

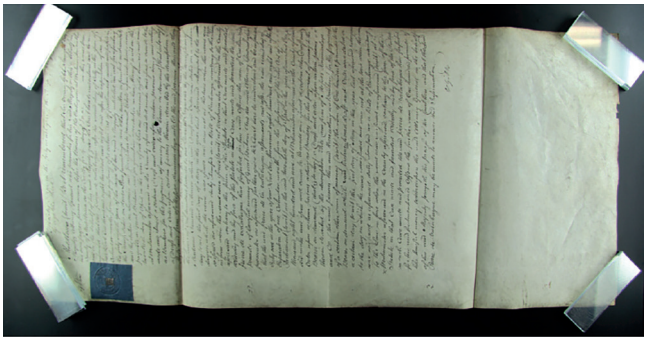

Figure 1. Image of the recto of the 18th century document written on parchment provided by The National Archives UK for this study

No information on previous conservation treatments or storage conditions was provided.

Atomic force microscopy (AFM)

The imaging of fibres extracted from the parchment was performed using an XE-100 (Park System, South Korea) and a Dimension 3100 (Bruker, USA) in contact mode with MSNL-10 probes (Bruker, USA).

Generally, the imaging scan rate was kept between 0.5 Hz and 2 Hz, depending on the sample surface and image scan size. Set-point and feedback gains were kept as small as possible (to avoid damaging the sample and to reduce the noise) and were adjusted accordingly for each image. A Nanosurf EasyScan 2 AFM in dynamic mode was also used to collect images directly on the historical parchment document surface (Figure 1) using nanosensors PPP-NCLR-20 probes, with a spring constant of 48 N/m and a resonance frequency of 190 kHz.

Scanning electron microscopy (SEM)

SEM imaging of the parchment was performed using a Philips XL30 FEG SEM (FEI, Eindhoven, Netherlands) with an accelerating voltage of 5 keV and a spot size of 3. A small piece of approximately 0.5 cm × 0.5 cm was sampled and fixed on a standard SEM Al stub (Agar Scientific, UK) using carbon adhesive tabs or Araldite® glues and left to air dry overnight. Samples were then gold-palladium sputter coated at 20 mA and 1.25 kV for 90 s (Palaron E5000 sputter coater).

Direct surface measurements

1. Proof of concept
   Before performing any direct measurement on parchment, which presents a very heterogeneous surface, a portable Nanosurf Easyscan 2 AFM operating in intermittent contact mode was used to image the varnished surface of a violin (see Figure 2). The proof of concept proved that it is possible to perform successful imaging on a rigid smooth surface and demonstrated the possibility of direct imaging with no sample preparation.
2. Imaging parchments
   A piece of modern parchment of approximately 5 mm × 5 mm

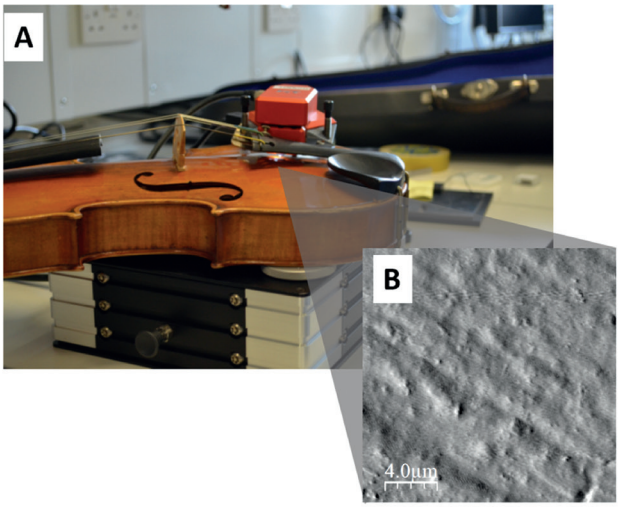

Figure 2. Nanosurf Easyscan 2 AFM head on a violin placed on an anti-vibration table (A) and AFM image of the surface of the violin (B)

was cut and mounted (with the flesh side up) on a microscope slide with double-sided tape and imaged using the Dimension 3100 in contact mode. Additionally, a portable Nanosurf Easyscan 2 AFM operating in intermittent contact mode was also placed directly on the 18th century parchment document. Finally, SEM imaging was finally performed on a fragment of all parchments to corroborate the AFM imaging with SEM analysis.

Histological preparation of parchment

Cross-sections were prepared using a cryotome (Bright Instruments Co Ltd, Huntingdon, Cambridgeshire, UK). A sample (5 mm × 5 mm) was cut from the original parchment sample and embedded in an OCT embedding matrix (Cellpath plc, Newtown, Powys, Wales, UK), a water-soluble glycol that quickly freezes below –10°C. Cross-sections with a thickness of 10 µm were cut perpendicular to the head-tail direction of the parchment with a disposable stainless steel knife. The obtained cross-sections were mounted on microscopical slides and stored at –80°C for 24 h. After acclimatisation to ambient conditions (25±5°C and 30±20% relative humidity (RH)), cross-sections were visually inspected at 200× magnification with an Olympus BX60 (Olympus Europe, Hamburg, Germany) and with a Dimension 3100 (Bruker, USA) AFM in contact mode.

Sampling of fibres

Fibres were scraped with a sharp scalpel from the flesh side of the parchment. The scraping was performed over an area of approximately 5 mm by 5 mm and removed about 20 µm from the sample thickness; the accumulated fibres were centred on the glass slide with a scalpel, a few drops of distilled water were added and the fibres were left to physisorb onto the glass slide. The samples were dried for at least 24 h at ambient conditions (25±5°C and 30±10% RH) before imaging with AFM (Dimension 3100) in contact mode.

Microsampling

The sampling protocol proposed by Dr Kathleen Mühlen Axelsson and Professor René Larsen, formerly from the School of Conservation in Copenhagen (The Royal Danish Academy of Fine Arts, Schools of Architecture, Design and Conservation), for the microscopic assessment of collagen fibres[22,28] was slightly modified. A small piece of parchment (approximately 1 mm^2) was cut and placed in the depression of a concave microscope glass slide. A few drops of double distilled water were added and the sample was left soaking for 10 min (Figure 3, panel A). After this wetting time, the grain side was separated from the flesh side (Figure 3, panel B) and bundles of fibres were pulled out from the flesh side with the help of a sharp needle and tweezers (Figure 3, panel C). The bundles of fibres were then placed on a clean, flat microscope slide in excess water, separated and left to physisorb onto a glass slide overnight at standard conditions (Figure 3, panel D).

Results

Application of AFM on parchment within the IDAP project

Figure 4 shows AFM deflection images of an unaged parchment sample prepared using different methods and compared to an untreated rat tail tendon (Panel A). Panel B shows an AFM deflection image of a parchment fibre scraped from the flesh side and physisorbed onto a glass slide, panel C shows an AFM image

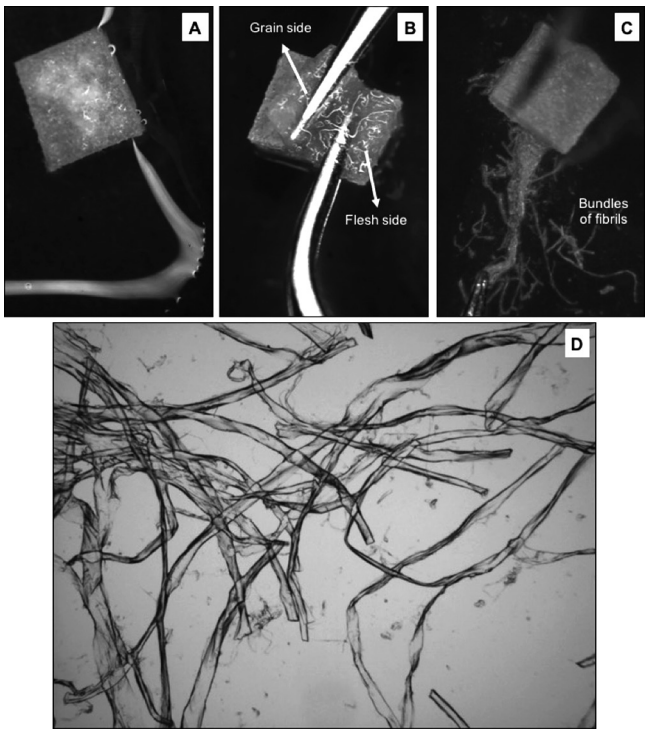

Figure 3. Microsampling for the extraction of collagen fibres from parchment: (A) soaking of parchment in water; (B) separation of grain side from the flesh side with the help of sharp tweezers; (C) pulling out of bundles of fibres from the flesh side; (D) extracted bundles of collagen fibres, still in water, observed under an optical microscope at a magnification of 100×

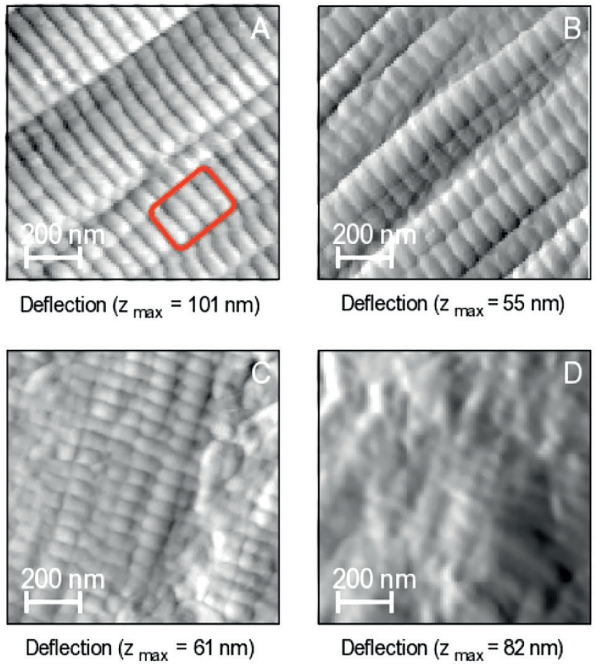

Figure 4. AFM deflection images of an untreated rat tail tendon (panel A) and unaged parchment sample (B to D) prepared using three different methods. Panel B shows an AFM image of parchment fibres scraped from the flesh side, panel C shows an AFM image recorded directly on the flesh side and panel D shows an AFM image of a cross-section prepared using a cryotome. The red rectangle highlights the D-banding periodicity. The images are from de Groot's PhD thesis[23]

recorded directly on the flesh side and panel D shows an image of a cross-section prepared using a cryotome. For measurements performed directly on the parchment surface or on scraped fibres, it was possible to observe the typical collagen banded fibril structure, with better results obtained by scraping fibres from the parchment surface. This structure was less clearly present in the AFM images of the cross-sections.

Improving the cross-section preparation might allow better results to be obtained; however, this approach would require a larger amount of sample, compared to other approaches, and result in a partial alteration of the samples[32] and it would not be possible to reuse the sample for further measurements.

Although good results were obtained when imaging directly on the parchment surface or scraped fibres, several drawbacks can be highlighted for these methods. Acquiring good AFM topographical images directly on the parchment surface can be challenging and time-consuming. Parchments in fact display uneven surfaces (due to different manufacturing processes, finishings applied and state of conservation) with an overall roughness at the meso-nanoscale, which might not be compatible with the z-range of commercial AFMs (12 µm for the Park XE 100 and 6 µm for the Dimension 3100).

As an example of how complex a parchment surface can be, representative FEG-SEM images for the new parchment and for the historical document are shown. Figure 5 panel A shows the flesh side of the new parchment; its surface appears uneven and it is covered by a matrix of sheared fibres that were not removed during the finishing of the parchment (the final stage of the parchment manufacture process)[15]. In panel B, a SEM image of the recto of the historical document is presented; in this image, one can observe that the parchment surface is smooth, with a visible fibre network, and it is covered by deposits (both biological and mineral, panel C).

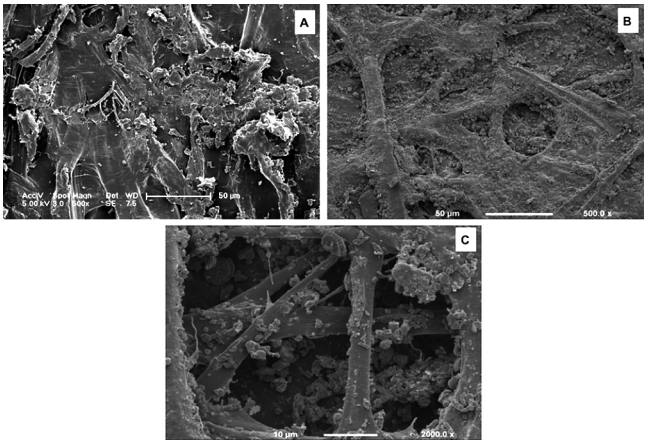

Figure 5. FEG-SEM image of the flesh side of the new parchment sample brought from Pergamena (panel A) and of the recto of the historical parchment (panels B and C)

Experimental work in the IDAP project was mainly performed on fibres scraped from the surface as this provided an optimum image. However, further refinements are needed in terms of sample preparation, especially for the analysis of historical parchment samples. For some of these, it is not always possible to distinguish between the grain and the flesh side and consequently the scraping of the fibres should be performed on both sides of the parchment, which may not be allowed and could have an impact on the object. Moreover, the action of scraping itself might damage the sampled fibres.

Combining the microscopic assessment of collagen fibres with AFM

In more recent AFM microscopes, the improvement in the optical set-up has made easier the possibility to track both the AFM probe and the sample surface at the same time. This is a significant technical improvement as it allows the probe to be positioned on specific areas of interest. This also makes possible the combination of the microscopic assessment of collagen fibres with AFM measurements. The modified protocol to the microsampling was used to prepare samples for AFM measurements by extracting bundles of collagen fibres from new, artificially-aged and historical parchment. Subsequently, as for the microscopic assessment[22,28], three different locations containing between 10-20 fibres were digitally photographed under an optical microscope and specific fibres upon which to perform AFM were selected. These areas were then relocated under the AFM.

Figure 6 shows an example for the new parchment bought from Pergamena. In panel A, bundles of collagen fibres are shown at a magnification of 100× and the red dots indicate the areas that were analysed by AFM; in panel B, an AFM image acquired on a specific fibre is shown. On this image, one can observe individual and well separated collagen fibres; according to the microscopic assessment of the collagen fibres, it is possible to recognise some of the characteristic features of damaged fibres. Flat fibres represent the majority of the fibre population under investigation, nevertheless one can also see the presence of split fibres and several 'pearls on a string' structures[28]. The application of AFM allows the user to increase the magnification of the area analysed and to see the ultrastructure of collagen fibres. In Figure 6, panel B, the AFM image was acquired on a fibre with an intact-like appearance. In the AFM images, it is possible to observe collagen fibrils aligned in register, which is one of the main characteristics of collagen scaffold morphology in 'undamaged' parchments, as reported by de Groot and elsewhere[23,33,34]. Additionally, individual fibrils display the characteristic D-banding periodicity that is expected from native collagen.

AFM imaging can be performed on all the different damaged fibre breakdown morphologies and this has allowed the damage detected to be correlated at the microscopic level with damage at the nanoscale[31]. However, the characterisation of damage morphologies, such as the twist and pearls or the 'butterfly' structures, is particularly challenging, especially when imaging in contact or intermittent contact mode. This is due to the nature of

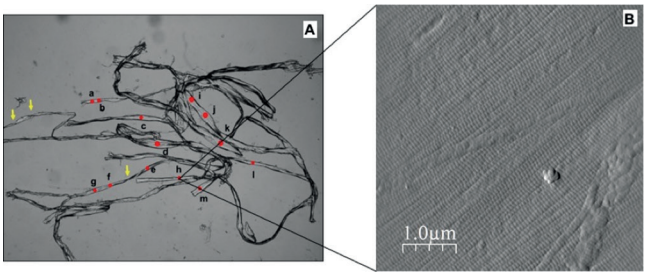

Figure 6. Panel A: Bundles of collagen fibres extracted from new parchment hide. The red dots indicate the area analysed by AFM, whilst the yellow arrows highlight the twist and pearls structure. The image was acquired with a light optical microscope at a magnification of 100×. Panel B: AFM deflection image acquired on a collagen fibre showing an intact-like appearance. The collagen scaffold is characterised by fibrils aligned in register with a visible D-banding

the sample in the twist region. The abrupt changes in the surface feature height cannot be easily handled by AFM and this leads to a certain degree of image distortion (the presence of lines or repeated geometric patterns across the image) or failure detection (due, for example, to tip convolution or differences in the sample topography that are too high). An example is reported in Figure 7, where two AFM images of a twist region are presented. In Panel B, one can observe the collagen structure on only half of the image, as the other half cannot be detected. By repositioning the probe (black rectangular) and reducing the scan size, it is now possible to observe the collagen structure in the twist region. In panel C, one can observe the presence of intact banded collagen fibrils (star), as well as the deteriorated structure (red arrows). Whilst thermal ageing induces the dehydration of the sample and this results in a shrinkage of the collagen structure[23,31,35], accelerated ageing involving temperature and humidity leads to different alterations of the structure. The latter induces the swelling of the fibrils and a pre-gelatinised state characterised by the loss of structure and formation of spherical aggregates or bubble-like features[36].

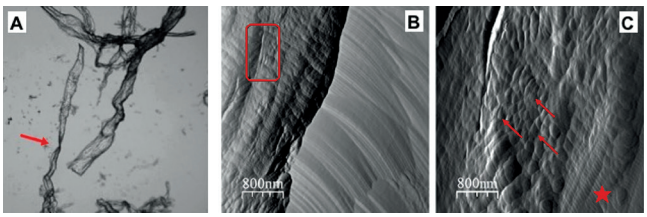

Figure 7. Optical (magnification of 100×) and AFM images of a twist and pearl structure. Panel (A) shows bundles of collagen fibres and the twist region that was analysed with AFM. Panel (B) is a first AFM image of the twist region, whilst panel C shows the corresponding AFM image acquired in the area highlighted by the red rectangle in panel (B). These images were recorded from extracted collagen fibres from a new parchment hide, aged at 80°C and 60% RH for 28 days, used in a collaboration project with Dr Kathleen Mühlen Axelsson and are also reported in her PhD thesis (original name sample A_p_Humox)

In Figure 8, an example of the correlation between damaged fibres and deterioration at the nanoscale is presented for the historical parchment sample. In panel A, one can observe bundles of collagen fibres under an optical microscope at a magnification of 100×. All the fibres exhibit damaged morphological features: one can observe flat, split fibres and that the amount of flat fibres and

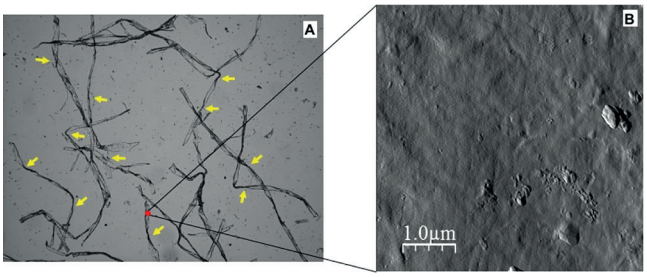

Figure 8. Panel A: Bundles of collagen fibres extracted from the historical parchment for The Kew National Archives (London, UK). The image was acquired with a light optical microscope at a magnification of 100× and the yellow arrows highlight the twist and pearl structure. Panel B: AFM deflection image acquired on a collagen fibre showing a damaged morphological appearance. The collagen scaffold in this location is characterised by the complete loss of its characteristic features

'pearls on a string' structure has significantly increased, based on a qualitative assessment, if compared to the new parchment. The AFM images acquired on a damaged fibre (panel B) show that the typical collagen scaffold topology is completely lost.

This kind of degradation has been previously reported in the IDAP project, where four damage categories were created based on the progressive loss of the banded fibril structure[24].

However, historical parchments can still present an intact collagen scaffold. An example is given in Figure 9, where AFM images acquired on randomly selected fibres from the historical parchment are shown. Looking at these different AFM images, it is possible to notice the presence of alterations of the collagen structure. These variations from the native structure can affect a single fibril or the entire area scanned and have also been observed on series of samples ranging from the 'model' collagen sample (rat tail tendon) to modern parchment exposed to acetic acid vapour or inorganic pollutants and historical parchments within the MEMORI project[37]. One can observe, for example, that the collagen scaffold is affected by a general disruption of its well-organised structure, also showing a shrinkage of fibrils along their alignment direction resulting in the wrinkling of the surface (Panels C and D). Moreover, it is also possible to observe the presence of deposit on the surface; these can be either mineral deposits or extraneous materials that were deposited on the fibres, perhaps during the sample preparation. The presence of these and other alterations of the collagen scaffold were further characterised and are currently used to create a new protocol to calculate the extent of degradation in parchment at the nanoscale[31].

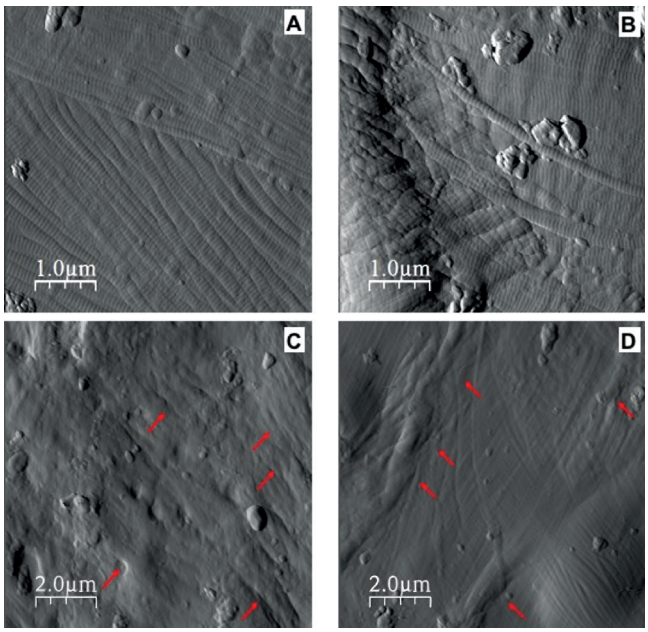

Figure 9. Representative AFM deflection images for the historical parchment sample, showing intact banded collagen fibril aligned in register (panel A), disruption of the well-organised collagen structure and the wrinkling of the surface (red arrows)

Although the combination of the microscopic assessment of collagen fibres with AFM still requires the original artefacts to be sampled, there are several advantages of adopting such an approach. These include the possibility of linking AFM results with information from other techniques (and more familiar to conservators). Moreover, it would be possible to have a more complete understanding of the state of degradation of the parchment artefacts at the level with which physical deterioration starts to occur as a result of chemical and other breakdown factors.

Towards the application of portable AFM (Nanosurf Easyscan 2) on parchment

With the advances in AFM instrumentation, small portable AFMs (such as Nanosurf EasyScan 2) are available on the market. These instruments present several advantages that make them suitable to be used by conservators as well. Part of the set-up of the instrument, such as laser alignment on the tip, is done automatically. Compared to Park XE-100 and Dimension 3100, which do not have the space in the sampling area to place a full document, the Nanosurf Easyscan 2 AFM can be used directly on the objects requiring examination (Figure 10, panel A). The use of soft plastic supports (the red arrows in panel B) protects the document from damage and allows the AFM to be placed directly on the parchment surface and to be moved without damaging the artefact. There are two small optical lenses at the top of the instrument that allow side-on viewing of the approach of the tip to the surface and the other allows viewing, at a low magnification, of the tip on the sample. The z-range for this instrument is, however, still limited to 14 µm.

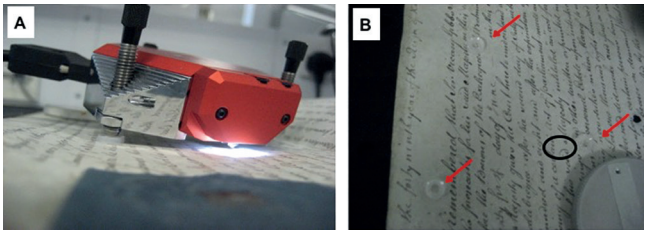

Figure 10. Panel A shows the set-up of the EasyScan with the AFM placed directly on the parchment document using soft plastic supports to protect the document from damage (highlighted with red arrows in panel B). The black circle in panel B shows the area imaged with AFM

Compared to the AFM images acquired from extracted collagen fibres from the same sample, direct imaging appears less clear. Difficulties are related, as previously mentioned, to the nature of the parchment surface, which, as seen in the SEM images (Figure 5), shows differences in the topography of the sample with the presence of a fibre network and mineral deposits. One of the areas raster-scanned with AFM is highlighted by a black circle in Figure 10, panel B; a handheld digital imaging accessory (http://www.supereyes.cc) was used to view the surface of the parchment. In this location, the parchment surface is characterised by the presence of a pre-gelatinised layer (Figure 11)[38]. This is uneven, with evidence of holes in the layer showing the underlying white layer, which results in tip convolutions and a loss of contact between the probe and the sample. The corresponding AFM images acquired in this area show the complete absence of the typical collagen structure.

The presence of this pre-gelatinised and underlying carbonate layer can cover the collagen scaffold, precluding its characterisation. There may be occasional losses that may permit viewing the collagen scaffold below. Evidence of the pre-gelatinised layer was also confirmed by Rabin *et al*[39] where measurements were performed directly on the surface of both new, artificially-aged and naturally-aged parchments. This revealed that damage first occurred on the parchment surface, leading to the formation of a glassy layer or pre-gelatinised layer. This film possibly has a protective function towards the bulk of the parchment, shielding the collagen structure lying underneath from further deterioration. Thus, in order to reveal the state of collagen in parchment, this upper surface layer would have to be removed, with an impact on the document.

In view of the difficulties of acquiring images directly on the

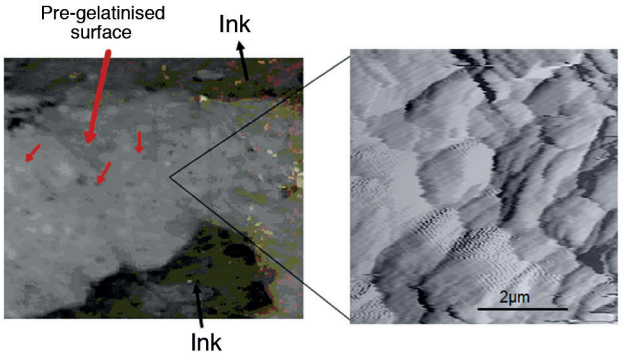

Figure 11. The handheld digital image USB microscope (Supereyes B008) shows the parchment surface in the area raster-scanned with AFM: the related image (on the left, magnification 50×) shows an area on the parchment between two letters, characterised by a pre-gelatinised state. This is confirmed by the related AFM image (on the right, scan size 5 µm) where the collagen scaffold is characterised by a complete loss of its typical structure, similar to Figure 7(c)

sample surface, in the present study it was possible to observe some correlation between the damaged structure, as seen in the images collected from extracted fibres and those directly on the parchment surface. In Figure 12, for example, in the image on the left (from extracted fibres) one can observe that the entire surface is characterised by the folding (or wrinkling as previously defined) of the collagen scaffold. This type of damage can also be observed in one of the images collected from the sample surface. In Figure 12 on the right, the red circle highlights the presence of folding of the surface.

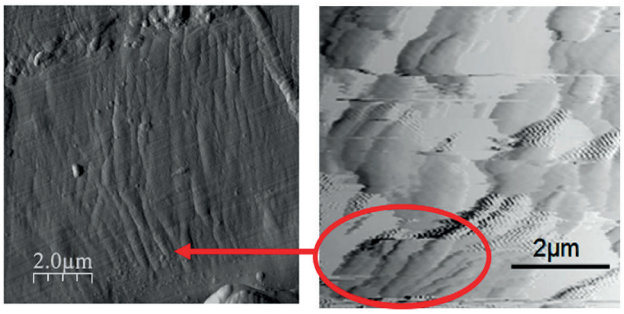

Figure 12. Example of damage (folding or wrinkling) as seen in the image recorded from an extracted fibre (on the left) and in the image directly recorded on the parchment surface of a similar dimension and showing a similar folding pattern (on the right, highlighted by the red circle, scan size 5 µm). The AFM image also shows problems with a loss of contact between the tip and sample in certain areas

Conclusions

In this study, we have demonstrated that AFM is capable of characterising the degree of degradation of collagen in historical parchments. The approach is non-destructive and is minimally non-invasive in that fibre samples are extracted. We have demonstrated that fibre morphology does reflect the state of damage in the collagen fibres; damage described in terms of 'twist' and 'pearl' showed changes in the ordered structure of collagen with fibril swelling, surface wrinkling and localised areas of surface gelatinisation. It has also been shown that the application of portable AFM for the characterisation of parchment documents is possible; however, the

information it provides might be limited due to the presence of a gelatinised surface layer. The damaged surface, however, does not necessarily reflect the degradation state of the bulk, as previously reported[38]. In the AFM images of fibres extracted from the historical parchment, intact portions of ordered collagen fibrils were observed in some of the analysed locations. The case study described here has shown that AFM does provide information on the state of the surface in a non-invasive way. There are also indications of structures in the preliminary AFM images recorded on the surface that were observed in both SEM and AFM images from extracted fibres. However, further work is required to develop a database of such images with a comparison with those obtained from extracted fibres. This would provide a useful database that would enable the AFM to be used to study the surfaces of collagen-based documents in museum and archival collections.

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