Collagen: levels of structure and alignment

Collagen structure and function

Collagen is the most abundant protein in the human body, accounting for around 30% of the total amount of protein. Collagen is a structural support for most tissues in the body as the extracellular matrix, and is particularly abundant in connective tissue. Skin, for example, is around 75% collagen, and collagen therefore has a vital role in many processes such as wound healing. Collagen production or mineralisation are the basis for the formation of cartilage, tendons or bones. Cells in all the other tissues of the body are also surrounded by finer collagen structures through the extracellular matrix, so collagen has a vital role also in cell proliferation, migration and differentiation.

Collagen is a family of around 20 related proteins, which form triple helices through three polypeptide chains winding around each other in a rope-like structure. These triple-stranded nanometre-sized protofibrils can bind together to form different types of higher level structures. The tough collagen fibres formed by Type 1 collagen give tendons or ligaments their high tensile strength, but other types of collagen form smaller, or more branched structures in the extracellular matrix. Collagen is involved in the structure or function of many tissues, so there are many diseases associated with collagen malfunction. A genetic defect called Alport syndrome affecting Type IV collagen, for example, causes malfunction of the glomeruli in the kidneys, as well as eye and ear problems, and generally leads to kidney failure.

The structure of Type 1 collagen fibrils is sketched schematically in Figure 1. Three polypeptide chains wind together to form a stiff helical structure. These collagen molecules then align along the helix axis and group as a bundle to form the collagen fibrils. These collagen fibrils can also align laterally to form bundles on a higher order of structure and make up the tough micron-sized collagen fibres found in ligaments. A characteristic feature of the collagen fibrils is their banded structure. The diameter of the fibril changes slightly along the length, with a highly reproducible D-band repeat of approximately 67nm.

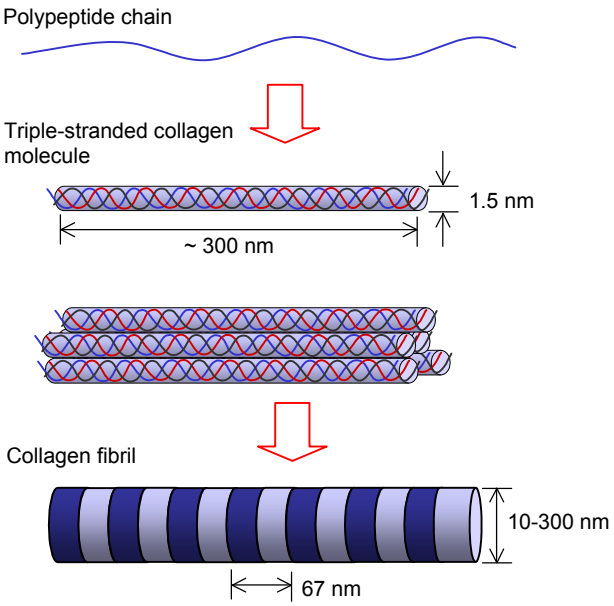


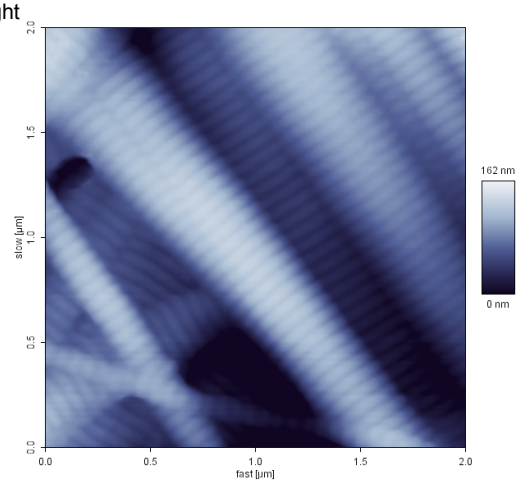
Fig 1. Schematic diagram of Type I collagen fibril structure. Helical collagen molecules form from three polypeptide chains, and these associate laterally to form collagen fibrils with a characteristic banded structure.

Aside from its natural function in the body, collagen has also been used in technical and medical applications as a model physiological surface for cell culture, implant biocompatibility and directed cell growth.

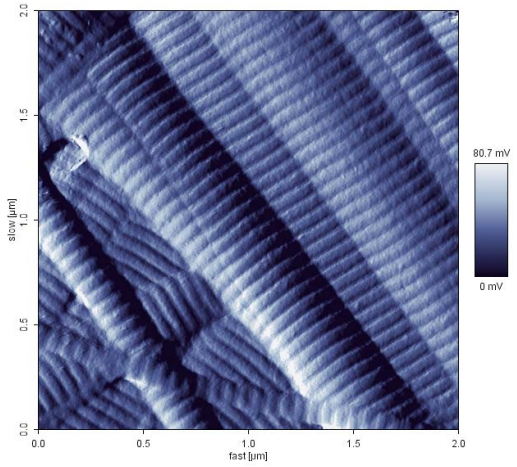
Naturally formed collagen tissue

The classic banded fibril structures can be seen clearly in rat tail collagen, which demonstrates the high degree of structure and regularity possible in collagen fibres. Figure 2 shows height and vertical deflection images of rat tail collagen fibres, for a 2 x 2 micron scan area. In this image, many of the fibres are lying parallel to each other, although a few are crossed over in the lower left hand corner. The cross-section image below shows a section along the central fibre axis. The red markers are 1345 nm apart, which corresponds to 20 D-band repeats. This gives a value for this sample of 67.3 nm for the repeat period. Changes in the repeat period due to various mutations can be important to measure accurately.

Height



Vertical deflection



Height cross-section

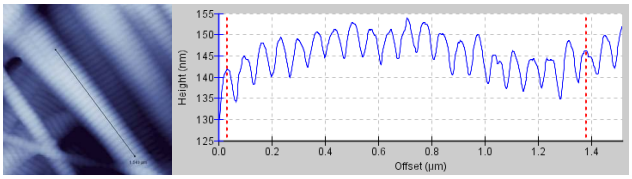
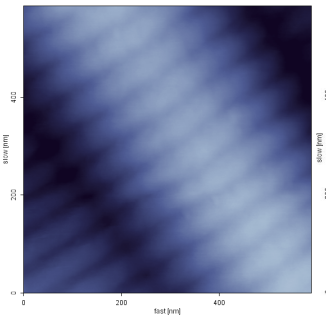


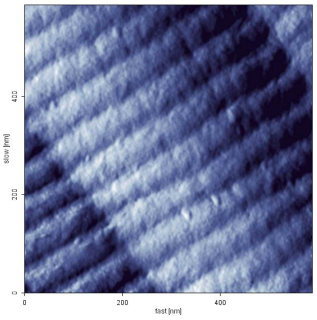
Fig 2. Height and vertical deflection images of a 2 x 2 micron scan area on a rat tail collagen sample. The cross-section shows 20 repeats of the D-banding marked with red lines, giving and average period of 67.3 nm.

Figure 3 shows smaller (600 x 600 nm) area scans of the same sample, again height and vertical deflection signals, to show the higher order structure that is visible. The third image is part of the height scan after it has been high-pass filtered to remove the background curvature of the fibre. This shows the smaller features more clearly, and the cross-section image shows the spacing of the small structures on the surface. The height scale for the cross-section is not absolute, since the background fibre curvature has been removed. The lateral size of the features is around 7 nm. Example features are marked with red lines with a separation of 6.7 nm.

Height



Vertical deflection



Height cross-section (background curvature removed)

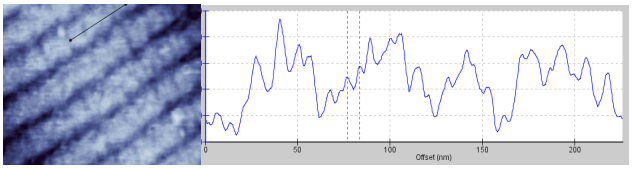


Fig 3. Height and vertical deflection images of a 600 x 600 nm area of a rat collagen fibril. Besides the transverse banding, there is also structure visible across the fibre, as shown in the cross-section in the lower part.

Deposited collagen fibrils – substrates for bone cell studies

Isolated collagen fibrils from a bovine tendon sample (deposited at low density on APTES-coated glass) are shown in Figure 4. The images are courtesy of Prof. M. Horton, Bone and Mineral Center of University College London and the London Centre for Nanotechnology. The optical image (top) shows the AFM cantilever and the larger collagen fibrils are also visible using optical phase contrast. The two large AFM images show height and vertical deflection signals for a 100 x 100 micron scan area. Most of the fibrils form smoothly curving structures. A smaller (5 x 5 micron) scan is also shown as a 3D height image, where the fibril periodicity can be seen.

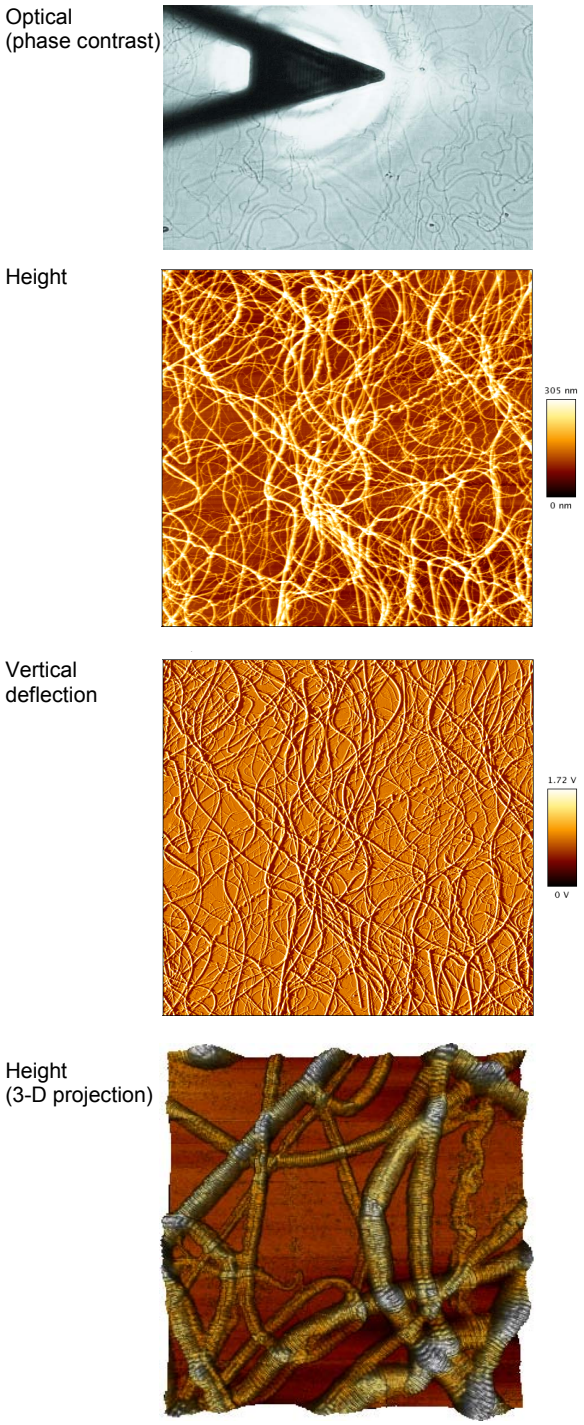


Fig 4. Bovine tendon collagen fibrils on APTES-glass, images courtesy of Prof. M. Horton, Bone and Mineral Center UCL and London Centre for Nanotechnology. Optical phase contrast (top), 100 x 100 micron scan area height and deflection AFM images (centre), and 5 x 5 micron 3D height image (bottom).

Collagen structures within natural bone

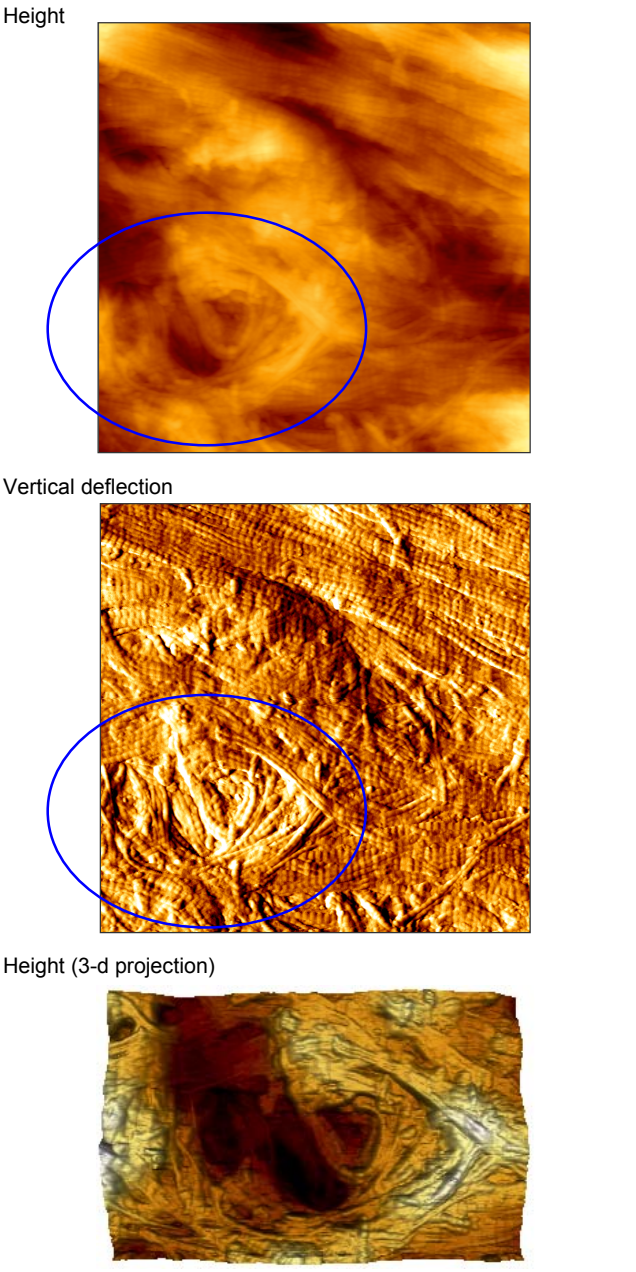


Fig 5. Bone collagen structure revealed by osteoclasts removing collagen and minerals from a slice of cortical bone. Images courtesy of Bozec and Horton [2], UCL and London Centre for Nan technology. 4 x 4 micron scan area for both large AFM images (521 nm z-range height, 300mV z-range deflection image). 3-D height projection zoom into osteocyte lacuna.

Despite the apparently stability of bone, in fact the bone in the skeleton of a living human is constantly being remodelled. Osteoblasts and osteoclasts are cells that are responsible for the formation and resorption of bone, and together they maintain a dynamic equilibrium in the skeleton of healthy adults [1,2]. Collagen has a vital role in these processes, since it forms the structural protein framework of bone, and also has a role in signalling between the osteoblasts and osteoclasts.

Figure 5 shows AFM images of a slice of cortical bone where the bone collagen structure has been revealed by osteoclasts removing collagen and minerals. Laterally associated collagen fibres with clear D-banding are seen in the topography and error images. An osteocyte lacuna is seen within the bone (ringed). Images courtesy of Bozec and Horton, UCL and London Centre for Nanotechnology [2]. The upper AFM images show a 4 x 4 micron area with a 521 nm z-range for the height image and a 300 mV z-range for the deflection image. The lower image shows a 3-D height projection zoom into the osteocyte lacuna, the resorption pit formed by removal of collagen and minerals. Using the AFM, the fibril arrangement, periodicity and diameter can be measured statistically, giving the possibility to study subtle differences between healthy or diseased tissues [2].

Deposited collagen film for implants

Various methods have been developed to deposit collagen onto materials to form biocompatible surfaces, or for directed cell growth. The collagen can be adsorbed relatively straightforwardly to surfaces such as glass, but it is more difficult to attach collagen stably to synthetic materials such as silicone. These kinds of composite structures can be used to promote wound healing and improve biocompatibility of materials for implants, particularly vascular grafts [3,4]. The crosslinked collagen can be treated with molecules such as heparin to reduce thrombosis activation, or fibroblast growth factor to promote endothelial cell seeding [3].

The images in Figure 6 show a 10 x 10 micron overview of a sample of collagen-coated silicone film (sample courtesy of M. J. B. Wissink, University of Twente, The Netherlands). The sample was imaged under phosphate buffered saline (PBS) solution (height and amplitude images for intermittent contact mode in liquid). The larger collagen fibrils can be seen lying in a smoother matrix. The collagen is very soft in liquid, since it contains a high proportion of water.

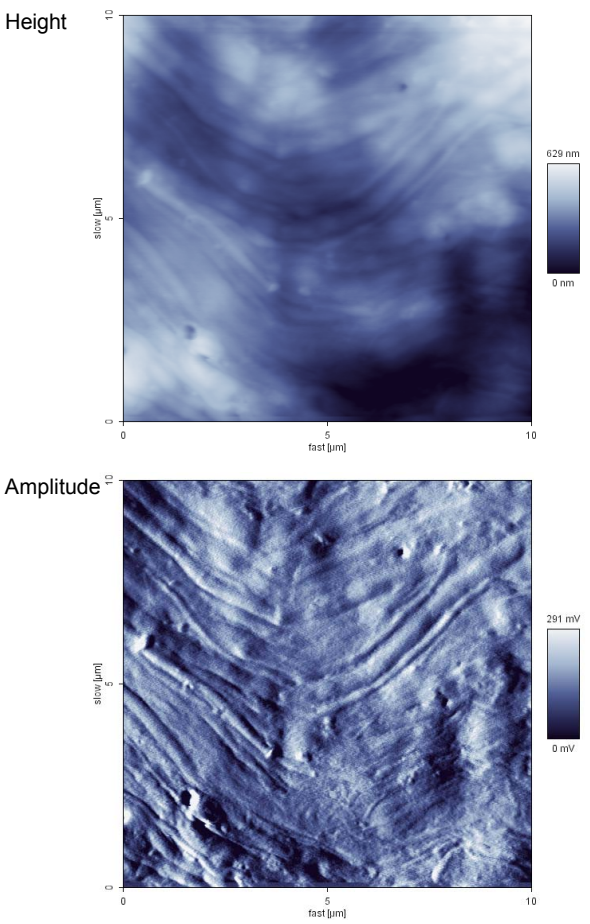


Fig 6. Intermittent contact mode image in liquid (10 x 10 microns) of a collagen-coated silicone film. Sample courtesy of M. J. B. Wissink, University of Twente, The Netherlands.

On smaller images, the details of the D-banding structure can be seen. Height, amplitude and phase images of the same sample are shown in Figure 7 for a smaller scan area (2 x 2 microns). The D-banding can be seen in all three images for the collagen fibres, which are lying diagonally across the image in the background matrix. Although the whole collagen layer is very soft, the 67 nm banding can be seen clearly.

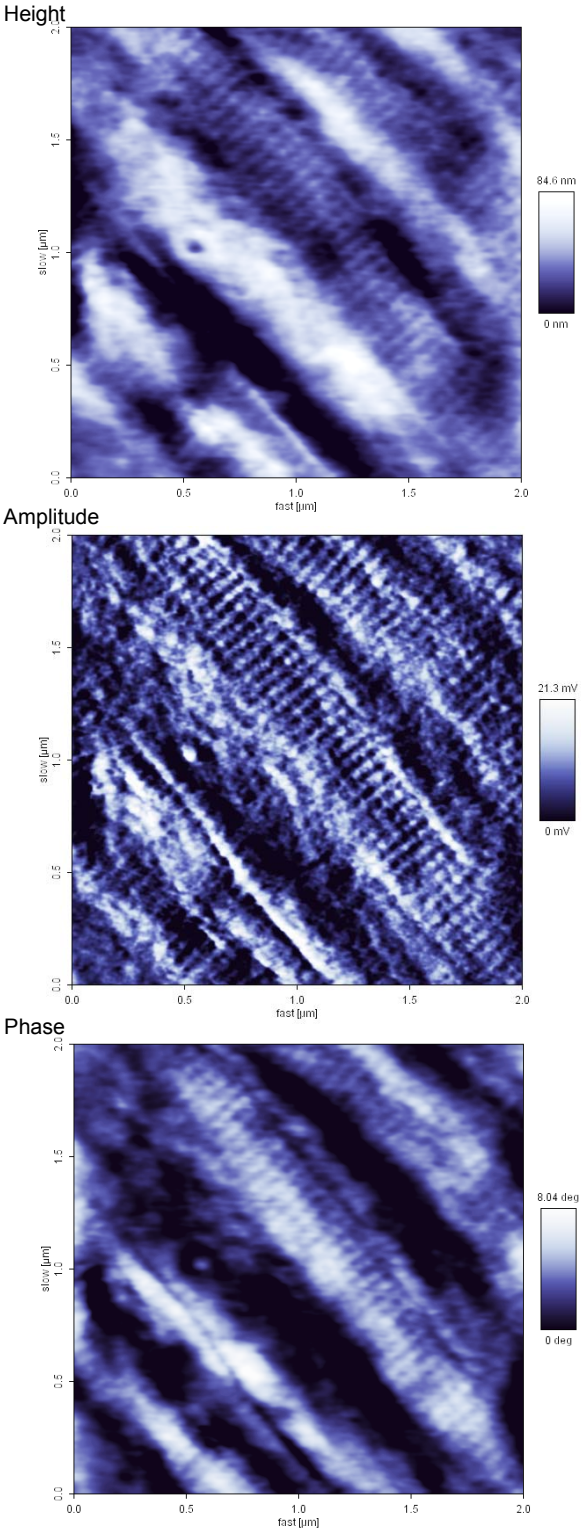


Fig 8. 2 x 2 micron intermittent contact mode scan of collagen-coated silicone in buffer (sample courtesy of M.J.B. Wissink, University of Twente, The Netherlands).

Aligned collagen layers and directed cell growth

Collagen molecules can be assembled and adsorbed onto mica supports to coat the surface evenly in a thin (~ 3 nm) flat layer [5]. Around five individual collagen molecules associate form microfibrils, with a lateral size of around 3-5 nm. A monolayer of these microfibrils can then be adsorbed to the surface to form a nanostructured, biologically active surface. These microfibrils are also likely to be an intermediate stage in the formation of the larger collagen fibres seen in natural tissue.

The first level of organisation is to align the collagen fibrils so that there is an overall orientation in the surface layer, which can be achieved through adsorbing under conditions of hydrodynamic flow [5]. Within a certain time after deposition, the orientation can also be manipulated using the AFM tip [6]. Figure 9 shows a 750 x 750 nm intermittent contact mode height image of unfixed collagen in buffer (z-range 5.5 nm). The sample is courtesy of A. Taubenberger, D.J. Müller Cellular Machines group, Technical University Dresden. The fibres are all oriented in the same direction, and the 67 nm D-repeat can be seen on the individual fibres. In this case there is no large-scale alignment of the D-repeat between adjacent fibrils.

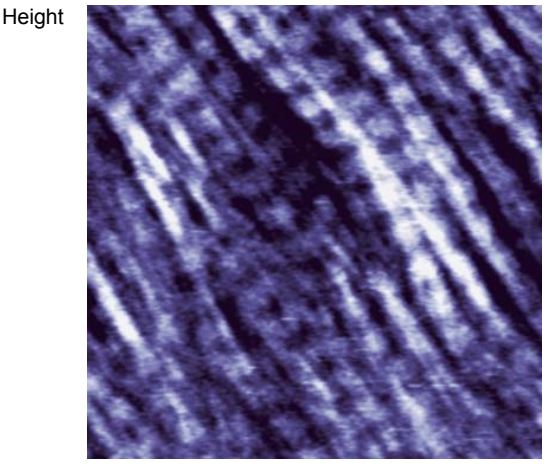
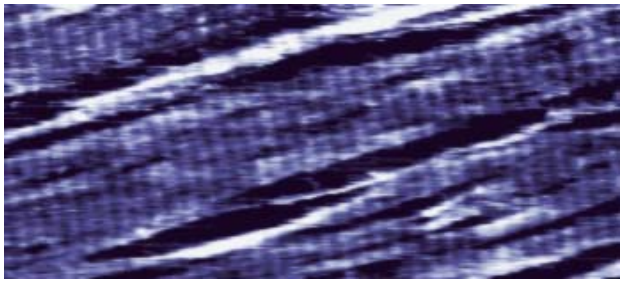


Fig 9. Intermittent contact mode image of unfixed collagen in buffer (750 x 750 nm, z-range 5.5 nm). Sample courtesy of A. Taubenberger, D.J. Müller Cellular Machines group, TU Dresden.

A further level of organisation can be achieved if the D-repeat banding of the collagen microfibrils is also aligned, as in natural tissue. The alignment or non-alignment of the D-repeats is sensitive to the ionic composition of the deposition buffer, and alignment is seen in solutions mimicking cytoplasmic or extracellular environments of eukaryotic cells [6]. In the height and vertical deflection images shown in Figure 10, the molecules have been deposited so that there is an overall alignment of the D-repeat bands of adjacent collagen fibrils. The images in Figure 10 are courtesy of A. Taubenberger, D.J. Müller Cellular Machines group, TU Dresden. In this sample, the collagen does not completely cover the surface and gaps can be seen where the glass surface is visible beneath.

Height



Vertical deflection

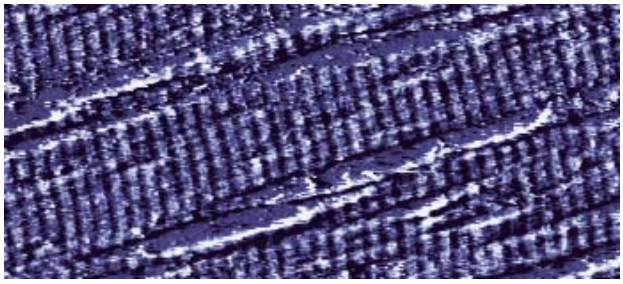


Fig 10. Aligned unfixed collagen fibrils in liquid. Images courtesy of A. Taubenberger, D.J. Müller Cellular Machines group, TU Dresden.

These surfaces have some of the nanostructural properties of natural collagen fibres, laid down as a thin coating. The alignment of the D-repeats also produces a bioactive surface. Fibroblast cells cultivated on the collagen where the fibrils are oriented, but not aligned, show no particular orientation of their shape or growth direction. When the D-repeats are also aligned, however, the fibroblasts show highly oriented motility along the axis direction, and have an elongated shape in this direction. The texture of the surface caused by large-scale orientation of the fibrils alone was not sufficient to control cell motility, and this suggests a biological function for the D-band alignment in guiding cell growth [7].

Conclusions

The AFM is a powerful tool for observing the levels of alignment and organisation in collagen structures. Natural collagen structures, isolated fibrils and novel biomaterials can all be studied under physiological conditions. These measurements can illuminate fundamental biological questions, as well as test new biocompatible or specifically bioactive composites.

Literature

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