Atomic force microscopy (AFM) has become one of the most widely used near-field microscopy techniques. Since its invention in 1986 [1], atomic force microscopy (AFM) has become one of the most widely used near-field microscopy techniques. Atomic force microscopy, one technique in a family of scanning probe microscopies (SPMs), has been established as a valuable imaging technique in fields such as physics, biology, and materials science. In favourable circumstances it can resolve surface detail down to the atomic level, with non-destructive probes. AFM can be used in situ to image the three-dimensional structure of biological specimens in a physiological environment. This enables real time biochemical and physiological processes to be monitored at a resolution similar to that obtained for the electron microscope [2].

In this article the usefulness of AFM in the field of dentistry will be demonstrated. Today's dental materials research focuses on the structure, surface roughness, chemistry and mechanical properties of the dentine substrate. The challenge facing the dental researcher is to investigate structure-property relationships of materials on even smaller scales. Although other techniques can be used to study these properties, the main attraction of AFM is that measurements can often be made with minimal sample preparation hence leaving the materials and their properties in their original state. Despite the obvious advantages of scanning probe microscopy (SPM) techniques, these have been applied in dental materials research only very recently. Marshall et al. pioneered the investigation of dentine surfaces with AFM [3] by examining the effect of demineralisation on dentine structure using contact mode AFM. Our research in this field includes studies of the morphology of dentine surfaces (Fig 1) and of materials used in dentistry after various clinical treatments. An other example is the alteration to the surfaces of orthodontic elastomeric modules and archwires after clinical use. An advantage of AFM is that it can also be used as a quantitative metrological tool. This significant feature is demonstrated in measurements on the dentine surface. Here the dimensions of dentine tubules (diameter and depth), and the surface roughness have been obtained using the AFM analysis software.

Various applications of AFM in dentistry are mentioned below. The importance of AFM for dental research is that analysis can be made with minimal sample preparation. Hence samples obtained directly after clinical treatment can be used intact, and clinical conditions can be recreated more realistically. However there are some criteria that must be met in the sample preparation, particularly regarding its planarity. The sample has to be flat, since SPM can only cope with limited height variations. For example, in the tapping mode of AFM it can only deal with up to a certain height variation. In our instrument this is 6µm. The sample size cannot be very large, simply because it cannot fit inside the scanner. The x-y scan area can be a maximum of 240µm x 240µm. In the case of dentine samples, non-destructive preparations avoid sample dehydration that leads to the collapse of collagen and thus surface alterations. The direct measurement of such surfaces provides essential information for understanding clinical treatments and for the development of new adhesive agents. Also, since AFM is non-destructive, the same samples can be sequentially imaged when subjected to various treatments, or they can be further integrated with many other destructive surface analytical techniques for correlational studies.

**AFM MODES UTILISED**

The traditional method for obtaining information about the microscopic dentine surface structure is scanning electron microscopy (SEM). This method, however, is not a direct surface technique since a conducting gold or carbon coating of the dentine is required for imaging. Furthermore, SEM does not allow the observation of water-containing components of the dentine since the sample chamber operates under high vacuum. A number of high quality studies have nevertheless been performed on human dentine, including inter alia the use of fixative methods. In general, dentine dehydration causes collapse of the demineralised collagen. A more recently developed variant of SEM, environmental scanning electron microscopy (ESEM), can be used to examine unfixed biological samples under low vacuum and wet conditions, although at a much lower obtainable resolution [4].

These disadvantages can be avoided when using scanning probe microscopy methods. The most common method used in dental research is contact mode AFM, where the probe is in permanent contact with the surface and the resulting image is a topographical map of the surface of the sample. However, the dragging motion of the probe tip, combined with adhesive forces between the tip and the surface, can cause substantial damage to both sample and probe and create artefacts in the recorded image. These experiments have usually been carried out in a pH buffered aqueous environment in order to alleviate the problem by reducing capillary forces between tip and dentine surface and to study alteration processes in real time. It has been shown, however, that tapping mode AFM can produce higher quality images with fewer artefacts [5]. In particular, tapping mode AFM has the advantage of avoiding any surface dragging effects of the tip on the surface by alternately placing the tip in contact with the surface to provide high resolution and then lifting the tip off the surface to avoid dragging the tip across the surface. This is important when delicate surface structures may be generated by procedures such as etching. Both methods of scanning were utilised in our research according to the nature of the substrate. These experiments have been carried out using a Nanoscope III, multimode SPM microscope (Digital Instruments).

AFM can be used to obtain quantitative...
information on surface changes. The analysis of the surface to quantify parameters such as dentine tubule diameter and depth, was performed with the ‘section analysis’ option of the AFM software. The roughness analysis software of the AFM can be used to obtain various roughness parameters of the altered dental materials being examined. These parameters, which include mean surface roughness (Ra), root mean square deviation (Rq), and maximum roughness depth (Rmax), quantify the amount of etching or degradation induced by the treatments being examined.

RESULTS

Metrological studies

The microscopic structure of the dentine surface plays a key role in the successful application of bonding agents used in restorative dentistry. In order to ensure good bonding between dentine and a bonding system, acid treatment of dentine is commonly employed as a first preparative step. Depending on the duration of the acid treatment, modification or removal of surface debris occurs resulting in the exposure of the underlying tubules at the surface. Severe demineralisation of the dentine can be expected. A better understanding of the micro-structure of treated dentine surfaces is thus of great importance for research focused on innovative methods of bonding between dentine and restorative materials.

In our research dentine surfaces etched with bonding agents used in clinical practice were examined [6]. These surfaces exhibit dentine tubules (Fig 2a). Figure 2b shows one typical dentine surface section analysis. The tubule depth and tubule diameter were measured, thus providing an indication of the potency of the agent applied and its effects. Surface roughness is a crucial factor influencing the adhesion of the bonding agent. Here, the ability to obtain at the same time both direct measurements of surface roughness from a dental surface that has not required any special treatment to allow imaging, and images of the surface to agree with the ability is a significant advantage offered by AFM. These metrological capabilities can also be used to study enamel erosion by acids [7]. These studies utilize the ability of the AFM to provide high resolution in the z-direction and precise measurement of surface heights.

Surface morphology

We have used contact mode AFM to study the surface of orthodontic wires obtained in vivo after clinical treatment (Fig 3) [8]. Similar measurements were made on elastic orthodontic chains [9], which are routinely used in orthodontics for the retraction of teeth. The extent of degradation of the chains after specific clinical treatment can be seen. The surface roughness of these chains is an essential factor that determines the effectiveness of chain-guided tooth movement. Our observations suggest that more frequent change of these devices may be required for better clinical results.

Novel modifications to the AFM also allow measurement of the mechanical properties of dentine at a resolution not previously possible. One type of modification in the measurement instrument consists of the replacement of a conventional silicon nitride tip, which is mounted on a silicon cantilever, with a diamond tip having significantly greater hardness mounted on a stainless steel cantilever having greater stiffness. A conventional AFM imaging assembly has a cantilever stiffness of about 1-2 Nm⁻¹; whereas, the stainless steel cantilever assembly has a measured stiffness of 646 Nm⁻¹. This development allows site-specific mechanical property measurements of the dentine constituents in the hydrated state, as well as evaluation of changes in properties, following applications of various bonding agents. The modified AFM tip [10] offers the advantage of measuring the viscoelasticity and stiffness of dentine whilst imaging it at the same time. Nano-hardness and modulus measurements have shown that there are differences in the properties of the intertubular component of dentine that are dependent upon location within the tooth [10].

These approaches, which have provided new and exciting insights into the structure-property relationships of dentine, should lead to better models of dentine treatment that will assist in the development of improved preventive and restorative treatment.

Materials applications

AFM can be used in many other areas of dental research. Examples include using AFM in fractographic investigations where the mode of fracture mechanism of composites has been studied. In addition to the properties of the natural tooth, investigations of new synthetic dental materials using surface techniques is an active field of dental research. One application was to examine in detail the surfaces of various adhesive clinical materials used in fillings to and show the particle arrangement of the composites [5,12]. AFM has been used to study the surface of dental ceramic materials in order to detect any signs of thermal alterations following treatment with a laser [13].

Also AFM has provided images of the surface topography of etched glass ceramic [14]. We have quantified image features, such as height and surface roughness on these ceramics. (Fig 6) These new ceramics consist of lithium disilicate crystals embedded in a glass matrix. Their surface properties, of fundamental importance to dentine-ceramic bonding, and in-vivo durability, remain largely unexplored. AFM was more suitable for evaluation of the crystal structure changes of fine ceramics during various surface treatments since localised measurement-damage to the test surface does not occur, owing to the light probe contact compared with the traditional method used for dental materials research of a stylus profiler [15].

CONCLUSIONS

We have shown here that AFM is valuable for...
gaining understanding of basic dentine structure and its variants, and particularly for understanding the effects of demineralisation. AFM permits the microstructural-mechanical properties relationship to be investigated at the sub-micron level without any invasive treatment of the surface. In fact, AFM is recognised as the only microscopy to date that can achieve nanometre resolution on dentine samples under native conditions. It also complements other methods, and can be used for quantitative measurements such as dentine tubule size and surface roughness. It has been shown to be an effective and versatile analytical tool for the dental materials scientist. The results obtained through AFM can help to towards selection of suitable materials for clinical use and the refinement of existing clinical practises. The growing number of publications related to AFM in the field of dentistry proves its usefulness.

Tapping mode AFM appears to be the more suitable tool for investigating dentine fine surface structure after treatment with acidic conditions by reducing tip-sample interactions such as those induced by conventional contact mode AFM.

For the dental research scientist, AFM has opened up new approaches to examine dental materials, both natural and synthetic. New developments in AFM will offer more opportunities in the near future. The challenge of observing changes in dental materials in situ can now be met by recently introduced fluid cell-type AFM configurations. This cell has flow-through capabilities, so that real-time monitoring of changes in the surface chemistry can be monitored. This will be an exciting area of dental research in the next few years.

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Figure 3: AFM micrograph of an orthodontic wire after a 3-week exposure to the oral cavity. Size 50 x 50 µm. Prior to exposure, the surface had a 2-range of 800 nm.

Figure 4: Flat areas of a fractured polymer matrix separated by fine lines (indicated by arrows A). Filler particles embedded in the polymer matrix are indicated by arrow B. Height data is presented on the left z-range 500 nm, and oscillation data 300 nm on the right.

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Figure 5: Surface of a dental composite material showing a compact particle arrangement.

Figure 6: 2D AFM micrograph of the IPS-Empress ceramic untreated. Image size 20 x 20µm.