In vivo nanoscale atomic force microscopy investigation of diatom adhesion properties

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Most state of the art adhesives fail to bond under wet conditions. Therefore, knowledge of the intrinsic properties of natural adhesives might give valuable information for future engineering approaches. This work investigates the adhesive that *Eunotia sudetica*, a species of benthic freshwater diatoms, produces to attach itself to a substrate. Atomic force spectroscopy under aqueous solution reveals the modular, self-healing properties of this natural adhesive. **MST/5209**

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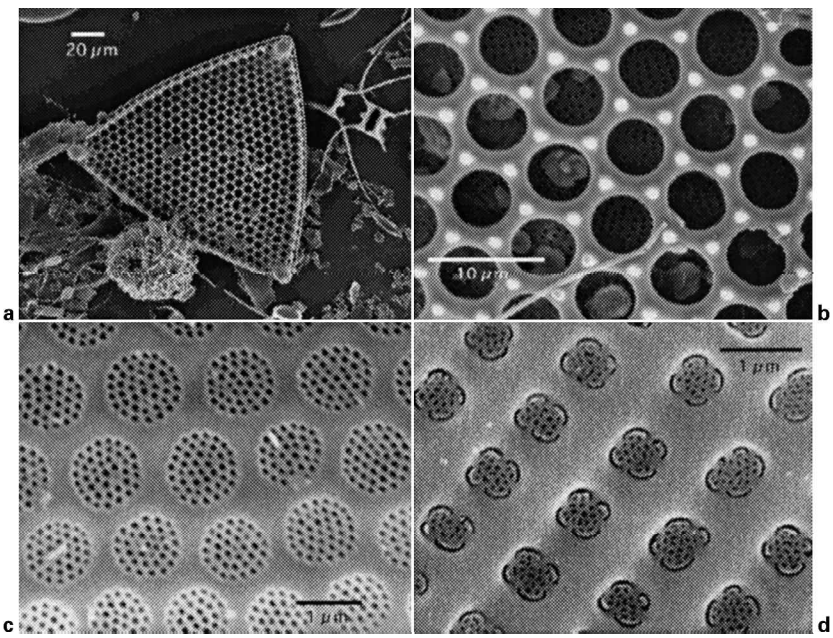
Introduction

Biomimetics is a new interdisciplinary field seeking to understand relationships between structures and functions of biological composites in order to design and synthesise new materials, possibly without the toxic residues characteristic of non-biological modes of industrial mass production.[1,2] Understanding the processes involved in biomineralisation may eventually allow to mimic these structures to produce optimised materials with minimal environmental impact. This research may lead to the synthesis of novel magnetic, electronic, magnetopharmaceutical or adhesive materials on a nanometre scale.

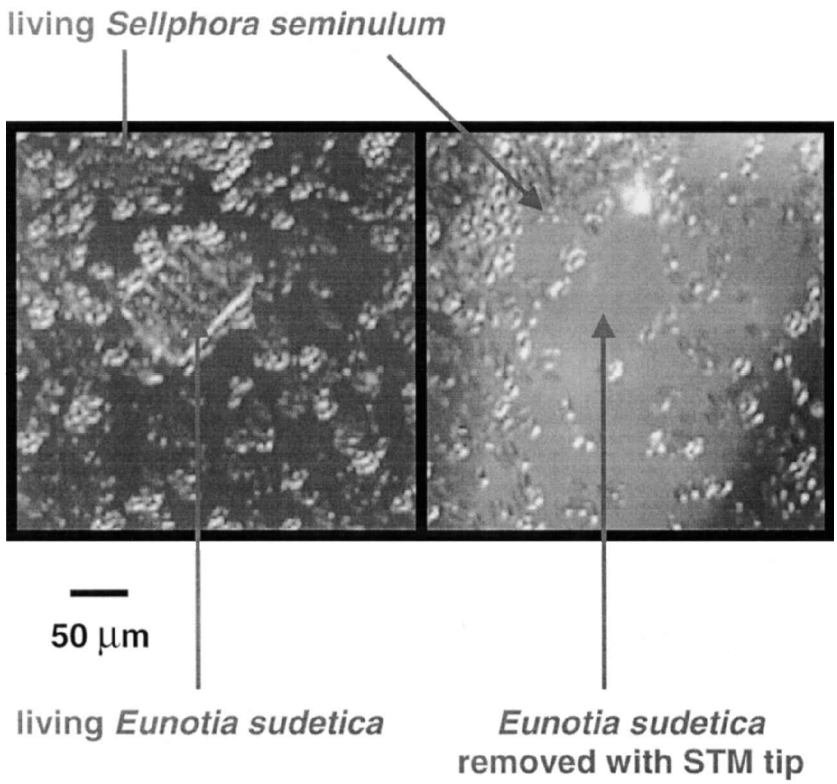
Diatoms are unicellular microalgae with cell walls consisting of a siliceous skeleton enveloped by an organic case. The skeletons exhibit an amazing diversity of nanostructured frameworks (Fig. 1, SEMs courtesy of M. A. Tiffany, San Diego State University, CA, USA). Diatoms live in various wet environments, like salt water or freshwater and on wet surfaces. They can either be freely floating (planctonic forms), or be attached to substrates like rocks or plants (benthic forms).

These unicellular organisms are interesting from the point of materials science and biomimetic studies, since they master challenges as diverse as building nanostructured glass shells with high load capacity (a problem interesting for lightweight structures architecture)[3] and engineering strong and robust adhesives that are stable in wet environments (most man made adhesives fail to bond in wet conditions, owing to chemical modification of the adhesive or its substrate).[4] Furthermore, diatoms excel at preventing dissolution of their silica shells in water owing to an organic layer (up to date technology is currently facing the problem that man made glass fibre reinforced polymers show rapid quality deterioration when used in water).[5] Currently, human chemical synthesis can not produce siliceous structures with



a and *b Triceratium favus*; *c Roperia tessellata*; *d Achnanthes brevipes*

1 Micrographs showing siliceous skeletons of three different marine diatom species (SEM)



2 Living diatoms covering glass slide: chain of *Eunotia sudetica* that is embedded in field of *Sellphora seminulum* is removed with help of scanning tunnelling microscope (STM) tip to expose natural adhesive to atomic force microscope (AFM) tip for acquisition of force *v.* distance curves and AFM imaging

the hierarchical structural detail of the diatom skeletons nor can ordered siliceous structures be produced synthetically under the benign conditions of diatom biomineralisation.

Atomic force microscopy (AFM) has opened the possibility of studying these interesting organisms *in vivo* with high spatial resolution.[6] The mechanical behaviour of the diatom adhesive under extension on a molecular level is investigated in this paper.

Materials and methods

Three different species of benthic freshwater diatoms (*Eunotia sudetica*, *Sellphora seminulum*, and a yet unidentified species) were selected for their strong adhesives by exposure of a greater plethora of species to highly selective environmental conditions.[6] *Eunotia sudetica* was then selected for this study, because its greater size (several tens of micrometres) simplifies the following micromanipulation and engage step (Fig. 2).

The diatoms where grown and studied in fluid culture medium[7] (Diatom medium, Culture Collection of Algae and Protozoa, UK), where they attach themselves onto glass slides immersed in this medium.

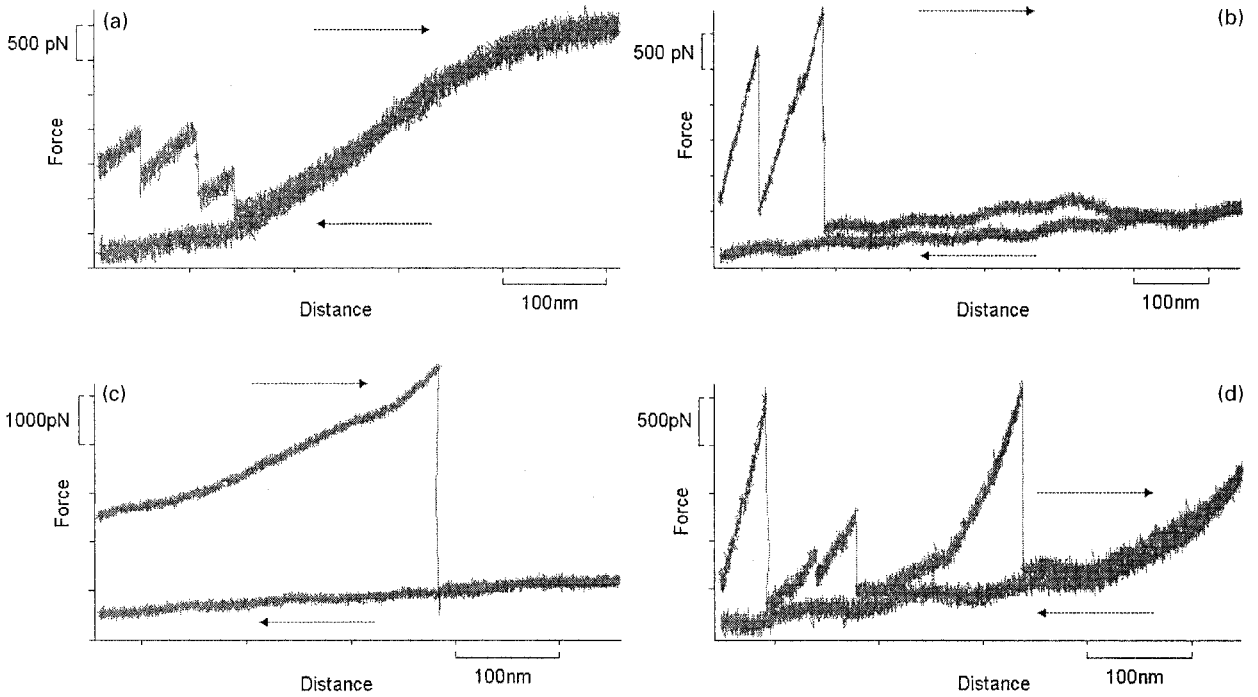
To access the adhesive *Eunotia sudetica* produces for its attachment, an *Eunotia sudetica* cell was mechanically removed using an scanning tunnelling microscope tip,[8] i.e. a sharpened wire, on a micromanipulator stage. Its former location is clearly marked by the gap in the surrounding *Sellphora seminulum* (Fig. 2).

An AFM (BioScope, Digital Instruments, Santa Barbara, CA, USA) was now engaged at this location under optical control (Zeiss Axiovert). The AFM[9] consists of a micromachined springy cantilever[10] (Si_3N_4, $k = 0{\cdot}06$ N m^{-1}, Digital Instruments, Santa Barbara, CA) with a sharp tip perpendicular to the cantilever. When a force is applied to the tip, the cantilever is deflected proportionally. A low spring constant of a cantilever is desirable so that large deflections are obtained with small forces of interaction. This deflection is detected by a laser beam that reflects off the cantilever.[11] The AFM is suitable for studies in water and aqueous solutions.[12] Besides imaging applications, the AFM can also be used for stress–strain experiments of small specimens, down to the size of single molecules. In these experiments, the cantilever tip is approached to the material under study. When retracting the cantilever from the specimen, it can occur that a small strand of the specimen stays attached to the AFM tip. Under further extension, the deflection of the cantilever represents the force exerted on the attached strand, and can be plotted as a function of extension.[13]

Force *v.* distance curves of the adhesive residue on the glass slide were now acquired for several hours (Figs. 3 and 4). Control experiments of the adjacent glass slide were acquired for comparison. All experiments were performed in fluid diatom growth medium.[7]

Results and discussion

Owing to the poor adhesion to the substrate, it is impossible to obtain stable images of most benthic diatom species with the AFM. The foundation of the authors' interest in the natural adhesives of the three diatom species is that they turned out to be AFM compatible. *Eunotia sudetica* and the yet unidentified species form chains which grow in parallel to the surface of the glass slide, whereas *Sellphora seminulum* grows in stacks pointing out from the surface of the glass, like little skyscrapers, tens of micrometres high. Phase imaging data on the adhesive produced by the yet unidentified species to build stable chains is presented elsewhere.[6] This work concentrates on force *v.* distance curves on the adhesive *Eunotia sudetica* produces to attach to the substrate.



a adhesive forces are still acting upon cantilever at maximum retraction of piezo; *b* complete unbinding takes place; *c* complete unbinding takes place; *d* adhesive forces are still acting upon cantilever at maximum retraction of piezo

3 Force *v.* distance curves performed on natural adhesive *Eunotia sudetica* produces to attach to substrate: sawtooth pattern structure of unbinding events is clearly visible in *a, b,* and *d*; wavy structures in *b* and *d* origin from interference of laser on glass slide; time between pulls several minutes; cantilever spring constant $k = 0{\cdot}06$ N m^{-1}; pulling velocity 1 μm s^{-1}

The elongation of the adhesive under stress takes place in a sawtooth pattern (Figs. 3 and 4); the force rises with extension, some unbinding takes place, yet the adhesive force is still acting and again increasing with extension, once more some unbinding takes place, but there is still some adhesive force, and the cycle starts again. The hysteresis observed after a complete pulling cycle demonstrates that work has been done. This work is irreversibly dissipated as heat, and the area between the retracting and the approaching parts of the curve quantifies this heat (Figs. 3 and 4). Dissipation mechanisms that contribute to this area are energy dissipation inside the pulled adhesive matrix, and energy dissipated either inside the cantilever itself or by fluid dynamic effects. Control experiments, i.e. force *v.* distance curves on areas of the glass slide which are not covered with the adhesive, exhibit negligible energy dissipation;[6] the area between the retracting and the approaching parts of a pulling curve in the control experiment is only a small fraction compared with the energy dissipation in the adhesive pulling experiments.

The sawtooth pattern elongation behaviour of the adhesive under stress may reflect the successive unbinding of intrachain loops or the successive release of interchain bonds holding a cross-linked multichain adhesive matrix together. Experimental data on the molecular mechanistic origin of the toughness of natural adhesives, fibres, and composites show that this strategy is even applied on the single molecule level.[14]

Consecutive acquisition of force *v.* distance curves on a small amount of the adhesive with the tip of the cantilever staying away from the bulk adhesive on the glass slide reveals 'self-healing' properties of the adhesive matrix, when the time between curve acquisition is longer than 30 s. First, the tip of the cantilever 'dips' into the natural adhesive remaining on the glass slide after removing the chain of *Eunotia sudetica*. The cantilever is then carefully brought to a position at a given distance away from the substrate, with some adhesive molecules attached to it (like in Fig. 3, top left and bottom right traces). From this new cantilever 'resting position', five consecutive force *v.* distance curves (Fig. 4) are acquired, pulling the adhesives even further up, away from the glass slide. In the control experiment, i.e. when the time between the acquisition of two consecutive force *v.* distance curves is less than 30 s, no more sawtooth pattern detaching events can be detected in consecutive pulls. This result seems to indicate that the natural adhesive *Eunotia sudetica* produces to attach to the substrate is 'self-healing' and that rebonding needs several seconds to occur.

Conclusions and outlook

Atomic force microscopy (AFM) has proven to be a valid method for *in vivo* studies of diatoms.[6,15] Force *v.* distance curves on the diatom adhesive reveal a sawtooth pattern structure which considerably differs from data obtained from current man made adhesives[6] and reveal self-healing properties. Further investigations on this adhesive with AFM single molecule pulling experiments and other methods might provide the scientific basis for the production of adhesives that are stable and robust in wet environments, properties that still pose considerable challenges to current man made adhesives.

Acknowledgements

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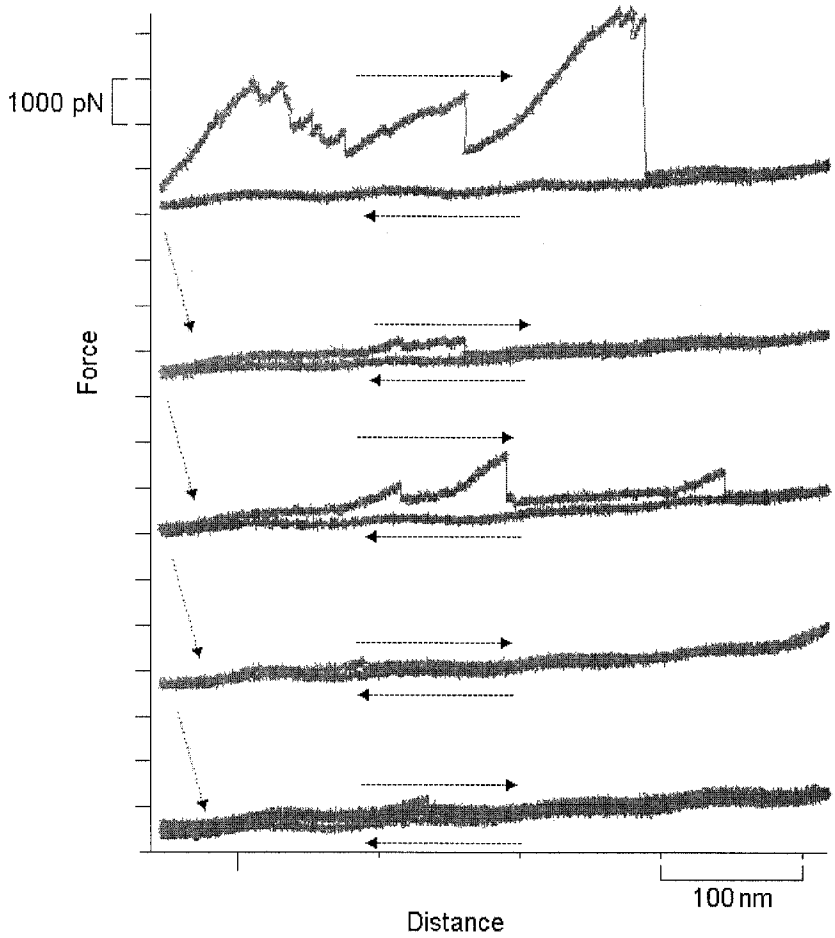

4 Five consecutive force *v.* distance curves performed away from surface on natural adhesive *eunotia sudetica* produces to attach to substrate: time between pulls 30 s; sawtooth pattern structure of adhesive unbinding characteristics clearly visible; if time between pulls is less than 30 s, no multiple unbinding events occur anymore

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