Bacterial attachment on optical fibre surfaces

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Optical fibres have received considerable attention as high-density sensor arrays suitable for both in vitro and in vivo measurements of biomolecules and biological processes in living organisms and/or nano-environments. The fibre surface was chemically modified by exposure to a selective etchant that preferentially erodes the fibre cores relative to the surrounding cladding material, thus producing a regular pattern of cylindrical wells of approximately 2.5 μm in diameter and 2.5 μm deep. The surface hydrophobicity of the etched and non-etched optical fibres was analysed using the sessile pico-drop method. The surface topography was characterised by atomic force microscopy (AFM), while the surface chemistry was probed by time-of-flight secondary ion mass spectrometry (ToF-SIMS). Six taxonomically different bacterial strains showed a consistent preference for attachment to the nano-scale smoother (Rq = 273 nm), non-etched fibre surfaces (water contact angle, θ = 106° ± 8°). In comparison, the surfaces of the etched optical fibres (water contact angle, θ = 96° ± 10°) were not found to be amenable to bacterial attachment. Bacterial attachment on the non-etched optical fibre substrata varied among different strains.

Keywords: optical fibre; micro-nano structured surfaces; bacterial attachment

Introduction

In recent years optical fibre bundles have been widely investigated as a base for developing sensors suitable for both in vitro and in vivo measurements of biological processes in living cells or nano-environments (Bernhard et al. 2001; Epstein et al. 2003; Stoddart and Brack 2007). Optical fibre arrays in a 96 element matrix have been commercialised by Illumina Inc. and are used to study gene expression and perform whole-genome SNP genotyping (Fan et al. 2004; Kuhn et al. 2004). Fibre bundles can also serve as a platform for constructing a highly sensitive bio-sensing system that can operate at the single cell level (Vo-Dinh and Kasili 2005; Fritzsche et al. 2009) or as live cell array biosensors for the detection of various chemical compounds (Biran et al. 2003). One of the most recent applications of such fibres is the design of whole-cell biosensors for environmental monitoring (Biran et al. 2003; Kuang et al. 2004). The latter application relies on random assembly of the cells by sedimentation, after which bacterial cells can remain viable, sustained by the reservoir of nutrients above the surface (Kuang et al. 2004). The array platform allows the physiological and genetic variabilities between cells and their response to various stimuli to be continuously monitored. The main concerns in designing a whole-cell biosensor is the immobilisation of live cells onto the fibre surface and maintaining their viability (Udd 1995; Gadelmawla et al. 2002; Lee 2003; Lee et al. 2005, 2009; Dhawan et al. 2009). This is mainly because most of the steps involved in cell immobilization frequently result in cell death or damage and subsequent impaired sensitivity. Moreover, in order to gain the full benefits from the optical fibre array sensor platform, it is important to ensure that collections of separated cells respond in the same way as a collection of unconstrained cells. Thus, a detailed understanding of the cell–fibre surface interactions is believed to be the key issue in the design of functional, long lasting and effective biosensing devices based on optical fibre substrata (Polwart et al. 2000; D’Souza 2001; Vo-Dinh and Stokes 2002; Biran et al. 2003; Vo-Dinh and Kasili 2005; White and Stoddart 2005; White et al. 2007).

Despite being the subject of intensive research for various applications in clinical diagnosis, biomedical and environmental monitoring, or industrial and food

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technology, very little is known about the interaction between the optical fibre surfaces and the biological materials (including bacteria) that they come into contact with. The present study aimed to investigate the effect that the surface characteristics of optical imaging fibre have on the retention pattern of medically and environmentally significant microorganisms of different taxonomic affiliations. Two types of optical fibre substrata were used in all experiments: standard, from now on referred to as 'plain' and chemically modified, referred to as 'etched'. Bacterial and fibre surface characteristics were analysed in order to better understand the characteristics that influence the extent of bacterial adhesion to the fibre substrata and the associated bacterial metabolic activity. Particular attention was given to the role the surface micro- and nano-scale morphology play in the bacterial adhesion process. Existing work has indicated that the nano-scale changes in surface roughness of various materials may have a significant effect on bacterial adhesion (Ivanova et al. 2008a, 2009; Mitik-Dineva et al. 2008b, 2009a, 2009b).

**Methods**

**Optical fibre surfaces**

The experimental surfaces were prepared from standard optical imaging fibre (FIGH 70-1300, Fujikura, www.fujikura.co.uk/fibre_optics/products/speciality_fibre/figh).

The optical imaging fibres used in this study were made from germanium doped silica glass cores surrounded by fluorine-doped silica cladding and consisted of ~70,000 individual single-mode fibres (known as pixels) fused together into a bundle that was coated with silicone resin. The total outer diameter of the final fibre bundle was about 1.3 mm (Figure 1d). Workable (5 mm long) fibre substrata were obtained by cleaving the fibre bundle received from the manufacturer, which yielded relatively smooth ($R_{\text{max}}$ up to 200 nm) flat surfaces on the fibre-end faces, as shown in the high-resolution SEM images presented in Figure 1a,b). In order to fabricate micro-structured surfaces, samples of the standard optical imaging fibre were further modified by chemical etching. This was achieved by immersing one half of the 5 mm long samples into buffered hydrofluoric acid (BHF) etching solution for 20 min (White and Stoddart 2005). Because the glass cores etch at a faster rate than the surrounding cladding, treating the fibre samples with BHF resulted in creating a general hexagonal pattern of cylindrical wells, approximately 2.5 μm in diameter and 2.5 μm deep, on the fibre surfaces (Figure 1).

Upon treatment all samples were rinsed with nanopure H$_2$O (18.2 MΩ cm$^{-1}$ Barnstead/Temolyne NANOpure Infinity water purification system), sterilised and stored under sterile conditions prior to inoculation. The rinse solution was tested for acidity with phenolphthalein and bromothymol blue indicator dyes to confirm that all of the BHF had been removed from the fibre surfaces.

The surface hydrophobicity of the substrata was determined via contact angle measurements using the sessile pico-drop method as described elsewhere (Taylor et al. 2007; Urquhart et al. 2008). Measurements were performed using nanopure water on a Krüss DSA 100 apparatus fitted with a piezo-doser head. The piezo-doser allowed small nanopure water droplets (100 pl) to be deposited onto the fibre facets (Taylor et al. 2007). Sample positioning and data acquisition took place automatically, with droplet side profiles being recorded (a dual camera system was used, one to record a side profile of a spot and the other to record a plan view to ensure that the water droplet was deposited at the center of each spot) for data analysis. Water contact angle (WCA) calculations were performed using nanopure water on a Kruess DSA 100 apparatus fitted with a piezo-doser head. The piezo-doser allowed small nanopure water droplets (100 pl) to be deposited onto the fibre facets. Measurements were taken over six areas of two samples of each fibre; in total 12 droplets on both, plain and etched fibre samples, were analysed. Results were averaged and standard deviation (SD) values were calculated (Table 1, Figure 2). The contact angles of formamide (Sigma) and diiodomethane (Sigma) were also measured using an FTA1000c (First Ten Ångstroms Inc) equipped with a nano-dispenser. An average of at least five measurements was taken for each solvent and titanium surface. Each measurement of a particular contact angle was recorded in 50 images in 2 s with a Prosilica Model Navitar 444037 camera, and the contact angle was determined as a result of images analysed using the FTA Windows Mode 32 software. The average contact angle for each of the three solvents on each surface was used to calculate the surface free energy and its components, based on the Lewis acid/base method (Öner and McCarthy 2000).

The topographical features of both the etched and plain fibre substrata were analysed using scanning electron microscopy (SEM) and AFM as described...
Table 1. Surface hydrophobicity, surface tension, surface free energy and AFM analysis of the roughness parameters for the non-etched and the etched fibre surfaces.

| Fibre surface | Water   | Diiodomethane | Formamide | TOT | LW | AB | T | Nm |
|---------------|---------|---------------|-----------|-----|----|----|---|----|
| Contact angle, $\theta$ (°) | 106.0 ± 4 | 96.0 ± 10 | 129.6 ± 5 | 102.3 ± 9 | 102.6 ± 3 | 101.3 ± 7 |
| Tension (mJ m$^{-2}$) | 9.18 | 9.66 | 1.67 | 7.89 | 7.51 | 1.77 | 3.34 | 4.22 | 4.22 | 4.38 |
| Roughness parameters, R$_a$ ± SD | 181 ± 2.86 | 1563 ± 3.53 | 237 ± 3.56 | 3428 ± 3.42 | 1741 ± 5.00 | 2399 ± 11.16 |
| R$_f$ ± SD |  |  |  |  |  |  |
| Nm |  |  |  |  |  |  |

SD = Standard deviation. *Contact angle of water, formamide and diiodomethane ($\theta_W$, $\theta_F$ and $\theta_D$ respectively). $^b$Lifshitz/van der Waals component ($\gamma^{LW}$), acid/base component ($\gamma^{AB}$), electron acceptor ($\gamma^{+}$) and electron donor ($\gamma^{-}$).

Figure 2. Typical images representing measured water contact angles on the as-received (a) and the etched (b) optical fibre surfaces.

elsewhere (Mitik-Dineva et al. 2008b, 2009a). High-resolution images of the samples containing adsorbed bacterial cells were taken using a FESEM (ZEISS SUPRA 40VP) at 3 kV with 1000×, 5000× and 20,000× magnifications. Images with 1000× and 5000× magnification were used to estimate the number of bacteria adhering to the fibre surfaces.

A scanning probe microscope (SPM) (Solver P7LS, NT-MDT) was used to image the fibre surface topography whilst also providing a quantitative analysis of the surface roughness (Table 1, Figure 3). The analysis was performed in the semi-contact mode, which reduces the interaction between the tip and the sample and thus allowed the destructive action of lateral forces that exist in contact mode to be avoided. The height of the surface features was measured with a resolution of a fraction of a nanometer and the surface roughness of the areas investigated could be statistically analysed using the standard instrument software (LS7-SPM v.8.58). Five samples of both etched and plain fibre surfaces were investigated. Each sample was briefly scanned to evaluate the overall homogeneity of the surface and then one typical topographical profile was studied in detail. Statistical data processing was performed using SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA). Single independent group T-tests were performed to evaluate the consistency of surface roughness parameters.

Reconstruction of interactive three-dimensional images

Interactive three-dimensional (3D) visualization of the fibre surfaces was undertaken using a custom C-code and the S2PLOT graphics library (Barnes et al. 2006; Ivanova et al. 2009). The input data files were in NT-MDT format and were read into the custom viewing tool (mdt-view) using a modification of the nt-mdt module of Gwyddion by Necas and Klapetek (http://gwyddion.net/, Version 2.12). NT-MDT files were then converted into a three-dimensional surface, coloured according to height, and displayed with the S2PLOT s2surpa function. Visualizations were exported from mdt-view to an intermediate VRML format, with textures for axis labels in TGA format. Textures were converted to PNG format and the VRML model was then imported into Adobe Acrobat 3D Version 8 to create an interactive figure, using the approach described by Barnes and Fluke (2008). Simple Java Script commands were used to provide additional functionality. The two interactive panels in Figure 3 can be viewed in the pdf format of this paper by clicking on either panel (C) or (F) with the mouse pointer, provided Adobe Reader Version 8.0 or higher is used to view this article. This opens a window where the surface can be examined interactively using the mouse to control the camera orientation and zoom level.

Time-of-Flight Secondary Ion Mass Spectrometry analysis

A Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used to investigate the surface chemistry of the two fibre surfaces and provide an insight into compositional differences that might have influenced the extent of bacterial adsorption. All measurements were performed using a ToF-SIMS IV instrument (ION-ToF GmbH, Munster, Germany) with a reflection analyser and a pulsed electron flood source for charge neutralization. Both positive and negative spectra were acquired from a 100 μm × 100 μm area. Samples were exposed to the atmosphere for less than 5 min during mounting in the TOF-SIMS instrument. All experiments were performed using a cycle time of 100 μs. A monoisotopic $^{69}$Ga$^+$ primary ion source was operated at 25 keV in the “burst alignment” mode, which afforded very high
spatial resolution at the expense of mass resolution and positive and negative spectra were acquired with a mass resolution typically larger than 6000 at $m/z = 27$, sufficient to identify most of the fragments (supplemental material). The resulting spectra acquired were analysed using the standard instrument software (Supplemental online material [Supplementary material is available via a multimedia link on the online article webpage]).

**Microorganisms, culture conditions and sample preparation**

*Escherichia coli* K12, *Staphylococcus aureus* CIP 68.5, *Pseudomonas aeruginosa* ATCC 9027; and three marine bacteria: *Cobetia marina* DSM 4741$^T$, *Pseudoalteromonas issachenkonii* KMM 3549$^T$, and *Sulfito bacter guttiformis* DSM 11458$^T$ were used in this study. The bacterial strains were obtained from American Type Culture Collection (ATCC, USA), Culture Collection of the Institute Pasteur (CIP, France) and German Culture Collections (DSM, Germany). Selected marine bacteria derived from our previous studies (Mitik-Dineva et al. 2009a). All bacteria were routinely cultured in a nutrient (Merck) or marine (Oxoid) agar and stored at $-80^\circ$C as described elsewhere (Ivanova et al. 2002). A fresh bacterial suspension was prepared for each of the strains grown overnight in 100 ml of nutrient or marine broth (in 0.5 l Erlenmeyer flasks) at 37$^\circ$C with shaking (120 rpm) prior to each experiment (Mitik-Dineva et al. 2009a). Bacterial cells were collected during the logarithmic phase of growth as confirmed by growth curves (data not shown). However, because cell densities varied after incubation for 12 h, the cell density of each strain was adjusted to OD$_{600} = 0.3$ in order to achieve an approximately similar number of cells in each sample (OD$_{600} = 1$ corresponds to $8 \times 10^8$ cells ml$^{-1}$ for E. coli, however, this estimate may vary for different bacteria). Therefore, the bacterial cell suspensions were further subjected to direct counting using a haemocytometer to confirm the

Figure 3. Typical 2D and 3D AFM images, and surface profiles of non-etched and etched fibre substrata. The first three rows show (A) 2D AFM image, (B) typical surface profile, and (C) 3D AFM image for the non-etched fibre substratum. The last three rows show (D) 2D AFM image, (E) typical surface profile and (F) 3D AFM image for the etched fibre substratum. Readers using version 8.0 or higher of Acrobat Reader can enable interactive, 3-dimensional views of the data by clicking on the Figure panels (C) or (F). Once enabled, 3-d mode allows the reader to rotate and zoom the view using the computer mouse.
number of bacterial cells for each strain used in experiments (Mather and Roberts 1998). On the day of the experiment, a 2 ml aliquot of log-phase bacterial suspension was adjusted to OD_{600} = 0.3 in nutrient/marine broth and kept in centrifuge tubes. Duplicate samples of both the as-received and etched fibres were placed into each of the tubes and were incubated for 12 h at room temperature (ca 22°C). After incubation, all samples were rinsed three times with sterilized nanopure H₂O, attached to glass supports and stored under sterile condition until needed. In SEM experiments, samples with adsorbed bacteria were initially coated with 20 nm gold thin films using a Dynavac CS300 according to the procedure developed previously (Mitik-Dineva et al. 2009b; Truong et al. 2009). The lower detection limit was estimated as 1.1 x 10³ cells mm⁻² according to Morono et al. (2009) using the following formula:

\[ n = \frac{T_{fov}}{C_{fov}} \ln(1 - p) \]

where \( n \) is the number of cells required giving a probability \( p \) (\( p = 0.95, 95\% \) chance to find one bacterial cell) of detecting a cell, \( T_{fov} \) is total area of fields of view, \( C_{fov} \) is the number of fields of view, and the total fibre area is 1.3267 mm².

Bacterial cell surface hydrophobicity was determined from a series of static contact angle measurements using the sessile drop method as described elsewhere (Mitik-Dineva et al. 2008a). The bacterial surface charge (Table 2) was determined via measurement of the electrophoretic mobility (EPM) of the cells. The EPM was converted to zeta potential using Smoluchowski’s approximation (Korenevsky and Beveridge 2007; Soni et al. 2007; Mitik-Dineva et al. 2008a). The zeta potentials of all six strains were measured as previously described (de Kerchove and Elimelech 2005; van Merode et al. 2007).

### Visualization and quantification of the bacterial adsorption and metabolic activity

Bacterial cell density quantification was performed using SEM images; bacterial morphological changes are not described in the results. Cell numbers from at least ten representative images/areas were transformed into a “number of bacteria per unit area” to allow the quantity of bacteria attaching to the substratum surface to be determined. The average densities have estimated errors of approximately 10–15% due to the local variability in the surface coverage.

Viable bacterial cells were visualised using SYTO®17 Red (Molecular Probes™, Invitrogen) (data not shown) and bacterial production of extracellular polymeric substances (EPS) on the fibre surfaces using Concanavalin A Alexa Fluor 488 Conjugate (Molecular Probes Inc.). This dye selectively binds to \(\alpha\)-mannopyranosyl and \(\alpha\)-glucopyranosyl residues in EPS (Goldstein et al. 1964). The EPS was visualised and analysed using confocal laser scanning microscopy (CLSM, Olympus Fluoview FV1000 Spectroscopic Confocal System). The fluorescence intensities were scanned at 543 for SYTO®17 Red and 488 for Alexa Fluor 488/Ex/Em.

### Results

#### Fibre surface characterisation

As indicated by the high resolution SEM (Figure 1) and AFM (Figure 3) images, the surface topography of the optical fibres had significantly changed as a result of the chemical etching. The differences in the surface topography are summarised in Table 1 and confirmed by a statistical analysis of the surface roughness parameters.

| Table 2. Surface hydrophobicity, electrophoretic mobility, zeta potential and retention profiles of bacterial cells on the non-etched and etched fibre surfaces after 12 h incubation. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Strain                          | Water contact angle \(\theta\) (°) | Electrophoretic mobility (\(\mu\)s⁻¹Vm⁻¹) | Zeta potential (mV) | Initial cell density (cells x 10⁸ mm⁻²) | Number of attached cells x 10³ mm⁻² | Non-etched fibre, SD | Etched fibre* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Pathogenic bacteria             |                 |                 |                 |                 |                 |                 |                 |
| *Escherichia coli*              | 33 ± 4          | −3.1 ± 0.6      | −38.4 ± 0.3     | 110 ± 20.8      | 2.98 ± 0.15     | < DL            |                 |
| *Pseudomonas aeruginosa*        | 43 ± 8          | −1.1 ± 0.1      | −14.4 ± 0.7     | 198 ± 34.4      | 1.15 ± 0.03     | < DL            |                 |
| *Staphylococcus aureus*         | 72 ± 8          | −2.8 ± 0.8      | −35.2 ± 1.0     | 205 ± 21.3      | 1.38 ± 0.04     | < DL            |                 |
| Marine bacteria                 |                 |                 |                 |                 |                 |                 |                 |
| *Cobetia marina*                | 75 ± 9          | −2.5 ± 0.6      | −32.5 ± 0.5     | 283 ± 50.0      | 55 ± 0.41       | < DL            |                 |
| *P. issachenkonii*              | 52 ± 3          | −2.9 ± 0.2      | −35.3 ± 0.2     | 301 ± 35.5      | 53 ± 0.29       | < DL            |                 |
| *S. guttiformis*                | 56 ± 4          | −3.4 ± 0.5      | −43.2 ± 0.2     | 118 ± 24.7      | 3.6 ± 0.02      | < DL            |                 |

SD, standard deviation; < DL, below detection limit. *E. coli, P. aeruginosa, S. aureus, C. marina, P. issachenkonii and S. guttiformis did not attach to the etched fibre surface.
The results indicated that the etched, micro-structured, honeycomb patterned fibre surface was significantly rougher than that of the non-etched fibre surface. All roughness parameters, including the average surface roughness (\(R_a\)) which represents the average/absolute deviation of the surface irregularity from the mean line over one sampling length, the root mean square roughness (\(R_q\)) defined as the SD of the distribution of surface height, and the peak-to-peak roughness (\(R_{\text{max}}\)) which represents the vertical distance between the highest peak and the lowest valley along the assessment length of the profile, appeared to be considerably higher on the etched fibre substrata than those of the non-etched samples as confirmed by statistical analysis (\(p < 0.05\)). The most significant increase, more than tenfold, was observed for the (\(R_q\)) which is believed to be more sensitive than the (\(R_a\)) parameter when considerable deviations from the mean line occur (as is the case in this instance) (Gadelmawla et al. 2002).

Analysis of the surface chemistry of the plain and etched fibres using ToF-SIMS revealed appreciable differences between the surface chemical characteristics of both substrata. In the case of the plain fibre, ToF SIMS analysis revealed the presence of silicone contamination deriving from the coating material with characteristic fingerprint of poly(dimethyl siloxane) (PDMS): \(m/z = 43: \text{CH}_3\text{Si}^+\), \(m/z = 73^+: (\text{CH}_3)_3\text{Si}^+\), \(147^+: (\text{CH}_3)_2\text{Si} – \text{O} – \text{Si}(\text{CH}_3)_2^+\), \(207^+: (\text{CH}_3)_2\text{Si} – \text{O} – \text{Si}(\text{CH}_3)\), \(281^+: (\text{CH}_3)_2\text{Si} – [\text{O} – \text{Si left}(\text{CH}_3)]^+\), \(149^- (\text{CH}_3)_2\text{Si} – \text{O} – \text{SiH CH}_3\text{O} – \) (Supplementary Figure S1). Positive and negative ToF-SIMS spectra, images and detailed analysis of both fibre substrata are presented in supplementary online materials.

The etching process resulted in a slight decrease in the fibre surface hydrophobicity, with the WCA decreasing from \(\theta = 106 \pm 4^\circ\) on the as-received, to \(\theta = 96.0 \pm 10.1^\circ\) on the etched fibre surface. Markedly, surface tension values remained very similar for both types of surfaces; 9.18 mJ m\(^{-2}\) and 9.66 mJ m\(^{-2}\) for as-received and etched fibres, respectively. The large deviation in the WCA measured on the etched surface is likely to be due to the variable position of the small drops relative to the topography for repeated measurements. Removal of silicone contamination after etching might also play a role in slight decrease of WCA on the etched fibre surface. The composite contact angle measured on the etched fibre surface (which is comprised of solid together with air filled pores) can be used to calculate the contact angle of the solid part of the etched surface using the Cassie-Baxter theory for non-wetting surfaces. However, it is recognised that there has been some debate as to the applicability of this equation for solid surfaces where the size of the surface roughness is small compared to that of the liquid–vapour interface, which is comparable to the size of the droplet used to measure the contact angle (Öner and McCarthy 2000; Gao and McCarthy 2007; Nosonovsky 2007). According to this theory, it can be assumed that the liquid droplet in contact with the etched fibre surface is in contact with both the solid surface and the air trapped in the pores formed by the etching process (Abdelsalam et al. 2005). The apparent contact angle (\(\theta^*\)) of the composite surface allows the contact angle of the solid itself (\(\theta\)) (ie in the absence of air filled voids) to be calculated according to the following relationship:

\[
\cos \theta^* = f_1 \cos \theta - f_2
\]

where \(f_1\) and \(f_2\) are the area fractions of the solid and air filled voids, respectively, with \(f_1 + f_2 = 1\). The area fraction of the air filled voids was calculated to be 0.20 (\(f_2\)), and since \(\theta^*\) was measured as 96 ± 10°, the contact angle on the solid itself (\(\theta\)) was calculated to be 83 ± 12°.

**Surface characteristics of the bacterial cells**

The surface hydrophobicity of the bacterial cells and surface charge (Table 2) varied amongst the species, probably reflecting the different chemical composition of the surface-associated EPS. WCA (\(\theta\)) values of 60° for bacterial cell surfaces were considered to be the borderline between hydrophilic and hydrophobic behaviour (Vogler 1998), which allows the observation that the surface of four of the studied strains, *E. coli*, *S. guttiformis*, *P. aeruginosa* and *P. issachenkonii*, exhibited slightly hydrophilic characteristics, whereas the cell surfaces of *C. marina* and *S. aureus* were found to exhibit hydrophobic characteristics.

The surface charge of each of the bacterial cells is presented in Table 2. It can be seen from the data presented that the least negatively charged bacterium was *P. aeruginosa* (\(\zeta = -14.4 \pm 0.7\) mV), whereas the most negatively charged bacterium was *S. guttiformis* (\(\zeta = -43.2 \pm 0.2\) mV). If these results are considered in the context of the suggested inverse correlation between cell surface charge and bacterial adhesion (Li and Logan 2004) and the electrostatic repulsion between negatively charged bacteria and negatively charged surfaces under commonly encountered pH conditions (Jucker et al. 1996), *S. guttiformis* would be expected to exhibit the weakest and *P. aeruginosa* the strongest attachment preferences to the negatively charged fibre substrata used in this study.

**Bacterial attachment patterns on the non-etched and etched fibre surfaces**

The number of retained bacteria on both fibre surfaces after 12 h incubation was determined and statistically...
analysed (Table 2, Figure 4, Supplementary online material Figure S2). It is clear that all six strains maintained their presence on the smoother, plain fibre substrata, but not on the etched surfaces. *C. marina* and *P. issachenkonii* were the most prominent colonizers with 55,000 and 53,000 attached cells mm$^{-2}$, respectively. The attachment patterns of the six bacterial strains can be seen on the high resolution SEM images (Figure 4).

Granular deposits of variable size were also detected for *E. coli*, *P. aeruginosa*, *P. issachenkonii* and *S. guttiformis* on both types of surfaces. These deposits are presumed to be EPS secreted by adhering cells and it is likely that they serve as primers that facilitate bacterial adhesion. The secretion of EPS by these strains during colonization of other surfaces has previously been reported (Goldstein et al. 1964; Ivanova et al. 2008a, 2008b, 2009; Mitik-Dineva et al. 2008b, 2009b, 2009a). Interestingly, *C. marina* and *S. aureus* cells, while also being successful colonizers of the non-etched fibre surface, did not produce EPS to the same extent as *E. coli*, *P. aeruginosa*, *P. issachenkonii* and *S. guttiformis*. A remarkably different bacterial response was observed on the etched fibre substrata. Although none of the tested strains were able to remain attached to the etched fibre substrata, varying quantities of EPS were still detected around and inside the wells.

EPS aggregations produced by *E. coli*, *P. aeruginosa* and *P. issachenkonii* were mostly located on and around the fibre cores, whilst granular EPS of different sizes produced by *S. guttiformis* (Figure 4f) was randomly deposited over the fibre cores and the surrounding cladding. Neither the SEM nor the CLSM analysis showed any *C. marina* or *S. aureus* cells remaining on either of the fibre substrata. In addition, the EPS produced by both strains was considerably less than that detected for the other strains.

**Discussion**

Even though the relationship between bacterial attachment patterns and cell surface characteristics (such as hydrophobicity and surface charge) has been studied intensively over the past few decades, the somewhat contradictory results have not allowed the formulation of a reliable correlation (van Loosdrecht et al. 1990; Li and Logan 2004). This is most likely due to the fact that bacterial survival strategies include a dynamic attachment process that is dependent on the presence, chemical composition and structure of extra-cellular surface components (Auerbach et al. 2000; Danese et al. 2000; Jain et al. 2007).

In the present study, the two strains with highly hydrophobic cell surfaces, *C. marina* and *S. aureus*, displayed rather different attachment tendencies. *C. marina* cells were successful colonizers of the as-received fibre substrata, while *S. aureus* cells did not attach onto these to any significant extent (Figure 4, Supplemental online material Figure 2S). Considering the electrostatic properties of bacterial cell surfaces, *S. guttiformis* cells, having the highest negative charge, would be expected to have the weakest propensity for attachment to both fibre substrata and *P. aeruginosa* the strongest propensity for attachment. However *S. guttiformis* cells were seen to attach in greater numbers than *P. aeruginosa* (Figure 4, Table 2). The most successful colonizers overall were *C. marina* and *P.
isachenkonii, both of which exhibited a significantly higher surface charge than \( P. \ aeruginosa \). The observation reported here that the bacteria responded differently on the same surfaces, is supported by data from earlier studies, where it was reported that bacterial cells can exhibit variable surface characteristics due to the presence of EPS on the outer surface, their constant dynamic motion and the presence or absence of molecular areas of variable polarity or charge (Li and Logan 2004; Vadillo-Rodriguez et al. 2004). The EPS material usually encapsulates the cell and/or is released into the liquid phase as planktonic EPS. These substances are believed to serve as promoting factors for bacterial attachment and biofilm formation (Beech et al. 1991; Favre-Bonte et al. 1999; Evans 2000). Nevertheless, an increasing amount of information suggests that the function of the EPS depends on its composition (Sutherland 2001). For example, capsular polysaccharides of \( Vibrio vulnificus \) are mainly composed of uronic acids, and contribute to an increase in negative charge and hydrophilicity (Wright et al. 1990) and hence inhibit the attachment of the bacterium. Other chemically different polysaccharides, eg those of \( P. \ aeruginosa \) composed of neutral sugars, may promote adhesion to the same surfaces (Yildiz and Schoolnik 1999; Wozniak et al. 2003).

In previous studies the authors have tested the attachment behaviour of a number of medically and environmentally challenging bacteria to various substrata, such as glass, polymer and titanium (Ivanova et al. 2008a, 2009; Mitik-Dineva et al. 2008b, 2009a, 2009b). Results obtained so far indicate that neither the cell surface hydrophobicity nor the charge correlates with the level of bacterial attachment. Hence neither of the cell surface characteristics studied here could provide a reasonable explanation for the different bacterial responses towards the two fibre substrata.

Substratum physico-chemical characteristics such as hydrophobicity, charge, chemistry and topography have also been intensively studied in an attempt to predict trends in bacterial attachment behaviour (Busscher and van der Mei 1997; Bos et al. 1999, 2000; Teixeira and Oliveira 1999; Pereira et al. 2000). The results of the present study also indicate that the only property of the imaging fibre substrata that is significantly affected by the chemical modification is the surface roughness. All of the other surface parameters remained nearly constant. Therefore the observed difference in the bacterial response to the two types of fibre substrata appears to be principally associated with the change in the surface structure. Importantly, irrespective of their taxonomic affiliations and species-specific characteristics, all of the studied strains attached to the smoother, plain fibre substrata, while no bacterial cells were retained on the etched fibre surfaces.

The current understanding of the effects of surface topography on bacterial adhesion, including the “attachment point” theory, suggests that bacteria prefer microscopic surface irregularities as the starting point for their attachment, as these provide shelter from unfavorable environmental influences (Howell and Behrends 2006; Riedewald 2006; Scardino et al. 2006; Whitehead and Verran 2006). However, recent work in this field, suggests that this might not always be the case (Ivanova et al. 2008a, 2009; Mitik-Dineva et al. 2008b, 2009b). In particular, nano-scale surface roughness may have a significant effect on bacterial adhesion, with differences in the surface roughness of just a few nanometers appearing to exert a strong influence on the cellular response to certain surfaces (Howell and Behrends 2006; Riedewald 2006; Whitehead and Verran 2006; Ivanova et al. 2008b, 2009; Mitik-Dineva et al. 2008b, 2009). The results presented here show that a variable number of bacterial cells were able to colonize the nano-scale rough, non-etched fibre substrata, whereas less smooth, micro-scale rough, etched fibre substrata might sustain cellular attachment below the detection limit. It is notable that the same adhesion tendency was observed for all of the tested strains on the etched fibres, regardless of their taxonomic affiliation and their cell surface characteristics.

In summary, an improved understanding of cell-surface interactions may facilitate the design and manufacture of optical fibre sensing surfaces with cyto-attractive or cyto-repellent characteristics, depending on the particular application requirements (Bos et al. 2000; Polwart et al. 2000; Bernhard et al. 2001; D’Souza 2001; Biran et al. 2003; Kuang et al. 2004; Howell and Behrends 2006; Riedewald et al. 2006). The results of this study suggest that plain FIGH-70-1300N optical fibres would make suitable substrata for the development of whole cell biosensors, due to the fact that they possess surface characteristics that allow bacterial attachment. On the other hand, the modified fibre surfaces were found to be amenable to bacterial attachment and might have cyto-repellent potential. They may be more suitable for the development of Surface Enhanced Raman Spectroscopy (SERS) substrata or optic probes where a bacteria-free environment is desirable. The results of this study will be of use for the construction of chemical sensors, whole-cell biosensors, SERS probes or other optical fibre instrumentation.

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