Bacterial interactions with optical fibre surfaces

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***Abstract*— despite recent advances in the technology, very little is known about the interactions between bacteria and the optical fibre surfaces. The work presented herein aims to determine the influence of surface characteristics and chemistry of standard optical imaging fibres used in biomedical applications on the attachment behavior of three medically and environmentally significant microorganisms (*Pseudomonas aeruginosa, Pseudolateromonas issachenkonii* and *Cobetia marina*).**

Keywords-bacteria, adhesion, optical fibres

I. INTRODUCTION

In the recent years numbers of novel techniques and instruments have been introduced in the areas of nano- and applied science. Some of these newly developed instruments exploit fibre optic technology. Although mainly used for applications in industries such as television, telecommunications, laser machining, dentistry and medicine, optical fibres can also be used as thermal, chemical, environmental or biological sensors [1]. While performing as either imaging or biosensing devices the fibre surface is inevitably exposed to various environmental perturbations including microbial colonization. Therefore, investigation of the cell-optical fibre surface interactions appears to be of critical importance when designing such instruments. Attachment patterns and bacterial metabolic activities of *P. issachenkonii*, *C. marina* and *P. aeruginosa* cells on optical fibre substrates were analyzed from series of scanning electron microscopy (SEM) and atomic force microscopy (AFM) analyses.

II. MATERIAL AND METHODS

A. Optical fibres

The experimental surfaces used for this study were glass optical fibres (FIGH-70-1300N, Fujikura Ltd). This is a standard optical imaging fibre, with approximately 70,000 picture elements and total outer diameter of 1.3 mm, made from germanium doped silica glass cores surrounded by fluorine-doped silica cladding. Two working substrates were prepared from the fibre, the so-called "as-received" substrates, obtained by cleaving the original fibre, and the "modified" substrates fabricated by exposing the as-received substrates to buffered hydrofluoric acid [2]. A detailed analysis of the fibres, including their surface wettability (θ), tension (γ), chemistry and roughness (R), was obtained through contact angle measurements, surface tension calculations, ToF-SIMS analysis, SEM and AFM.

B. Microorganisms - culture conditions and sample preparation

P. issachenkonii, *C. marina* and *P. aeruginosa* bacterial strains were selected for the study. All bacteria were routinely cultured in a marine (Oxoid) or nutrient (Merck) agar and stored at -80°C. Prior to each experiment, a fresh bacterial suspension was prepared for each of the strains. On the day of the experiment, an aliquot of 2 mL 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 as-received and etched fibres were placed into each of the tubes and were incubated for 12 hours at room temperature (*ca*. 22°C). After incubation, all samples were rinsed three times with sterilizedH_2O (18.2 $M\Omega cm^{-1}$ Barnstead/Thermolyne NANOpure Infinity water purification system), were attached to glass supports and stored under sterile condition until needed.
Bacterial cell surface hydrophobicity was determined from a series of water contact angle (WCA) measurements using the sessile drop method as described elsewhere [3]. Bacterial surface charge was determined via measurement of the electrophoretic mobility (EPM) of the cells and converted to zeta potential using Smoluchowski's approximation [3].

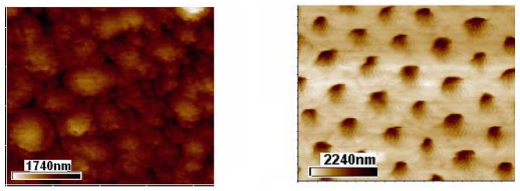
C. Visualisation and quantification of bacerial adsorption and metabolic activity

Bacterial attachment patterns and their morphological changes were observed using SEM. 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 substrate surface to be determined. The average densities have estimated errors of approximately 10-15%, due to local variability in the coverage.

III. RESULTS AND DISCUSSION

A. Fibre surface characteristics

Fibre surface characteristics, such as topography, wettability, charge and surface chemistry were all investigated. Results indicated that the fibre surface topography has significantly changed after the exposure to the etching solution (Figure 1).



(a) (b)

Figure 1. Typical 2D AFM images of the as-received (a) and modified (b) fibre substrates (scanned areas 4 µm x 4 µm).

As seen by the AFM images, the difference in the chemical structure between the fibre components resulted in the modified optical fibre surfaces displaying a general hexagonal pattern of cylindrical wells approximately 2.5 µm in diameter and 2.5 µm deep. Roughness parameters from both surfaces that support the visually observed differences in the surface topography are presented in Table I. All roughness parameters appeared to be considerably higher on the etched fibre substrates than the non-etched samples as confirmed by statistical analysis (p<0.05). The surface roughness increased more than tenfold for the R_q (235 on the as-received and 3425 on the modified fibre surface) which is believed to be more a sensitive indicator of roughness than the average surface roughness parameter (R_a) when considerable deviations from the mean line occur (as the case in this instance) [4].

TABLE I. SURFACE WETTABILITY, TENSIONS AND ROUGHNESS OF THE AS-RECEIVED AND THE MODIFIED FIBRE SURFACES.

Type of analysis	As-received	Modified
θw[a] (°)	107.2	106.9
θdm (°)	129.6	102.6
θf (°)	102.3	101.3
γ^{LWb} (mJ m^{-2})	1.67	7.89
γ^{AB} (mJ m^{-2})	7.51	1.77
γ^{+} (mJ m^{-2})	3.34	0.18
γ^{-} (mJ m^{-2})	4.22	4.38
R_a (nm)	180 ± 9	1563 ± 77
R_q (nm)	235 ± 9	3428 ± 173
R_{max} (nm)	1740 ± 84	2400 ± 96

[a]θ_W, θ_F and θ_D: water, formamide and diidomethane contact angles respectively.

[b]Surface free energies components: Lifshitz-van der Waals (γ^{LW}), acid/base (γ^{AB}), electron acceptor (γ^{+}) and electron donor (γ^{-}) components

Table I also combines the surface wettability results which indicate that exposure of the fibre surfaces to the etching solution decreased the surface hydrophobicity, with the contact angle reducing from θ=106.0° ± 3.7° on the as-received, to θ=96.0° ± 10.1°on the modified fibre surface.

The surface chemistry of both fibre surfaces was explored by ToF-SIMS analysis, which did not reveal appreciable differences between both substrates (data not shown). The most abundant component on both surfaces was found to be Si, followed by SiC_3H_9, $SiCH_3$, CH_3 and Na, representing approximately 70% of the elemental composition for both surfaces. The only apparent difference was the lesser representation of germanium and higher concentrations of fluorine on the modified surface. This change was expected, since the precise effect of the etching process was to remove some of the germanium ions from the fibre surface. The higher concentrations of fluorine are most likely residual fluorine from the etching solution remained on the surface. Both surfaces showed an appreciable contamination by adventitious carbon.

With respect to the fibre surface charge, it is well accepted that materials mainly based on silica, such as the optical fibre used here, always carry some negative charge on their surface when in contact with water or air [5].

B. Bacterial surface characteristics

Bacterial surface wettability and charge were also measured. As the data presented in Table II indicate, both bacterial surface characteristics varied amongst the species, probably reflecting differences in chemical composition of surface-associated extra-cellular polymeric substances.

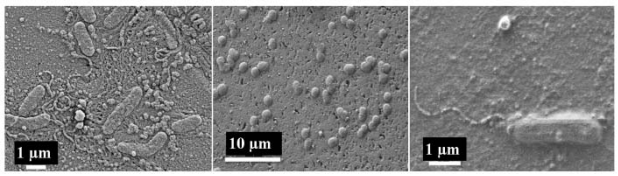
TABLE II. WCA, EPM AND BACTERIAL RETENTION PROFILRS ON THE AS-RECEIVED AND MODIFIED FIBRE SURFACES

Strain	WCA (θ)	EPM (µs^{-1} V cm^{-1})	Number of attached cells × 10^3 mm^2	
			As-received fibre	Modified fibre
P. aeruginosa	43 ± 8	-1.1 ± 0.1	1.2± 0.03	Below detection limit
C. marina	75 ± 9	-2.5 ± 0.6	55 ± 0.41	Below detection limit
P. issachenkonii	52 ± 3	-2.9 ± 0.2	53 ± 0.29	Below detection limit

WCA (θ) values of 60° for bacterial cell surfaces were considered to be the borderline between hydrophilic and hydrophobic behavior [6], which allows the assumption that the surface of *P. aeruginosa* and *P. issachenkonii* exhibited slightly hydrophilic characteristics, whereas *C. marina* cells were found to be more hydrophobic. This would suggest that *C. marina* cells would be expected to attach in highest numbers on both fibre substrates as they both exhibited hydrophobic characteristics. It is also clear that the least negatively charged bacterium was *P. aeruginosa* (EPM = -1.1 µs^{-1} V cm^{-1}), whereas the most negatively charged bacterium was *P. issachenkonii* (EPM = -2.9 µs^{-1} V cm^{-1}). If these results are considered in the context of the suggested inverse correlation between cell surface charge and bacterial adhesion [7] and the electrostatic repulsion between negatively charged bacteria and negatively charged surfaces, the *P. issachenkonii* would be expected to exhibit the weakest and *P. aeruginosa* the strongest attachment preferences to the negatively charged fibre substrata used in this study.

C. Bacterial attachment patterns

The results presented clearly indicate that after 12 h incubation all three strains maintained their presence on the smoother, as-received fibre substrates, but not on the etched, modified, surfaces. *C. marina* and *P. issachenkonii* were the most prominent colonizers with 55,000 and 53,000 attached cells per mm^2, respectively. Their attachment patterns are presented on the high resolution SEM images in Figure 2.



(a) (b) (c)

Figure 2. Typical SEM image of *P. issachenkonii* (a), *C. marina* (b) and *P. aeruginosa* (c) cells and EPS deposits on the as-received fibre surface.

The SEM images presented also indicate the presence of granular extracellular polymeric materias (EPS) deposits of variable size for *P. aeruginosa* and *P. issachenkonii*. Similar results were also obtained using confocal laser scanning microscopy after fluorescent labeling (data not shown). It is suggested that the EPS deposits secreted by adhering cells may serve as primers that facilitate bacterial adhesion. The secretion of EPS by these strains during colonization of other surfaces has previously been reported [8, 9]. Interestingly, *C. marina* cells, while also being successful colonizers of the non-etched fibre surface, did not produce EPS to the same extent as the other two strains.

On the other hand, 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 fluorescently labeled EPS were still detected by CLSM.

Although a number of studies has addressed the effects of various surface parameters deriving from both bacteria and substrata in terms of their effect on bacterial attachment, contradictory results have not allowed the formulation of a reliable correlation Results presented herein suggest that whilst surface chemistry, wettability and charge remained almost unaffected by the etching process, the fibre surface roughness and topography were significantly affected. Therefore the observed difference in the bacterial response to the two types of fibre substrates can be attributed to the change in the surface topography. Importantly, irrespective of their taxonomic affiliations and species-specific characteristics, all of the studied strains attached to the smoother, as-received fibres, while no bacterial cells were retained on the modified fibre surfaces.

IV. CONCLUSION

Existing understanding of the effects of surface topography on bacterial adhesion suggest that bacteria prefer microscopic surface irregularities as the starting point for their attachment, as these provide shelter from unfavorable environmental influences [10-13]. However, it has recently been demonstrated that nano-scale changes in the surface roughness may have stimulating effect on bacterial adhesion [8, 9]. In a similar manner, the results presented here demonstrate that variable number of bacterial cells were able to colonize the nano-scale rough, as-received fibre substrates, whereas no cells were detected on the micro-scale rough, modified fibre surfaces. It is notable that the same adhesion tendency was observed for all of the tested strains, regardless of their taxonomic affiliation and their cell surface characteristics.

The results of this study suggest that a thorough understanding of cell-surface interactions will be of interest to those designing and manufacturing biosensing devices, SERS probes or other optical fibre instruments with cyto-attractive or cyto-repellent characteristics, depending on the particular application requirements.

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