
REMOVAL OF BACTERIA FROM SOLID SURFACES BY WIPING WITH NONWOVEN FABRICS

Nicholas William Moses Edwards

Submitted in accordance with the requirements for the degree of
Doctor of Philosophy

The University of Leeds
School of Design

April 2017

Under the supervision of Professor Stephen Russell and Dr Parikshit Goswami

The candidate confirms that the work submitted is his own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 2 contains work also included in the publication: Goswami, P and Edwards, NWM (2017) "Plasma-Based Treatments of Textiles for Water Repellency - Low-Pressure, Atmospheric and Dielectric Barrier Discharge Methods" in Waterproof and Water Repellent Textiles and Clothing. ISBN 9780081012123.

In the above work, N Edwards carried out the review work and was joint author of the book chapter. P. Goswami carried out editing and proof-reading of the text was joint author of the book chapter.

Chapter 4 contains work also included in the publication: Edwards NWM, Best EL, Connell SD, Goswami P, Carr CM, Wilcox MH, Russell SJ (2017) "Role of surface energy and nano-roughness in the removal efficiency of bacterial contamination by nonwoven wipes from frequently touched surfaces", Science and Technology of Advanced Materials, 18:1, 197-209, DOI: 10.1080/14686996.2017.1288543

In the above work, N Edwards carried out all of the practical and analytical work and was lead author of the paper. E.L. Best provided assistance with microbiology techniques and analysis; S.D. Connell provided assistance with atomic force microscopy analysis; P. Goswami, C.M. Carr, M.H. Wilcox and S.J. Russell carried out proof-reading and editing of the text.

Chapter 5 contains work also included in the publication: Edwards NWM, Best EL, Goswami P, Wilcox MH, Russell SJ (2017) "Factors affecting Removal of Bacterial Pathogens from Healthcare Surfaces during Dynamic Wiping", Textile Research Journal (Accepted).

In the above work, N Edwards carried out all of the practical and analytical work and was lead author of the paper. E.L. Best provided assistance with microbiology techniques and analysis; P. Goswami, M.H. Wilcox and S.J. Russell carried out editing and proof-reading of the text.

This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

©2017 The University of Leeds and Nicholas William Moses Edwards.

The right of Nicholas William Moses Edwards to be identified as Author of this work has been asserted by him in accordance with the Copyright, Designs and Patents Act 1988.

Contents

List of Figures	vii
List of Tables	x
Table of Equations	xii
List of Abbreviations	xiii
Acknowledgements	xiv
Abstract	1
Chapter 1	3
Introduction	3
1.1 Hospital Acquired Infection	3
1.2 Aims and Objectives	8
1.3 Thesis Structure	8
Chapter 2	11
Literature Review	11
2.1 Infection and Pathogens	11
2.1.1 Bacteria	13
2.1.1 Peptidoglycans and the cell wall	17
2.2 Adhesion to surfaces	20
2.2.1 Bacteria-substratum interaction	21
2.2.2 Biofilms	25
2.3 Disinfection and decontamination	28
2.3.1 Biocides	28
2.3.2 Resistance to disinfection and decontamination	33
2.4 Wiping Dynamics	36
2.4.2 Studies of Wiping in Relation to Infection Control and HCAs	42
2.4.3 Manufacture, Structure and Properties of Industrial Wet Wipes for Healthcare Settings	45
2.4.4 Nonwoven wipe formation	49
2.4.5 Bonding Process	51
2.4.6 Wetlaid	53
2.4.7 Substrates	54
2.5 Modification of nonwoven wipe substrates by plasma treatment	56
2.5.1 Thermal plasma	57

2.5.2 Non-thermal plasma	58
2.6 Atmospheric Plasma Treatment.....	59
2.6.1 Corona Plasma Treatment.....	59
2.6.2 Glow Discharge Plasma	60
2.6.3 Dielectric Barrier Discharge Plasma	60
2.7 Low Pressure Plasma	60
2.8 Surface Modification with Plasma	61
2.8.1 Physical.....	61
2.8.2 Chemical	61
2.9 Summary.....	63
Chapter 3	65
Experimental Materials and Methods	65
3.1 Preparation of Nonwoven Wipe Substrates	65
3.2 Plasma functionalisation of nonwovens	69
3.3 Measurement of Surface Energy	71
3.4 Model Healthcare Surfaces	72
3.5 Surface roughness of fibres and cleaning surfaces	72
3.6 Mass spectrometry	73
3.7 Biocide and neutraliser	74
3.8 Bacterial strains	76
3.9 Measurement of bacterial removal efficiency	76
3.10 Agar Diffusion Plate Test	77
3.11 Recontamination of Cleaned Surfaces	78
3.12 Residual Antimicrobial Activity	78
3.13 Orthogonal Array Design of Experiments (OATs) and Taguchi Analysis.....	79
3.14 Electrophoretic mobility of bacterial cells	83
3.15 Streaming potential of nonwoven wipe substrate fabrics.....	84
3.16 Surface wetting tension	85
3.16.1 Contact angle	85
3.16.2 Contact angle hysteresis.....	87
3.17 Scanning electron microscopy (SEM)	87
3.17.1 Bacteria residing on fibre surfaces	87
3.17.2 Morphological features of model healthcare surfaces and fibres in the nonwoven substrates	88

3.17.3	Solid surface area fraction at the wipe-surface interface	88
3.18	Statistical analysis	89
Chapter 4		90
	Role of surface energy and nano-roughness on the bacterial removal efficiency of nonwoven wipes from a solid surface	90
4.1	Introduction	90
4.2	Experimental Design	92
4.3	Results and discussion	93
4.3.1	Plasma treatment	93
4.3.2	Wetting behaviour of the wiping surface	107
4.3.3	Bacterial removal from solid surfaces	108
4.3.4	Streaming potential of nonwoven fabric substrates and zeta potential of bacteria	109
4.3.5	Removal of bacteria via adpression	110
4.3.6	Dynamic wiping	111
4.4	Interaction of bacteria with wipe fibre surfaces under dynamic wiping conditions	117
4.5	Summary	130
Chapter 5		132
	Factors affecting Removal of Bacteria from Solid Surfaces during Dynamic Wiping	132
5.1	Introduction	132
5.2	Experimental Design	134
5.3	Results and Discussion	135
5.3.1	Optimum process parameters	135
5.3.2	Bacterial removal efficiency trends	139
5.3.3	Fibre composition of the wipe substrate	147
5.4	Summary	148
Chapter 6		151
	Recontamination of Solid Surfaces and Residual Activity following Wiping with Biocidal Liquid-Impregnated Nonwoven Wipes	151
6.1	Introduction	151
6.2	Experimental Design	153
6.3	Results and Discussion	154
6.3.1	Analysis of Surfaces	154
6.3.2	Residual Antimicrobial Activity	166

6.3.3 Bacterial Removal and Surface Recontamination	168
6.4 Summary.....	178
Chapter 7	180
Conclusions.....	180
7.1 General Conclusions	180
7.2 Recommendations for Further Work	184
References.....	186

List of Figures

Figure 2-1. Example of a typical hospital patient-care area, and the numerous different surfaces contained within (43).....	12
Figure 2-2 Schematic of Gram-positive and Gram-negative bacteria. Gram positive bacteria have a thick layer of peptidoglycan. Gram negative bacteria have a thin peptidoglycan layer and an outer membrane. Structures in (parentheses) are not found in all bacteria. Adapted from “Medical Microbiology” (57).....	14
Figure 2-3. Structure of N-acetylglucosamine. (Created in ChemDraw Pro).	18
Figure 2-4. Structure of N-acetylmuramic acid. (Created in ChemDraw Pro).....	19
Figure 2-5. Peptidoglycan monomer. (Created in ChemDraw Pro). “n” denotes repeating unit.....	19
Figure 2-6. The surface organelles of a bacterial cell.	22
Figure 2-7. Surface interactions affecting bacterial adhesion.....	24
Figure 2-8. Surface property changes caused by close proximity of planktonic bacterial cells. (A) pH; (B) osmolality; (C) flagella inhibition.....	24
Figure 2-9. Scanning Electron Micrograph of a native biofilm on a steel surface. Reproduced from Donlan (130).....	26
Figure 2-10. Benzalkonium chloride structure, where n=10-16. (Created in ChemDraw 12).....	31
Figure 2-11. Hydrolysis of β 1-4 glycosidic bond by Benzalkonium Chloride. (Created in ChemDraw 12).....	32
Figure 2-12. Force diagram for sphere adhesion and removal from a flat plane during wiping. Adapted from Verkouteren et al. (168).	36
Figure 2-13. Polypropylene repeating unit where n is number or repetitions. Figure created using ChemDraw 12.....	55
Figure 2-14. Parallel and anti-parallel chains.	55
Figure 2-15. Definitions and characteristics of technical plasma processes used for material processing.	57
Figure 3-1. Hydrolace pilot line.	68
Figure 3-2. Diener Plasma Reactor.....	70
Figure 3-3. KRÜSS K100 tensiometer.....	71
Figure 3-4. Ion-ToF SIMS unit.	73
Figure 3-5. Malvern Zetasizer nano-ZS.	83
Figure 3-6. Anton-Paar SurPASS EKA.	84

- Figure 3-7. Illustration of contact angles formed by sessile drops on smooth, homogenous solid surfaces. θ indicates contact angle, γ_{lv} indicates liquid-vapour interface tension; γ_{sv} indicates solid-vapour interface tension; γ_{sl} indicates solid-liquid interface tension.....86
- Figure 4-1 Surface energy of nonwoven wipe substrate fabrics vs. C_2F_6 low temperature, low pressure plasma exposure time, measured via the OWRK method (274). \triangle = O_2 treated lyocell; \times = C_2F_6 treated lyocell; \diamond = Untreated lyocell; \bullet = untreated PP. Fit line and data points for C_2F_6 treated lyocell, unless specified otherwise. Data is the mean of five replicates. Error bars = Standard deviation. n=5.....95
- Figure 4-2. Negative ion ToF-SIMS spectra of untreated and C_2F_6 plasma treated lyocell nonwoven fabrics. Mass range $m/z = 0-360$. n=5. ..97
- Figure 4-3. AFM micrograph of untreated fibre surface - Untreated lyocell fibre surface, R_a 0.18 nm101
- Figure 4-4. AFM micrograph of plasma treated fibre surface - C_2F_6 plasma-treated lyocell fibre surface after 1 minute exposure, R_a 1.36 nm102
- Figure 4-5. AFM micrograph of plasma treated fibre surface - C_2F_6 plasma-treated lyocell fibre surface after 4 minute exposure, R_a 1.6 nm.103
- Figure 4-6. AFM micrograph of plasma treated fibre surface - C_2F_6 plasma-treated lyocell fibre surface after 20 minute exposure, R_a 2.27 nm104
- Figure 4-7. AFM micrograph of plasma treated fibre surface – O_2 plasma-treated lyocell fibre surface after 20 minute exposure, R_a 3.81 nm105
- Figure 4-8. AFM micrograph of untreated fibre surface - Untreated PP fibre surface, R_a 2.64 nm106
- Figure 4-9. Bacterial removal by wiping. (A) Mean *E. coli* removal by adpression method; (B) Mean *S. aureus* removal by adpression method; (C) Mean *E. faecalis* removal by adpression method. (D) Mean *E. coli* removal by dynamic wiping; (E) Mean *S. aureus* removal by dynamic wiping; (F) Mean *E. faecalis* removal by dynamic wiping. Error bars represent standard error of the mean (SEM). n=9115
- Figure 4-10. (L) SEM micrograph of *E. faecalis* on untreated PP fibres within the nonwoven fabric sample after dynamic wiping.129
- Figure 5-1. Removal efficiency of wipe vs. fabric basis weight, with linear best fit. A - C 100% lyocell wipes, D - F 100% PP wipes. Error bars indicate standard deviation, * indicates significant difference ($p>0.05$).141
- Figure 6-1. (C) SEM of PMMA healthcare surface sample wiped with biocide.....157
- Figure 6-2. (C) SEM of steel healthcare surface sample wiped with biocide.....160
- Figure 6-3. (C) SEM of Ceramic healthcare surface sample wiped with biocide.....163

Figure 6-4. Total bacterial recontamination of surfaces of healthcare surfaces of different average roughness (R_a) by nonwoven lyocell wipes, calculated according to Chapter 3 section 3.11. n=3.....174

Figure 6-5. Total bacterial recontamination of healthcare surfaces of different average roughness (R_a) by PP nonwoven wipes, calculated according to Chapter 3 section 3.11. n=3.....175

List of Tables

Table 1-1. Definitions for commonly used terms	6
Table 2-1. Examples of common wet wipe formulations for solid surface disinfection and decontamination.....	30
Table 2-2. Examples of bacterial intrinsic resistance mechanisms to biocides. Adapted from Masri et al. (158).....	34
Table 3-1. Orthogonal array parameters arranged in a 3**3 Taguchi array.	80
Table 3-2. Optimum process parameter (OPP) calculation scheme and results.	82
Table 4-1. Mean contact angles and wetting tensions of PMMA wiping surfaces according to organic load, as measured by goniometry (section 3.16). n=3.....	107
Table 4-2. Mean streaming potential of nonwovens. S.D. indicates standard deviation. n=4.....	109
Table 4-3. Mean zeta potential of bacteria. S.D. indicates standard deviation. n=3.....	110
Table 4-4. Comparison of static wiping vs. dynamic wiping via paired-sample t test.	116
Table 5-1. Bacterial removal efficiency results for the polypropylene and the lyocell wipes for E. coli, S aureus and E. faecalis. n=3.	138
Table 5-2. Slope and intercept for removal efficiency vs. basis weight graph best fit lines from Figure 1.	140
Table 5-3. Optimised results for fabric basis weight, liquid addition and wiping pressure.....	146
Table 5-4. Solid (fibre) area fraction at the wipe surface interface. Means that do not share a grouping letter are significantly different from each other. S.D. is standard deviation. n=5.	147
Table 6-1. EDX analysis of biocide and control PMMA (Perspex) samples (Figure 6-1). n=3.	158
Table 6-2. EDX analysis of biocide vs control steel samples (Figure 6-2). n=3.....	161
Table 6-3. EDX analysis of biocide vs control ceramic samples (Figure 6-3). n=3.....	165
Table 6-4. Residual antimicrobial activity of biocide. “+” value indicates increase in bacterial recovery versus control, “-“indicates a reduction in the number of bacteria recovered versus control. SEm indicates standard error of the mean. Steel (R) denotes rough steel samples. n=3.	167

Table 6-5. Contact angle, wetting tension and roughness of model surfaces. Steel (R) denotes rough steel samples, Steel (S) denotes smooth steel samples. R_a means that do not share a “grouping” letter are significantly different - ANOVA with a post hoc Tukey’s test (p < 0.05). n=3.....169

Table 6-6. Removal of bacteria and recontamination of surfaces of different material compositions by a lyocell nonwoven wipe.172

Table 6-7. Removal of bacteria and recontamination of surfaces by polypropylene nonwoven wipe.173

Table of Equations

$pB = 4. \sigma 1. \cos \theta dp. max$	Equation 2-1	39
$DWAC = tw1 - tw2tw2 \times 100$	Equation 2-2.....	39
$DWE = VSVC \times 100$	Equation 2-3.	40
$DWE = (tw1 - tw2)d VC \times 100$	Equation 2-4	40
$WPRA = \frac{Collected\ particles}{Total\ particulate\ challenge} \times 100$	Equation 2-5.	40
$K'e = 1.1nC d^3 D^2 p^{1.5} v b m p w^{0.5}$	Equation 3-1.....	67
$R = \frac{Cct - Cwt}{Cct} \times 100$	Equation 3-2.....	77
$\gamma l v \cos \gamma \theta = \gamma s v - \gamma s l$	Equation 3-3.....	86
$FP_{psi} = \frac{F_{pixels}}{F_{pixels} + V_{pixels}} \times 100$	Equation 3-4	89

List of Abbreviations

- AFM - Atomic force microscopy
- ANOVA – Analysis of variance
- C. difficile* - Clostridium difficile
- dH₂O – Deionised water
- DWAC - Dynamic wiping absorbent capacity
- DWE – Dynamic wiping efficiency
- E. coli* – Escherichia coli
- E. faecalis* - Enterococcus faecalis
- EDX - Energy-dispersive X-ray spectroscopy
- EPS – Exopolysaccharide(s)
- HAI – Hospital acquired infection
- HCAI – Healthcare associated infection
- MRSA - Methicillin-resistant Staphylococcus aureus
- NHS – National Health Service
- OATS – Orthogonal array testing strategy
- P. aeruginosa* - Pseudomonas aeruginosa
- PBS – Phosphate buffered saline
- PMMA - Poly (methyl methacrylate)
- PP – Polypropylene
- S. aureus* - Staphylococcus aureus
- SEM - Scanning electron microscopy
- SEm – Standard error of the mean
- ToF-SIMS - Time-of-flight secondary ion mass spectrometry

Acknowledgements

I have many people to thank for helping me during the course of my PhD – I will attempt to name some of them below.

Firstly, my PhD supervisors, without whom I would never have made it to this final stage: Professor Stephen Russell and Dr Parik Goswami. Thanks for the comments, editing, direction, assistance and making sure I did not go off on too many tangents.

I would like to thank the Clothworkers' Foundation and the Clothworkers' Centre for Textile Materials Innovation for Healthcare for their generous financial support, without which I could never have completed my studies.

Professor Chris Carr and Giles Crowley have been instrumental in providing materials, developing a relationship with industry and analysing ToF-SIMS results. Professor Mark Wilcox allowed me use of his infection control laboratory and Dr Emma Best provided crucial guidance and assistance in developing the methodologies used to analyse bacterial removal.

Other university staff have provided invaluable help. Manoj Rathod for assistance with sample manufacture; Dr Simon Connell for help with atomic force microscopy; Gareth Griffiths for use of his workshop; Algy Kazlauciusas for his SEM and EDX facilities; and Martin Fuller for help with SEM analysis.

Finally, friends and colleagues past and present as well as my parents, brother and girlfriend for emotional support and sharing a few beers when the need often arose.

Abstract

Healthcare associated infections are responsible for substantial patient morbidity, mortality and economic cost. Infection control strategies for reducing rates of transmission include the use of nonwoven wipes, with or without a biocidal liquid, to remove pathogenic bacteria from frequently touched surfaces. Considerable research has been conducted on the role of biocides in disrupting microbes such as bacteria, but less is known about the influence of wiping surfaces with nonwovens regarding their removal. This research considers the role of intrinsic and extrinsic factors on the removal of bacterial contamination from model healthcare surfaces. The extent to which systematic changes in wipe fibre surface energy and nano-roughness influence removal of bacteria from a polymer surface in dry wiping conditions was studied. Nonwoven wipe substrates composed of two commonly used fibre types, lyocell (cellulose II) and polypropylene (PP), with different surface energies and nano-roughnesses, were experimentally manufactured. The surface energy and nano-roughness of lyocell substrates were modified by either oxygen or hexafluoroethane plasma treatment. Static wiping of an inoculated surface under dry conditions produced bacterial removal efficiencies of between 9.4 colony forming unit (CFU) % and 15.7 CFU % versus control, with no significant difference ($p < 0.05$) in the relative removal efficiencies of *E. coli*, *S. aureus* or *E. faecalis*. Dynamic wiping increased peak wiping efficiencies to >50 CFU % versus static wiping ($p < 0.05$), depending on fibre type and bacterium. Under dynamic wiping conditions, nonwoven wipe substrates with a surface energy closest to that of the contaminated surface produced the highest *E. coli* removal efficiency, while the associated increase in fibre nano-roughness

abrogated this trend with *S. aureus* and *E. faecalis*. Considering both intrinsic and extrinsic factors of wiping and design factors on the removal of pathogenic bacteria, the single most important parameter affecting bacterial removal efficiency was impregnation with biocidal liquid ($p < 0.05$). However, dynamic wiping in the dry state and with water alone without biocide still resulted in substantial removal. Bacterial removal was therefore not conditional on the presence of a biocide. For 100% lyocell wipes impregnated with biocidal liquid, removal of *E. coli*, *S. aureus* and *E. faecalis* improved by increasing the fabric basis weight and hand weight wiping pressure to their maximal values (150 g.m⁻² and 13.80 kN.m⁻² respectively). For 100% polypropylene wipes, the same conditions maximised the removal efficiency of *S. aureus*. For *E. coli* and *E. faecalis*, a reduction in the hand weight wiping pressure to 4.68 kN.m⁻² was required to maximise the removal efficiency with 100% polypropylene. Generally, the lyocell wipes were more effective in removing bacterial contamination than 100% polypropylene wipes. The removal and destruction of pathogenic bacteria partly by wiping relies on their transfer to fibre surfaces within the wipe. The extent to which the surface properties influences specific bacterial removal was investigated in terms of polymer composition and surface roughness, as well any residual antimicrobial activity conferred to the surface by the biocide. It was determined that there was no significant difference in removal of *E. coli*, *S. aureus* and *E. faecalis* from plastic, ceramic or metal surfaces by either 100% lyocell or 100% polypropylene nonwoven wipes ($p < 0.05$) during wet wiping. No significant residual antimicrobial activity was seen from the biocide deposited on clinical surfaces after wiping ($p < 0.05$). Therefore, regular disinfection of clinical surfaces, with a “one wipe, one surface” policy should be implemented.

Chapter 1

Introduction

1.1 Hospital Acquired Infection

A hospital acquired infection (HAI), healthcare associated infection (HCAI) or nosocomial infection is defined as any infection occurring within forty eight hours of hospital admission, three days of hospital discharge, or thirty days of an operation (1). Such infections are a serious consequence of hospitalisation (2) and are a major cause of both morbidity and mortality.

In 2003, HCAs affected approximately 10% of all hospital in-patients, and delayed discharge by an average of eleven days in the UK (3). In 2011 a parliamentary briefing found that patients with HCAs cost the hospital three times as much as patients without HCAI (4). In the latest impact assessment, HCAs caused five thousand deaths per year in the UK (5) at a cost to the NHS of £1 Billion per annum (5). Widening the focus to the European Union, in a recent assessment, HCAs were directly associated with more than thirty seven thousand deaths and four million affected patients per annum (6). The direct financial cost to hospitals has been placed at €5.4 Billion, while the wider costs to the economy have been estimated to be €7 Billion, associated with lost working hours (7). Additionally, there is a significant risk of cross infection to both patients and clinical staff (8, 9).

Immunocompromised patients, such as those admitted to intensive care units (10) are less capable of fighting infection due to a weakened or non-functional immune system (11). Consequently, such patients have a higher incidence of severe infections and represent a significant proportion of those affected by HCAs (12, 13).

Solid surfaces in hospitals of which there are innumerable types are generally composed of metals, polymers, ceramics and glasses and can be subject to varying degrees of microbiological contamination depending on their location. Of particular concern are frequently touched surfaces, such as bedrails, trolleys, furniture, doors and curtains because of the possibility of cross-infection, particularly around vulnerable patients. Common types of microbiological contamination include bacteria, yeast, mould, fungi, virus, prions, protozoa and their toxins and by-products (14). These hospital surfaces become contaminated because of factors such as poor hand hygiene, direct contact with body secretions or fluids, aerosol contamination, contact with airborne microorganism that settles after disturbance of another contaminated surface, equipment or article or vector transmission (15, 16). It is known that these surfaces can act as a reservoir for a large variety of microorganisms (17).

If not frequently cleaned by means such as wiping with biocides such contamination can promote unwanted transmission of pathogens, within and beyond the contaminated location (18-22).

Bacteria are amongst the most important microbiological contaminants and exist in either free-floating (planktonic), or substratum-attached (sessile) states (23). Surface attachment and subsequent biofilm formation is a key survival mechanism (24). The process of attachment anchors the microorganism in an

environment that is nutritionally advantageous (23), and provides increased resistance to chemical and physical insults (25, 26).

Evidence from a large number of investigations, including studies modelling transmission routes (18), microbiologic studies (19), observational epidemiologic studies (20), intervention studies (21), and outbreak reports (22) has found that critical patient care surfaces contaminated with pathogenic bacteria contribute to the transmission of HCAs.

At least 20-30% of HCAs are considered to be preventable by appropriate hygiene and control programmes (6), and so the effective removal of pathogens from surfaces in critical patient care areas is key (27). One currently employed strategy is to clean contaminated surfaces using nonwoven fabric wipes, either alone or in combination with detergents or biocides (28). Convenience is a major benefit, compared to separate dispensing of the biocidal liquid, followed by wiping to clean or remove the residual liquid.

In the context of this thesis, the definitions for “Cleaning”, “Disinfection” and “Decontamination”; and the types of cleaning product; are taken from NHS (29) or Centre for Disease Control (30) guidelines and are outlined in Table 1-1.

Table 1-1. Definitions for commonly used terms

Term	Definition
Cleaning	A process that removes dirt, dust, large numbers of microorganisms and the organic matter using detergent and warm water or disposable detergent wipes, such as blood or faeces that protects them. Cleaning is a pre-requisite to disinfection or sterilisation.
Disinfection	This is a process of removing or killing most, but not all viable organisms. The aim of disinfection is to reduce the number of micro-organisms to a level at which they are not harmful. Spores are not destroyed.
Decontamination	A general term used to describe the destruction or removal of microbial contamination to render an item or the environment safe and free from viable microorganisms. The term decontamination includes sterilisation, disinfection and cleaning.
Biocide	Refers to chemical agents that kill microorganisms. These general terms includes disinfectants, antiseptics and antibiotics. Biocides generally react with proteins, specifically essential enzymes of microorganisms. Actions may include oxidation, hydrolysis, denaturation or substitution. When a killing action is implied, the suffix <i>-cide</i> (e.g. biocide, bactericide, virucide, sporicide) is used, while <i>-static</i> (e.g. bacteriostatic, virostatic, sporostatic) is added when an organism's growth is merely inhibited or it is prevented from multiplying.
Disinfectant	A product applied directly to an inanimate object. It destroys or irreversibly inactivates most pathogenic microorganisms, some viruses, but not usually spores.
Detergent	Disperse and remove soil and organic material from surfaces allowing a disinfectant to reach and destroy microbes within or beneath the dirt. These products also reduce surface tension and increase the penetrating ability of water, thereby allowing more organic matter to be removed from surfaces. Some disinfectants have detergent properties.

Pre-moistened wet-wipes are used to disinfect surfaces in environments commonly interacted with by patients, visitors and staff, such as the intensive care unit (31). Therefore, it is crucial that these wipes are effective in removing and killing potential pathogens on healthcare surfaces quickly. A range of biocides is currently employed, which when impregnated into nonwoven wipes can be delivered to contaminated surfaces to physically remove contamination and abrogate any microbiological activity. In such a wet wipe product, the effective removal of the pathogenic compounds contaminating solid surfaces in critical patient care areas is crucial if reduced rates of HCAI are to be achieved by breaking existing routes of transmission.

Owing to the increase in antibacterial resistance, considerable focus has understandably been placed on the role of alternative biocides or detergents to decontaminate surfaces (32, 33). Whilst there has been extensive study of biocidal formulations and the efficacy of commercial wipes in the clinical setting, there is a paucity of academic research exploring the fundamental factors that modulate the cleaning efficiency of biocidal wipes in contact with solid surfaces. Specifically, there are few fundamental studies that seek to elucidate the underlying mechanisms of particle capture and disinfection during solid surface cleaning processes relevant to clinical settings.

The manner by which microorganism contamination (particularly bacterial) are removed, disrupted and retained by fibrous media such as nonwoven wipes is therefore poorly understood at the present time (3, 34). Improved knowledge of how wipes interact with surfaces contaminated with a range of different bacteria found in hospital settings is essential if improvements to wipe design parameters

and cleaning performance are to be achieved. Addressing this knowledge gap is the motivation for the present work.

1.2 Aims and Objectives

The main aim of this research is to develop understanding of key parameters affecting wiping efficiency of nonwoven wipes when applied to the cleaning of solid surfaces contaminated with bacteria. Specifically, the focus is on compositional and structural aspects of a nonwoven wipe. The specific objectives are to:

- Critically review relevant microbiological as well as compositional and structural factors influencing bacterial wiping efficiency.
- Determine the influence of fibre surface properties, specifically surface energy and nano-roughness on the removal of bacteria.
- Determine the influence of wipe construction, biocide addition and wiping pressure on bacterial removal efficiency.
- Determine the influence of surface properties on recontamination by a pre-contaminated nonwoven wipe.
- Determine whether residual antimicrobial activity is conferred to a surface by using a typical nonwoven wipe impregnated with a biocidal liquid.

1.3 Thesis Structure

This thesis is organised into 7 chapters. Following a critical review of literature in Chapter 2, which outlines the relevant microbiological, compositional and structural factors influencing bacterial wiping efficiency, and identifies gaps in

knowledge, Chapter 3 details the raw materials and methods used to enable the experimental work that is reported in subsequent chapters. There are three experimental chapters each of which explores either intrinsic (wipe composition and structure) or extrinsic factors (e.g. biocide loading, hand wiping pressure, surface type) factors with the purpose of understanding the degree to which bacterial wiping efficiency is affected.

To enable robust study of the key factors, all wipes were manufactured in-house to enable their composition and structure to be strictly controlled, and a proprietary biocide from a single source was employed. Methods of preparing and conducting the dynamic wipe experiments followed recently developed protocols. In this way, it was possible to minimise confounding factors that may otherwise be present when studying the performance of wipes whose full polymer and chemical composition, processing history and physical properties are either unknown or not comparable with other test samples.

Chapter 4 considers bacteria adhesion and the extent to which interfacial contact with wipes can remove them from a model surface, including in circumstances where a biocide is not present. This chapter focuses on surface energy and nanoroughness of the wipes and their influence on bacterial removal from a model healthcare surface.

Chapter 5 reports a systematic study of intrinsic and extrinsic wipe factors using an orthogonal array design of experiments, to determine the effects of wipe basis weight (area density), liquid addition and wiping pressure on bacterial removal from a model healthcare surface. This enables ideal wipe composition and usage conditions to be recommended.

Chapter 6 explores the factors affecting recontamination of wiped surfaces and the residual antimicrobial activity of surfaces wiped in the presence of a biocide. This allows recommendations to be made regarding re-use of wipes. General conclusions from the experimental work carried out and recommendations for further work are detailed in Chapter 7.

Chapter 2

Literature Review

2.1 Infection and Pathogens

To fully understand how wipes are likely to interact with microorganisms such as bacteria it is important to consider the structure and properties of such microbes. Microorganisms, microscopic single- or multi-cellular organisms, are ubiquitous, being present in almost every environment on Earth (35). In terms of human health and disease, the majority of microorganisms are either harmless or beneficial, with a minority being actively harmful or considered to be pathogens.

Pathogens are defined as infectious agents that can cause disease in a host organism (36). Common types of pathogenic microbiological organisms include bacteria, yeast, mould, fungi, virus, prions, protozoa and their toxins and by-products (14). The microorganisms cause disease as they invade a host, multiply in close association with the host's tissues and cause damage. The capacity of a microorganism to cause disease reflects its relative pathogenicity. This is particularly relevant in hospitals, where many of the patients are immunocompromised so are more susceptible to any infection (37).

Bacteria are a domain of microscopic (μm scale), single celled prokaryotic organisms present in most environments on the planet (38),(39). They have a number of shapes ranging from spheres to rods and spirals. They were among the first life forms to appear on Earth, and are present in most of its habitats.

Pathogenic bacteria will be focus of this review as they can cause a multitude of different infections, ranging in severity from sub-clinical (no detectable clinical symptoms) to fulminant (infections that occur suddenly and intensely) (40), and are responsible for a large percentage of HCAs. Indeed, in a study by the National Healthcare Safety Network of 621 U.S. hospitals between January 2006 and October 2007, 87% of the 33,848 pathogens reported were bacterial in nature (41, 42).



Figure 2-1. Example of a typical hospital patient-care area, and the numerous different surfaces contained within (43).

Non-porous low-maintenance solid surfaces are commonplace in the clinical setting (44) (Figure 2-1). High touch environmental surfaces present in hospitals include stainless steel (45), plastic surfaces (46), or ceramics (47). These surfaces will be subject to multiple instances of contamination by bacterial pathogens, which can persist on surfaces for months and can therefore be a continuous source of transmission if no regular preventive surface disinfection is performed (48). The most common bacterial pathogens include *S. aureus*, MRSA, *E. coli*, *E. faecalis*, *S. aureus*, *C. difficile*, and *P. aeruginosa* (41, 49). The main form of resistance to environmental stresses, such as those encountered by pathogens during a disinfection regime, is the formation of biofilms (50).

2.1.1 Bacteria

Bacteria are traditionally divided into two main categories, Gram-positive or Gram-negative, based on retention of a gentian violet Gram-stain (51), which is indicative of the cell membrane structure. As indicated in Figure 2.1-2, this is due to the presence or absence of an outer lipid membrane, which affects resistance to biocides, drugs and antibiotics due to cellular permeability (52). These are fundamental cell characteristics (53). Those which retain the stain are described as Gram-positive and those which do not are Gram-negative (54).

Bacteria exist in one of two states, either planktonic or sessile (55). The planktonic (free-floating) state allows the bacterial population to spread and proliferate, while the sessile (substratum-attached) state bacteria are highly resistant to environmental stresses (56).

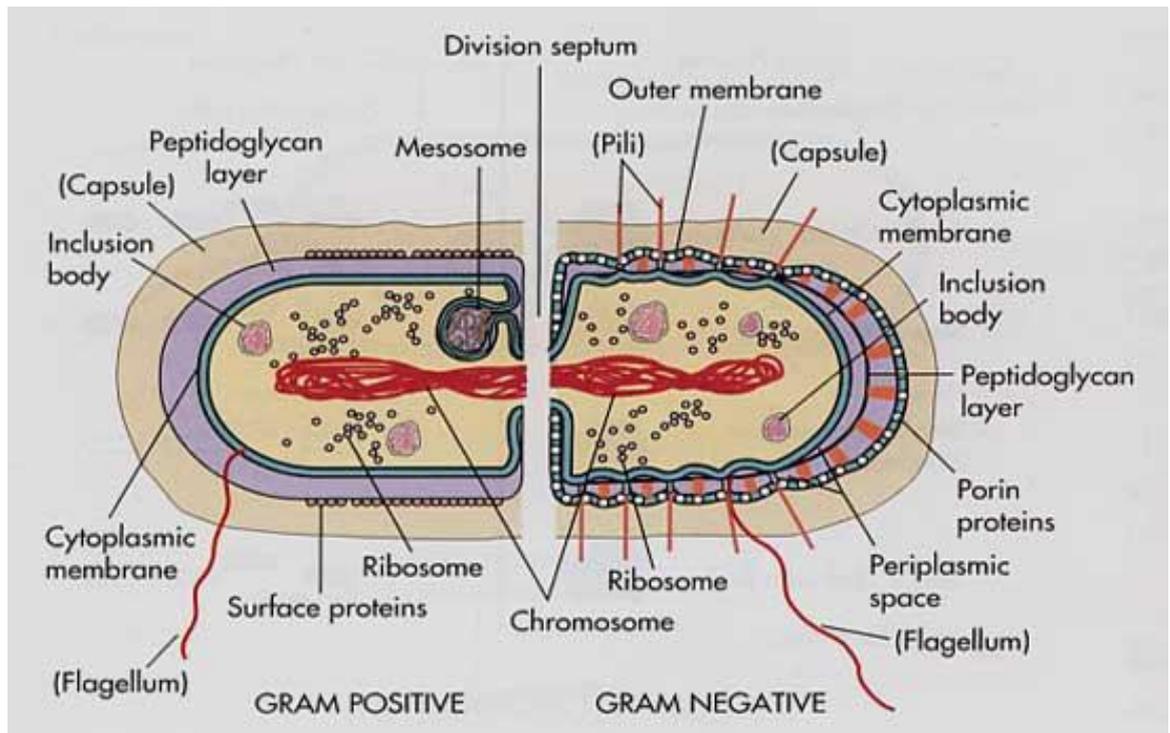


Figure 2-2 Schematic of Gram-positive and Gram-negative bacteria. Gram positive bacteria have a thick layer of peptidoglycan. Gram negative bacteria have a thin peptidoglycan layer and an outer membrane. Structures in *(parentheses)* are not found in all bacteria. Adapted from “Medical Microbiology” (57).

The scope of this review is limited to those Gram-positive and Gram-negative bacteria commonly found on surfaces in the clinical or healthcare environment. Gram-positive and Gram-negative bacteria are frequently implicated in healthcare associated infections and therefore it is instructive to focus on the structure and properties of these particular microorganisms. For a biocide molecule to reach its target site, the outer layers of a cell must be crossed. The nature and composition of these layers depend on the organism type and may act as a permeability barrier, in which there may be a reduced uptake of the biocide (58).

2.1.1.1 Gram-positive

Gram-positive bacteria have an outer membrane composed of a 20–80 nm thick layer of peptidoglycan (sugars and amino acids) (59) and an inner cytoplasmic membrane (60) (Figure 2-2). This protective layer encapsulates the inner content of the cell and is a target for many biocides and antibiotics (61). This is relevant in terms of infection control as this peptidoglycan layer is not an effective barrier to the entry of biocides, as in *Staphylococci* (58); or the growth rate and nutrient availability to the cell can have an effect on the cellular sensitivity to biocidal agents (62).

2.1.1.1.1 Pathogenesis

Streptococcus and *Staphylococcus* are both sphere shaped, while *Listeria*, *Corynebacterium*, *Bacillus* and *Clostridium* are all rod shaped (bacilli), with the latter two also being spore forming. Spores are not part of the bacterial sexual cycle; they are resistant structures used to ensure survival under unfavourable conditions (63).

Certain types of the *Bacilli* and *Clostridia* family form endospores (64) – these are resistant to chemical denaturation, heat, radiation and desiccation and are usually produced in response to a lack of nutrients (65). A notable example of Gram-positive endospore-forming bacteria is *C. difficile*, which causes mild to severe diarrhoea and in some cases pseudomembranous colitis (66). These pathogens represent a challenge to aseptic procedures and cleaning regimens (67). Endospores are the most resistant of all types of bacteria to antiseptics and disinfectants, owing to their structure. Endospores consist of an exosporium, surrounding a spore coat that itself surrounds the cortex, plasma membrane and

cytoplasm. The spore coat is resistant to many biocides, as it excludes large toxic molecules (68).

Without proper disinfection and decontamination, these pathogens particularly endospores, will contaminate surfaces generating a reservoir for HCAI transmission.

2.1.1.2 Gram-negative

In contrast to Gram-positive bacteria, Gram-negative bacteria have an outer cell membrane of lipopolysaccharide and protein, a median peptidoglycan membrane and an inner plasma membrane, all distinct and separated by periplasmic spaces (60) (Figure 2-2). The peptidoglycan layer of Gram-negative bacteria is much thinner than that found in Gram-positive species (69), however, they are more resistant to antibiotics, due to the relatively impermeable outer envelope that they possess (70). This outer lipopolysaccharide and protein layer stops certain drugs, antibiotics and biocides from penetrating the cell, partially accounting for why Gram-negative bacteria are generally more resistant to biocides than Gram-positive bacteria. This means that Gram-negative bacteria colonising clinical surfaces are more resistant to hospital disinfection and decontamination regimen.

2.1.1.2.1 Pathogenesis

The pathogenicity of these bacteria is related to the composition of the cell's outer envelope (71). For example, the lipopolysaccharide component has been shown to trigger immune system activation in humans (72).

Acinetobacter are commonly found on equipment in critical patient care areas (73). They can cause a range of diseases in immunocompromised patients (74).

A baumannii is the most common pathogenic variant (75), causing pneumonia,

meningitis, urinary tract infections and wound infections (74). *Acinetobacter* can also colonise without causing infection or symptoms, especially in open wounds (76). *A. baumannii* can persist under a range of environmental conditions - it is multidrug-resistant and can survive desiccation (74).

In a 2003 study focussing on intensive care units, Gram-negative *Bacilli* were associated with 23.8% of bloodstream infections, 65.2% of pneumonia cases, 33.8% of surgical site infections and 71.1% of urinary tract infections (77). Regarding these Gram-negative *Bacilli*, data collected from 118 intensive care units throughout Northern Europe show that *Enterobacteriaceae* and *P. aeruginosa* were the most common cause of HCAs (78). In the studied intensive care units which participated in this study, there was a high incidence of reduced susceptibility of antibiotic amongst Gram-negative bacteria (79). Supporting this evidence, a 2003 study into HCAs undertaken by the National Nosocomial Infections Surveillance System, found that antibiotic resistance of Gram-negative pathogens were significantly increased (77). This supports the increasing body of evidence that suggest more effective strategies are needed to control the selection and spread of these resistant organisms. Further resistance methods are highlighted in section 2.3.2.

2.1.1 Peptidoglycans and the cell wall

Peptidoglycan is an essential structural element of the cell wall in many bacteria (80, 81). It forms a layer that surrounds the cytoplasmic membrane, and is composed of glycan strands (82) and short peptide chains (83) of 3-6 amino acids (84).

The glycan strands are composed of N-acetylglucosamine (GlcNAc) (Figure 2-3) and N-acetylmuramic acid (MurNAc) (85) (Figure 2-4) linked by β 1-4 glycosidic bonds (86) (Figure 2-5). This type of linkage favours formation of straight chain polysaccharides which are optimal for structural purposes (87), therefore peptidoglycan provides strength to the cell and resists the osmotic pressure of the cytoplasm (88).

There is a high diversity in the sequence of crosslinking peptides between different bacterial species (80), for example Gram negative *E. coli* contains D-alanine, D-glutamic acid, and meso-diaminopimelic acid (89); while Gram positive *S. aureus* contains L-alanine, D-glutamine, L-lysine, and D-alanine (90). These D-amino acids control the cell wall's peptidoglycan composition, amount, and strength via their incorporation into the peptidoglycan polymer and by regulatory enzymes, controlling synthesis and modifications (91, 92). This can also affect the penetration of any biocides into the cell, thereby determining the intrinsic resistance of a bacterial species or strain to a given biocide.

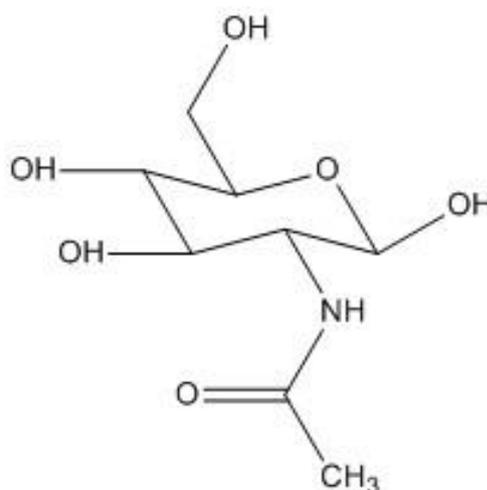


Figure 2-3. Structure of N-acetylglucosamine. (Created in ChemDraw Pro).

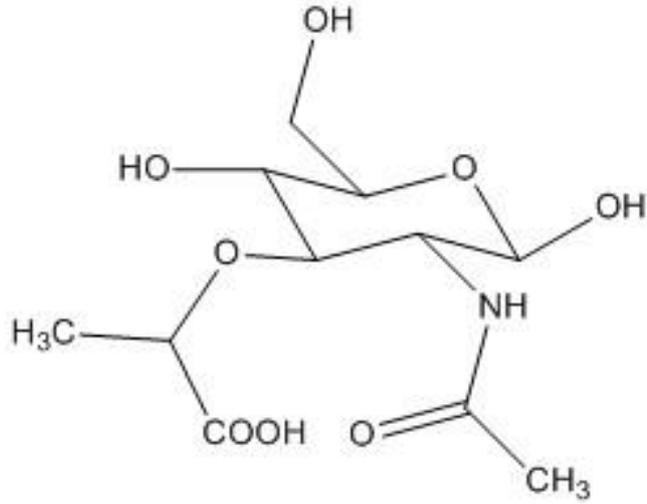


Figure 2-4. Structure of N-acetylmuramic acid. (Created in ChemDraw Pro).

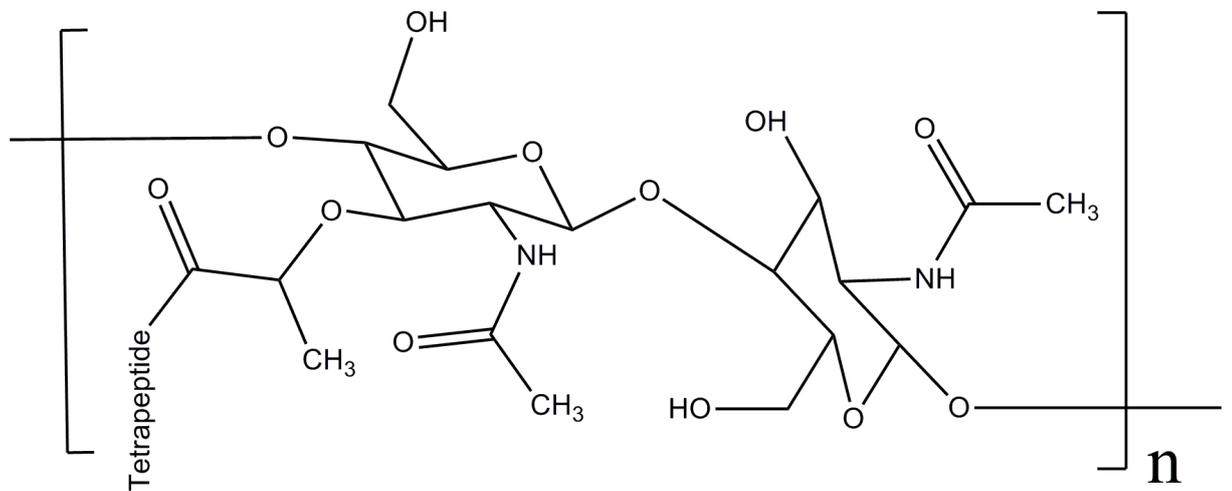


Figure 2-5. Peptidoglycan monomer. (Created in ChemDraw Pro). “*n*” denotes repeating unit.

2.2 Adhesion to surfaces

The prevalence of different types of microbial contamination in healthcare settings is dependent on the bacterium's ability to adhere to different surfaces. In healthcare settings, non-porous low-maintenance solid surfaces are commonplace (44) and consist of various chemical compositions and surface finishes.

High touch environmental surfaces present in hospitals include stainless steel (45), polymeric surfaces (46), ceramics and multi-layer or laminated surfaces (93). These surfaces are subject to multiple insults, i.e. contamination may occur multiple times and decontamination will typically involve numerous cycles of cleaning and disinfection over the course of the operational lifetime (94, 95). In other words, the surfaces are not only exposed to microbial activity but also significant chemical treatment, which may or may not chemically modify the structure or morphology of the surface. Therefore, it cannot necessarily be assumed that the characteristics of these surfaces will remain uniform over this time due to physical damage or chemical degradation. For example, the biocide used in these cleaning regimens can affect the surface properties in terms of chemistry, charge, hydrophobicity and topography (61). Naturally, any change in these properties is likely to alter the way in which bacteria interact and adhere to a surface (24).

Understanding "bacterial adhesion" is fundamental to understanding how pathogens adhere to surfaces in the hospital environment. The extent of contamination on high-touch environmental surfaces in clinical environments is often unknown (96).

2.2.1 Bacteria-substratum interaction

Surface adhesion and the subsequent biofilm formation is a key bacterial survival mechanism (24). The process anchors the microorganism in an environment that is nutritionally advantageous (23), and provides the cell with increased resistance to stressors such as biocides (25).

Planktonic bacteria typically exist suspended in a bulk fluid (24). In relation to a surface they are in one of three states: In the bulk liquid, unaffected by the surface; In a near-surface environment in the bulk liquid, where hydrodynamic effects from the surface can influence the cell (97); or in a near-surface constrained environment, where both hydrodynamic and physiochemical properties of the surface can influence the cell (98). Motile bacteria will attach to a surface regardless of the velocity of the bulk fluid (24).

After contact between the cell and the solid surface has been made, attachment occurs - first reversibly then irreversibly (99). Surfaces to which bacteria attach can be pristine, soiled or coated with a conditioning film (100). The following text will assume a pristine surface to avoid complication (101).

2.2.1.1 Initial attachment

The initial attachment of the bacterium is reversible and occurs over the course of minutes (102). The surface of an individual bacterial cell has several organelles - the curli, the pili and the flagella that facilitate this interaction with solid surfaces (Figure 2-6 and Figure 2-7) (24).

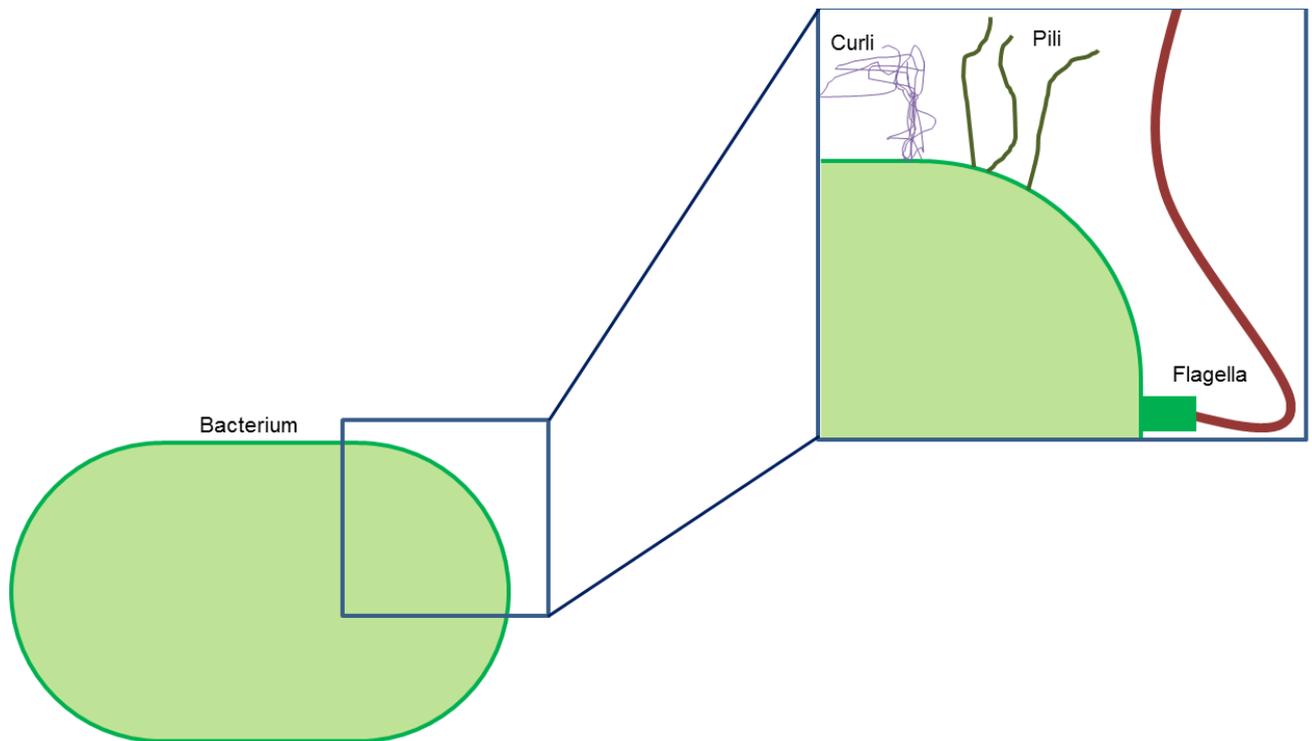


Figure 2-6. The surface organelles of a bacterial cell.

Curli are amyloid fibres produced by many *Enterobacteriaceae* as part of their extracellular matrix (103). Knockout mutation studies focusing on the curli-expression-activating genes in *E. coli* have shown that curli are morphological structures of major importance to surface attachment (104).

The pili are hair-like appendages found on the surface of many bacteria. In Gram-negative bacteria, pili are formed by non-covalent interactions between pilin subunits. In contrast, Gram-positive pili are typically formed by covalent polymerisation of the adhesive pilin subunits (105). Pilus-deficient variants of *Influenza* have a notable reduction in their ability to adhere in comparison to the wild type (106). These results are consistent with those seen in *E. coli* (107) and *cholera* (108), indicating the pili are used to attach a bacterial cell to host tissue or a solid surface.

Flagella are the main locomotory apparatus of bacterial cells. They can also function in a sensory role, with particular sensitivity to extracellular temperature and chemicals (109). Flagellum-minus and paralysed-flagellum mutant strains of *L. monocytogenes* have been proven to be defective in abiotic surface adherence versus the wild type (110). Similar observations are seen in both *E. coli* (107) and *P. aeruginosa* (111). These results demonstrate that cell motility, mediated by the flagella, is a requirement for initial surface adhesion and any subsequent biofilm development. This cell motility is required for initial interaction with and for movement along a surface (112).

Close proximity of bacteria to a surface cause changes in flagella rotation that is sensed by the cells (24). This leads to repositioning of the cell body to maximise attachment to the surface (113). Alongside flagella inhibition, pH and the osmolality of the surface also affect the adhesion of bacteria to the surface (Figure 2-8) (24).

Differing surface topographies result in differing degrees of steric hindrance to bacterial organelles when they are in close proximity to said surface; so the ability of bacteria to adhere will change between different surfaces (114).

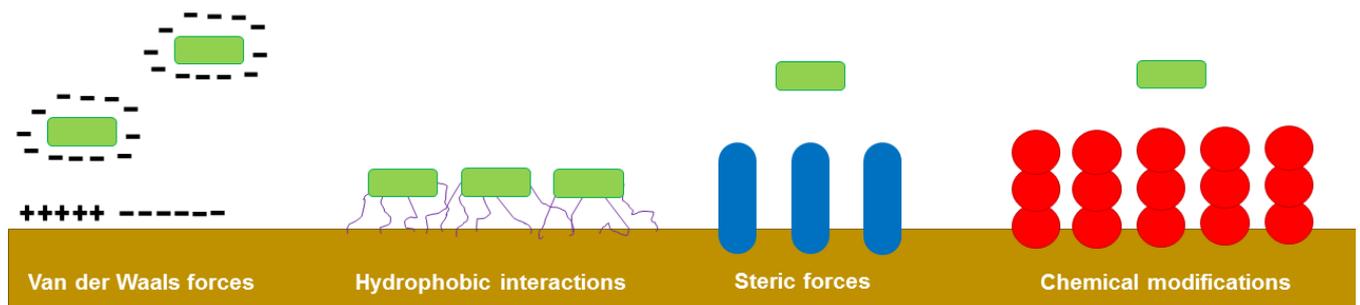


Figure 2-7. Surface interactions affecting bacterial adhesion.

Irreversible attachment is the secondary stage and occurs on a time scale of several hours (24), (97). Transition from reversible to irreversible attachment is caused by certain proteins specific to each type of bacteria (115). Irreversible adhesion also involves van der Waals interactions between the hydrophobic region of the outer cell wall and the surface the cell is attached to (116).

The surface energy of bacteria is typically smaller than the surface energy of the bulk liquid; this means cells will favour attachment to hydrophobic surfaces (117). Conversely, cells will favour attachment to hydrophilic surfaces when the surface energy of the bacterium is greater than the surface energy of the bulk liquid (117).

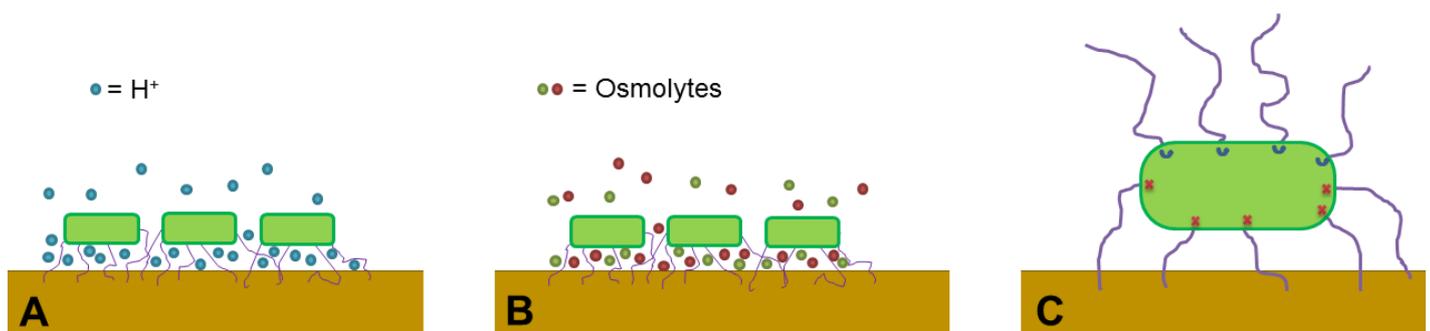


Figure 2-8. Surface property changes caused by close proximity of planktonic bacterial cells. (A) pH; (B) osmolality; (C) flagella inhibition.

2.2.2 Biofilms

Once attached to a surface, bacteria do not randomly aggregate together. They instead form organised communities (biofilms) with specialised signalling methods and unique phenotype expression (118) when free floating microorganisms aggregate and attach to a surface (119).

These biofilms are microbiologically derived sessile communities of cells that are adhered to a biotic or an abiotic substratum (120). They can be beneficial, such as in sewage treatment in a bioreactor (98) or for degradation of hazardous soil particles (121). However, biofilms are also a major cause of solid surface contamination (122) and are increasingly recognised as the cause of a range of human infections, including endocarditis, pneumonia in cystic fibrosis and those affecting prosthetic devices and implants (123).

Biofilms are embedded in a matrix of high molecular weight compounds, known as extracellular polymeric substances or exopolysaccharides (EPS) (Figure 2-9) (124, 125). This EPS determines both the structural integrity and physiochemical properties of the biofilm (126), while enhancing survival in the face of environmental stress (127).

Biofilms are ubiquitous in that over 99% of microbial life is found in the form of a biofilm (128). There is also evidence of biofilm formation early in the fossil record - approximately 3.25 billion years ago (129), supporting the theory that biofilm formation is a fundamental process for the survival of microorganisms.

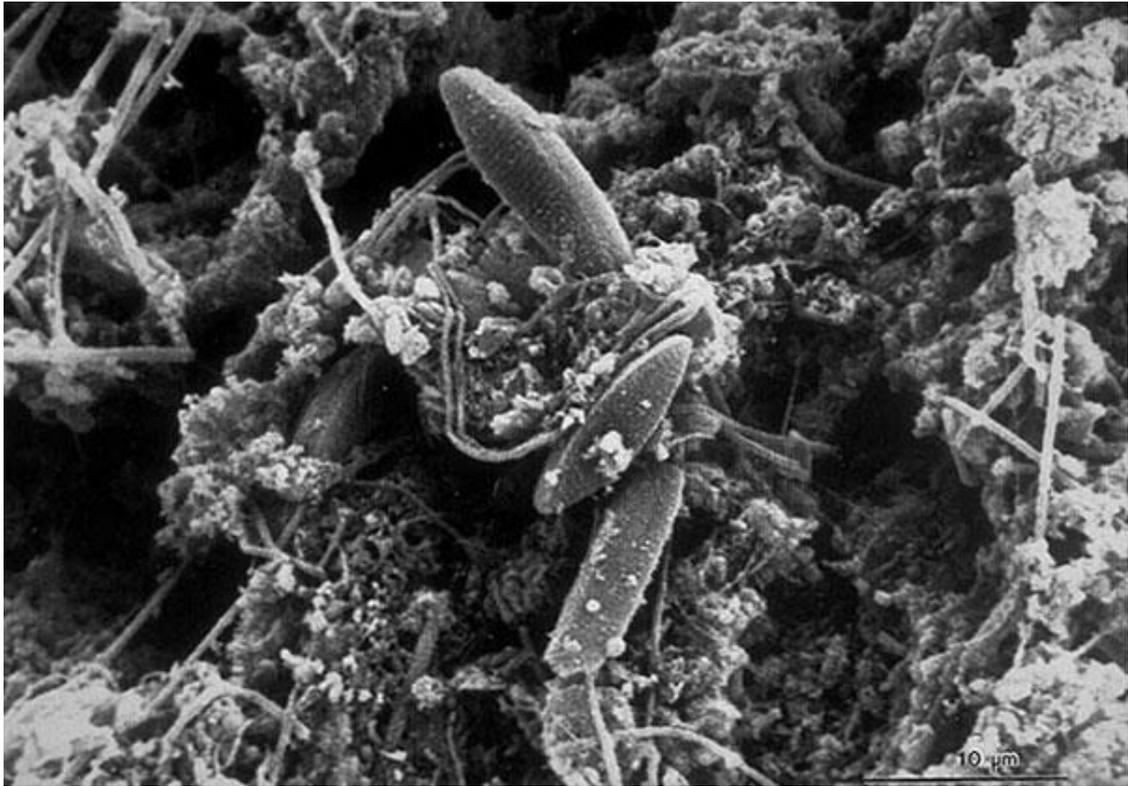


Figure 2-9. Scanning Electron Micrograph of a native biofilm on a steel surface. Reproduced from Donlan (130).

Adhesion and subsequent attachment to a surface is a survival advantage to a cell or community of cells, which allows the cells to persist in a nutrition rich environment (108), increasing their resistance to environmental stresses (131). In contrast, one disadvantage of cell attachment to surfaces is inhibition of cell motility (24).

Current understanding of the interaction of bacteria with surfaces remains incomplete (24). Further research is needed to understand the “conditioning layer” of proteins that often precedes bacterial attachment to a surface that initially resists the attachment of cells (132) such as those containing quaternary ammonium salts (133).

Bacteria can attach to a range of surface materials, including metals (134) and polymers (115). This is important as common hospital surfaces have a diverse array of topographies and chemistries (45). Any organic load or soiling will affect these properties and can also affect cell adhesion (135), again highlighting the need for further study.

Disruption of bacterial adhesion, by an antimicrobial active surface or regular decontamination and disinfection, has applications in a range of areas in the clinical field and beyond; including agriculture, biomedicine and industrial processing (24). If bacterial adhesion is to be reversed and biofilm formation prevented, wiping of the surface needs to be frequent and bacterial removal efficiency needs to be effective (136).

2.3 Disinfection and decontamination

When using wipes to reduce bacterial loading on surfaces they are commonly used in conjunction with or impregnated with an active agent in the form of a biocide. The wipe therefore acts as a delivery medium for the biocide, and potentially as a collecting medium for organic material that is collected during the wiping process. It is important to recognise the role of the biocide and the mode of action as it influences design considerations in the wipe.

2.3.1 Biocides

Decontamination of solid surfaces can be achieved by the use of chemical compounds such as biocides (137). A biocide is defined as:

“...any microorganism or chemical substance which exerts a controlling influence on a harmful organism, via biological or chemical means...” (138).

This influence can be to deter, render harmless or destroy the organism in question.

The bactericidal effect of a biocide is determined by the overall damage to the target sites within the microbial cell. This is the η -value or minimum inhibitory concentration; representing the antimicrobial efficacy of the biocide (139). The minimum inhibitory concentration is the minimum concentration of a biocide required to have an inhibitory effect on bacterial, fungal or viral growth (140). This is a basic measurement of the activity that a biocide has against an organism (141). The minimum bactericidal concentration is a similar concept; it is the lowest concentration of antimicrobial that will prevent the visible growth of an organism (140).

A disinfectant is a product applied to non-living objects, such as solid surfaces, to destroy microorganisms. Biocides can be used as disinfectants (138). The term “antimicrobial” describes any substance of natural or synthetic origin which (at low concentrations) kills or inhibits the growth of microorganisms (142). The term “antibiotic” is used as to describe antibacterial agents when used to treat bacterial infections in both humans and animals (143). When “antibiotic resistance” of an organism is mentioned in papers and discussions, it is synonymous with antibacterial resistance (144).

Biocides often contain surfactants that are wetting agents, which reduce the surface tension of the liquid and allow it to spread out on a surface. They have molecular structures that include both hydrophilic and hydrophobic parts. Adsorption of a surfactant to a solid surface exposes lyophilic functional groups (145). Cationic surfactants can provide softening, antistatic, soil repellent or antibacterial properties (146). Those with shorter hydrophobic tails often have biocidal properties, while those with longer tails tend to be very substantive on surfaces.

The antibacterial mechanism employed by quaternary ammonium compounds is destabilisation of the cell’s cytoplasmic membrane, which leads to leakage and cell death (147). Non-ionic surfactants have an uncharged hydrophilic group (148) and are suited to cleaning purposes (146). An example of this is C₉₋₁₁ Pareth-5; an ethoxylate of a fully saturated C₉₋₁₁ alcohol (149). It functions as a water-soluble non-ionic surfactant, wetting agent and detergent (149). Commonly used hard surface pre-impregnated wet wipes and their biocides are listed in Table 2-1,.

Table 2-1. Examples of common wet wipe formulations for solid surface disinfection and decontamination.

Manufacturer	Product	Biocide	Claims/General Information
VWR	Kleriwipe	Chlorine; Quaternary Ammonium Compounds	"...sterile, binder-free, low particulate..."
Gama Healthcare	Clinell alcohol wipes	70% w/w Isopropyl Alcohol	"...act as a rapid disinfectant for medical devices, surfaces and equipment with proven bactericidal action, they kill 99.999% of germs including <i>E. coli</i> , <i>Enterococcus</i> , <i>Pseudomonas</i> and MRSA according to EN1276 and EN13727."
	Clinell sporicidal wipes	Sodium Percarbonate (<50% wt.); Citric acid (<20 % wt.)	"...the wipes produce peracetic acid as the active ingredient which will kill all germ groups thus providing a direct and safe alternative to chlorine products. Clinell Sporicidal Wipes are designed for surface disinfection..."
PAL	Medipal 3 in1 Disinfectant Wipes	Didecyl-dimethylammonium chloride (DDAC); (N-(3-Aminopropyl)-N-dodecylpropane-1,3-diamine) (Triamine)	"DDAC has been developed to give improved biocidal activity, stronger detergency and a lower level of toxicity compared to the previous three generations of Quaternary Ammonium Compounds..." "Triamine acts as a cell disruptor. It is effective against both gram positive and gram negative bacteria as well as enveloped viruses such as Hepatitis -B. It is a low toxicity, non-tainting and non-corrosive compound combining both detergent and disinfectant properties"
Techtex	Clinitex Hard Surface Alcohol Wipes	70% w/w Isopropyl Alcohol BP	"...effective against a range of bacteria including MRSA, <i>E-Coli</i> , <i>Salmonella</i> , <i>Typhimurium</i> , <i>Listeria Monocytogenes</i> , <i>Pseudomonas Aeruginosa</i> , <i>Staphylococcus Aureus</i> and <i>Enterococcus Faecium</i> ...independently tested for their efficacy and used on-contract by the NHS and in other healthcare environments..."
Amity	IPA Wipe	Isopropyl alcohol	"...a fast drying cleaning and degreasing agent for use in a wide variety of applications including healthcare, electronics and other environments..."
	Virusolve+	Quaternary Ammonium Compounds; Biguanides	"...formulation is highly effective as a cleaner and disinfectant against bacteria, mycobacteria, fungi, viruses and spores..."
Robinson Healthcare	Readiwipes Wet	70% w/w Isopropyl alcohol	"...suitable for cleaning and disinfecting hard surfaces and ideal for use in many clinical and food preparation areas..."

Another example of a commonly used biocide is Benzalkonium chloride. This is a quaternary ammonium compound and cationic surfactant (Figure 2-10). It has a high affinity for membrane proteins (150) owing to its surface-active structure (151) and has been shown to deposit on surfaces (152). Quaternary ammonium compounds with alkyl chain of lengths C12 and C14 (the dodecyl and myristyl alkyl derivatives) show the greatest biocidal activity (153, 154).

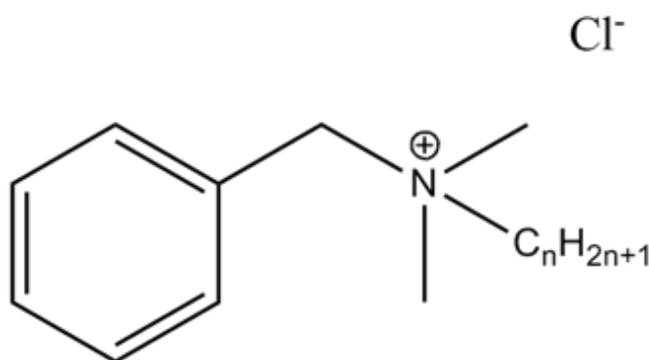


Figure 2-10. Benzalkonium chloride structure, where $n=10-16$. (Created in ChemDraw 12).

Peptidoglycan is an essential structural element of the cell wall in many bacteria (80, 81). It forms a layer that surrounds the cytoplasmic membrane, and is composed of glycan strands (82) and short peptide chains (83) of 3-6 amino acids (84).

Benzalkonium chloride is known to disrupt cell membranes by catalysing the hydrolysis of the β 1-4 glycosidic bond between the GlcNAc and MurNAc in the structural peptidoglycan (Figure 2-11). This action leads to the breakdown of cell membrane and exposure of the cytoplasm of cell to external environment.

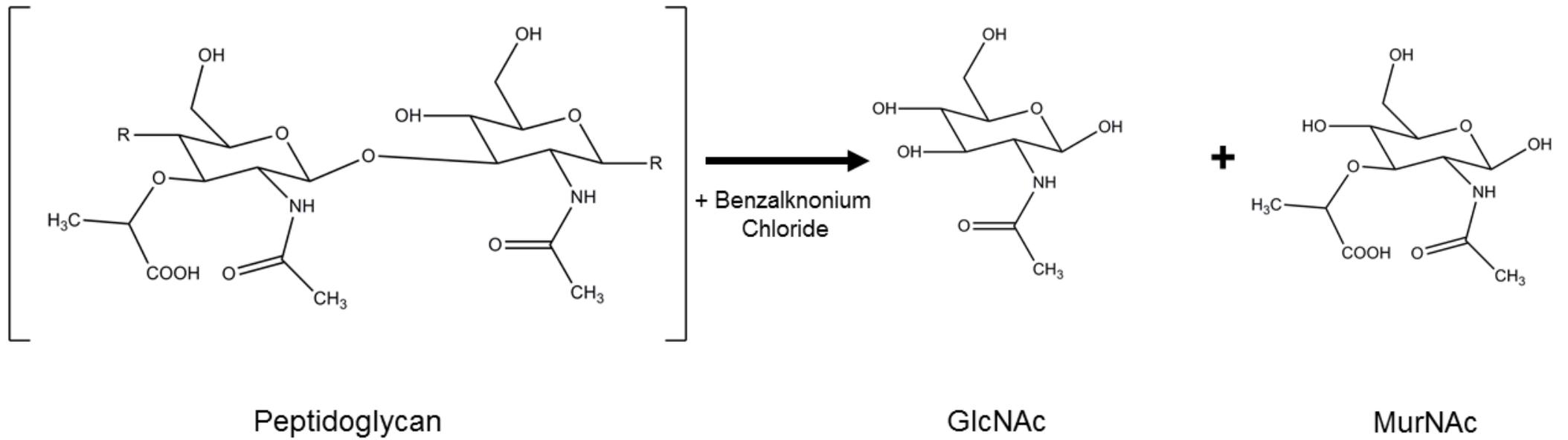


Figure 2-11. Hydrolysis of β 1-4 glycosidic bond by Benzalkonium Chloride. (Created in ChemDraw 12).

This causes a breakdown of the homoeostasis (equilibrium) between internal and external environment of cell and as a result the cell dies. Disruption to peptidoglycan synthesis will also give similar results.

2.3.2 Resistance to disinfection and decontamination

As outlined in section 2.1, peptidoglycans provide strength to bacterial cells, so in theory, targeting these structures will lead to cell lysis and death. However, the real picture is far more complex.

Bacteria employ a variety of resistance methods. Strains can mutate over time and become resistant to a specific antibiotic. Evolution of resistant strains is a natural phenomenon that happens when microorganisms are exposed to an antimicrobial agent (155). This is analogous to natural selection in the animal kingdom.

Some bacteria acquire resistance traits from other bacteria, others by becoming resistant following mutations in the chromosomal gene that codes for the target of the inhibitory compound (156). Bacteria respond to the use of biocidal agents such as disinfectants or antibiotics by producing progeny that are resistant to these substances and their mechanisms of action (156). This resistance is particularly evident in the healthcare setting – hence the growing trend of increasing antibiotic resistance (157).

Table 2-2. Examples of bacterial intrinsic resistance mechanisms to biocides. Adapted from Masri *et al.* (158).

Bacteria	Example(s) of Biocide	Mechanism of resistance
Gram-negative	Quaternary ammonium compounds	Outer membrane acts as complex barrier to biocides and antibiotics, regulating and/or preventing passage to target regions
Mycobacteria	Chlorhexidine, Glutaraldehyde	Waxy cell wall prevents adequate biocide entry
Bacterial spores	Phenolics	Spore coat and cortex act as barrier to uptake of compound
Gram-positive	Chlorhexidine	Glycocalyx (extracellular polymeric material) associated with reduced diffusion of biocide

In addition, some bacteria produce dormant, resistant spores [159] that can survive hostile environments such as pressure, heat, desiccation, radiation and chemicals (Table 2-2) [64].

2.3.2.1 Surface attachment and resistance

Surface attachment of cells (section 2.2) is commonplace in microorganisms (24). A key biofilm phenotype is the higher degree of resistance shown to biocides and antimicrobial agents vs. the same bacteria in planktonic form (124). This is due to a multitude of factors, including the exopolysaccharide (EPS) (127), multidrug efflux pumps (25), a slower cell growth rate (127) and changes in gene expression (159). Also, colonising bacteria (on a surface, for example) are exposed to prolonged selection pressure (143).

This resistance is important - recent evidence suggest that adherent cells in a non-biofilm state have a biocide resistance profile comparable with that displayed by cells embedded in a biofilm (160). This resistance phenotype is reversible, as

the cells become susceptible to biocides after detachment from the surface (24) (161).

Suggested mechanisms for this resistance phenotype include:

- The attachment stabilises the cell membrane (162);
- The attachment causes the cell's metabolic rate to reduce to a similar level to that of the G0 (stationary phase) of bacterial growth (163);
- Bacterial membranes have a net negative charge - a reduction in this caused by surface attachment serves to stabilise the cell (162).

Efflux pumps allow biofilms to regulate their internal environment by pumping solutes across the otherwise impermeable cell membrane, meaning that signalling molecules, metabolites and toxic substances (i.e. antimicrobials) can be removed (124). Multidrug efflux pumps can extrude antimicrobial agents from bacterial cell (25). In *E. coli* they can extrude chemically unrelated antimicrobials (164), while in *P. aeruginosa* they extrude ofloxacin (73). However, their exact role is still unclear.

Stress resistance is an intrinsic part of microbial survival, presenting a major problem in the treatment of HCAs (165-167). Attachment of bacteria to surfaces is a survival advantage known to promote resistance, so disinfection and decontamination of solid surfaces is a crucial step in disrupting this relationship. By impregnating the wipe with a biocide, there is an opportunity not just to deliver the active compound, but also to physically remove organic and microbial material, together with the residual biocide such that the overall loading of the surface is reduced.

2.4 Wiping Dynamics

The basic principles of removing an ideal spherical particle from an ideal uniform surface involves a balance of forces as shown in Figure 2-12. Wiping is a dynamic and mechanically complex process, which is very challenging to model without over-simplification.

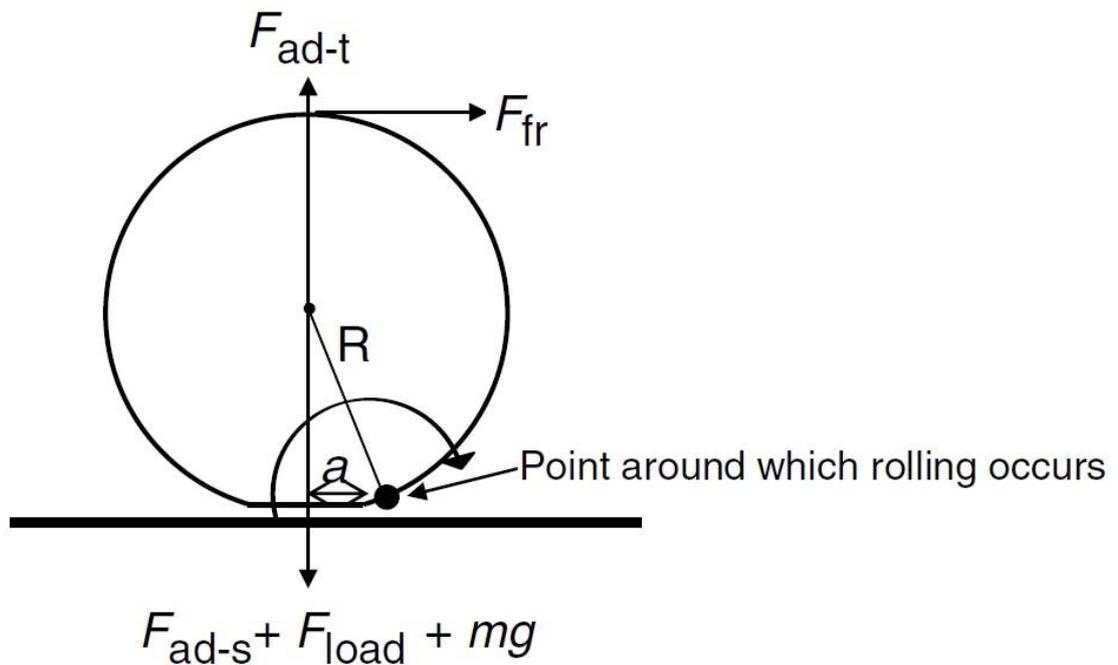


Figure 2-12. Force diagram for sphere adhesion and removal from a flat plane during wiping. Adapted from Verkouteren *et al.* (168).

F_{ad-s} represents the adhesive forces between surface and particle;

F_{ad-t} represents the attraction force between the wipe and the particle;

F_{fr} represents the frictional force between the particle and the wipe;

F_{load} represents additional forces, such as gravity;

mg is the weight of the particle (mass x gravity);

R is radius of the particle, a is the area of contact between particle and surface.

Energy input (mechanical, thermal and chemical) is required in such a cleaning process (169) and the operational mechanisms are complex. The physical

mechanism of wiping action exerts pressure on a surface (96) and the removal (detachment) of particulate matter from the surface depends on the balance of forces between the particle, surface and wipe.

Particles are lifted from the surface when the force of adhesion to the wipe (F_{ad-t}) is greater than the force of adhesion to the surface (F_{ad-s}) and the particle's weight (mg). Displacement or rolling of the particle (around the point indicated in Figure 2-12) occurs when the removal moment of momentum is greater than the adhesion moment of momentum (168).

Hydrodynamic drag will also affect particle capture from a surface (168). This is dependent on several factors including the roughness of the surface (170) and the particle size (171), which in practice is likely to be a distribution of sizes depending on the nature of the particulate material.

However, the factors outlined in Figure 2-12 are based on an "ideal" spherical particle on a flat surface (168) and not a bacterium on a clinical surface, which may not be entirely flat. Bacteria will adhere to surfaces with different affinities and attachment strengths (24) (section 2.2.1) such that modelling bacterial removal by wiping is highly complex.

During a dynamic wiping process, mechanical forces are introduced between the wipe and the contact surface including those linked to pressure, friction and shear. This brings the wipe and specifically its component fibres and biocidal liquid in to direct contact with bacteria residing on the surface to be cleaned. The combination of these forces during dynamic wiping presents conditions for detachment of bacteria from the surface, as well as disruption of the bacterial cell due to the presence of the biocide. In practice, cell disruption may occur within

the wipe itself, after the bacterium has been detached or on the contaminated surface itself, depending on the biocidal mode of action and the reaction kinetics.

One of the major advantages of pre-moistened nonwovens (wet wipes) impregnated with a biocide is that this format allows for the retention and delivery of a large liquid volume (~150-350% w/w), as well as its retention post wiping, due to the inherent absorbency of such a high porosity (P) structure (P~80-98%) (172). In addition to the solid surface provided by the fibres, the liquid volume provides a second medium in which bacteria can be captured and removed from the contaminated surface, provided the wipe is able to retain or reabsorb residual liquid during wiping.

The capillary mechanisms governing liquid absorbency and retention in nonwovens are well known and have been extensively studied in relation to fibre composition, porosity and fibre orientation (173-177). For example, the Laplace equation describes the wetting of a porous system such as a nonwoven fabric (Equation 2.4-1) (178).

$$p_B = \frac{4 \cdot \sigma_1 \cdot \cos \theta}{d_{p.max}} \quad \text{Equation 2-1}$$

Where p_B is wetting pressure; σ_1 is surface tension of the wetting liquid (N.m-1); θ is contact angle (°) and $d_{p.max}$ is maximum pore diameter (m).

From equation 2-1, the wetting pressure is dependent on the nonwoven wipe's maximum pore diameter and the surface tension of the incident liquid.

2.4.1.1 Measures of wiping efficiency

There are various methods of measuring wiping efficiency in the laboratory setting, not all of which have been developed for the study of bacterial removal. The dynamic wiping absorbent capacity (DWAC) describes the change in weight of the wipe before and after the wiping process. It is essentially a measure of liquid absorbency from the solid surface (179) and is described by Equation 2.2:

$$DWAC = \left(\frac{t_{w1} - t_{w2}}{t_{w2}} \right) \times 100 \quad \text{Equation 2-2}$$

Where, t_{w1} is the wet weight of the wipe (g)
 t_{w2} is the dry weight of the wipe (g).

The DWAC will be affected by the fibre composition and its moisture absorbency as well as by the nonwoven substrate, which controls the pore volume available for liquid sorption. The latter is modulated by the method of nonwoven production and the process conditions used to make the wipe substrate (180). Also, the quantity of biocide retained in and released by the nonwoven wipe is determined

by the size; thickness; composition; layering; and absorbent capacity of the wipe structure (96, 181).

The dynamic wiping efficiency (DWE) also characterises the amount of liquid absorbed from the surface during wiping, but it is expressed as a ratio of the original liquid challenge presented to the wipe rather than solely by the change in the weight of the wipe (182).

The DWE is determined as indicated in Equation 2.3 and 2.4.

$$DWE = \frac{V_S}{V_C} \times 100 \quad \text{Equation 2-3.}$$

Or,

$$DWE = \frac{(t_{w1} - t_{w2})}{d V_C} \times 100 \quad \text{Equation 2-4}$$

Where V_S is the liquid absorbed (g);

V_C is the liquid challenge (g);

d is the density of the liquid challenge (e.g. water = 0.997 gm⁻³).

The wet particle removal ability (WPRA) determines the removal of particulate matter from a surface contamination by a wipe (183) as defined by Equation 2.5.

$$WPRA = \frac{\text{Collected particles}}{\text{Total particulate challenge}} \times 100 \quad \text{Equation 2-5.}$$

Note that the extent of particle removal during the wiping process is also influenced by any particles and fibres that are left behind by the wipe on the surface (182, 183). Practical application of DWAC, DWE and WPRA for assessing the removal of bacteria from surfaces is extremely limited because of their reliance on detecting changes in weight or mass rather than particle counts.

The liquid content and level of saturation of the wipe also influences particle pick up. Whilst the presence of liquid can be advantageous in terms of particle pick-up, assuming electrostatic forces are not required, too much liquid can interfere with particle removal. Saturated wipes can leave a surface with more extraneous contaminants than optimum level of liquid content (184). A pre-wetted wipe below saturation point is reported to result in fewer particle contaminants remaining on the wiped surface (184) since liquid released by the wipe during wiping can be more readily reabsorbed. Reabsorption is valuable because during wiping, target contaminants may be dissolved or dispersed within the liquid, aiding its removal from the surface. In studies focussed on wet particle removal efficiency, a key aspect regarding structure-function was the ability of materials to wipe surfaces dry, i.e. to leave behind no residual liquid on the surface (182).

As the interaction between biological contaminants and fibrous media such as nonwoven fabrics is poorly understood at present (34) a detailed analysis of this interaction remains challenging and there are surprisingly few studies directly considering bacterial cell-fibre interactions in wiping.

Elsewhere, it is known that increasing surface-area-to-volume ratio can be advantageous with regard to the attachment and proliferation of living cells (185). This promotes the attachment of cells via physical and biological interactions, and a similar effect is likely to be applicable to the removal of microorganisms by

wipes. A high solid surface area provides additional scope for direct immobilisation of bacteria by adhesion and thereby yielding more effective disinfection (186). The solid surface area provided may be readily modulated by changing fibre diameter or fibre cross-sectional shape such that is possible to achieve a higher surface-area-to-volume ratio for a similar cross-sectional area (185).

2.4.2 Studies of Wiping in Relation to Infection Control and HCAs

Previous studies have tended to focus on whether the biocide incorporated into the wipe kills the pathogen rather than considering the interaction between the contaminated surface, the nonwoven, pathogen and biocide (96). Several factors are of particular importance in healthcare environments.

A major factor is the contact time between the wipe and the surface (28) during the wiping process and the kinetics of the biocide's activity. Unlike situations where the biocide may be applied separately and then wiped later, when wet wipes are used, the contact time is likely to be shorter because introduction of the biocide and wiping-clean take place in one continuous process lasting seconds not minutes. This is important because the biocide incorporated into the wipe requires a certain contact time to effectively disinfect the surface by acting on the bacterial cell (187).

A typical contact time in a clinical environment operating with a formal cleaning schedule is between 10 - 30 s (188). The contact time is in practice likely to vary significantly and the total area treated during that time will not be uniform. This has been an area of debate in the infection control community, because the efficacies claimed by commercial manufacturers for their biocide-loaded wipes

are based upon much longer contact times of about 2 min (96). This raises questions as to the actual efficiencies being achieved in clinical settings, since unless the biocide has a longer-term residual effect, it will be delivered and removed from the surface within a maximum of 30 s during a typical wiping cycle. This is particularly important if the surface is wiped dry such that there is no apparent liquid loading left on the surface. Under these circumstances it is debatable whether any residual biocidal function will remain.

Surface wiping (176) and the removal of bacteria by wipes has been investigated by Williams *et al.* (28) and Ramm *et al.* (189). These studies have described reproducible methodologies for assessing wiping efficiency, but the focus has been on the macro-scale removal of bacteria in the presence of detergent or biocide, rather than on the fundamental micro- or nano-scale interactions between the fibres in the wipe, bacteria and contaminated surface.

The pressure applied during wiping is also known to affect the efficiency and has to be within an appropriate range. Williams *et al.* suggested a force of 0.98 N after observation of *in situ* usage (28). Clearly, in practice the hand wiping pressure is a difficult variable to control, alongside the wiping action itself (96), which entirely depends on user practice. The degree of surface soiling, otherwise known as the organic load, also has a direct effect on the efficiency of the wipe. Increased organic load correlates with a decrease in the amount of bacteria removed by wiping (28).

Multiple use of a single disposable wipe or use of the wipe over a large area has been shown to reduce the biocidal activity (190) and can lead to cross contamination (43), or even the spreading of pathogens over a larger surface

area (96). Unfortunately, even good wipe practice can leave residual bacterial contamination on surfaces (191), such that removal is not be complete.

Addition of an aqueous liquid such as a biocide to a nonwoven wipe can substantially improve the removal of particles up to a limit dependent on the absorptive capacity of the nonwoven structure (176). During dynamic wiping, shear and compressive forces are applied, facilitating the transfer of bacteria to the wipe and overcoming the adhesive forces between bacteria and surface (182).

Based on current trends (192), there is a risk that prolonged usage of biocides will result in increased bacterial resistance (193). Therefore, novel ways of keeping a surface free from contamination are desirable. These issues could at least be partly mitigated by improving the inherent design and performance of healthcare wipes to enhance their microorganism removal efficiency with much reduced biocide loadings.

Researchers have studied the removal efficiency of both organic and inorganic particulates from a variety of surfaces using fabrics (28), but few have focused on the underlying mechanisms of microbial removal from the types of solid surface found in the clinical environment (67). In addition to this, little is known about the relative ease by which different microbes can be removed from different types of solid surface (24), and there is very little information available regarding the efficiency of wipes against bacterial biofilms (96). This is important as a range of microorganisms, surfaces and extents of contamination are present in both the clinical and wider environments.

There have been few fundamental studies to elucidate the underlying mechanisms of particle capture and disinfection during wiping of surfaces. This

is an important knowledge gap, as evidence from a large number of investigations, including studies modelling transmission routes (18), microbiologic studies (19), observational epidemiologic studies (20), intervention studies (21), and outbreak reports (22) has found that critical patient care surfaces contaminated with pathogenic bacteria contribute to the transmission of HCAs. These HCAs cause morbidity, mortality and an increased financial burden; 20-30% of these are considered to be preventable through appropriate hygiene and control programmes (6).

2.4.3 Manufacture, Structure and Properties of Industrial Wet Wipes for Healthcare Settings

Healthcare providers commonly procure nonwoven wipes for hard surface cleaning as part of their infection control strategies, and it is commonly the case that economic cost as much as function is a key driver. Nonwovens are well suited to single use, disposable wipes because of their low production costs (194) and such wipes provide a high level of convenience as well as reducing the possibility of cross contamination (195).

Pre-moistened wipes represent approximately 71% of the total wipes market (196). In addition to general surface wiping, which is the focus herein, pre-moistened wet wipes are also routinely used for disinfecting medical devices, such as catheters, where there are increasing concerns about biofilms.

In practice, wipe substrates are produced using a variety of nonwoven technologies including spunmelt-thermal bonding, drylaid-hydroentangled, wetlaid-thermal bonded, wetlaid-hydroentangled and hybrids. The physical properties and liquid handling characteristics provided in the fabrics produced by each production route are substantially different, but ultimately, they are utilised

by distributors to fulfil the same purpose. The focus herein is on hard surface wiping, where surfaces refer to floors, bed rails, furniture, benches, work-tops, door handles and so on, and not regulated medical devices.

2.4.3.1 Wipe Products

Common healthcare surface wet-wipe products are listed in Table 2-1. Typical biocides incorporated into such wipes include quaternary ammonium compounds; Chlorhexidine; 70% w/w solutions of Isopropyl alcohol; or chlorine. Biocide selection is dependent on the claims made by the wipe manufacturers, which can include the terms “sporicidal” or bacteriostatic”, amongst others, the target environment level (e.g. ICU, outpatient’s clinic) and target organism.

2.4.3.2 Markets

The major markets for nonwoven wipes used to clean clinical surfaces are Europe, USA and Asia. In Western Europe, the largest five consumers are the UK, Germany, France, Italy, Spain and Scandinavia, which collectively account for about 86% of the nonwoven healthcare wipes market. In Eastern Europe, Russia, Poland and Turkey account for about 80% of the market. Asia trails behind North America and Western Europe globally in terms of the production and use of nonwoven wipes. In Asia, Japan, China and India account for about 83% of the market, with China accounting for over 58% of this market (197). Proctor and Gamble [200], Kimberley Clark [201] and PGI, now Berry Plastics [202] are among the largest commercial manufacturing firms with multiple filed patents in the field of nonwoven wipes. In addition to well-known brands, there is a multitude of private-label suppliers, many of whom rely on third party converters to manufacture their nonwoven wipes (Table 2.3 1) for major markets. The trend for replacement of non-disposable medical fabrics with disposable ones, such as

nonwoven healthcare surface wipes in South America, Africa and Asia reflects a rapidly expanding market in these regions [205].

2.4.3.3 Trends

There is a predicted worldwide trend for a reduction in the basis weight of nonwovens, including healthcare surface wipes, to save material and shipping costs. This is affecting both the production of spunmelt (continuous filament) nonwoven as well as staple fibre nonwoven substrates both of which are found in healthcare wipe products. For example, hydroentangled wipes with a basis weight as low as 35 g.m^{-2} are replacing airlaid nonwovens that have a feasible minimum basis weight of around 55 g.m^{-2} (198). This is technically possible as hydroentangled nonwovens can be made from carded substrates containing longer fibres than is possible in airlaying, resulting in stronger fabrics than can be achieved by thermally bonded airlaids. Similarly, lighter-weight hydroentangled fabrics can be produced with satisfactory mechanical properties than is possible by chemically bonded airlaids or light-weight needlepunched fabrics. Additionally carding rather than short fibre airlaying permits the ability to produce lighter webs and a wider range of basis weights, i.e. between 40 and 150 g.m^{-2} (199).

There is also evidence to suggest that pulp fibre airlaid webs produce more rigid fabric structures than fabrics produced from carded webs. Therefore, carding is a preferred manufacturing route for the production of healthcare wipes.

Wipes produced by hydroentanglement have excellent strength due to the frictional resistance that can be generated within the fibrous network; a relatively low machine direction/cross direction strength ratio, when based on a carded web, and they are dimensionally stable. Their penetration into the wipes market has also been aided by their low bending modulus and smooth surface, which

collectively leads to textile-like “handle” and good drape. Owing to the mechanism of bonding and interaction with the water jets, fibre ends are effectively buried in the structure and there is generally less fibre breakage during the process, as compared to needlepunching leading to a low linting or fibre shedding propensity. It also gives uniformity, consistency, softness and excellent absorbency to the resulting fabrics, depending on the fibre composition. For these reasons, hydroentangling is one of the most commonly employed manufacturing technologies for the manufacture of wet wipes and will be used in this work.

Around 50% of the global nonwoven wipes market uses hydroentanglement as the method of web consolidation, after web formation by carding (200). Conventional wipe manufacturers often employ a blend of fibre types to provide different performance functions. A disinfectant wipe, for example, will require satisfactory wet strength to resist disintegration during removal from the pack and during use as well as sterility and absorbency (201). Such wipes impregnated with antimicrobial agents also need to be stable with common biocidal liquids (202). The effect of hydroentangled wipe composition, structure and basis weight on bacterial removal is currently unknown and therefore is worthy of further investigation.

Typical wipe biocide loading (liquid add-on) levels vary dependent on the substrate, but can be between 100-450% weight:weight (w/w) (203-205). From the patent literature, PP is often used in surface wipes, as part of composite nonwoven wiping structure (206, 207) and may be modified to increase its wettability (208, 209). Lyocell is also commonly used in nonwoven wipes (210, 211), for example 60 g.m⁻² disposable cellulosic nonwoven wipes are commonly encountered in various wipe sectors (212).

It is known that a fabric comprising a blend of PET and lyocell fibres can produce a particle removal DWE >89% at 10 ml challenge or a DWE > 70% at a challenge volume, representing 130% of the sorptive capacity of the wipe (210). Viscose rayon is another example of an absorbent cellulose II material commonly found in nonwoven wipes (213, 214), but it may need to be blended with a synthetic fibre such as PET to compensate for its relatively low wet strength, compared to lyocell.

As is the case in the academic literature, there is a dearth of experimental data in the patent databases regarding the underlying interactions between nonwoven wipe structure, the contaminated surface and bacteria residing on surfaces, which further highlights the need for fundamental study in this area.

2.4.4 Nonwoven wipe formation

Nonwoven wipe production is classified according to the method of web formation (181). This is the stage during which linear fibres are transformed into loosely-arranged planar networks (215). Final textile properties such as weight per unit area are also established in this phase of production (181). The manufacturing processes reviewed in brief here are limited to those commonly used to make disinfectant wipes.

2.4.4.1 Carding

Carding is a mechanical process that uses wire pins embedded in a sturdy flexible backing (the card roller) to disentangle, clean and mix fibres into a continuous web, which can then be further processed (216, 217). The web is held together by fibre-fibre friction and a low degree of mechanical entanglement. Carding can blend different types of fibres to create a mixed web(218).

2.4.4.2 Airlaid

Airlaid web formation is a method of drylaid web formation, suitable for making heavy webs of short fibres (215). Fibres are dispersed into an air stream and condensed onto a moving belt by using pressure or a vacuum, forming a web. This can then be thermally bonded or spray bonded with resin and cured. Different types of fibre can be overlaid to give a composite substrate, with different layers having specific properties. Compared with other drylaid processes, airlaid webs are less dense, softer and have no laminar structure.

2.4.4.3 Extrusion formed

Extrusion formed (spunlaid, spunmelt or polymer laid) manufacturing processes are linear. Typically, polymers with both a high molecular weight and broad molecular weight distribution such as polypropylene or polyester (or blends thereof) are used (219).

Properties of a spunbond nonwoven are influenced by the web structure, the bonding conditions and properties of the fibres themselves (220). Spunbonded webs are ideal for use in the medical setting due to their breathability; fibre resistance to fluid penetration and impermeability to bacteria (221). Alongside these characteristics lint free structure, fray resistance and liquid retention capacity are all properties found in spunbonded nonwovens that are desirable in wipes (220).

Meltblowing confers high liquid retention capacity due to high void content (porosity) alongside stability to heat and chemicals. Products of this process are typically used in disposable and medical applications (222).

2.4.5 Bonding Process

Nonwoven webs can be consolidated by chemical, thermal or mechanical means. The bonding process used effects the properties of the finished product such as fluid retention and resistance to abrasion. This section will be limited in scope to typical bonding processes used in the manufacture of wipes.

2.4.5.1 Chemical bonding

In the chemical bond process, a chemical binder is used to interlock fibres in a web. First, a binder is applied to a web; any moisture or solvent is removed; resulting in the formation of strong bond between the binder and the nonwoven web. The amount of binder used generally ranges from 5% to as much as 60% of the web by weight(223).

Binders are used to improve certain characteristics of a finished product – for example, strength and antimicrobial properties are two desired characteristics in a wipe – a binder can be selected accordingly(224). Nonwovens consolidated by other means can be subject to secondary chemical bonding, to change product appearance or properties(225).

2.4.5.2 Thermal bonding

Thermally bonded nonwovens employ thermoplastic component – present as a binding fibre, powder or web - to bond fibres together. A hot calendar or through-air oven is typically used to heat the thermoplastic component until it becomes viscous or melts; whereupon it flows to fibre-fibre crossover points, forming bonding regions after subsequent cooling (225).

Properties of the finished product include uniform bonding throughout and strong bond points, resistant to environmental stress and solvents (226). This is suited

to wipe manufacture. In addition, thermal bonding is environmentally friendly due to lack of latex binder and lower energy consumption than techniques such as hydroentanglement (227).

2.4.5.3 Mechanical bonding

2.4.5.3.1 Hydroentanglement

Hydroentanglement is a mechanical web bonding process, wherein arrays of fine, high-pressure water jets are used to consolidate a web of fibres (228). The water jets strike and displace the fibres in the web, causing them to rotate around and/or interlock with neighbouring fibres (229). Inter-fibre frictional forces give the finished nonwoven density and strength (230). Higher pressures can also split fibres into micro- and nano-fibres (231).

Hydroentanglement is favoured in the manufacture of wipes because of the uniformity, consistency, softness and excellent absorbency for contaminants conferred to the finished product (173). The low lint exhibited by hydroentangled wipes is another desirable characteristic for a product used to decontaminate critical patient areas (232). Good drape and relatively high strength are other major benefits of using this technique, alongside resistance to delamination (233).

2.4.5.3.2 Needle punch

The needle punch technique is a mechanical bonding process that involves mechanically orienting and interlocking the fibres of spunlaid and drylaid webs. Multiple barbed felting needles pass in and out of the web multiple times, thereby interlocking the constituent fibres. Physical properties of the end product depend on the constituent fibre, the fibre arrangement in the structure and the level of

consolidation the web is subject to(234). The number, arrangement and type of needles used to bond the web influence the web consolidation and structure

Needlepunch produced wipes are generally very strong with moderate softness and absorbency. One major disadvantage to the needlepunch method of production is its relatively slow speed - therefore it is less commercially viable when compared to techniques such as hydroentanglement (~150m/minute vs. ~1000m/minute) or the wetlaid process.

2.4.6 Wetlaid

The wetlaid process is analogous to paper production methods – the end goal is to produce a product with textile-like characteristics (i.e. flexibility and strength) at the rate of paper manufacturing.

In wetlaid nonwoven production, short fibres are suspended in a fluid medium; the fibres are deposited from this slurry onto a screen; the fluid is removed and a web is formed. Wood pulp is the main raw material used in the wetlaid sector.

Like nonwovens, paper also consists of fibre webs; however hydrogen bonds between these fibres mean that the consolidation is so complete that the entire sheet comprises one unit (235). The formal distinction between wetlaid nonwovens and wetlaid papers outline by the European Disposables and Nonwovens Association is as follows - a material shall be regarded as a nonwoven if:

“...more than 50% by mass of its fibrous content is made up of fibres (excluding chemically digested vegetable fibres) with a length to diameter ratio of greater than 300; or if the conditions in (a) do not apply then, if the following conditions are fulfilled: more

than 30% by mass of its fibrous content is made up of fibres (excluding chemically digested vegetable fibres) with a length to diameter ratio greater than 300 and its density is less than 0.4 g cm⁻³" (236).

Wetlaid nonwovens exhibit a high degree of uniformity, flexibility and strength. However, drawbacks of the wetlaid process include the high cost of thermally bonding any synthetic fibres used (237).

2.4.7 Substrates

Based on the patent and academic literature, two polymers were selected for further analysis and experimental work. These were polypropylene and lyocell, due to their common use in industry and different wetting properties, outlined below.

2.4.7.1 Polypropylene

Polypropylene (PP) is a linear hydrocarbon polymer with the molecular formula C_nH_{2n} (Figure 2-13). It is hydrophobic and regarded as chemically inert to many solvents, acids and bases (238). PP can therefore provide the "scrubbable" surface of a nonwoven wipe (239).

PP has previously used in the field of infection control, for example in nonwoven surgical drapes, shown to be impenetrable to bacteria for up to 30 minutes (240). Additionally, PP is frequently used for a range of disposable apparatus in clinical settings, including bed pans and surgical trays. PP scaffolds are typically used to repair wounds such as hernias (241).

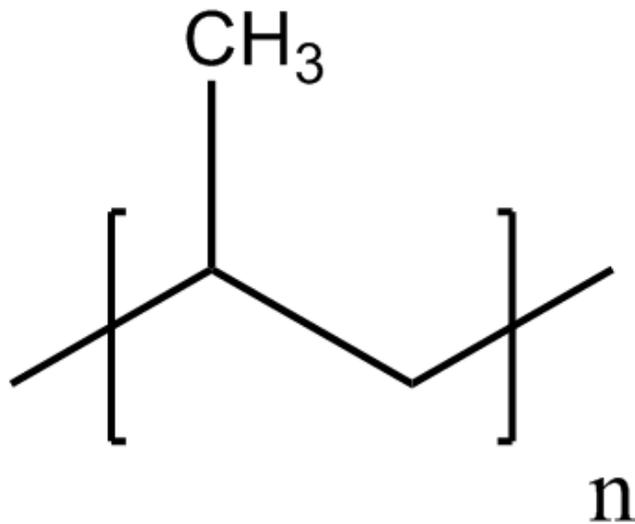


Figure 2-13. Polypropylene repeating unit where n is number or repetitions.

Figure created using ChemDraw 12.

2.4.7.2 Regenerated cellulosic fibres

Cellulose is polymer of $\beta(1-4)$ linked glucose residues, abundant in nature (242). Cellulose II differs from native cellulose (cellulose I) in that it is man-made. The regeneration process alters the hydrogen bonding pattern of the cellulose, changing the cellulose chain direction from parallel (cellulose I) to anti-parallel (cellulose II). This means that the chains are oriented in the opposite directions in cellulose II (242) (Figure 2-14).

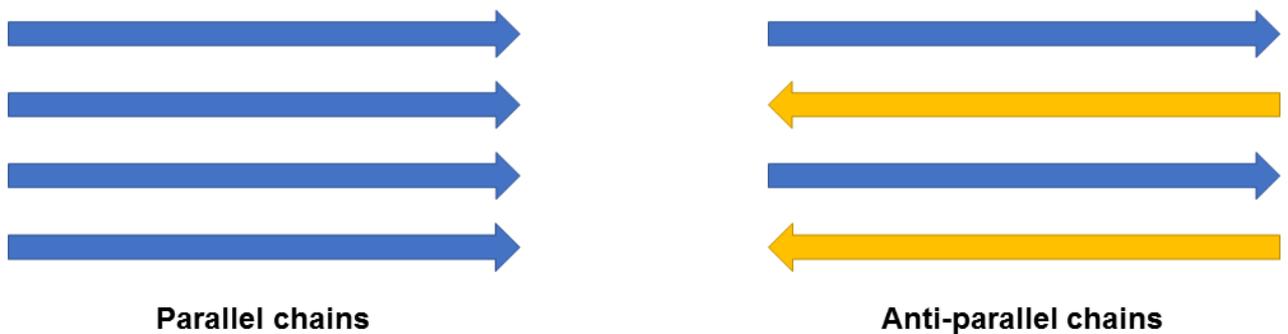


Figure 2-14. Parallel and anti-parallel chains.

Viscose (243), and Lyocell (244) are regenerated cellulose fibres, reclaimed from wood or bamboo pulp. Viscose is reclaimed via the reaction of cellulose I with carbon disulphide and a base, such as sodium hydroxide (245); while Lyocell is reclaimed by use of *N*-Methylmorpholine *N*-oxide (246).

The high wet strength demonstrated by Lyocell, is a desirable characteristic in a wipe (247), while the hygroscopic nature of these fibres allows excellent absorbency (248).

2.5 Modification of nonwoven wipe substrates by plasma treatment

Given the importance of being able to store and retain large volumes of biocidal liquid within the wipe and the fact that fibres may come in to direct contact with bacteria adhering to solid surfaces, it is important to consider key methods of modifying surface characteristics.

Nonwovens, particularly spunmelts composed of PP intended for use in hygiene applications are commonly modified by the addition of wetting agents to improve liquid holding. An alternative approach known to influence surface wetting is plasma treatment, but this is just one of many possible surface modifications.

According to Buyle, for fabric, fibres, nonwovens and other textile materials, plasma treatment can be used to (249):

- Impart hydrophilic properties;
- Increase adhesion;
- Influence printability and dyeability;
- Change the electrical conductivity;
- Impart hydrophobic and oleophobic properties;

- Apply antibacterial agents;
- Modify fibre surface roughness.

First described by Langmuir in 1928, plasma, also considered as the fourth aggregation state of matter (250), can be described as a mixture of partially ionised gases where the constituents are achieved by external energy addition (251). Plasmas are generally classified as hot/thermal and cold/non-thermal depending on the temperature of the plasma zone.

A concise overview of different technical plasma processes used for material processing is given in Figure 2-15.

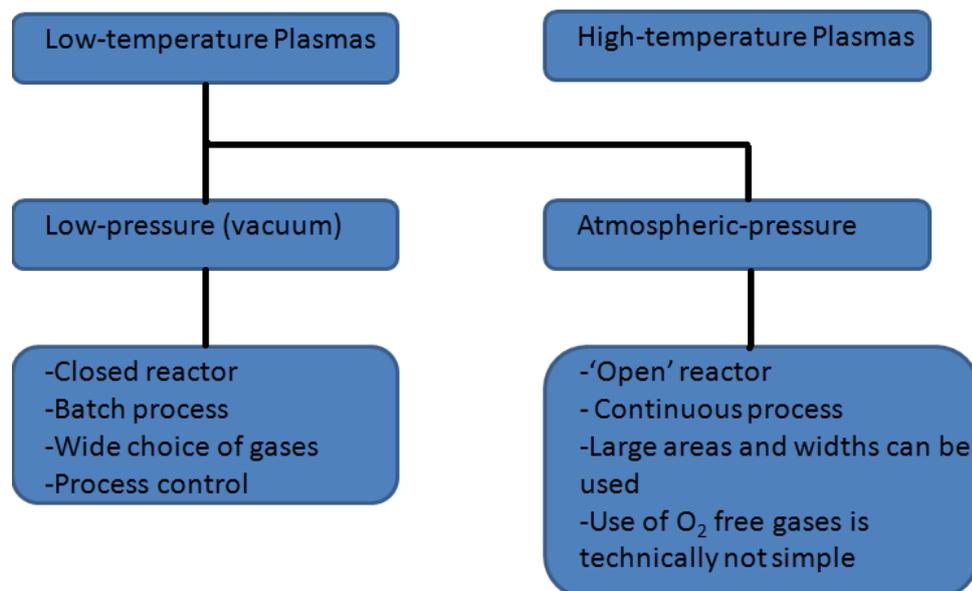


Figure 2-15. Definitions and characteristics of technical plasma processes used for material processing.

2.5.1 Thermal plasma

In thermal plasma, the gas density is sufficiently high and so the frequency of collisions between electrons, ions, and neutral species composing the plasma is such that an energy exchange is possible. So, thermal plasmas are characterised

by thermal equilibrium between all the different species contained in the gas. Temperatures in the order of thousands of degrees Celsius are reached in thermal plasmas, so they are not suitable for surface modification of heat-sensitive polymeric and nonwoven materials (252).

2.5.2 Non-thermal plasma

Cold plasmas can be produced at room temperature by applying an electrical field over two electrodes with a gas in-between, or by inducing radiofrequency (RF) resonant current in a coil (252). The electrons acquire higher energies than ions and molecules, and, due to low density of gas, collisions are rare and thermal equilibrium is not reached (253). These electrons make up $> 1/1000000$ of the total mass, their influence is negligible and the plasma has a low overall temperature making this treatment type suitable for use in surface modification of textile materials.

The main advantages of non-thermal plasma treatments include (254):

- The electrons can cleave covalent bonds at the surface of the treated material, producing physical and chemical modification at the surface (on the sub- μm scale) without changing the bulk properties of the material;
- Compared to conventional finishing processes, plasma treatment consumes minimal chemicals and no costly drying process is required;
- The plasma processes has high environmental compatibility;
- Low temperature plasma can be applied to most kinds of fibre, nonwoven or polymeric material.

Regarding large scale plasma treatment lines, the “Plasmatreat” pilot workshop includes a conveyor system with integrated plasma for treating widths up to 300 mm and processing speeds of up to 400 m min⁻¹ for treatment of textiles and nonwovens. Some atmospheric plasma treatment systems can handle widths of 2 m at speeds of 20 m min⁻¹ (254). Sub atmospheric and low pressure plasma systems tend to be batch processes (249).

2.6 Atmospheric Plasma Treatment

Atmospheric plasma treatment is, as the name suggests, carried out under ambient conditions. This is advantageous as material can be processed continuously, so this system can be integrated into an existing textile processing or treatment line. Atmospheric plasma treatment can be split into corona treatment, dielectric barrier discharge and glow discharge (255).

2.6.1 Corona Plasma Treatment

Corona plasma treatment uses a low temperature corona discharge plasma to impart changes in the properties of a textile surface. A corona discharge is an electrical discharge resulting from the ionisation of a fluid around an electrically charged conductor. Corona treatment can improve the spinnability, strength, and abrasion resistance of a fabric (256). Corona treatment can increase the hydrophilicity of a nonwoven material, especially when used in combination with a pre-treatment (257), or if the treatment is repeated multiple times (258).

2.6.2 Glow Discharge Plasma

Glow discharge plasma is created by the passage of electric current through a low-pressure gas. This type of treatment can be used to sterilise nonwoven filters (259).

2.6.3 Dielectric Barrier Discharge Plasma

Dielectric barrier discharge plasma is an electrical discharge between two electrodes, separated by an electrical insulator that can be polarised by an applied electric field (dielectric). This is used to clean or modify substrates, including nonwovens (260, 261). This usually involves RF to microwave frequency, high voltage AC current (262).

2.7 Low Pressure Plasma

Low pressure plasma is a cost effective, environmental friendly technique used to modify the surface of polymeric materials, such as nonwovens, on the micro- to nano- level. The material to be treated is placed in a vacuum chamber to facilitate the low pressure (typically below 500 mT). The advantages of this type of treatment include no heat, minimal surface ablation, a uniform 3-D treatment, repeatable results and little to no environmental or health concerns (263). It can be used to modify the mechanical properties of nonwoven fabrics (264). Low temperature, low pressure plasma treatment is advantageous for treatment of nonwovens as it is both uniform and reproducible, especially when compared to traditional textile treatment methods (265).

2.8 Surface Modification with Plasma

Plasma treatment can be used to modify nonwoven materials either chemically, physically or by a mixture of both.

2.8.1 Physical

Physical modification of nonwoven substrates by plasma treatment can take place, generally via etching.

2.8.1.1 Etching

Plasma etching is material removal from a surface via a plasma process. This involves a sample being treated with an appropriate plasma gas mixture being pulsed at a sample. The plasma source, known as etch species, can be either charged (ions) or neutral (atoms and radicals). Applications include the micro-structuring of nonwoven fibre surfaces (264). O₂ plasma can be used to etch material surfaces, conferring nano-texture to polymeric fibres, particularly in nonwovens (266, 267). O₂ plasma therefore provides potential to increase both the surface energy and surface roughness of fibres in a nonwoven fabric. This is further explored in Chapter 4.

2.8.2 Chemical

Chemical modification of nonwoven substrates by plasma treatment can occur via a number of methods, outlined in the following sections.

2.8.2.1 Plasma enhanced vapour deposition (PEVD)

Plasma enhanced vapour deposition is used to deposit thin solid films on a substrate, from the gas state. Plasma is created from this gas by either DC, or more commonly radiofrequency (RF; AC) discharge in the reaction chamber.

PEVD different to plasma grafting; PEVD is a one-step in-situ method, while plasma grafting has separate radical-forming and graft-polymerisation steps (268). Plasma polymer films are often deposited by PEVD (269). Plasma deposition is limited to very thin layers (up to 200 nm) on the individual fibres. This method can be used to functionalise nonwoven fabric surfaces (270).

For example, hexafluoroethane (C_2F_6) plasma can deposit CF_x radicals on fibres to form hydrophobic finishes (254). C_2F_6 plasma is therefore a potentially useful means of reducing the apparent wettability of inherently hygroscopic materials such as those composed of regenerated cellulose. There is the possibility to increase both surface roughness of fibres within a nonwoven fabric, while decreasing the surface energy, allowing the study of the effects of surface roughness and surface energy on bacterial removal, especially when compared and contrasted to other treatments, such as O_2 plasma. This is further explored in Chapter 4.

2.8.2.2 Plasma cleaning

Plasma treatment can be used to remove contaminants from a fibre surface, e.g. desizing of cotton but is not directly relevant to the work included in the present study. It is mentioned here only to ensure completeness in the discussion.

2.8.2.3 Grafting

Grafting of copolymers involves fixing polymeric chains to a structurally different polymeric substrate, to change surface functionality whilst preserving bulk mechanical properties. Grafting copolymers to a textile surface can be facilitated by using atmospheric or pressure-dependent plasma processing, allowing tailoring of the nonwoven to a specific end, including modification of hydrophobicity (271) and functional finishing (272).

2.9 Summary

In respect of removing and collecting bacteria from solid surfaces during wiping in healthcare settings, the purpose is to overcome the adhesion between the pathogenic bacterium and the support surface. It is inevitable that contamination will take place because bacteria adhere to surfaces as a survival mechanism. This places them in a nutritionally advantageous environment and allows biofilm formation (56). The efficiency of removal by wiping as a form of decontamination is related to the properties of the wipe, soil and surface (96). If a representative environmental surface is to be selected for standardised assessment of decontamination efficacy of wipes, it should have both representative topography and also be widely available.

Nonwoven fabrics are extensively used as wipes for removing soils from a surface and those wipes impregnated with a biocidal agent are considered ideal for use in the clinical environment as part of an infection control strategy. In previous studies, the fundamental underlying mechanisms of bacterial capture have not been thoroughly investigated. Instead, the focus has been on whether there are bacteria present on the fabric, rather than how the two interact during the wiping process itself, or how the design of the wipe influences these interactions. These factors are both intrinsic and extrinsic and can be modulated to potentially control wiping efficiency by adjusting fabric structure and dimensions, fibre surface chemistry and fibre chemical composition. A range of characterisation methods can be employed to study particle capture behaviour during dynamic wiping some of which have been recently developed specifically for evaluation of biocidal impregnated wipes intended for use in healthcare.

Given the paucity of fundamental studies, there is significant scope for research into the interaction between pathogens and nonwoven media, particularly with respect to the clinical or healthcare environment. The complete picture regarding the role of wipe substrate design on the interaction with bacteria has yet to be fully understood and this merits further study, particularly with the rising prevalence of nosocomial infection. The benefits of research into pathogen-fibre interactions could also include the development of new disinfection regimens; the improvement of quality of life in the general and immunocompromised population; and cost savings for the NHS during a time of global austerity.

Chapter 3

Experimental Materials and Methods

Detailed in this Chapter are the raw materials, methods of wipe substrate production, general test methodologies and characterisation techniques used in the experimental work that follows. Additional experimental details are included in subsequent chapters where they relate to specific experiments.

3.1 Preparation of Nonwoven Wipe Substrates

Nonwoven fabric production can be classified in part according to the method of web formation (181). This is the initial stage during which fibres are transformed into loosely-arranged planar networks (215). Final dimensional properties such as weight per unit area are established during this phase of production (181).

Preparation of wipe substrates for the experimental work was guided by industrial norms. As detailed in section 2.4.3, application of inherently hydrophobic fibres such as PP and inherently hydrophilic regenerated cellulose fibres such as lyocell to manufacture surface wipes is common, despite obvious fundamental differences in moisture relations. [204]. Webs were prepared by carding followed by mechanical bonding in the form of hydroentanglement.

Hydroentangling can be used to create nonwovens with a wide range a variety of basis weights from both PP and lyocell fibres, for example, between 50 g.m⁻² and

150 g.m⁻². Thereby, the effect of both fibre type and wipe basis weight can be readily studied.

To ensure satisfactory control of wipe substrate properties and enable reliable comparisons of wiping behaviour, fabric samples were manufactured in-house, using pilot-scale nonwoven manufacturing processes at the University of Leeds.

The manufacturing procedure was designed to replicate as closely as possible that commonly used to prepare wipe substrates in an industrial context. Wipe substrates were produced based on carding and hydroentangling, which is one of the most commonly employed industrial methods. Hydroentanglement is favoured in the manufacture of wipes because of the uniformity, consistency, softness and excellent absorbency of the fabrics (218), depending on the fibre composition.

Wipes were prepared using two different fibre types, both of which were sourced by commercial producers. Polypropylene fibre (T133 HY-Entangle, Fibervisions; Varde, Denmark) of 1.7 dtex linear density, 40 mm fibre length and lyocell fibre (Lenzing; Grimsby, UK - 1.7 dtex, 38 mm fibre length, dull) were mechanically pre-opened using a Fearnought (Tatham Ltd.; Bradford, UK) prior to carding. These fibres were selected as representative hydrophobic and hydrophilic raw materials (respectively) that are commonly found in the wipes market.

Parallel-laid webs of 60 g.m⁻² (Chapters 4 and 6) or parallel-laid webs of 50 g.m⁻², 100 g.m⁻² and 150 g.m⁻² (Chapter 5) were manufactured using a 0.5 m wide single cylinder and single doffer, worker-stripper card (Tatham Ltd.; Rochdale, UK). Carding involves mechanical fibre disentanglement and mixing resulting in

the formation of a continuous web of uniform weight per unit area, and which can then be bonded by various means (216, 217).

Hydroentanglement is a mechanical web bonding process, wherein arrays of columnar, high velocity water consolidate the web as well as displace and entwine fibres to increase frictional resistance and therefore the strength of the fabric (228-230). At higher water pressures, e.g. >100 bar can begin to fibrillate fibres such as lyocell into micro- and nano-fibres (231). The degree of bonding during hydroentangling can be characterised by the kinetic energy consumed by the process (176) (see Equation 3.1):

$$K'_e = \frac{1.1nC_d^3D^2p^{1.5}}{v_bmp_w^{0.5}} \quad \text{Equation 3-1.}$$

Where,

K'_e = Kinetic energy of the water jets applied to a mass of web ($J.m^{-2}$)

n = Number of water jets per unit width in the jet strip ($jets.m^{-1}$)

C_d = Water discharge flow coefficient (valued at 0.66 based on measurements taken using the hydroentanglement unit at the Nonwovens Research Group, Leeds University)

D = Diameter of the holes in the jet strip (m)

p = Water jet pressure ($N.m^{-2}$)

v_b = Velocity of the web/conveyor under the jet ($m.s^{-1}$)

m = Area density of the web ($k.m^{-2}$)

ρ_w = Density of water ($1000.kg.m^{-3}$)

Each carded web was hydroentangled using a 0.5 m wide Hydrolace pilot line (Figure 3-1), whilst supported on a woven conveyor (ca. 24% open area, mean aperture size $0.7.m^2$) at specific energy of $3.47.MJ.kg^{-1}$ (Chapters 4 and 6) or

4.86 MJ kg⁻¹ (Chapter 5). The higher specific energy enabled all three web weights (50 g.m⁻², 100 g.m⁻² and 150.g.m⁻²) used in that set of experiments to be bonded without mechanically damaging the lightest web due to excessive energy input.

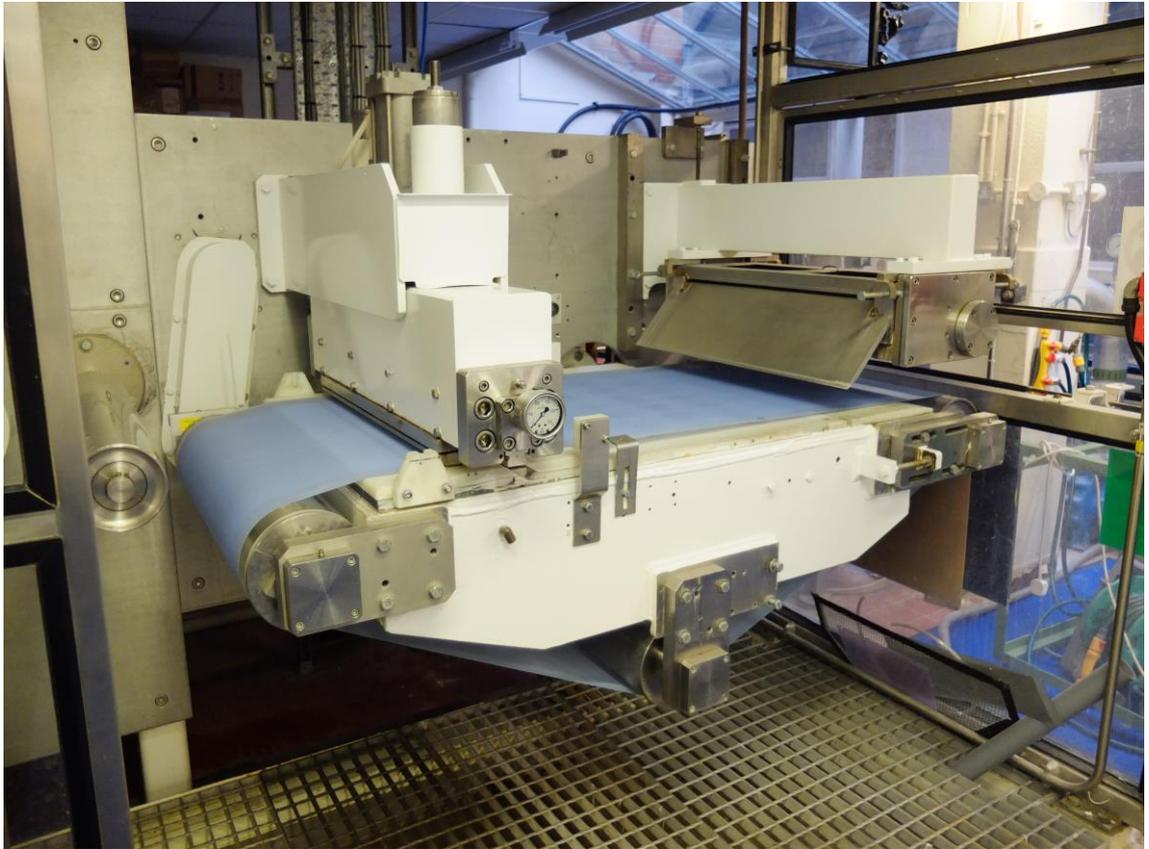


Figure 3-1. Hydrolace pilot line.

The fibres selected for the experimental work were specifically intended for hydroentangling and therefore contained low foaming fibre finishes. The hydroentangling process is known to be highly effective at removing residual fibre finish because of the action of the jets and high volume of water that passes through the web. Nevertheless to ensure all residual fibre finish was removed from the fabric prior testing and characterisation, all samples were scoured in a Roaches Rotohose rotary drum dyeing machine (Roaches, UK) for 15 min at

60°C with using 1 g.dm⁻³ non-ionic detergent (Hostapal NIN; Clariant Produkte GMBH; Frankfurt, Germany) and 2 g.dm⁻³ sodium carbonate at a liquor ratio of 20:1 (273). Fabrics were then thoroughly rinsed and line-dried prior to further treatment or testing.

3.2 Plasma functionalisation of nonwovens

Following fabric formation, modification of PP and lyocell fibre surface properties was carried out using low pressure low temperature plasma treatment to provide a range of samples with different surface energies. Lyocell fabrics were exposed to hexafluoroethane (C₂F₆) or oxygen (O₂) gas (BOC; Manchester, UK) in a PICO low temperature low pressure plasma coater (40 kHz; Diener GmbH; Ebhausen, Germany - Figure 3-2). Treatment was carried out at 150 W power and 12 cm³ min⁻¹ gas flow rate. Exposure times were 30 s and 1, 2, 3, 4, 5, 10 or 20 min for C₂F₆ and 20 min for O₂ to provide a range of surface characteristics. These treatment times were selected after preliminary experiments where the wetting properties of the plasma treated nonwovens were crudely assessed using a Pasteur pipette and ~0.5 ml distilled water. This preliminary work is not reported herein as it was replaced by tensiometry, which is a far more accurate method to analyse surface energy of nonwoven samples (section 3.3).



Figure 3-2. Diener Plasma Reactor.

Initial pressure in the chamber was 0.15 Torr. Post-exposure, samples were left to condition in a standard textile testing environment (Temperature $21^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and relative humidity $65\% \pm 5\%$) for at least 24 h before further analysis or testing.

3.3 Measurement of Surface Energy

Surface energies for each of the nonwoven fabric samples were evaluated using the Owens, Wendt, Rabel and Kaelble method (OWRK) (274). Wetted length was calculated using *n*-hexane (Sigma Aldrich, UK), and contact angle values were calculated with ethanol, 1-octanol, cyclopentanol (all purchased from Sigma Aldrich, UK) and distilled water. Experiments were performed using a KRÜSS K100 tensiometer (KRÜSS GmbH; Hamburg, Germany - Figure 3-3). Five replicates for each sample were performed.



Figure 3-3. KRÜSS K100 tensiometer.

3.4 Model Healthcare Surfaces

High touch solid surfaces found in hospitals include stainless steel (e.g. bed rails) (45) plastics (e.g. desk surfaces) (46) and ceramics (e.g. sinks) (47). As such, surfaces composed of poly (methyl methacrylate) (PMMA; Perspex) surface tiles (registered to ISO 9001), Grade 304 stainless steel or ceramic tiles were selected for evaluation in these studies to replicate those found in hospital settings. These were alcohol-sterilised (ethanol) and inspected to ensure freedom from any defects prior to use in the experimental work.

3.5 Surface roughness of fibres and cleaning surfaces

Fibre surface roughness and the surface roughness of the model cleaning surfaces were measured using Atomic Force Microscopy (AFM). A Dimension Fastscan atomic force microscope (Bruker, US) was used in contact DC mode to probe the surface of both in ambient conditions:

- a) Lyocell; C₂F₆ treated lyocell fabric samples (1 min, 4 min and 20 min treatment times); O₂ treated lyocell (20 min); and PP. Fibre samples were mounted on a 10 mm diameter circular metal disc using epoxy resin, or;
- b) Steel, ceramic and PMMA model healthcare cleaning surfaces.

Nanoscope Analysis v1.5 software (Advanced Surface Microscopy, Inc., US) was used to evaluate the resulting data. Three replicates were performed for each sample, and representative images are shown where relevant.

3.6 Mass spectrometry

To determine changes in surface chemistry associated with plasma treatment, time-of-flight secondary ion mass spectrometry (ToF-SIMS) (Intertek MSG, Wilton, UK) analysis of untreated lyocell and the 1 min, 4 min and 20 min C₂F₆ plasma treated lyocell nonwoven fabrics was performed using an Ion-ToF-SIMS IV unit (ION-TOF GmbH; Münster, Germany - Figure 3-4) with a Bi⁺ source and an ion dose of less than 1×10^{12} ions cm⁻². Both positive and negative ion spectra were acquired from a 200 μm x 200 μm area in the mass range $m/z = 0-1500$. The fabric sample was handled with sterile stainless steel tweezers and stored in clean aluminium foil, prior to analysis.



Figure 3-4. Ion-ToF SIMS unit.

3.7 Biocide and neutraliser

A large variety of biocide formulations are in industrial use although many share similar chemical compositions. For the present experiments, one commercially produced biocide intended for use with healthcare wipes was selected as a representative model system. This formulation was supplied direct by the manufacturer and was of known composition and history.

3.7.1.1 Biocide composition

The biocide selected for these experiments was a proprietary blend composed of a non-ionic surfactant (C₉-C₁₁ ethoxylated alcohol Pareth-5), a cationic surfactant (Benzalkonium chloride), and various buffering agents and sequesterants. The mode of action of such biocides is outlined in section 2.3.1. A 1:20 dilution of the stock biocide solution, mixed with deionised water (dH₂O) was known to pass the EN 1276 “Quantitative Suspension Test of Bactericidal Activity of Chemical Disinfectants” test, giving a 5 log₁₀ (99.999%) reduction of the pathogenic bacteria *S. aureus*, *E. coli*, *E. hirae* and *P. aeruginosa* inside 5 minutes (275). The biocide surface tension was 37.5x10⁻³ N.m⁻¹ at 20°C; the viscosity was 1.35 mPa·s (60 rpm at 2.7% torque); and the pH was 9.98.

3.7.1.2 Neutraliser toxicity and efficacy tests

A neutraliser stops the action of the biocide to facilitate a bacterial removal evaluation as part of an experiment. In the present work, the neutraliser was manufactured according to the methodology outlined by Ramm *et al.* (189). The compositions that was prepared consisted of 30 g.dm⁻³ saponin, 30 g.dm⁻³ polysorbate 80, 3 g.dm⁻³ azolectin from soybean, 1 g.dm⁻³ L-histidine, 5 g.dm⁻³

sodium dodecyl sulphate and 5 g.dm⁻³ sodium thiosulphate (all Sigma Aldrich, UK) made up to 1 l in distilled deionized water.

The toxicity of the neutraliser and its ability to arrest the activity of the biocide was tested according to the method outlined by Knapp *et al.* (276). This involved adding 1 ml of a 1x10⁸ CFU·ml⁻¹ bacterial cell suspension of either *E. coli*, *S. aureus* or *E. faecalis* in to 9 ml of neutraliser. The suspension was vortex mixed and left for 5 min. A control experiment was performed alongside this, where 1 ml of the bacterial suspension was added to 9 ml of deionized water. Viable counts were performed on test and control suspensions using an appropriate agar. Test and control counts were compared to determine whether exposure to the neutraliser caused any significant decrease in CFU·ml⁻¹. The neutraliser was considered toxic if a $\geq 1 \log_{10}$ decrease was observed in the test colony count.

The ability of the neutraliser to quench the activity of the biocide was tested. 1 ml of the biocide was added to 8 ml of neutraliser and vortex mixed. After 5 min, 1 ml of a bacterial suspension containing 1x10⁸ CFU·ml⁻¹ was added and vortex mixed. A control experiment was performed alongside this using 8 ml sterile distilled water instead of neutraliser. Viable counts of both control and test suspensions were performed. The neutraliser was considered effective if $\leq 1 \log_{10}$ reduction was observed in the neutralized biocide suspension.

3.7.1.3 Addition of the water and biocide to the wipe substrate

To add liquid lotion, the wipes were soaked in either 10 ml 1:20 biocide or dH₂O (control) for 10 min before being run through a Werner Mathis mangle (2.2 bar pressure, 4 m.min⁻¹) to remove excess liquid as per Berendt *et al.* (277). The target liquid pickup (or “add-on”) weight was 150% w/w for both the biocide and dH₂O, on all wipe basis weights, in both the PP and the lyocell wipes. This was

based on the maximum liquid pick-up that could be retained with the hydrophobic PP wipes.

3.8 Bacterial strains

To provide a detailed experimental assessment of the bacterial removal efficiency using different wipe substrates, a range of bacteria associated with HCAs were selected. The microorganisms used in this study were *E. coli* (ATCC 25922), *S. aureus* (ATCC 29213) and *E. faecalis* (ATCC 29212) supplied by the Leeds Teaching Hospitals NHS Trust Microbiology department (LG); Leeds, UK). Strains were cultured according to previously published methods: *E. coli* according to Hsu *et al* (2013) (278), *S. aureus* according to Holinka *et al* (2013) (279) and *E. faecalis* according to Gallardo-Moreno *et al* (2002) (280).

3.9 Measurement of bacterial removal efficiency

Experimental assessment of the wiping efficiency of samples was undertaken based on a previously developed protocol. Removal of bacteria from the contaminated surface was tested using methodology adapted from Williams *et al.* (28). Bacterial cells were suspended in phosphate buffered saline (PBS); the solutions optical density was measured at $\lambda = 600$ nm; and the solution adjusted to McFarland standard 0.5, equivalent to an approximate cell density of 1×10^8 CFU·ml⁻¹ (281); 0.3 g·dm⁻³ bovine serum albumin (BSA) w/v was added to the final solution.

Alcohol-sterilised poly (methyl methacrylate) (PMMA) surface tiles (registered to ISO 9001), Grade 304 stainless steel or ceramic tiles were inspected to ensure freedom from any defects. These were then inoculated with 20 μ l bacterial cell

culture suspended in phosphate buffered saline (PBS) with 0.3 g.dm⁻³ bovine serum albumin (BSA).

To simulate static wiping, a 20 mm diameter of the nonwoven fabric was pressed against the inoculated surface tile for 10 s with an applied force of 150 g. For dynamic wiping, a 900 mm² section of the test fabric was attached to a 20 mm diameter boss, and fixed to a Caframo BDC2002 overhead stirrer (Caframo Limited, Ontario, Canada). This was rotated at 60 r.min⁻¹ for 10 s at 4.68 kN.m⁻² applied pressure (Chapter 4 and 6) or at either 0.68 kN.m⁻², 4.68 kN.m⁻² or 13.80 kN.m⁻² applied pressure against the inoculated surface tile, dependent on the Orthogonal Array Testing Strategy parameters (Chapter 5).

If biocide was used, surfaces were transferred to the neutraliser solution (see section 3.7.1.2), and shaken at 150 r.min⁻¹ for 5 min. Bacteria removal efficiency was calculated as in Equation 3.2.

$$R = (C_{ct} - C_{wt}/C_{ct}) \times 100 \quad \text{Equation 3-2}$$

Where R = Removal efficiency (CFU %);

C_{ct} = Bacterial colonies recovered from control tile; and,

C_{wt} = Bacterial colonies recovered from wiped tile.

3.10 Agar Diffusion Plate Test

Textiles were assessed for antimicrobial activity according to ISO 20645:2004 (282). Briefly, specimens of the material to be tested are placed on two-layer agar plates. The lower layer consists of a culture medium free from bacteria and the upper layer is inoculated with the selected bacteria. The textiles are tested on both sides. The level of antibacterial activity is assessed by examining the extent

of bacterial growth in the contact zone between the agar and the specimen and, if present, the extent of the inhibition zone around the specimen.

3.11 Recontamination of Cleaned Surfaces

Recontamination of surfaces was measured according to the method outline by Ramm *et al.* (189). After the application of a wiping cycle to a contaminated surface (section 3.9), subsequent transfer of bacterial contamination on to three consecutive sterile surfaces of the same type, e.g. steel to steel and PMMA to PMMA, was measured together with the effect of the dynamic wiping mechanism (60 r.min⁻¹ for 10 s at 150 g ±10 g applied force). Surfaces were transferred to the neutraliser solution and shaken at 150 r.min⁻¹ for 5 min. Bacterial colonies were enumerated using appropriate agar. A minimum of 3 replicates was performed.

3.12 Residual Antimicrobial Activity

Assessment of the residual antimicrobial activity of the biocide on sample surfaces was based on a modified Association of Official Analytical Chemists dilution method (283). Steel, ceramic or PMMA tiles were inoculated with 20 µl of the biocide. This amount was selected based on observations in previous wiping experiments (preliminary work). This was spread over the surface with an L-shaped hockey stick (VWR 612-1561) using five back and forth sweeps left and right, up and down then left and right, and allowed to dry in ambient conditions for 20 min.

These tiles were then inoculated with bacteria using the same method described in Section 3.9, but without simulated wiping. The results were compared with a control tile, with no biocide addition. Any significant bacterial death on the biocide

surface compared to the control surface was therefore attributed to the residual antimicrobial activity of the biocide left on the surface.

3.13 Orthogonal Array Design of Experiments (OATs) and Taguchi Analysis

This method is applied in scientific analysis when the number of parametric inputs, of factors, into the system is too large to allow a full factorial analysis, but is sufficiently sensitive to identify main effects (281). Standard full factorial ANOVA of the three bacterial species and two wipe fibre types tested with all combinations of basis weight, biocide liquid addition and wiping pressure, with the required number of replicates would have resulted in four hundred and eighty-six test specimens. Using OATs, the number of test specimens was reduced to one hundred and sixty-two.

An L9 3**3 orthogonal array, generated using the Taguchi method, was used to analyse the optimum wiping conditions for removal of pathogenic bacteria from a poly(methyl methacrylate) model surface (displayed in table 3.1).

Experimental factors and levels were selected based on preliminary experiments and industrial norms. Fabric basis weights of 50 g.m⁻², 100 g.m⁻², and 150 g.m⁻² were chosen to approximate the range of basis weights found in commercially available nonwoven healthcare surface wipes.

Table 3-1. Orthogonal array parameters arranged in a 3**3 Taguchi array.

Orthogonal array parameters			
Sample	Fabric basis weight (g.m ⁻²)	Liquid addition	Wiping pressure (kN.m ⁻²)
A1	50	Dry	0.69
A2	50	Water	4.68
A3	50	Biocide	13.80
A4	100	Dry	4.68
A5	100	Water	13.80
A6	100	Biocide	0.69
A7	150	Dry	13.80
A8	150	Water	0.69
A9	150	Biocide	4.68

The wipes were tested both in the dry state, after impregnation with distilled water (dH₂O control), or the biocide. In this way, it was possible to determine the relative influence of each parameter on bacterial wiping efficiency in a manner that has not been previously reported. The conditions used for the addition of water or biocide to the wipes are given in section 3.13.1.3.

Wiping pressure refers to the pressure applied to the wipe when in contact with the inoculated surface. A wiping pressure of 0.69 kN.m⁻² is the equivalent of 1 kg of exerted force from an average sized human hand, which can be termed the “hand-weight” [261]. A pressure of 4.68 kN.m⁻² between the wipe and the surface is equivalent to a “hand-weight” of 6.79 kg. This value was selected for the experiments as it reflects the values adopted by other researchers, obtained by extrapolating the 150 g “exerted weight” reported by Ramm *et al.* [191] in their wiping experiments.

For these studies, a higher pressure representing vigorous wiping of 13.80 kN.m^{-2} wiping pressure was also selected, which is the equivalent of a 20 kg “hand-weight”. In practice, higher values would not normally be encountered in normal healthcare cleaning situations, so the selected range of hand pressures approximates realistic conditions. Optimum process parameters were then calculated according to **Table 3.2**. The process parameter being optimised by this array is bacterial removal %, with a greater removal % value being optimum. The output values “A1-A9” from (Table 3 2) are used to calculate the optimum values of fabric basis weight, liquid addition and wiping pressure, giving the greatest bacterial removal. B1-B9 are the sums used to calculate the OPP. The OPP is the greatest of the “B” values for the given parameter. C1-3 are the “difference” values. The largest “C” value shows which of the parameters in the array has the greatest effect on bacterial removal %.

Table 3-2. Optimum process parameter (OPP) calculation scheme and results.

Optimum process parameter calculation			
	For fabric basis weight	For liquid addition	For wiping pressure
$\Sigma 1$	$B1 = A1 + A2 + A3$	$B2 = A1 + A4 + A7$	$B3 = A1 + A6 + A8$
$\Sigma 2$	$B4 = A4 + A5 + A6$	$B5 = A2 + A5 + A8$	$B6 = A2 + A4 + A9$
$\Sigma 3$	$B7 = A7 + A8 + A9$	$B8 = A3 + A6 + A9$	$B9 = A3 + A5 + A7$
Optimum Process Parameter (OPP)	Greatest of B1, B4 and B7 (Value of basis weight for relevant experiment – from “Orthogonal array parameters” in Table 3-1).	Greatest of B2, B5 and B8 (Value of biocide/dry/water for relevant experiment – from “Orthogonal array parameters” in Table 3-1).	Greatest of B3, B6 and B9 (Value of “exerted weight” for relevant experiment – from “Orthogonal array parameters” in Table 3-1).
Difference	$C1 = (\text{Greatest value of B1, B4, B7}) - (\text{Smallest value of B1, B4, B7})$.	$C2 = (\text{Greatest value of B2, B5, B8}) - (\text{Smallest value of B2, B5, B8})$.	$C3 = (\text{Greatest value of B3, B6, B9}) - (\text{Smallest value of B3, B6, B9})$.

3.14 Electrophoretic mobility of bacterial cells

The electrophoretic mobility of bacterial cells was determined as an indication of their electric surface charge, and was measured using a Malvern Zetasizer nano-ZS (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom - Figure 3-4).

Briefly, a 1:10 dilution of stationary-phase bacterial cell culture was made in Tryptic soy broth and gently vortexed to ensure thorough mixing. A 1 ml sample was aliquoted into the cuvette and inserted into the measurement chamber. Measurements were taken in triplicate for each bacterial strain.

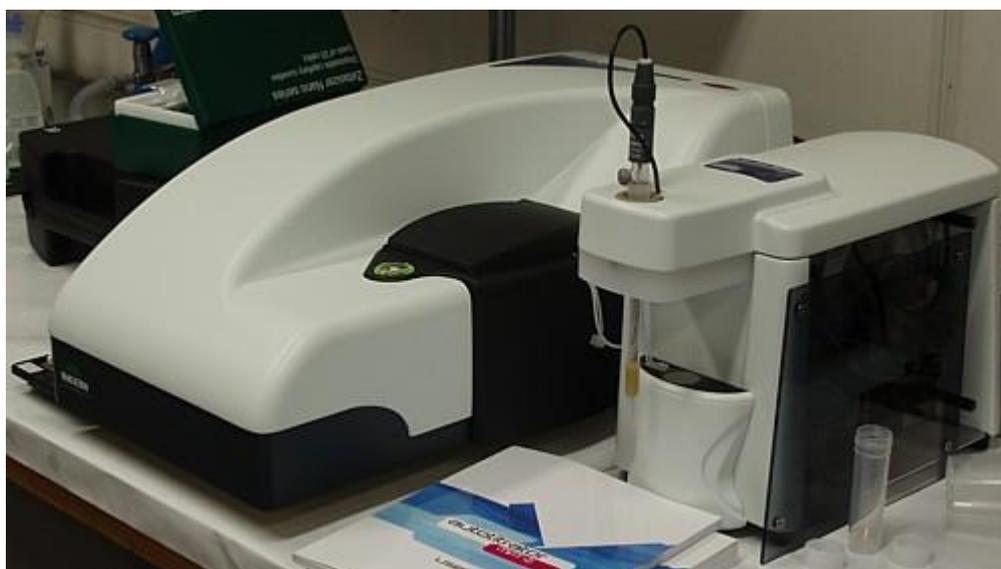


Figure 3-5. Malvern Zetasizer nano-ZS.

3.15 Streaming potential of nonwoven wipe substrate fabrics

The streaming potential of the nonwoven fabrics used to make wipes for the wiping experiments was measured according to Coday *et al.* (284), using a commercial electrokinetic analyser (SurPASS, Anton-Paar GmbH, Austria - Figure 3-6). Nonwoven fabric samples were mounted on a SurPASS adjustable-gap cell that accommodates small planar samples with a rectangular size of 200 mm². The cell was mounted on the electrokinetic analyser and the hydraulic system and gap between the membranes were flushed with deionized water for 2 min. The system was drained and flushed twice with electrolyte solution (ACS-grade potassium chloride KCl - Fisher Scientific) to ensure that all deionized water was removed. The electrolyte solution was replaced and recirculated for at least 30 min. This neutralized any localised charge on the Ag/AgCl electrodes and minimised variability in streaming potential measurements (285).

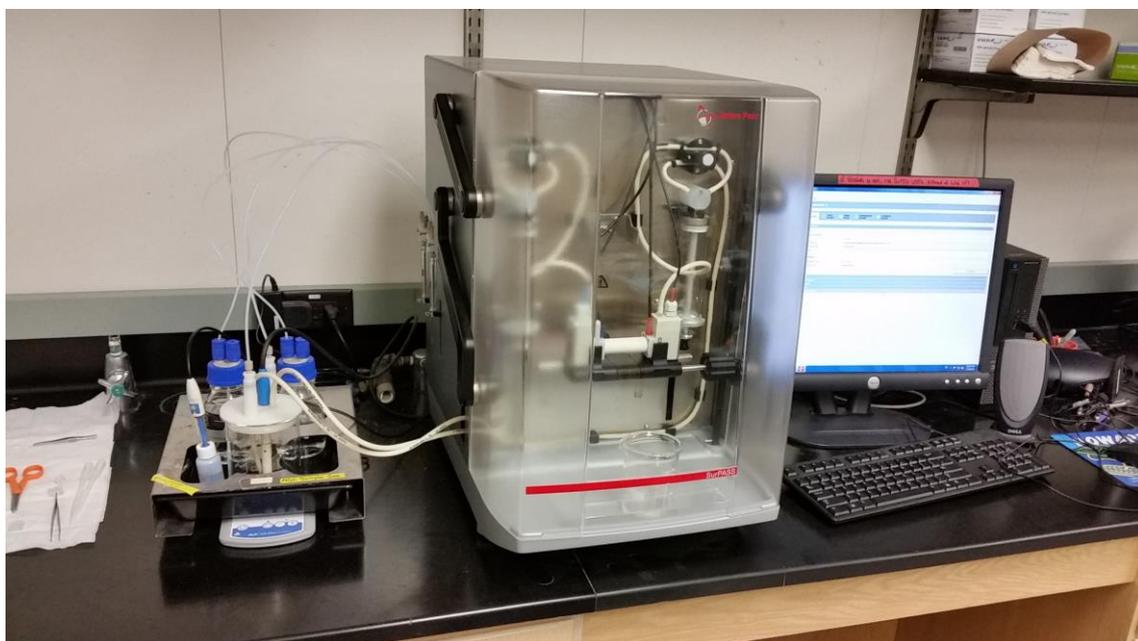


Figure 3-6. Anton-Paar SurPASS EKA.

All streaming potential measurements were performed at 20 °C with an average gap height of 116 ± 2 μm . At least eight streaming potential measurements (four flowing from left to right and four from right to left) were recorded and then averaged to calculate the zeta potential.

3.16 Surface wetting tension

The wetting behaviour of each of the model healthcare surfaces, i.e., the steel, ceramic and PMMA test surfaces was measured with milli-Q water using an FTÅ 1000 contact angle goniometer (First Ten Ångstroms; Portsmouth, Virginia, US). Tiles of each of the materials were tested either sterile, or following inoculation with 20 μl of either 0.015 $\text{g}\cdot\text{m}^{-2}$ or 0.15 $\text{g}\cdot\text{m}^{-2}$ bovine serum albumin (BSA) in phosphate buffered saline (PBS) and subsequent air-drying, or tested either sterile or following inoculation with 20 μl of either 0.015 $\text{g}\cdot\text{m}^{-2}$ BSA in PBS and subsequent drying (chapter 6).

3.16.1 Contact angle

The wettability of a surface is an important property of the material, particularly when it is brought in to contact with another medium such as a liquid or bacterium. The contact angle is influenced by the chemical composition and the geometry of the surface (286). Generally, wetting behaviour is determined by the relation of the interfacial energies between the solid substrate and the liquid (Y_{sl}), between the solid and gaseous atmosphere (Y_{sv}), and between the liquid and the atmosphere (Y_{lv}).

The contact angle is described by Young's equation (287, 288):

$$\gamma_{lv} \cos \gamma_{\theta} = \gamma_{sv} - \gamma_{sl} \quad \text{Equation 3-3.}$$

Where,

γ_{θ} = Young's contact angle;

γ_{lv} = liquid-vapour interface tension;

γ_{sv} = solid-vapour interface tension;

γ_{sl} = solid-liquid interface tension.

A low contact angle ($\theta < 90^\circ$), is indicative of a hydrophilic surface where surface wetting is favourable, such that the fluid will spread over a large area on that surface. Higher contact angle values ($\theta > 90^\circ$) indicate more hydrophobic surfaces where wetting of the surface is unfavourable such that the fluid minimises its contact forming a compact liquid droplet (289, 290) (Figure 3-7).

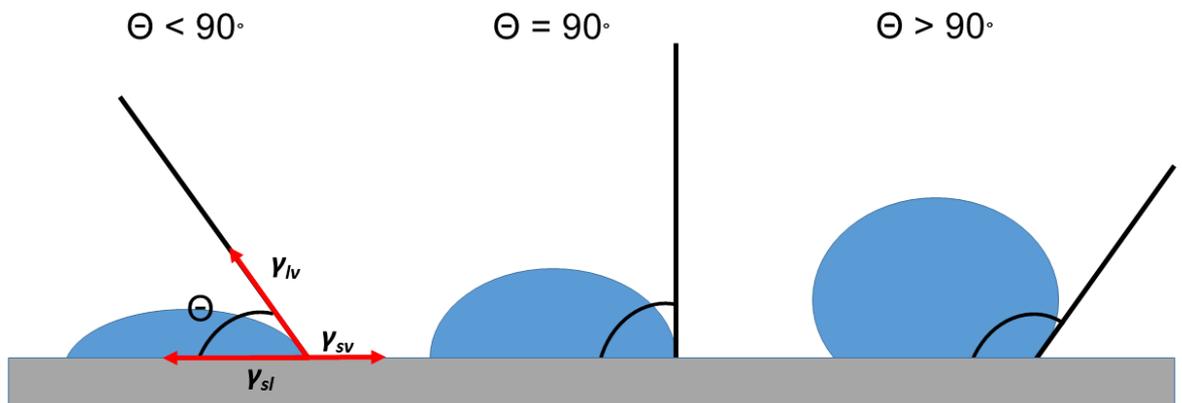


Figure 3-7. Illustration of contact angles formed by sessile drops on smooth, homogenous solid surfaces. θ indicates contact angle, γ_{lv} indicates liquid-vapour interface tension; γ_{sv} indicates solid-vapour interface tension; γ_{sl} indicates solid-liquid interface tension.

In sessile drop contact angle measurements, the angle formed between the liquid/solid interface and the liquid/gas interface is the contact angle (measured via the liquid phase) (291, 292). Direct optical contact angle measurement is the most widely used method of contact angle measurement (293).

3.16.2 Contact angle hysteresis

Surface wetting is not just a static state (290) particularly on a porous medium such as a nonwoven that consists of both solid and void volume. Contact angle hysteresis is the difference between the advancing (maximal) and receding (minimal) contact angles. This can arise due to the roughness and heterogeneity of a surface (294, 295), as Young's equation does not take surface topography into account (296). This "equilibrium" contact angle reflects the relative strengths of the molecular interactions between the solid, liquid, and vapour interfaces (297). Therefore, goniometry was used to assess samples with 20 μ l of distilled water (dH₂O).

3.17 Scanning electron microscopy (SEM)

SEM analysis was employed to explore the surface features of the model healthcare surfaces, the surface features of the fibres in the wipe substrates and the bacteria residing on the fibre surfaces following wiping.

3.17.1 Bacteria residing on fibre surfaces

Scanning electron microscopy (SEM) was employed to image explore surfaces and fabric samples before and after completion of each wiping test (section 3.9). Bacteria were fixed on the samples using a 2 h incubation in 2.5% w/v glutaraldehyde, 0.1 M phosphate buffer; then washed twice in 0.1 M phosphate

buffer for 30 min. Post-fixing was performed in 1% osmium tetroxide in 0.1M phosphate buffer for 12 h. Samples were dehydrated using an ascending acetone series – 20, 40, 60, 80 and 100% w/w for 30 min each.

Samples were critical-point dried using a Polaron E3000 critical point dryer (Quorum Technologies Ltd., East Sussex, UK), with liquid carbon dioxide as the transition fluid. Samples were mounted on 13 mm pin stubs, which were coated with 5 nm platinum using a 208HR high resolution sputter coater (Cressington Scientific Instruments Ltd., Watford, UK). Samples were imaged using a Quanta 200F FEG-ESEM (FEI; Hillsboro, Oregon, US) with an accelerating voltage of 3 kV, a working distance of 11.9 mm and a typical magnification of 20,000x.

3.17.2 Morphological features of model healthcare surfaces and fibres in the nonwoven substrates

Samples were gold coated using a Quorum Q150RS sputter coater Quorum Technologies Ltd.; East Sussex, UK). A JEOL JSM-6610 LV scanning electron microscope (SEM) (JEOL Ltd.; Tokyo, Japan) was then used to image the surface samples, with an accelerating voltage of 5 kV, a working distance of 8 mm and a typical magnification of 750x. Energy-dispersive X-ray spectroscopy (EDX) analysis was carried out using an Oxford Instruments INCA Xmax80 EDS Spectrometer (Oxford Instruments PLC; Abingdon, UK).

3.17.3 Solid surface area fraction at the wipe-surface interface

FIJI image analysis software (298) was used to analyse SEM images of nonwoven wipe samples to calculate the presented 2D fibre area at the wipe-bacteria-surface interface, according to equation 3-4 (images not shown).

During the coating and imaging, the wipes were subject to negligible pressure, so this did not influence the calculated values at the surface.

$$FP_{wsi} = (F_{pixels} / (F_{pixels} + V_{pixels})) \times 100 \quad \text{Equation 3-4}$$

Where FP_{wsi} = solid surface of fibre present at the at the wipe-bacteria-surface interface;

F_{pixels} = pixels in image which represent wipe fibres;

V_{pixels} = pixels in image which represent void space.

$(F_{pixels} + V_{pixels})$ = total pixels in SEM image.

3.18 Statistical analysis

All presented data are the results of at least three independent replicates. A one-way analysis of variance (ANOVA) at the 95% confidence interval with a post hoc Tukey's test was performed or a paired-sample *t*-test was conducted where appropriate. All analyses were completed in MINITAB software, version 16 (Minitab Inc., Pennsylvania, US).

Chapter 4

Role of surface energy and nano-roughness on the bacterial removal efficiency of nonwoven wipes from a solid surface

4.1 Introduction

As discussed in Chapter 1, an important currently employed strategy is to disinfect and decontaminate healthcare surfaces using nonwoven fabric wipes in combination with detergents or biocides (28). Whilst this is widely practised, the underlying interactions governing the removal of bacteria by nonwoven fabrics remains poorly understood (3, 34).

Considerable focus has understandably been placed on the role of the biocide or detergent used in combination with the wipe to decontaminate surfaces (32, 33). However, dry-wiping may also have a role to play in contributing to effective pathogen removal. Rapid removal of bacteria from surfaces (and subsequent disposal of the contaminated fabric) without biocidal usage does not counteract the selection pressure surface colonising bacteria are exposed to, but can reduce total pathogen numbers on a surface (299). This can therefore be important in combatting the spread of HCAs.

Bacterial adhesion to abiotic surfaces is known to be influenced by physicochemical and electrostatic interactions between the cell and surface (300). Hydrophobicity is one of the key factors influencing this interaction (301), and the importance of surface nano-roughness in respect of adhesion has also

been identified as an influential factor (302, 303). Surface wiping (176); and the removal of bacteria from solid surfaces by wipes has been investigated by Williams *et al.* (28) and Ramm *et al.* (189). These studies are notable as they have described reproducible methodologies for assessing wiping efficiency. However, the focus was on the macro-scale removal of bacteria in the presence of detergent or biocide, rather than on the fundamental micro- or nano-scale interactions between the fibres in the wipe, bacteria and contaminated surface. It is of interest to decouple the effects of the detergent or biocide and the wipe fabric itself to understand the role that dry wiping might have in decontaminating solid surfaces.

Accordingly, the aim of this chapter was to determine the role of fibre surface energy and surface roughness in removing bacteria from a model healthcare surface in the dry state, before impregnation with a liquid biocide or detergent. In this way the basic design attributes of the wipe fabric can be explored in the context of bacterial decontamination.

To assess the potential role of surface energy, an inherently hydrophilic regenerated cellulose fibre (lyocell) and an inherently hydrophobic fibre, polypropylene (PP) were selected as raw materials for wipe fabric production. PP is a linear hydrocarbon polymer with the molecular formula C_nH_{2n} . Lyocell is a regenerated cellulosic fibre (244) reclaimed from wood or bamboo pulp, regenerated by use of *N*-methylmorpholine *N*-oxide (246).

Furthermore, samples of the hydrophilic, high surface energy lyocell fabric were also functionalised by plasma exposure to:

- a) Reduce surface energy, and;
- b) Increase surface nano-roughness.

The untreated and treated lyocell fabrics were then evaluated together with fabrics composed of low surface energy, high surface nano-roughness PP fibres. These polymers were also of particular interest because of their relevance to industrial practice, where both cellulose-based and PP-based substrates are commonly encountered.

As is well-known, the hygroscopic nature of cellulosic fibres such as lyocell result in excellent absorbency (248), while the high wet strength of lyocell (247) is a desirable characteristic in a wet wipe. Meanwhile, PP is chemically inert to many solvents, acids and bases (238) and can effectively provide a high wet strength for the surface of a wipe, which may be required to undergo vigorous scrubbing (239).

4.2 Experimental Design

Nonwoven wipe samples of 60 g.m⁻² were prepared from PP or lyocell fibres according to the method described in section 3.1. Plasma treatment of lyocell samples with the objective of modifying their surface energy was performed according to the procedures given in section 3.2.

Analysis of samples was carried out according to the methods given in sections 3.6, 3.14, 3.15, 3.16 and 3.17.1. Bacteria removal from model healthcare surfaces was assessed using the method described in sections 3.7 and 3.9.

PMMA was selected as the model surface for these experiments as it is representative of those commonly found in healthcare environments. They were prepared according to section 3.4.

4.3 Results and discussion

4.3.1 Plasma treatment

Exposing polymer surfaces to plasma is an established method of surface modification (268), and is particularly well-documented as a method of adjusting surface energy, or the free energy per unit area ($\text{mJ}\cdot\text{m}^{-2}$) (304). This is usually carried out to influence physical phenomena such as wetting and adhesion between dissimilar materials. Depending on the type of plasma, the surface energy of a surface can be either increased or decreased.

In the context of wiping efficiency, the adhesion between the bacterium and a typical healthcare surface, e.g. a PMMA surface tile, as well as with the fibres in the wipe, is of interest since the relative forces will influence the nature of the attachment and reattachment behaviour.

By adjusting plasma exposure time (Figure 4-1), lyocell fabric samples were produced with significantly different surface energies ($p>0.01$) ranging from $128.1 \text{ mJ}\cdot\text{m}^{-2}$ for a 20 min O_2 exposure to $17.1 \text{ mJ}\cdot\text{m}^{-2}$ for a 20 min C_2F_6 exposure, the latter value being close to that obtained for untreated PP fabric ($19.2 \text{ mJ}\cdot\text{m}^{-2}$), which is an inherently hydrophobic polymer.

With the lyocell samples treated with C_2F_6 plasma an initial increase in surface energy was observed for exposure times of 30 s - 3 min, followed by a large decrease in surface energy for the 4 and 5 min exposed samples (Figure 4-1). This is a consequence of the increased surface roughness caused by the plasma

exposure (see Figures 4-4; 4-5 and 4-6). Untreated lyocell is wettable, with a water contact angle of over 90° , therefore, an increase in the fibre surface roughness will increase both the wettability and the surface energy of the lyocell (305).

Longer plasma exposure times can be expected to increase the nano-roughness of the fibre surface, as the sample is exposed for greater time in an energetic environment (306). It should be noted that plasma exposure alters both the surface energy and nano-roughness of a sample and therefore it is practically difficult to de-couple the two phenomena.

After a short exposure time (≤ 3 min), it is likely some surface chemical functionalisation occurs via oxidative fluorination, as confirmed by the ToF-SIMS spectra (Figure 4-2), resulting in an increase in surface polarity. However, as gaseous fluorine is strongly electronegative and oxidative (307), with extended C_2F_6 plasma exposure an increase in F^- or CF_x surface functionalisation can be expected to reduce surface energy (254). This is evident from the 4 min onwards treated sample data (Figure 4-1). There is also an increase in the nanoscale fibre surface roughness, associated with the reactive species in the plasma (Figure 4-3 - Figure 4-8). These reactive species, typically F^- , bombard the fibre surface (308), causing initial etching and the associated change in nanoroughness. In the ≤ 3 min samples, this etching the small degree of fibre surface functionalisation cause an increase in nano-roughness. From 4 min onwards, there was a marked increase in functionalisation, causing the decrease in surface energy seen in Figure 4-1.

Note that there was a greater influence of the O_2 plasma exposure on the nanoscale fibre roughness compared with that obtained with C_2F_6 for the same

exposure time (Figure 4-6 and Figure 4-7). The effect is to increase surface energy in the lyocell samples as a result of etching (309). O_2 causes more etching than the C_2F_6 , as there is relatively little deposition during low temperature, low pressure O_2 plasma treatment, and so no balance to be struck between deposition and ablation/etching on the fibre surface during this treatment.

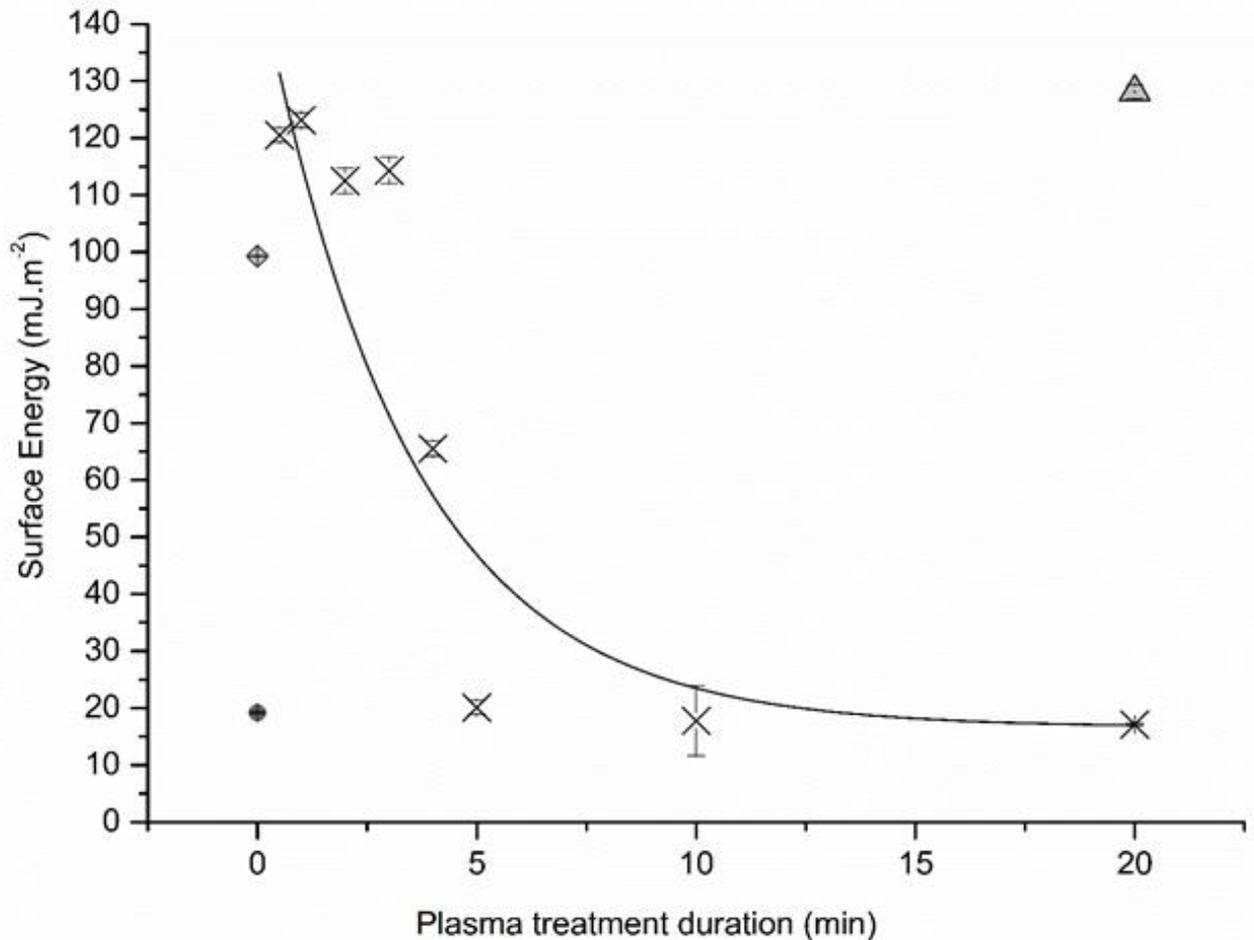


Figure 4-1 Surface energy of nonwoven wipe substrate fabrics vs. C_2F_6 low temperature, low pressure plasma exposure time, measured via the OWRK method (274). \triangle = O_2 treated lyocell; \times = C_2F_6 treated lyocell; \diamond = Untreated lyocell; \bullet = untreated PP. Fit line and data points for C_2F_6 treated lyocell, unless specified otherwise. Data is the mean of five replicates. Error bars = Standard deviation. $n=5$.

Functionalisation of the fibre surfaces conferred by C_2F_6 plasma exposure was explored by means of the semi-quantitative ToF-SIMS technique. Examination of the negative ion spectra of all four C_2F_6 plasma exposed lyocell samples (Figure 4-2) indicates the presence of the C_2H^- peak at $m/z = 25$, which can be used as an internal reference peak for monitoring surface compositional changes.

Examination of the C_2F_6 plasma exposed lyocell samples indicated that the surface F^- and CF_3^- peak intensities increase with plasma exposure time. However, during the low pressure RF plasma exposure, the C_2F_6 gas is fragmented producing F^- and CF_x^- radicals (310). These radicals, in addition to reacting with the cellulosic surface, can also polymerise on the cellulosic fibre surface. The presence of $C_3F_7^-$, $C_4F_9^-$, $C_5F_{11}^-$ and $C_6F_{13}^-$ on the 20 min plasma exposed lyocell ToF-SIMS spectrum confirms that marked plasma-enhanced chemical vapour deposition and polymerisation of CF_3^- have occurred. The C_nF_{2n+1} species are more abundant for lower n values. These results and the resulting increase in hydrophobicity at the fibre surface are consistent with the observed decreases in surface energy between the 1 min, 4 min and 20 min exposure times.

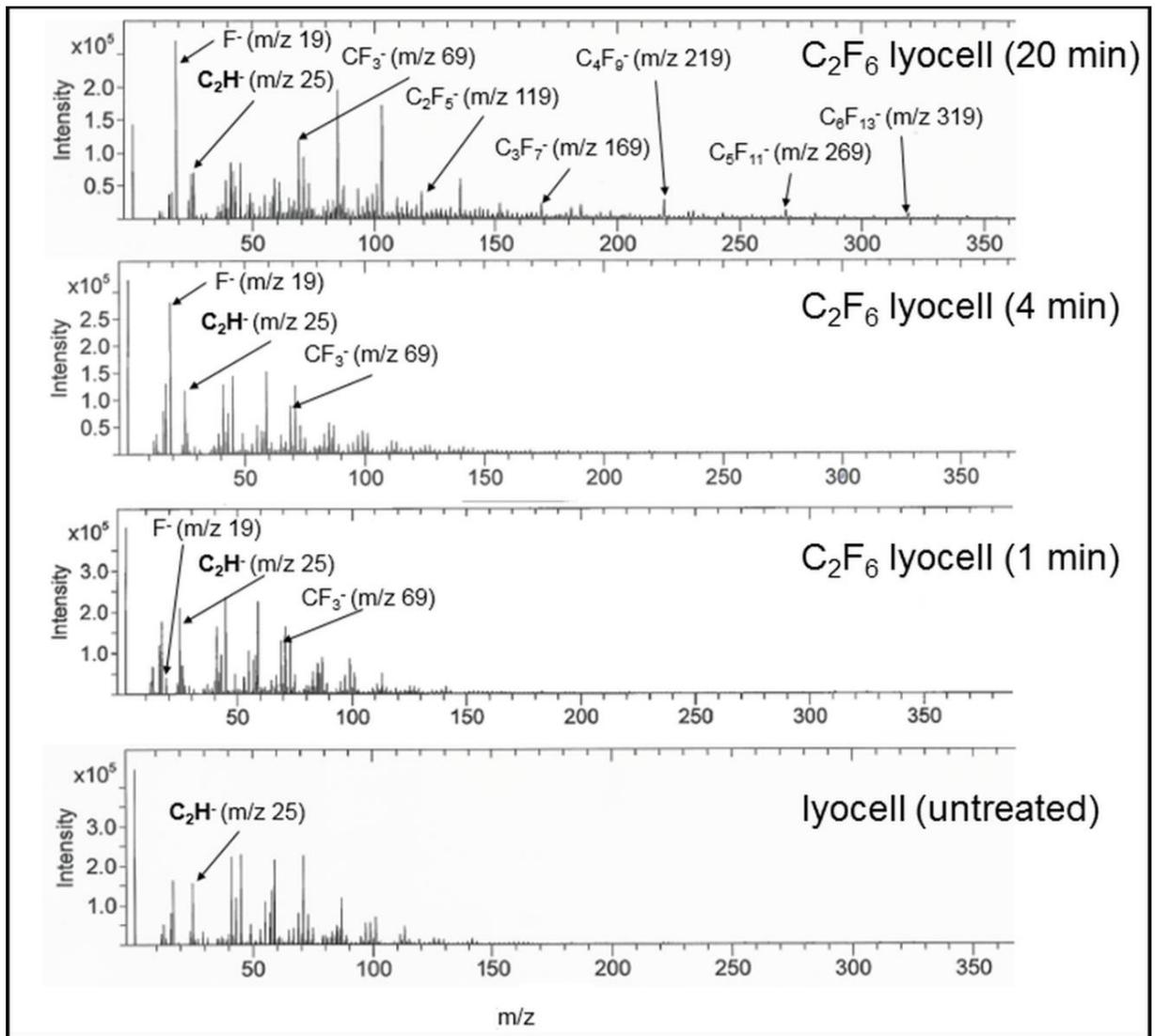


Figure 4-2. Negative ion ToF-SIMS spectra of untreated and C_2F_6 plasma treated lyocell nonwoven fabrics. Mass range $m/z = 0-360$. $n=5$.

The surface roughness of the nonwoven fabrics was measured via atomic force microscopy (AFM). R_a is the arithmetic average of the roughness profile, and was calculated at 3 μm resolution. The untreated lyocell fibre (Figure 4-3) shows a smooth, flat surface with no discernible nano-irregularities (R_a 0.18 nm). However, C_2F_6 plasma treatment of lyocell increased the fibre surface roughness (Figure 4-4, Figure 4-5 and Figure 4-6), progressively with increased plasma exposure time (R_a 1.36 nm for 1 min, 1.60 nm for 4 min exposure time compared to R_a 2.27 nm for 20 min exposure time).

The surface morphology of the 1 min treated lyocell fibre sample (Figure 4-4) was similar to that of the 4 min C_2F_6 treated lyocell fibre sample (Figure 4-5). However, there was less functionalisation on the fibre in the shorter treatment time, surface evidenced by the greater peak intensity for the F^- peak for 4 min sample versus the 1 min sample in Figure 4-2. This is linked to the observed difference in surface energy evident in Figure 4-1.

The lyocell fibre surfaces revealed exposure of the underlying fibrillar structure of the fibre, but after extending the treatment time to 20 min, nodular structures were evident (Figure 4-6). This reflects the progressive modification of the fibre surface structure, starting with the outermost part of the fibre, during plasma exposure.

In low pressure plasma exposure, surface etching occurs as fibres are bombarded with charged ions and electrons, subjecting their surfaces to a physical sputtering effect alongside chemical effects (253). The sputtering can lead to micro- or nano-roughness on the fibre surfaces, eventually exposing the underlying fibre structure by progressively removing surface material as a result of the energy input during the plasma exposure process (311). Low temperature plasma techniques are surface selective in this regard (312).

Etching is more commonly observed with non-polymerising gases such as O_2 than with depositing gases such as C_2F_6 (255), hence the higher surface roughness observed in lyocell fibres treated for 20 min in O_2 compared with the same period in C_2F_6 (Figures 4-7 and 4-6). In the former, many irregularities with nodular structures, and substantial roughness (R_a 3.81 nm) were observed compared to the unexposed control (R_a 0.18 nm). This has been observed by Kale and Desai and is attributed to plasma etching (313). In contrast the 1 min, 4 min and 20 min C_2F_6 exposed lyocell samples exhibited fibre surfaces resembling tree bark, with micro-fissures being evident, whereas the O_2 plasma exposed lyocell sample exhibited a more granular morphology. The untreated PP fibres (Figure 4-8) exhibited randomly distributed irregularities, as a result of the melt spinning fibre manufacturing process (314), with a R_a value of 2.64 nm.

Plasma exposure therefore results in modification of fibre surface morphology via physical etching, introducing nano-roughness, as well as chemical modification. Both can modify surface wetting and therefore, adhesion behaviour. Initially, the surface roughness increased as a direct result of fibre etching in the RF plasma; evidenced by the increase in surface energy from the 30 s to 3 min exposure time (Figure 4-1).

The chemical functionalisation necessary to reduce surface energy (with CF_x -) does not occur quickly and the polymerisation rate is dependent on the chemical activity of the plasma (315). This functionalisation takes roughly 4 minutes to occur to a sufficient degree to reduce the surface energy, as evidenced by Figure 4-1 and Figure 4-2.

As expected, the lyocell fibre surfaces subjected to the longest C_2F_6 exposure time exhibited the highest degree of functionalisation and the lowest surface

energy. The differences in fibre surface morphology (i.e. increase in surface nano-roughness after the same treatment time) between the C₂F₆ and O₂ exposed surfaces after 20 min exposures are due to the gasses used with the C₂F₆ plasma providing a polymerisable deposition while the O₂ plasma is a non-depositing surface modification. Longer plasma exposure times also increased the nano-roughness of the fibre surface (306).

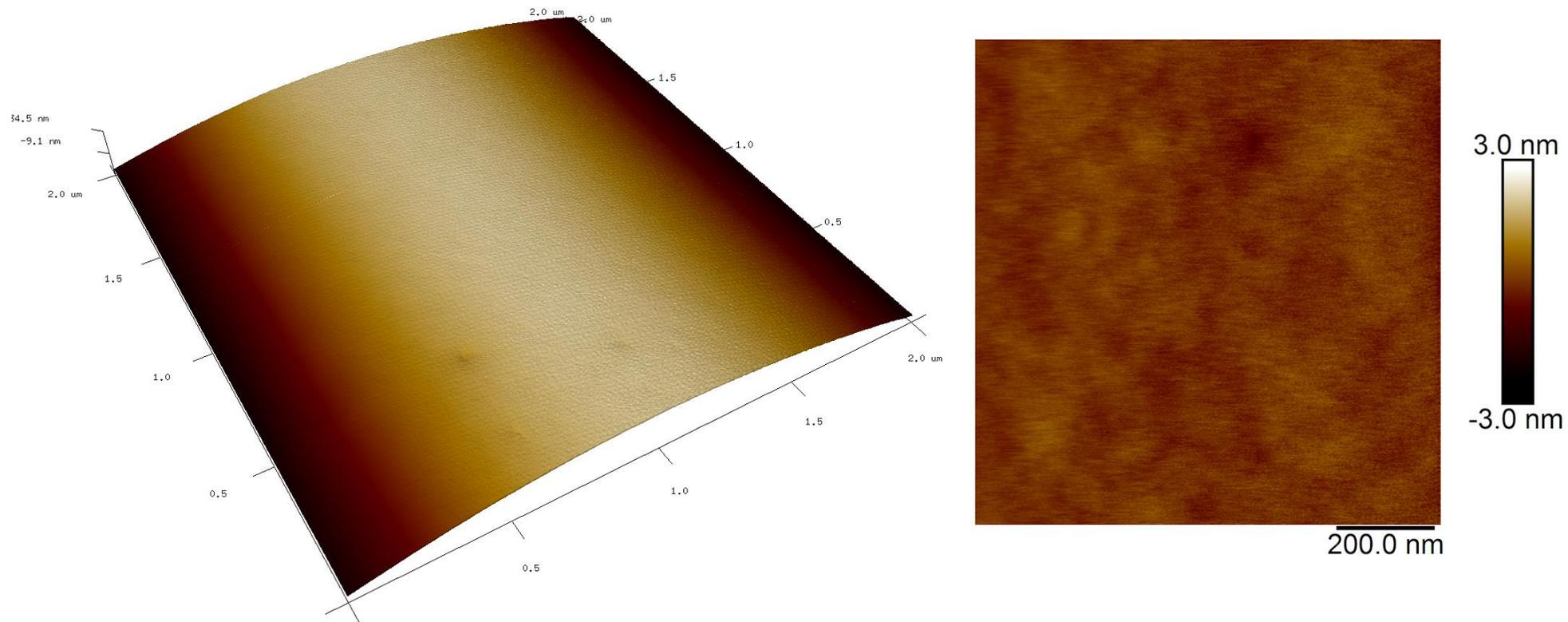


Figure 4-3. AFM micrograph of untreated fibre surface - Untreated lyocell fibre surface, R_a 0.18 nm

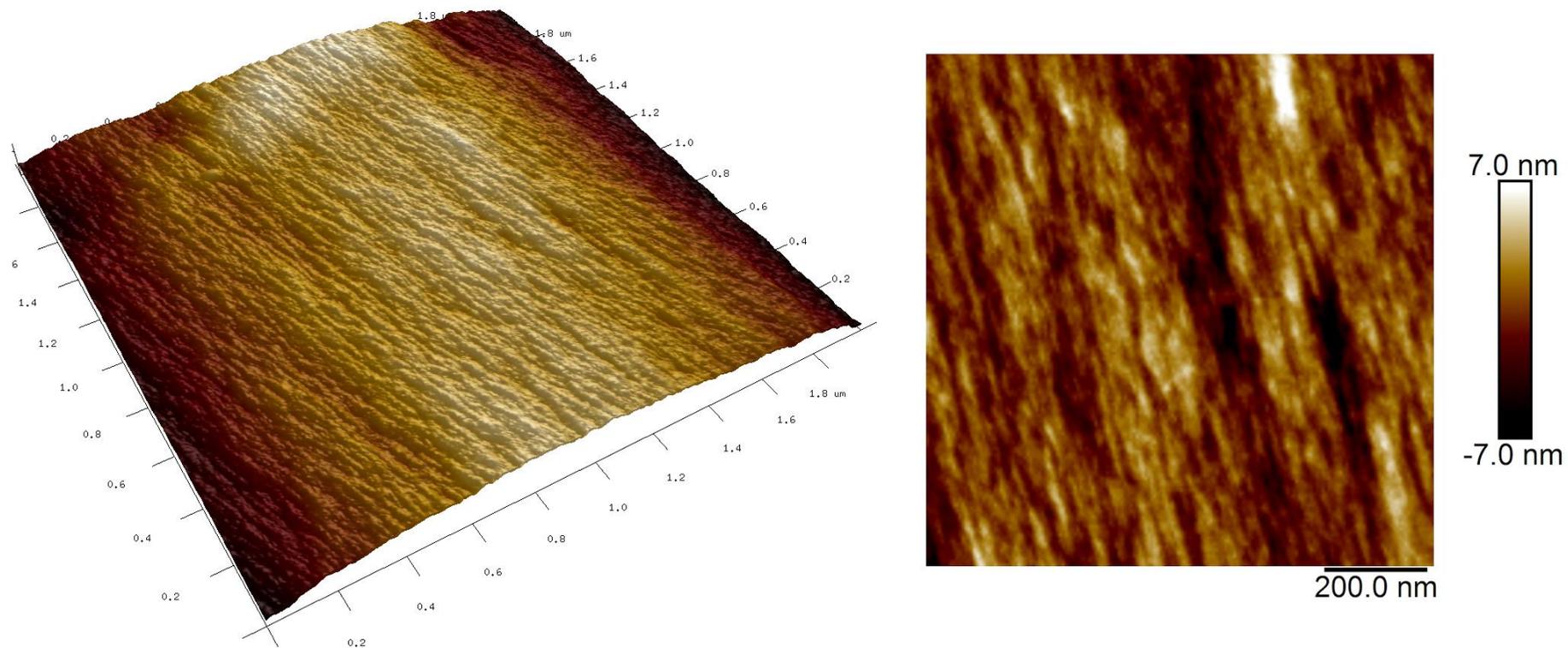


Figure 4-4. AFM micrograph of plasma treated fibre surface - C_2F_6 plasma-treated lyocell fibre surface after 1 minute exposure, R_a 1.36 nm

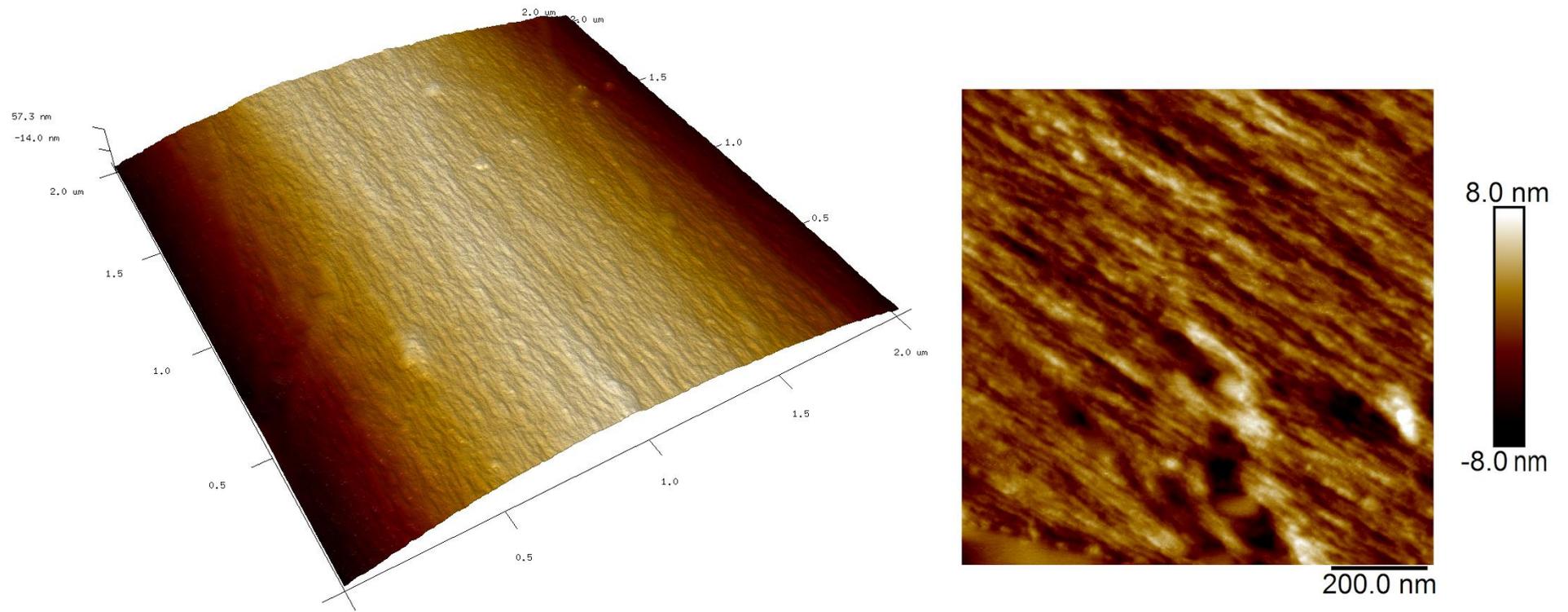


Figure 4-5. AFM micrograph of plasma treated fibre surface - C_2F_6 plasma-treated lyocell fibre surface after 4 minute exposure, R_a 1.6 nm

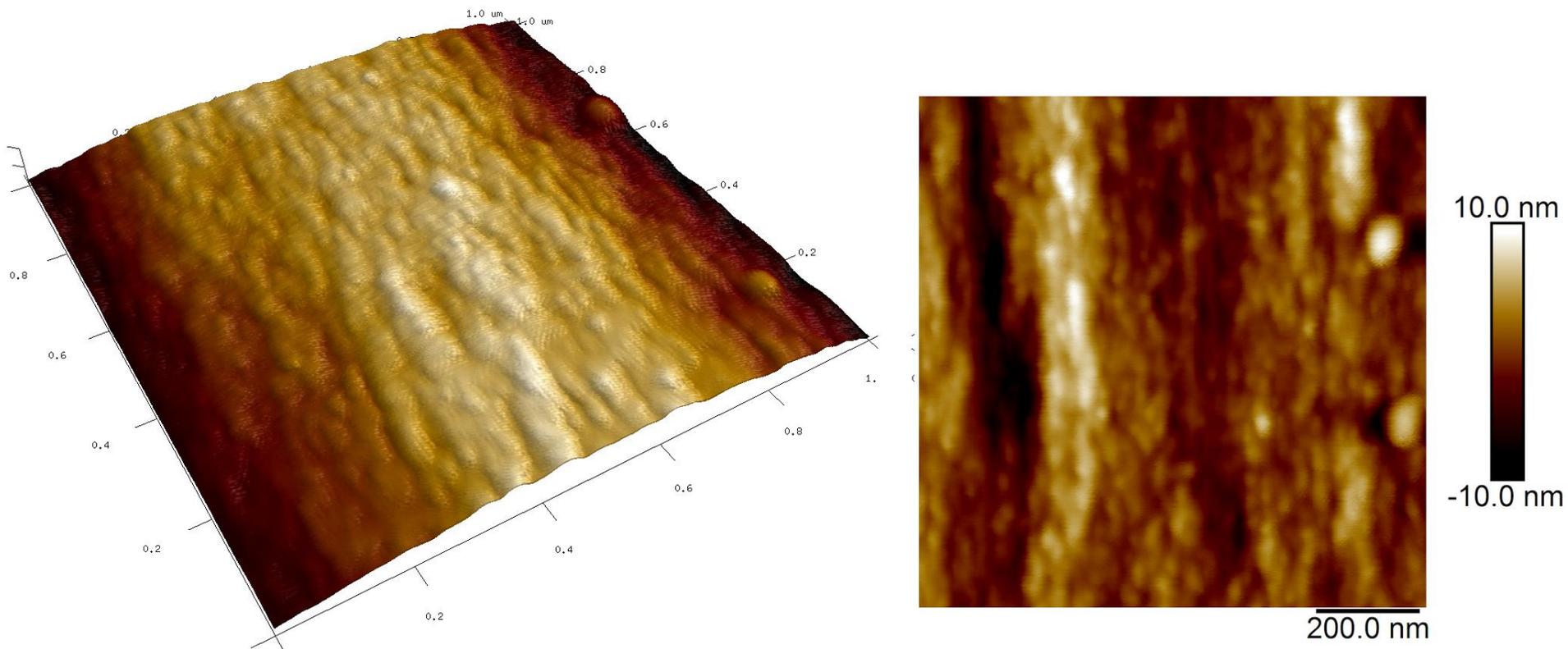


Figure 4-6. AFM micrograph of plasma treated fibre surface - C_2F_6 plasma-treated lyocell fibre surface after 20 minute exposure, R_a 2.27 nm

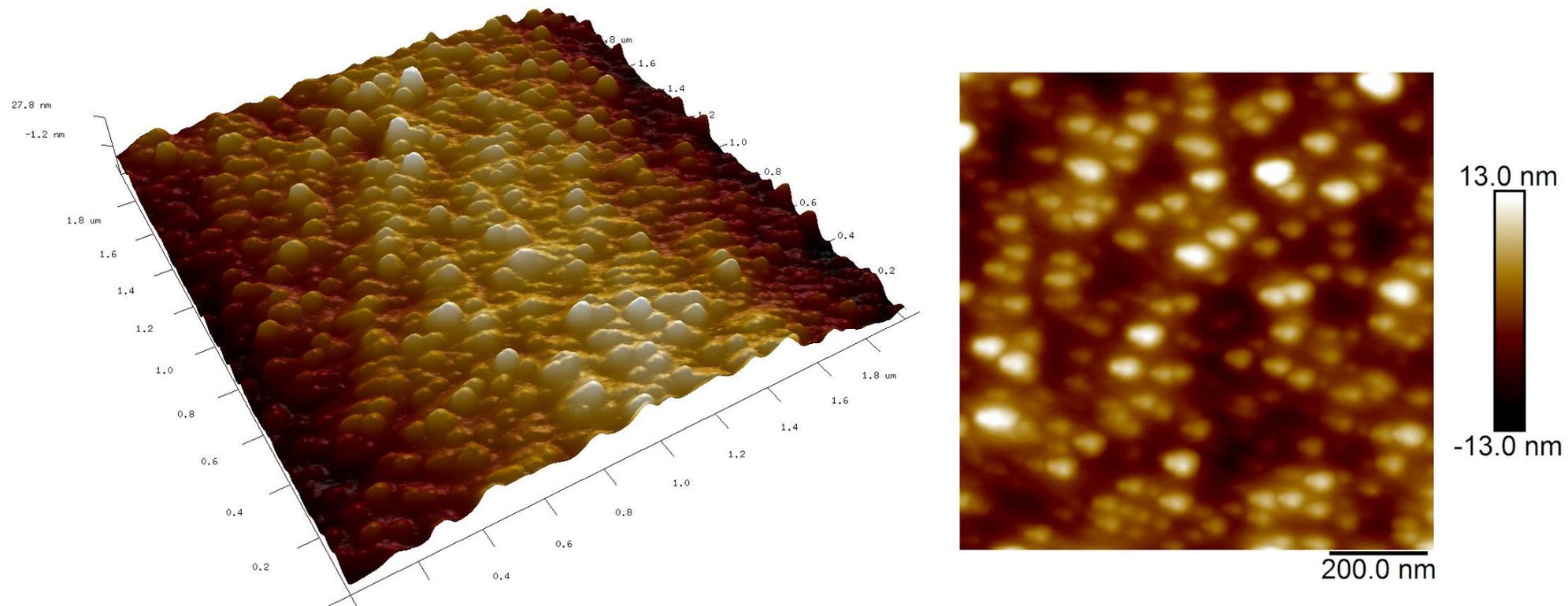


Figure 4-7. AFM micrograph of plasma treated fibre surface – O₂ plasma-treated lyocell fibre surface after 20 minute exposure, R_a 3.81 nm

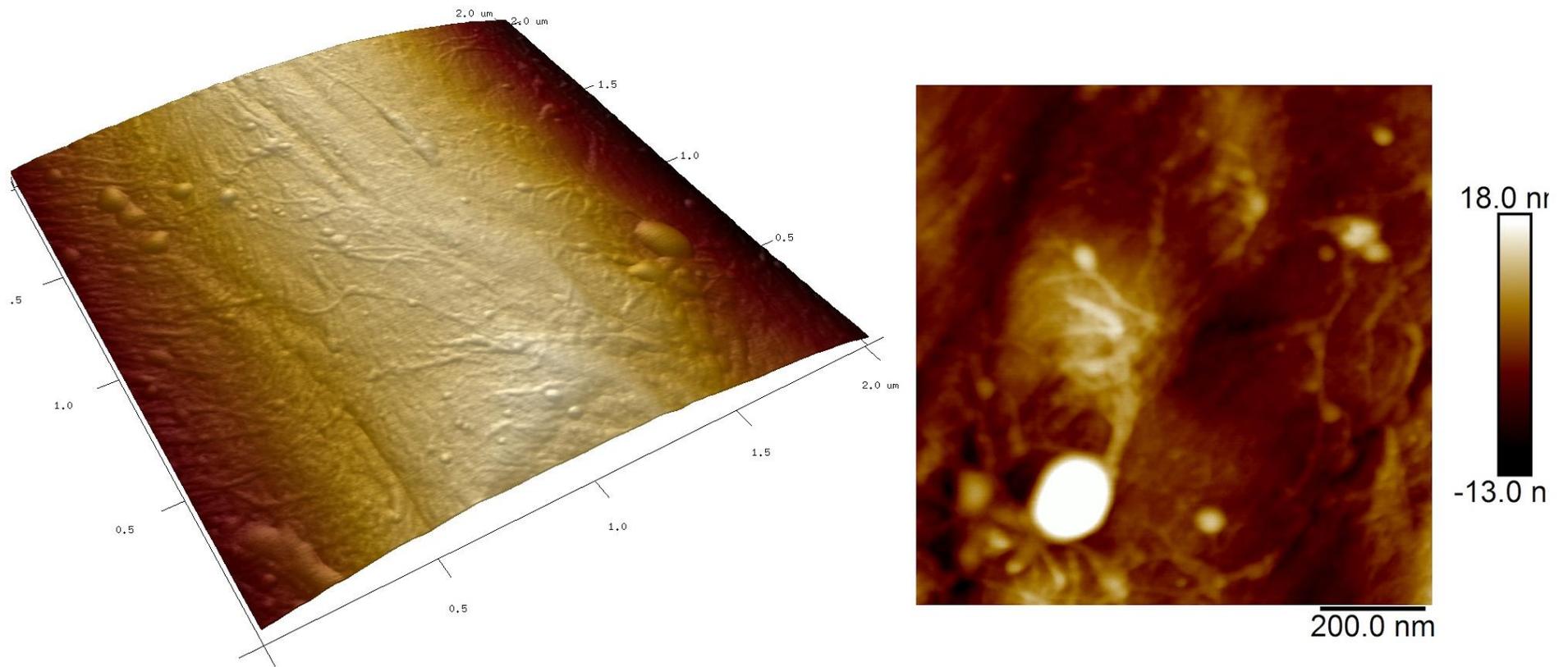


Figure 4-8. AFM micrograph of untreated fibre surface - Untreated PP fibre surface, R_a 2.64 nm

4.3.2 Wetting behaviour of the wiping surface

The water contact angle on the wiping surface can be expected to change according to the bacterial contamination situated upon it because of change in chemical and physical properties. Any such changes were measured using contact angle goniometry before and after the surface was contaminated with the simulated organic loads the preparation of which is described in section 3.16. An increase in the organic load increased the water contact angle and decreased the wetting tension (Table 4-1).

Light and heavy organic loads have been previously simulated in wiping studies using 0.015 g.m⁻² BSA and 0.15 g.m⁻² BSA respectively (28, 32). In this study, light organic load conditions were adopted, as they more accurately represent the conditions seen in the hospital environment.

Table 4.3 1 indicates an increase in the contact angle and a decrease in the wetting tension as the level of organic load increased.

Table 4-1. Mean contact angles and wetting tensions of PMMA wiping surfaces according to organic load, as measured by goniometry (section 3.16). *n*=3.

Organic load	Water contact angle	Wetting tension
Unsoiled surface (Alcohol sterilised)	29.2°	63.5 mJ.m ⁻²
Low organic load (0.015 g.m ⁻² BSA)	62.3°	33.8 mJ.m ⁻²
High organic load (0.15 g.m ⁻² BSA)	81.4°	10.9 mJ.m ⁻²

While this was expected given the chemical nature of BSA (protein), the salts in the PBS will also deposit on the surface, leading to an increase in surface

roughness [300], increasing the contact angle and decreasing the wetting tension of the PMMA surface.

4.3.3 Bacterial removal from solid surfaces

Wiping experiments using nonwoven substrates manufactured in house, according to the methods in section 3.1, using industrially applicable nonwoven manufacturing processes, were undertaken to determine the influence of nano-roughness and fibre surface energy on the bacterial removal using the “low organic load” conditions outlined in Table 4-1.

4.3.3.1 Antibacterial activity

The agar diffusion plate test can be used to determine the effect of antibacterial agents applied to textiles (316), as described in section 3.10. In this instance it was used to assess whether the plasma functionalisation of the fibres in the nonwoven substrates led to a biocidal effect.

The PP, untreated lyocell, 20 min C₂F₆ treated lyocell and 20 min O₂ treated lyocell fabric samples all demonstrated “insufficient” antibacterial effects against *E. coli*, *S. aureus* and *E. faecalis* according to the guidelines provided in the ISO 20645 method (282) . Therefore, none of the fabrics were found to be inherently biocidal before or after plasma exposure, based on this assay and relatively short timescale for contact with the bacteria on the contaminated surfaces.

4.3.4 Streaming potential of nonwoven fabric substrates and zeta potential of bacteria

The measured streaming potential of the nonwoven wipe samples assessed using the method described in section 3.15 were all slightly negative (Table 4-2), though not significantly different from each other (ANOVA, post hoc Tukey's test $p < 0.05$). Values from 0 to ± 5 mV suggest rapid coagulation or flocculation, i.e. the nonwoven material is not stable in the KCl solution. This is expected as the nonwoven material is not dispersed in the KCl solution.

Table 4-2. Mean streaming potential of nonwovens. S.D. indicates standard deviation. $n=4$.

Nonwoven sample	Streaming potential (mV)	S.D.
lyocell	-1.51	0.53
PP	-2.52	2.3
C ₂ F ₆ treated lyocell	-2.76	0.93
O ₂ treated lyocell	-1.43	1.47

Charge is a factor known to influence bacterial adhesion. Bacterial cells have a net negative charge, with Gram-negative being more negatively charged than Gram-positive (317). Gram-negative bacteria have an outer membrane that contains phospholipids and lipopolysaccharides, imparting a strong negative charge, while Gram-positive bacteria have phosphate-containing teichoic acids linked to either the peptidoglycan layer or to the underlying plasma membrane. The zeta potential of the bacteria were all negative (Table 4-3), however there was no significant difference between those of *E. coli*, *S. aureus* or *E. faecalis* (ANOVA, post hoc Tukey's test $p < 0.05$).

Table 4-3. Mean zeta potential of bacteria. S.D. indicates standard deviation. $n=3$.

Bacterium	Zeta potential (mV)	S.D.
<i>E. coli</i>	-13.6	0.5
<i>S. aureus</i>	-13.45	0.15
<i>E. faecalis</i>	-11.69	0.36

As there were no significant differences found between the nonwoven substrates or the bacteria, changes in zeta potential or streaming potential could be discounted when analysing bacterial removal modulated by different wipe conditions.

4.3.5 Removal of bacteria via adpression

To decouple the influence of the physical wiping mechanism, which involves complex forces and shear from the direct interaction between the contaminated surface and the fibres in the wipe, a static adpression experiment was performed using the method detailed in section 3.9. To clarify, all further C+F₆ experimental results were carried out using the 20 min C₂F₆ treated lyocell samples.

All bacterial removal values regardless of the type of nonwoven substrate were relatively low with none exceeding 16 CFU %. There was no significant difference between the four wipes in terms *E. coli* (Figure 4-9A), *S. aureus* (Figure 4-9B) or *E. faecalis* (Figure 4-9C) removal efficiency. However, the removal efficiencies for *E. coli* were generally higher than those for *S. aureus* and *E. faecalis* ($p > 0.01$). This may be due to the *E. coli* surface appendages that were observed during dynamic wiping, Figure 4-10 A-D, which allow the bacterium to overcome unfavourable surface topographies and to adhere better to fibres under adpression conditions than either *S. aureus* or *E. faecalis*.

Adpression involves physical contact between the face side of the wipe with the bacterial contamination residing on the contaminated surface, however, in the presence of applied pressure alone, the force of adhesion between the fibres and bacteria are insufficient to overcome the resisting forces within the bacterial contamination itself or the adhesion with the model wipe surface. Consequently, more substantial bacterial transfer to the fibres in the wipe surface is inhibited.

4.3.6 Dynamic wiping

Bacteria will adhere more preferably to a surface which is of a surface energy closest to their own, i.e. hydrophobic bacteria will adhere better to hydrophobic surfaces and hydrophilic bacteria to more hydrophilic surfaces (301). Therefore, it may be hypothesised that a reduction in surface energy of a lyocell fibre to a value closer to that of a surface soiled with a mixture of bacteria and proteins (the simulated organic load) will increase the bacterial removal efficiency of the lyocell fabric during wiping.

To explore this hypothesis, it was anticipated the 20 min C₂F₆ treated lyocell and PP samples would have surface energies closest to the wetting tension value of the soiled PMMA surface used in the wiping experiments. Additionally, it was anticipated the control and O₂ functionalised lyocell would have much greater surface energies than the wetting tension of the PMMA surface, so could be considered less favourable surfaces for bacterial adhesion. Dynamic wiping experiments were conducted according to the method detailed in Chapter 3 section 3.9.

The dynamic wiping data revealed markedly higher removal efficiencies than were achieved under adpression conditions, highlighting the importance of

mechanical forces acting during a wipe cycle. The PP and 20 min C₂F₆ exposed lyocell samples did remove significantly more *E. coli* (Figure 4-9D) than the other substrates, while there was no significant difference in removal between the different substrates for either *S. aureus* (Figure 4-9E) or *E. faecalis* (Figure 4-9F) (all ANOVA, post hoc Tukey's test, $p < 0.05$).

As expected the O₂ treated lyocell fabrics exhibited the lowest removal efficiency for all bacteria, due to surface energy and surface roughness values unfavourable for bacterial adhesion. The hydrophobic PP and the 20 min C₂F₆ exposed lyocell wipes exhibited significantly greater removal efficiency of *E. coli* (ANOVA, post hoc Tukey's test $p < 0.01$).

Additionally, plasma-treated lyocell samples displayed significantly higher bacteria collection in light of their increased hydrophobicity (reduced surface energy) and consequent enhanced interaction with the hydrophobic cell wall of *E. coli*.

These findings can be considered in light of the fact that the bacteria present in the simulated soil are in an environment that favours adhesion to surfaces of a similar surface energy. Essentially, the relative force of adhesion between bacteria and the contaminated surface on which they reside influences the force that is required to facilitate removal and adhesion to the fibres in the wipe during wiping (182).

In contrast to adpressure conditions, in dynamic wiping shear as well as compressive forces are applied, which will assist transfer to the fibre surfaces, overcoming the adhesive forces between bacteria and surface (182). This removal threshold is less likely to be reached during the adpressure experiments, as evidenced by the lower bacterial removal values.

Given their “favourable” surface energies, bacterial adhesion to the PP and 20 min C₂F₆ lyocell fabrics, was expected to be greater than to the O₂ treated lyocell or unexposed lyocell substrates. However, both the PP and 20 min C₂F₆ lyocell wipe fibres in addition to lower surface energy also had increased surface nano-roughness compared with the unexposed lyocell. This additional factor is important, as the thinner and more fluid outer membrane of Gram-negative bacteria such as *E. coli* will adapt and adhere better to the wipe’s increased fibre nano-roughness. This allows greater contact between the surface of the bacteria and the wipe, leading to an improved removal of the bacterial cells from the abiotic surface. This is shown by the observed increased *E. coli* removal efficiency values for the PP and 20 min C₂F₆ lyocell wipes.

In contrast, owing to their thicker peptidoglycan outer membranes and reduced conformation to the roughened fibre surfaces, no significant difference was observed between the removal efficiencies of the two Gram-positive bacteria (*S. aureus* and *E. faecalis*) under dynamic wiping conditions. The relatively low bacterial removal efficiency of the unexposed lyocell and the O₂ exposed lyocell substrate during the dynamic wiping experiments can also be attributed to their unfavourable high surface energy values relative to the wiping surface (Figure 4-1).

Although both surface energy and nano-roughness change with plasma treatment, the Gram-negative *E. coli* can better adapt to the unfavourable surface roughness than the Gram-positive *S. aureus* and *E. faecalis*. Therefore, for the favourable surface energy/unfavourable surface roughness (for bacterial adhesion and removal) PP and C₂F₆ treated lyocell nonwovens, more *E. coli* is removed than *S. aureus* or *E. faecalis*. The untreated lyocell has unfavourable

surface energy, while the O₂ treated lyocell nonwoven has both unfavourable surface energy and unfavourable surface roughness.

Substantial differences were observed between static (adpression) and dynamic wiping (Table 4-4). Based on a paired-sample *t*-test at the 95% confidence interval, dynamic wiping removed significantly more bacteria than static wiping irrespective of fabric treatment and the resulting surface energy. This is to be expected, as greater energy input is associated with dynamic wiping (169), and the applied forces involve shear as well as compression. The results confirm that dry wiping without the use of biocidal liquid or detergent can still remove bacteria from contaminated surfaces, supporting the conclusions of Wren *et al.* (318) and Koh *et al.* (176).

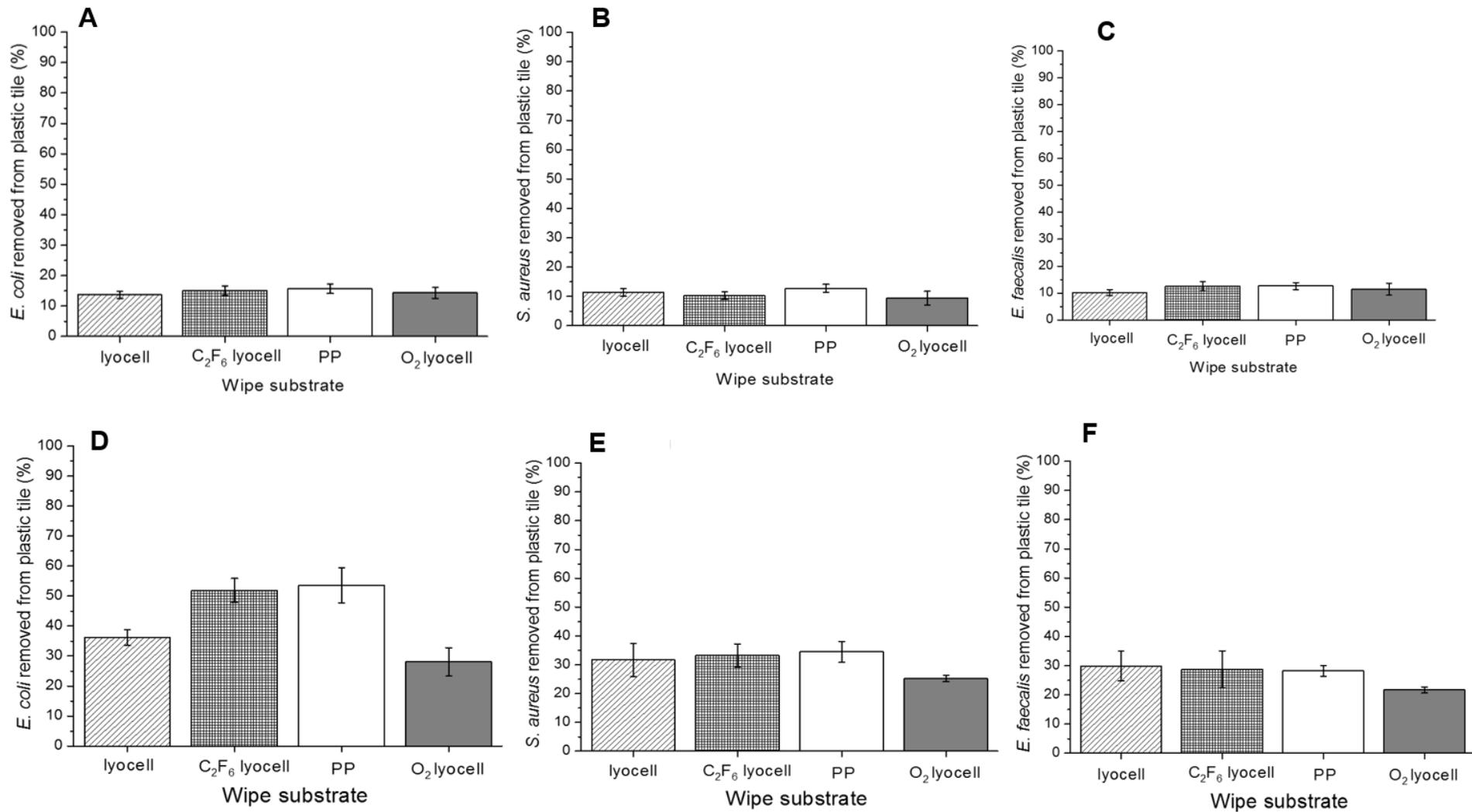


Figure 4-9. Bacterial removal by wiping. (A) Mean *E. coli* removal by adpression method; (B) Mean *S. aureus* removal by adpression method; (C) Mean *E. faecalis* removal by adpression method. (D) Mean *E. coli* removal by dynamic wiping; (E) Mean *S. aureus* removal by dynamic wiping; (F) Mean *E. faecalis* removal by dynamic wiping. Error bars represent standard error of the mean (SEm). $n=9$

Table 4-4. Comparison of static wiping vs. dynamic wiping via paired-sample *t* test.

	Wipe Sample	Significant difference	<i>p</i> value
<i>E. coli</i>	lyocell	+++	<0.001
	C ₂ F ₆ lyocell (20 min)	+++	<0.001
	O ₂ lyocell (20 min)	+	0.002
	PP	+++	<0.001
<i>S. aureus</i>	lyocell	+	0.008
	C ₂ F ₆ lyocell (20 min)	++	0.005
	O ₂ lyocell (20 min)	+++	<0.001
	PP	+++	<0.001
<i>E. faecalis</i>	lyocell	+	0.025
	C ₂ F ₆ lyocell (20 min)	+	0.009
	O ₂ lyocell (20 min)	+++	<0.001
	PP	+	0.001

The observed changes in surface energy and nano-roughness in lyocell fibres resulting from plasma exposure can be expected to influence bacterial adhesion (303, 319).

In Gram-positive bacteria, surface roughness has the ability to limit the number of anchoring points, reducing the surface area in contact with the membrane, which impairs adhesion. In contrast, the outer membrane in Gram-negative bacteria is both more fluid and thinner than the outer peptidoglycan layer of Gram-positive bacteria (317).

Previous adhesion studies indicate that increasing the nano-roughness of a steel surface can significantly decrease the attachment of *S. aureus*, but has no effect

on the adhesion of *E. coli* (302). Nano-patterning on Cicada wings has been found to be biocidal to Gram-negative bacteria, but not to Gram-positive bacteria adsorbed onto their surfaces, because of differences in cell membrane thickness (320). One important distinction is that in these previous studies, data was obtained over 24 h and involved no dynamic wiping mechanism. By contrast in the present work, the contact time was only 10 s, with dynamic wiping at a 60 r.min⁻¹ rotation speed. This may explain why there was no evidence of nano-roughness induced biocidal activity, with differences between samples being confined to the bacterial removal efficiency.

The bacterial cell suspension solution was adjusted to the McFarland standard 0.5 - equivalent to an approximate cell density of 1x10⁸ CFU·mL⁻¹ (281), indicating there would be 2x10⁶ cells in the 20 µl of suspension inoculated onto the PMMA tile. This is likely to be artificially high (321), though necessary for bacteria quantification. These findings are of significance to HCAI research as the present work demonstrates the *in vitro* impact of physical wiping alone where at present there is little reported information.

4.4 Interaction of bacteria with wipe fibre surfaces under dynamic wiping conditions

It is evident in Figure 4-10 that the bacteria are collected mainly on fibre surfaces, rather than between the fibres themselves. Agglomerations of bacteria were randomly distributed along the fibre surfaces, and regions in which a single bacterium was present were also in evidence.

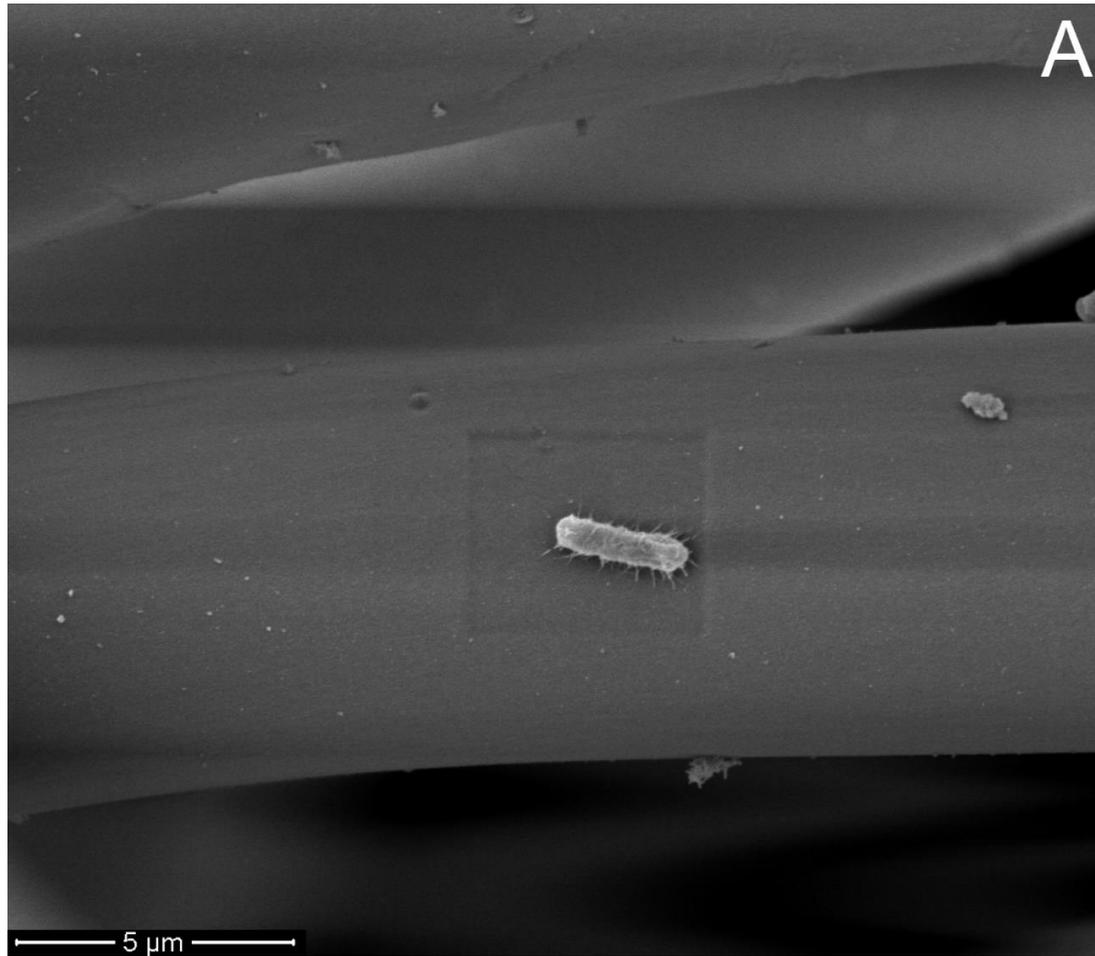


Figure 4.10 (A). SEM micrograph of *E. coli* on untreated lyocell fibres within the nonwoven fabric sample after dynamic wiping.

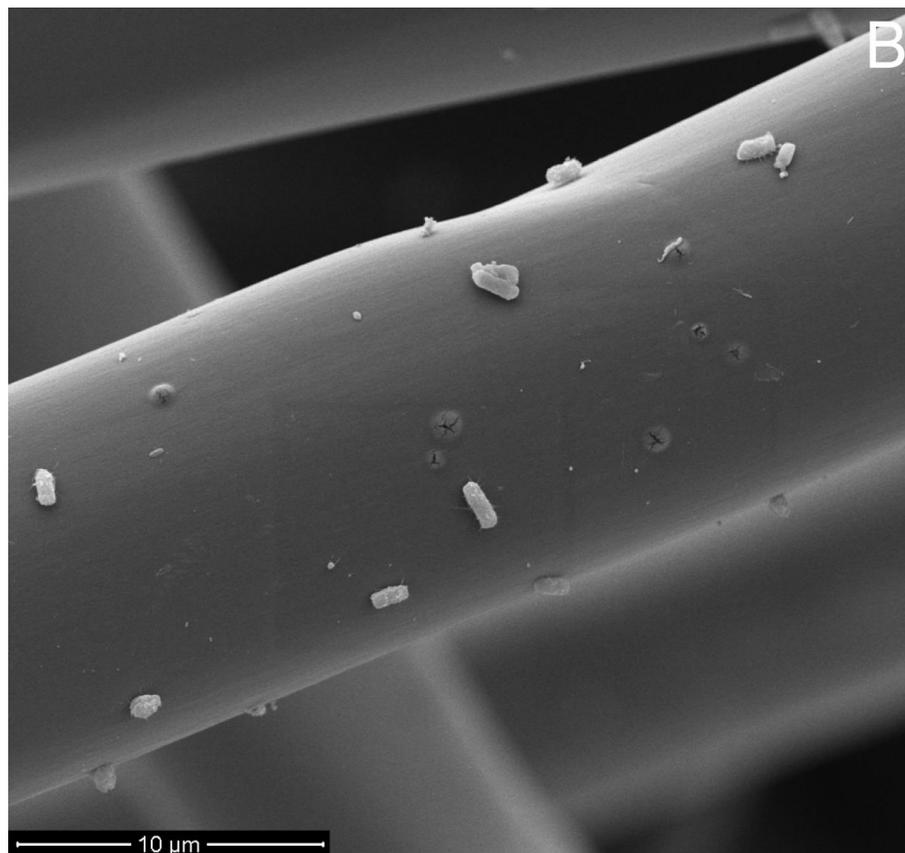


Figure 4.10. (B). SEM micrograph of *E. coli* on 20 min C_2F_6 exposed lyocell fibres within the nonwoven fabric sample after dynamic wiping.

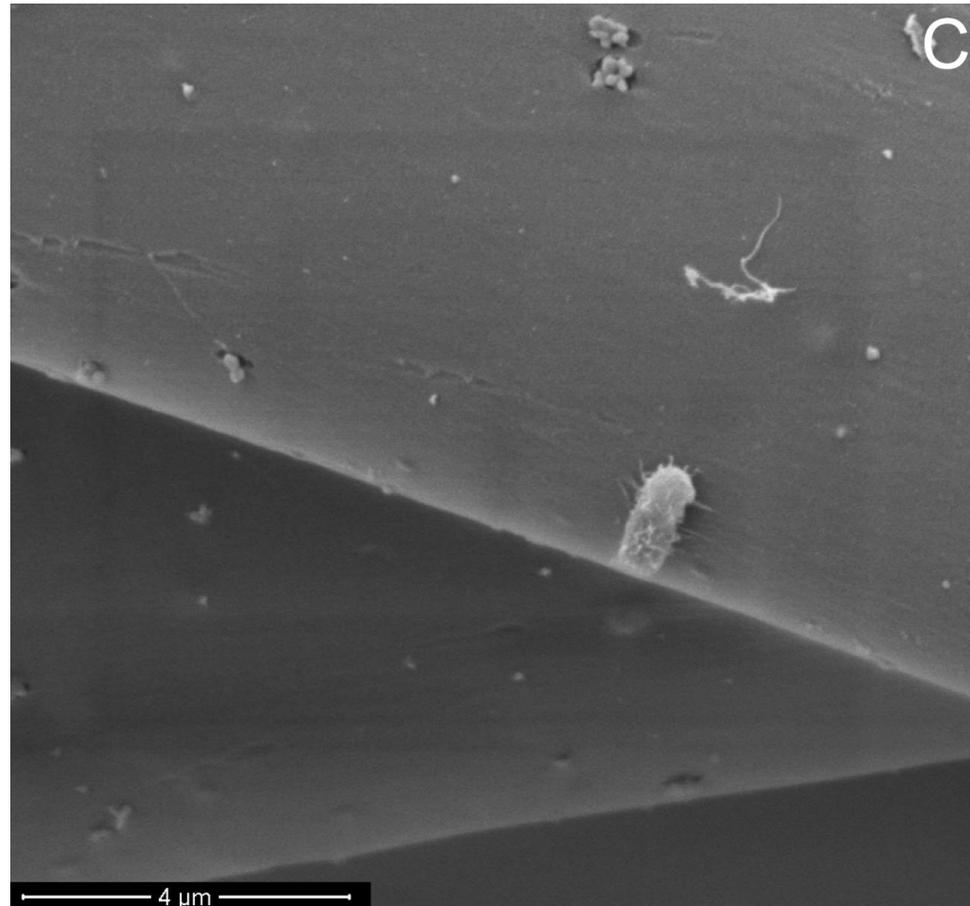


Figure 4.10. (C). SEM micrograph of *E. coli* on 20 min O₂ exposed lyocell fibres within the nonwoven fabric sample after dynamic wiping.

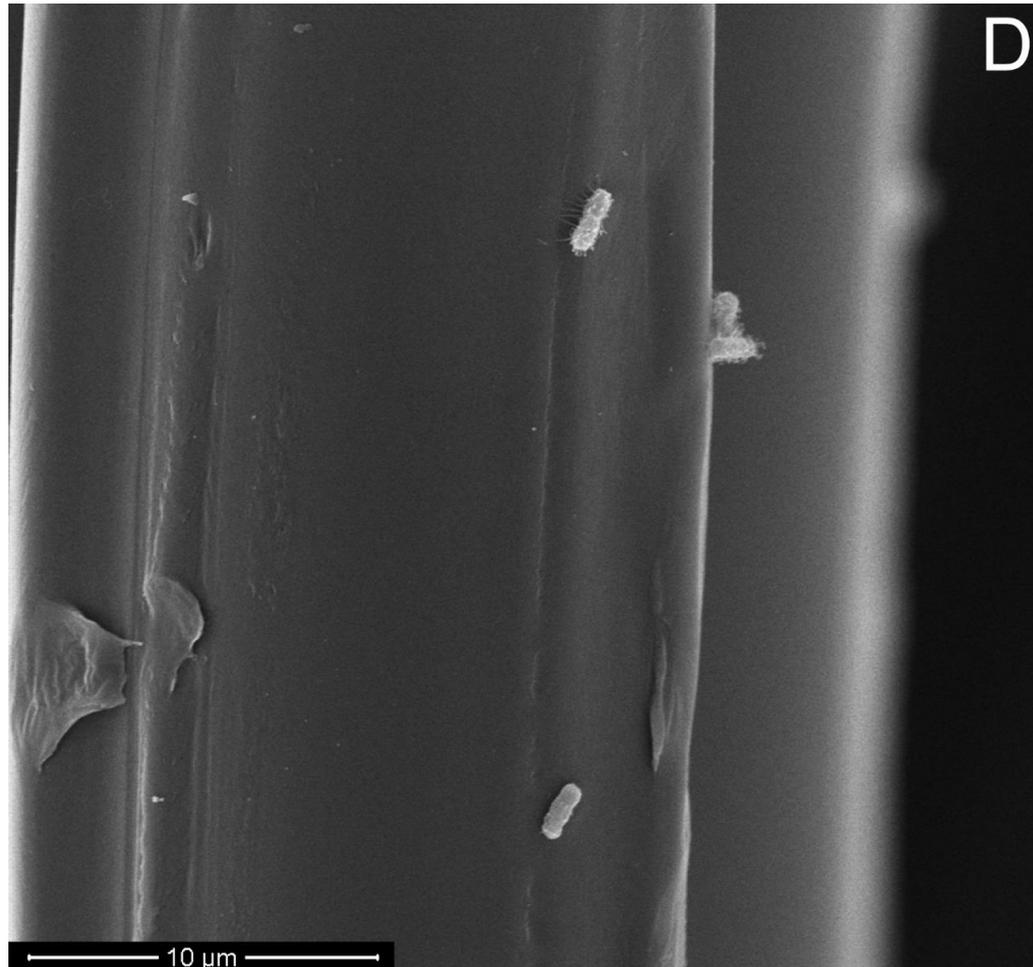


Figure 4.10. (D). SEM micrograph of *E. coli* on untreated PP fibres within the nonwoven fabric sample after dynamic wiping.

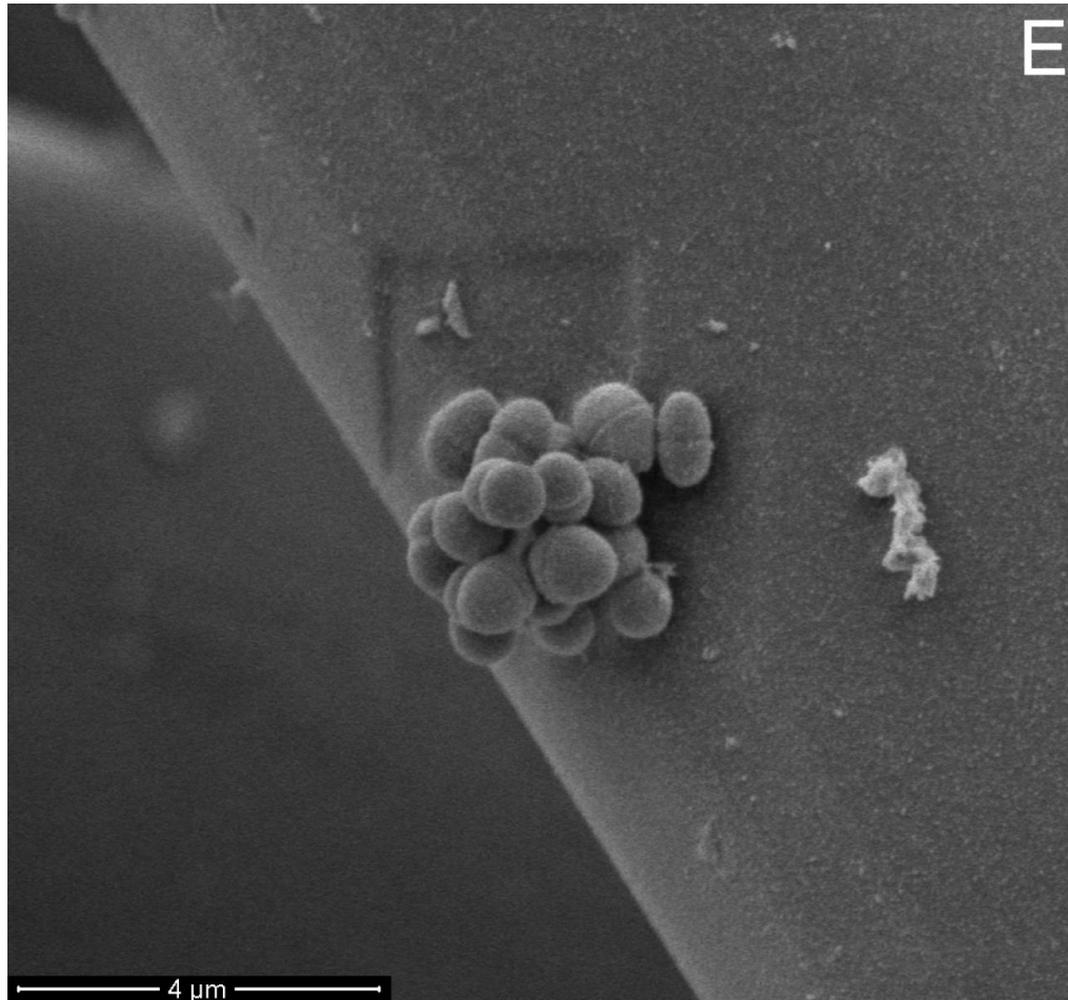


Figure 4.10. (E). SEM micrograph of *S. aureus* on untreated lyocell fibres within the nonwoven fabric sample after dynamic wiping.

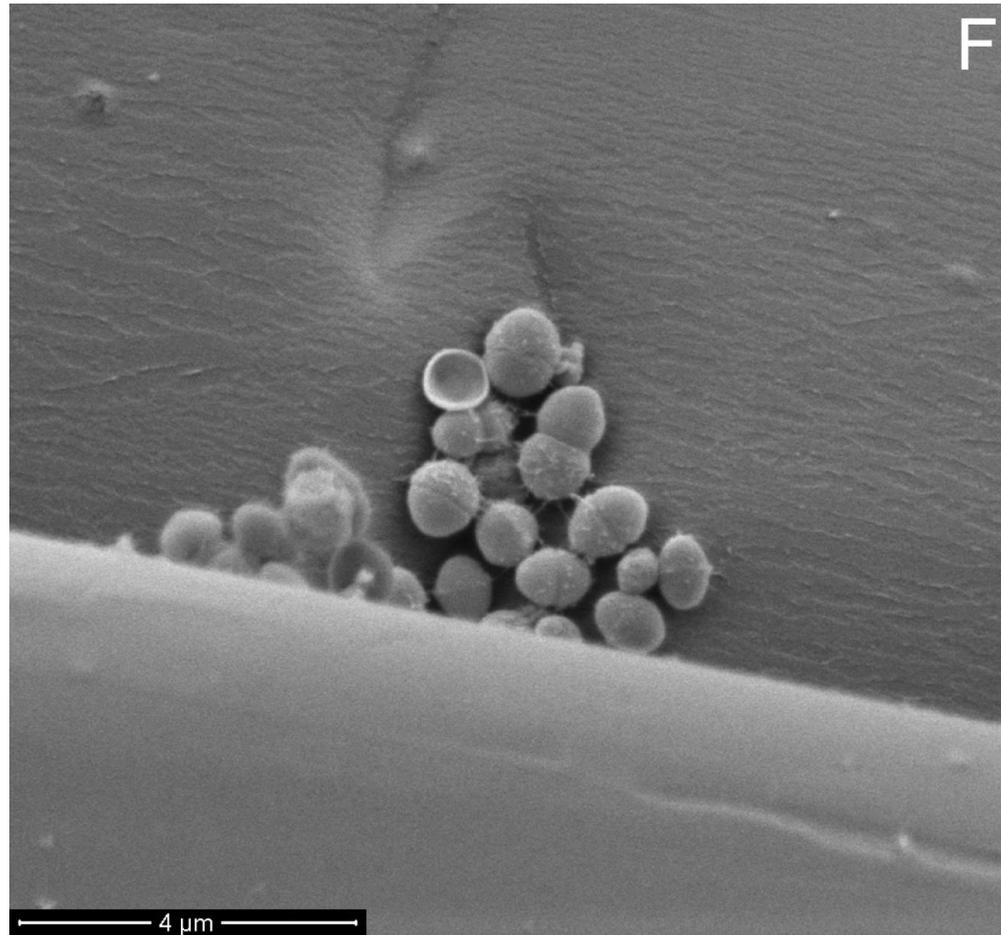


Figure 4.10. (F). SEM micrograph of *S. aureus* on 20 min C_2F_6 exposed lyocell fibres within the nonwoven fabric sample after dynamic wiping.

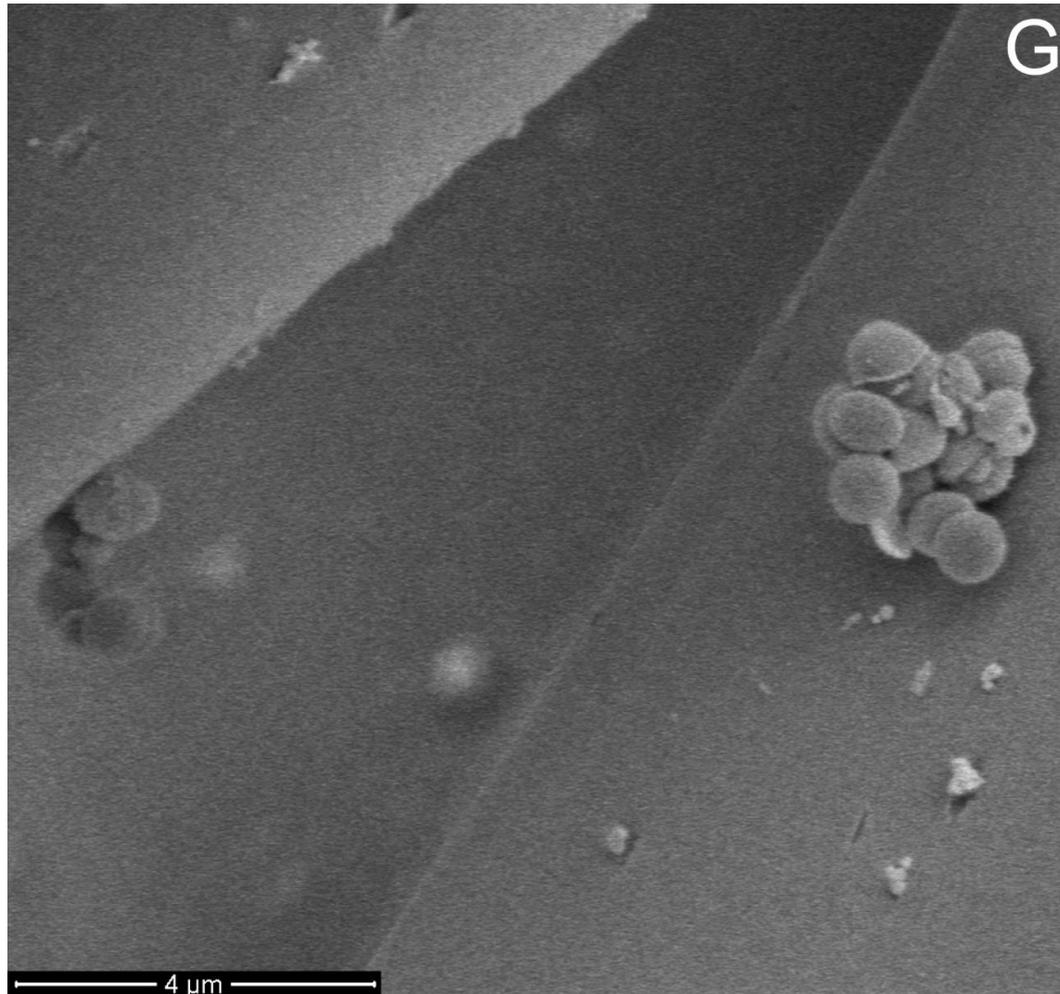


Figure 4.10. (G). SEM micrograph of *S. aureus* on 20 min O₂ exposed lyocell fibres within the nonwoven fabric sample after dynamic wiping.

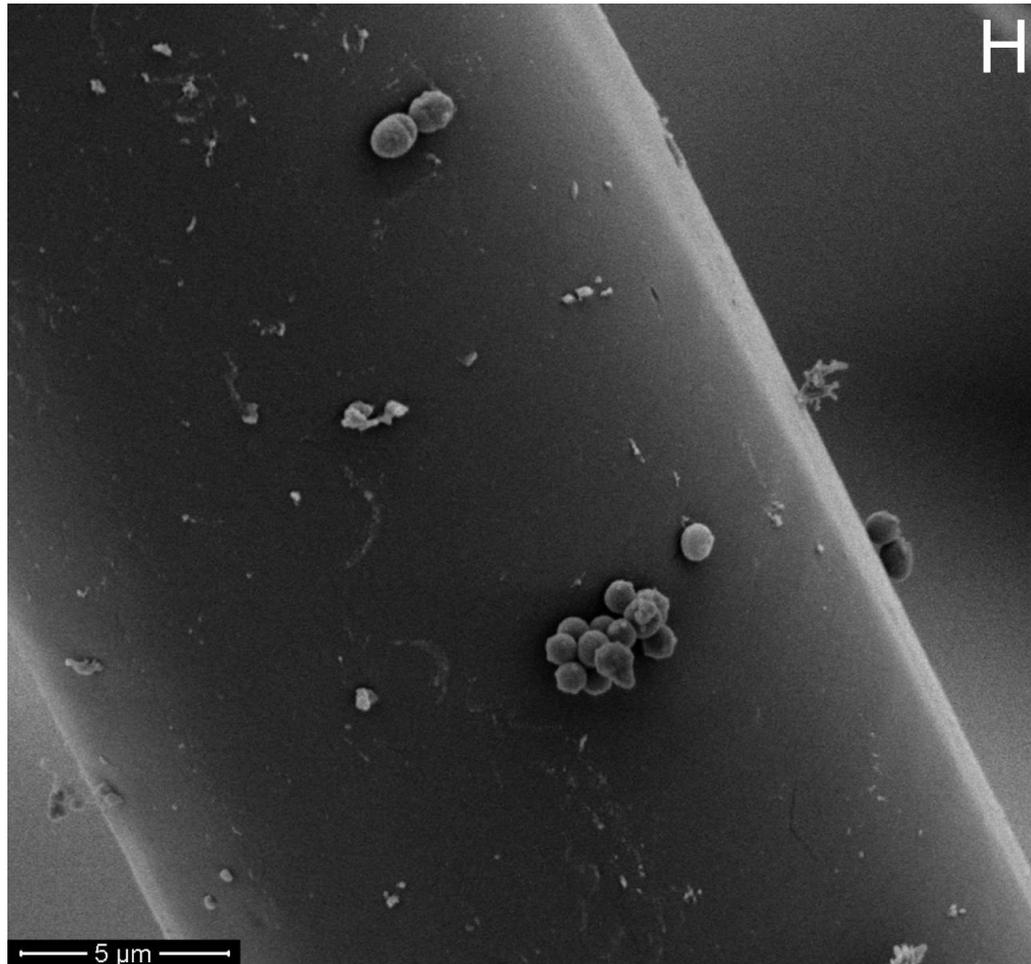


Figure 4.10. (H). SEM micrograph of *S. aureus* on untreated PP fibres within the nonwoven fabric sample after dynamic wiping.

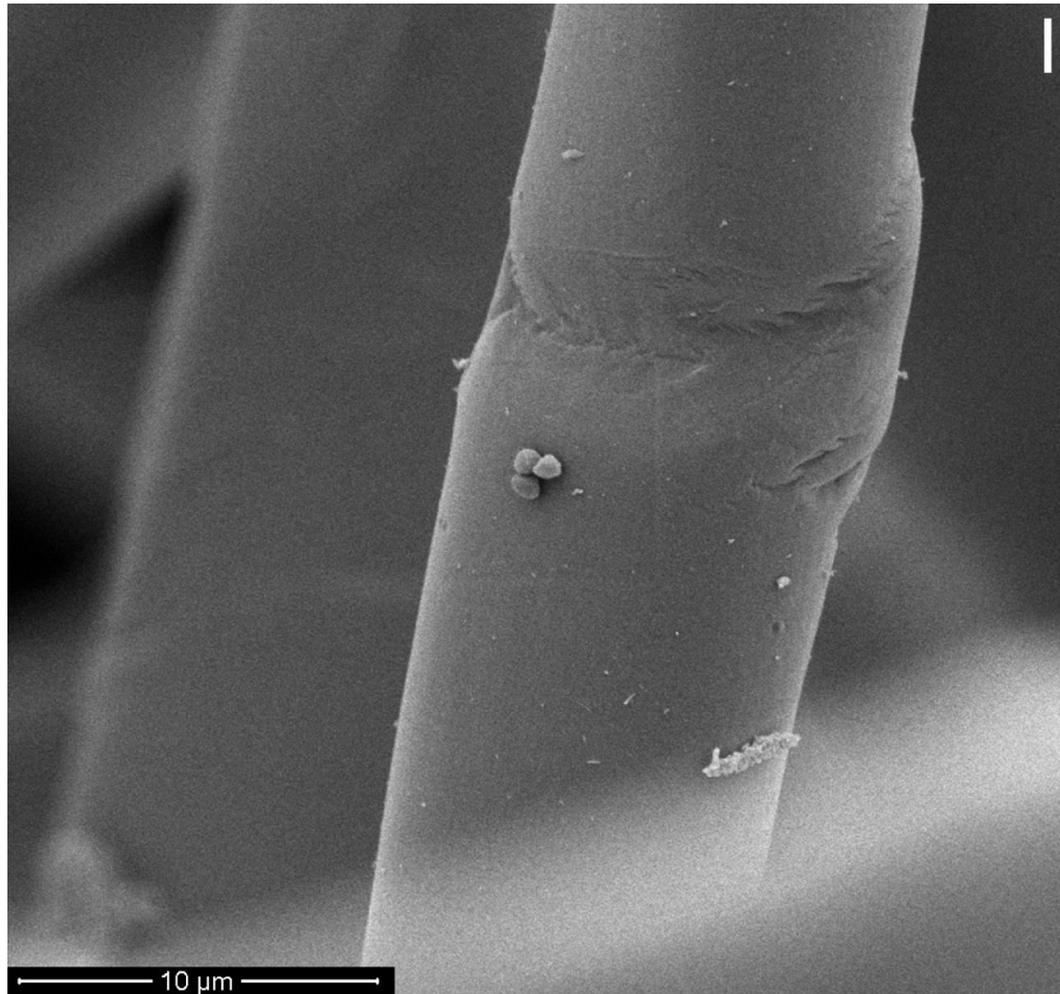


Figure 4.10. (I). SEM micrograph of *E. faecalis* on untreated lyocell fibres within the nonwoven fabric sample after dynamic wiping.

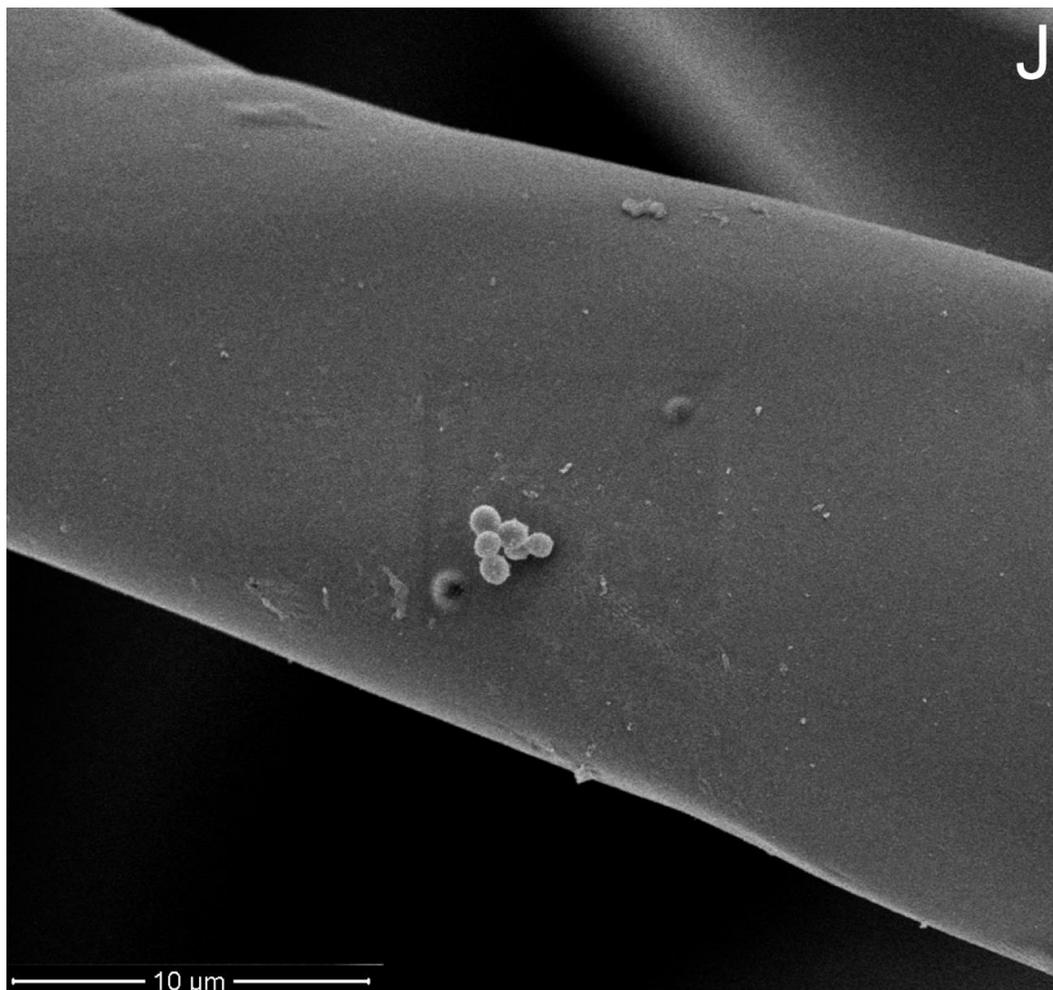


Figure 4.10. (J). SEM micrograph of *E. faecalis* on 20 min C₂F₆ exposed lyocell fibres within the nonwoven fabric sample after dynamic wiping.

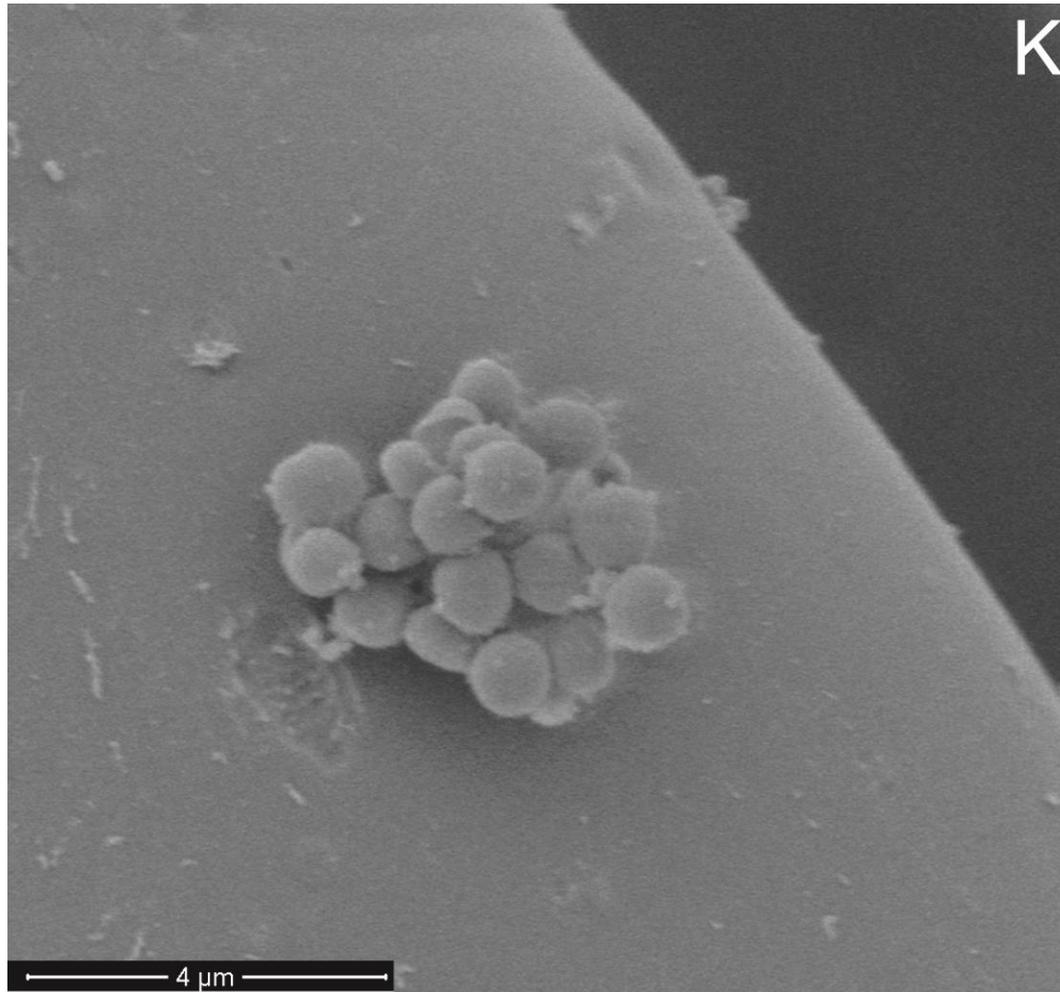


Figure 4.10. (K). SEM micrograph of *E. faecalis* on 20 min O₂ exposed lyocell fibres within the nonwoven fabric sample after dynamic wiping.

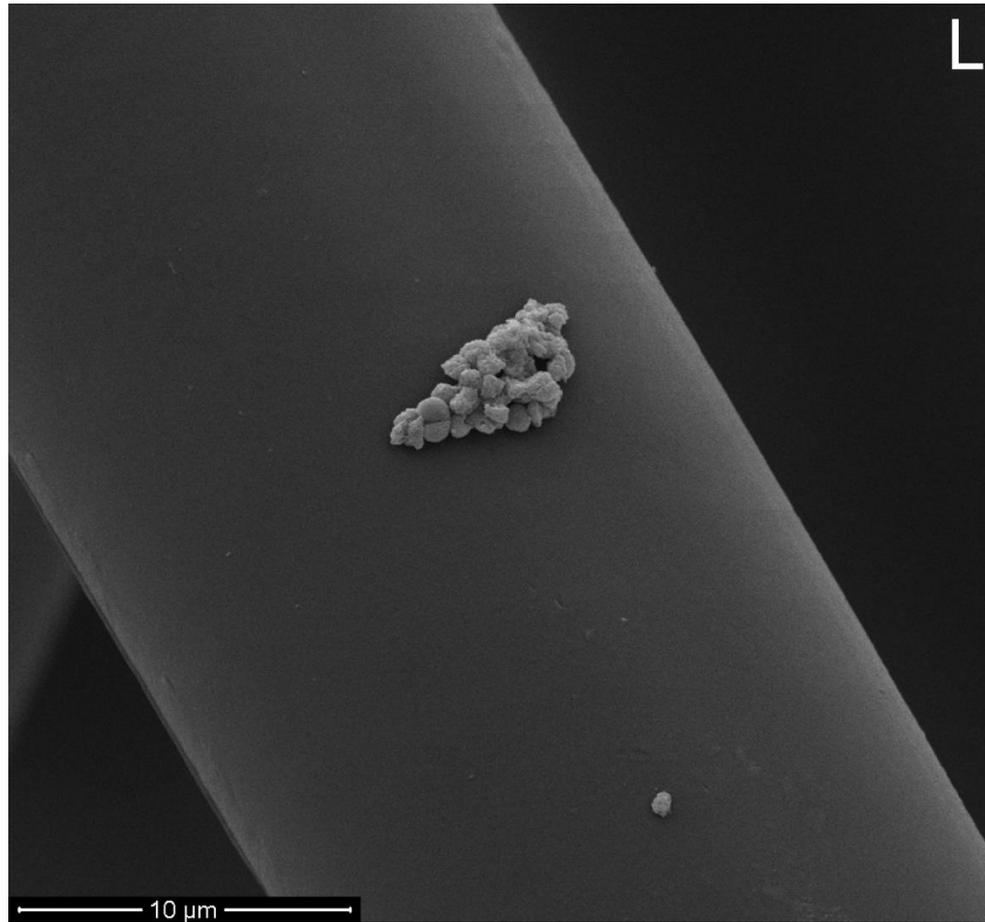


Figure 4-10. (L) SEM micrograph of *E. faecalis* on untreated PP fibres within the nonwoven fabric sample after dynamic wiping.

No notable variations in removal behaviour were observed for different bacteria in terms of their distribution along individual fibres after wiping, irrespective of the fabric type, i.e. in terms of fibre composition or plasma exposure. The SEM images are not all to the same scale, as they were to indicate presence of bacteria on wipe fibres alone.

E. coli tended to adhere to fibre surfaces as a single bacterium, while *S. aureus* and *E. faecalis* tended to adhere in colonies. *E. coli* surface appendages can be observed in Figure 4.10 A-D, which is indicative of flagella acting as structural elements, enabling the bacterium to overcome unfavourable surface topographies [307]. This further explains the relatively high removal efficiency of *E. coli* by the hydrophobic PP and 20 min C₂F₆ exposed lyocell wipe fabrics.

Gram-negative bacteria are known to facilitate adhesion through pili or fimbriae [308, 309], but, as expected, no appendages from adhered *S. aureus* were observed (Figure 4.10 E-H) [310]. Pili were observed with *E. faecalis* (Figure 4.10 I-L) and these appendages are known to contribute to adhesion and biofilm formation, particularly in endocarditis and urinary tract infections [311]. Only the fibres present at the surface of the wipe were imaged due to limitations in the available instrumental set-up, so no conclusions can be drawn regarding the extent to which there was bacterial penetration into the interior of the wipe.

4.5 Summary

Removal of pathogenic bacteria from abiotic surfaces using nonwoven wipes is a stratagem commonly used by healthcare providers to reduce the extent of bacterial contamination. The relative surface energies of the wipe fibres and the

contact surface have been found to influence dry wiping efficiency, as well as the surface roughness of the fibre, but the impact depends on the type of bacterium.

Reduction of the surface energy of lyocell nonwoven fibres by C₂F₆ plasma exposure increased the removal efficiency of *E. coli*, but there was no significant effect on the elimination of Gram-positive bacteria (*S. aureus* and *E. faecalis*).

This suggests that modification of the surface energy of a nonwoven can increase the removal of bacteria, providing that the bacterial cell membrane conformation can adapt to the changes in surface nano-roughness caused by the plasma treatment.

Interestingly, adpression alone is capable of removing a small proportion (up to 16%) of bacteria residing on a contamination surface, whereas dynamic wiping removes significantly more bacteria than adpression for all bacteria and wipe types. Bacteria are found to adhere to fibres in a wipe during a wiping process without either biocide or detergent. This is practically significant as it clearly demonstrates that a biocide is not strictly necessary for the removal of bacteria from surfaces. If the wipe is subsequently disposed of in an appropriate manner, dry wiping could to some extent contribute to reducing transmission of HCAs.

Chapter 5

Factors affecting Removal of Bacteria from Solid Surfaces during Dynamic Wiping

5.1 Introduction

Nonwovens are porous fibre assemblies containing fibres arranged mostly in the x-y plane [252]. They can be produced from hygroscopic or hydrophobic fibres and are often impregnated with an aqueous biocidal liquid. Liquid loadings on nonwoven substrates to make commercial wipes are typically of the order of 150-350% by weight, with much of the liquid volume being held in the interstitial pore volume between the fibres. These loading values are typically below the absorbent capacity of the structure to ensure minimal liquid is lost during converting, packaging and storage of the wipe prior to use. It also ensures that liquid released during use of the wipe is reabsorbed facilitating the ability to wipe the surface dry.

The basic dimensional properties of a nonwoven fabric include the basis weight ($\text{g}\cdot\text{m}^{-2}$), the thickness (mm) and porosity, which is the ratio of void volume to total fabric volume. The porosity in particular is an important influence on the total liquid absorptive capacity of the wipe because it dictates the void volume that is available for liquid absorption or storage.

For the characterisation of wiping performance, Williams *et al.* (28) and Ramm *et al.* (189) developed reproducible methods for analysing bacterial removal from

surfaces by wipes. However, previous studies have typically focused on comparing the performance of commercially sourced wipes, whose structure and properties have not been directly comparable due to differences in the way they are manufactured. Consequently, understanding the role of specific wipe design parameters on wiping performance has not been possible. To address this limitation in practice requires access to facilities, which enable the manufacture of experimental nonwoven wipe substrates. Given the dearth of previously reported studies in the academic literature relating to the effects of modulated changes in wipe properties on bacterial removal efficiency, a systematic experiment was therefore conducted.

In the present research, it has been previously shown that the mechanical mechanism of wiping with a dry nonwoven fabric is able to remove a proportion of the bacteria present on a surface (Chapter 4) (322), but impregnation of the nonwoven wipe with an aqueous medium can also be expected to substantially improve the removal of particles up to a limit, depending on the absorptive capacity of the fabric (176).

Cleaning regimens alone may be ineffective in eliminating pathogens from surfaces (323). Therefore biocides, more specifically, antimicrobials, are used for the control of organisms considered harmful to human health. These pre-impregnated, pre-moistened or “wet” wipes provide significantly higher cleaning-regimen compliance when used by staff and lead to a rapid cleaning and disinfection process (324).

During dynamic wiping, shear and compressive forces are applied, assisting transfer of bacteria to the wipe fibre surfaces and overcoming the adhesive forces between bacteria and the surface on which they reside (182). Changing the

wiping pressure can therefore be expected to affect the balance of these forces and the resulting bacterial removal efficiency.

As a basis to develop improved biocidal wipes products, there is a need for a controlled investigation into the effect of the wipe basis weight, liquid loading and applied pressure during wiping on the disinfection of abiotic plastic surfaces. Accordingly, experiments were conducted herein to explore factors relating to the basic design attributes of the wipe itself as well as the wiping action, all of which can be expected to influence the bacterial removal efficiency. In these experiments, each of these parameters were controlled in the laboratory to provide a basis for systematic study.

5.2 Experimental Design

To investigate the key intrinsic and extrinsic factors leading to the greatest bacterial removal efficiencies, an orthogonal array testing strategy (OATS) was employed as described in Chapter 3, section 3.13 (325).

Owing to the 24 h incubation time required to assess bacterial removal, adoption of the OATs methodology enabled a three-fold reduction in the number of test specimens required, reducing the total duration of the experiment from approximately six months to two months.

Fabric samples were prepared from lyocell and PP fibres, using the specifications and methods explained in section 3.1, such that both an inherently hydrophilic regenerated cellulose fibre (lyocell) and an inherently hydrophobic fibre (PP) were evaluated.

Wipe fabric basis weight values were selected to encompass the range commonly found in commercially available nonwoven wipes, as indicated in section 3.1. Wiping pressures were selected based on those produced by an average sized human hand and the median value reported in the literature (189) as described in section 3.13, while the influence of a biocidal liquid was compared with distilled water (dH₂O) and dry controls using add-on values and methods of impregnation detailed in section 3.7.1.3. The combinations of basis weight, biocide and wiping pressure can be found in section 3.13; Table 3-1 and Table 3-2.

5.3 Results and Discussion

The influence of key wipe parameters on bacterial removal efficiency was studied in relation to each type of bacterium in conditions of dynamic wiping.

5.3.1 Optimum process parameters

The output response variables from the orthogonal array (OATs) were the removal efficiency percentages of *E. coli*, *S. aureus* or *E. faecalis* from the model surface during simulated dynamic wiping (Table 5-1). These values were then selected as inputs in the orthogonal array to determine optimum parameters for the wipes, viz. basis weight, liquid addition and pressure during wiping (Table 3-1 and Table 3-2). Testing was conducted according to the orthogonal array (Table 3-1). The bacterial removal (CFU %) values in row A9 in Bold are the highest removal values for a given bacterium given by the “within array” analysis, and match the optimum combination of fabric weight, liquid addition and wiping pressure predicted by the orthogonal array. The underlined bacterial removal (

CFU %) values in row A9 are the highest removal values for a given bacterium given by the “within array” analysis , but not the optimum combination of fabric weight, liquid addition and wiping pressure predicted by the orthogonal array.

For the PP nonwoven, the predicted optimum process parameters were determined. In other words, the wipe manufacturing parameters predicted by the orthogonal array to give the highest removal efficiency of bacteria from the surface (Table 3-2 section 3.13) for both *E. coli* and *E. faecalis* were 150 g.m⁻² basis weight, in combination with the biocide and 4.68 kN.m⁻² pressure during wiping. This was confirmed by the OATS output values in Table 5-1, sample A9 – 81.67% removal of *E. coli* and 77.78% removal of *E. faecalis*, the highest removal values found for each bacterial strain during the testing.

For the *S. aureus*, the optimum wiping pressure was predicted to be 13.80 kN.m⁻², with 150 g.m⁻² wipe basis weight in combination with the biocide. This was confirmed by further testing, i.e. using a 150 g.m⁻² PP nonwoven with biocide and 13.80 kN.m⁻² pressure while wiping a surface contaminated with *S. aureus* gave a mean removal value of 74.4 CFU %, which was higher than any removal value obtained using combinations of wiping pressure, basis weight and liquid loading from the orthogonal array.

Table 5-1 shows the orthogonal array testing result and the highest removal efficiency value for within-array testing for *S. aureus* was 71.8 CFU % (test row A9).

For the lyocell nonwoven, a basis weight of 150 g.m⁻² basis weight in combination with the biocide and 13.80 kN.m⁻² wiping pressure were found to be optimum process parameters for all bacteria in terms of removal efficiency. This is also evident by comparing the data for conditions outside the orthogonal array – that

is, by assessing the bacterial removal efficiency using a given combination of wipe basis weight, liquid addition and wiping pressure, by measuring these separately. The mean removal efficiency values obtained were 88.74 CFU % for *E. coli*, 88.31 CFU % for *S. aureus* and 86.52 CFU % for *E. faecalis*, all of which were higher than any of the array outputs for the given bacteria (see underlined values in Table 5-1, row A9).

Table 5-1. Bacterial removal efficiency results for the polypropylene and the lyocell wipes for *E. coli*, *S aureus* and *E. faecalis*. *n*=3.

Sample	PP nonwoven			lyocell nonwoven		
	<i>E. coli</i> removal (CFU %)	<i>S. aureus</i> removal (CFU %)	<i>E. faecalis</i> removal (CFU %)	<i>E. coli</i> removal (CFU %)	<i>S. aureus</i> removal (CFU %)	<i>E. faecalis</i> removal (CFU %)
A1	44.64	36.60	29.17	34.19	36.90	32.10
A2	61.66	59.20	57.82	50.00	42.37	42.38
A3	65.66	72.00	58.61	87.46	74.40	75.21
A4	57.85	43.33	44.69	38.24	36.32	39.74
A5	68.03	63.27	65.88	60.00	69.08	70.94
A6	75.53	68.47	77.42	79.64	80.09	82.22
A7	59.83	51.67	54.64	68.01	69.41	71.16
A8	69.53	68.02	69.49	78.22	79.21	74.24
A9	81.67	<u>73.06</u>	77.78	<u>87.74</u>	<u>82.88</u>	<u>84.35</u>

5.3.2 Bacterial removal efficiency trends

The bacterial removal efficiency was considered as a function of fabric basis weight for each bacterium and each substrate material (Figure 5-1), by taking an average of the data from the three basis weight values (i.e. from Table 5-1, results from **A1-A3** for 50 g.m⁻², **A4-A6** for 100 g.m⁻², and **A7-A9** for 150 g.m⁻²).

Although usage of biocide was the most influential parameter in terms of increasing bacterial removal efficiency, the results suggested utilisation of higher basis weight would also improve removal efficiency, which can impact dry wiping as well and wet wiping. This is significant as dry wiping has shown to be effective in bacterial removal from surfaces (sections 4.3.5 and 4.3.6)

The differences in bacterial removal efficiency between the lowest and highest basis weight wipes containing both lyocell and PP for *E. coli*, *S. aureus* and *E. faecalis* were all significant at $p < 0.05$ (unpaired *t*-test).

There was a persistent trend of increasing bacterial removal efficiency with increasing fabric basis weight for all bacteria, in both the PP and lyocell wipes, though the effect was more pronounced with the lyocell wipe, as the gradients of the best fit lines are steeper (Figure 5-1). Based on these data it was clear that increasing the wipe basis weight, irrespective of fibre content, can therefore be expected to improve bacterial removal efficiency. This because increasing the basis weight increases the holding capacity for the biocide, itself largely aqueous.

Liquid add-on during biocide (or water) addition to the wipe was 150% weight to weight for all wipes, so heavier basis weight wipes will have the same percentage of biocide but more “actual” biocide. Therefore, there is more likelihood of either a bacterial “kill” on the contaminated surface or bacterial removal from the contaminated surface.

It is shown in section 4.3.6 Figures 4.9 A-L that bacteria interact with and adhere directly to the fibres in dry wipes. Therefore, if more fibres are present at the wipe-contaminated surface interface, there is a greater likelihood of bacterial adhesion and removal. Heavier basis weight wipes were shown to remove more bacteria without liquid addition, following the same trend as with the biocide-containing wipes.

Table 5-2. Slope and intercept for removal efficiency vs. basis weight graph best fit lines from Figure 1.

	Bacteria	Slope	Intercept
lyocell	<i>E. coli</i>	0.25	39.86
	<i>S. aureus</i>	0.27	37.12
	<i>E. faecalis</i>	0.26	37.18
PP	<i>E. coli</i>	0.12	50.35
	<i>S. aureus</i>	0.13	45.84
	<i>E. faecalis</i>	0.18	41.35

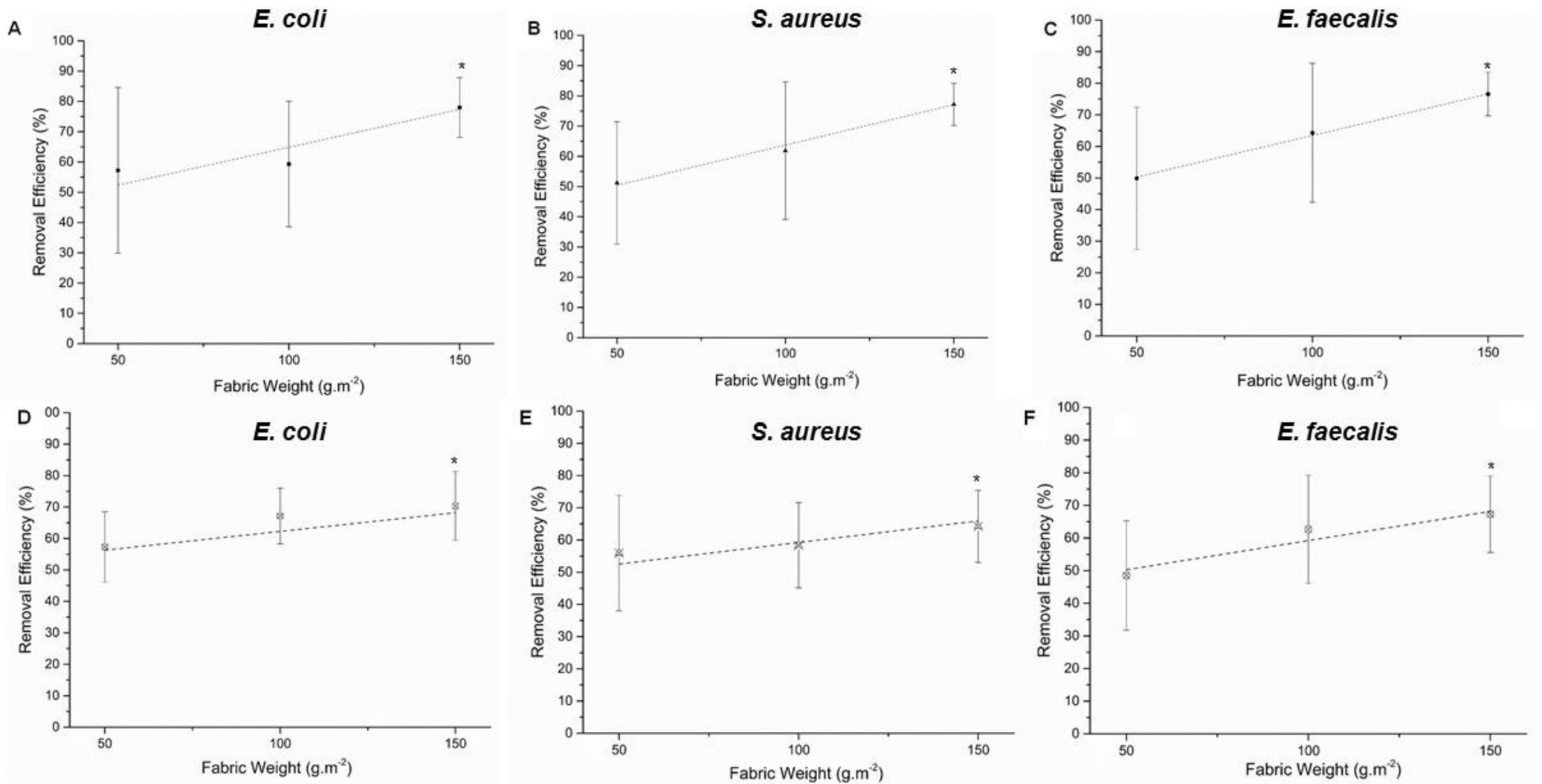


Figure 5-1. Removal efficiency of wipe vs. fabric basis weight, with linear best fit. **A - C** 100% lyocell wipes, **D - F** 100% PP wipes. Error bars indicate standard deviation, * indicates significant difference ($p > 0.05$).

The parameter with the greatest “C” value (Table 5-3, calculated according to Table 3-2) is the parameter that has the greatest effect on the removal efficiency. For all bacteria and both wipe fibre compositions, this was the liquid addition parameter (C2). This means that the addition of a biocide to a wipe has the greatest effect on bacterial removal (CFU %) of any of the parameters investigated. The main effects on bacterial removal efficiency were determined by ANOVA. For the PP wipe, liquid addition had the most significant effect on removal of *E. coli* ($p < 0.01$); *S. aureus* and *E. faecalis* (both $p < 0.05$), confirming the differences observed in the OATS. The PP fabric basis weight also had a significant effect on *E. coli* removal ($p < 0.01$).

For Table 5-3, the values highlighted in bold show the level of the optimum process parameter. OPP* denotes a set of optimum process parameters that have been confirmed by testing outside of the orthogonal array. Cells highlighted in **Red** indicate the largest “C” (“difference”) value. This is the variable that has most impact on bacterial removal

Similarly, for the lyocell wipe, biocide liquid addition had the largest effect on removal of *E. coli* ($p < 0.05$); *S. aureus* and *E. faecalis* (both $p < 0.01$), which was in agreement with the OATS differences. The lyocell basis weight and wiping pressure both had a significant effect on the removal of *S. aureus* ($p < 0.05$ and $p < 0.01$ respectively) and *E. faecalis* (both $p < 0.05$). Increase of basis weight for either wipe type will also increase dry wiping removal of biocide.

Note that the improvement in wiping efficiency due to the addition of the biocidal liquid might also be partly due to the presence of a liquid phase, and not just the fact that it is a biocidal liquid. The addition of water alone can substantially

increase bacteria removal from the surface by providing a transport medium in which bacteria can be suspended and transported the interstitial pore spaces within the wipe fabric structure.

In general, the presence of a biocide liquid in the wipe during the wiping process is therefore important to ensure effective removal of bacteria from hard surfaces. Since bacteria are attached to the surface, there will be an energy threshold that must be overcome to remove them. Whilst it is reasonable to assume that increasing wiping pressure will assist in overcoming these forces by providing greater energy to the surface (169), via applied forces such as shear and compression, it is apparent that a high wiping pressure cannot substitute for the presence of a biocide liquid.

Initially, during wiping, the role of the biocidal liquid is likely to relate to its inherent surfactancy and the consequent reduction in surface tension, which improves surface wetting (326, 327). Note that in the present study, the surface tension of the biocide liquid was roughly half that of water (328) (Section 3.7.1.1). Consequently, an increase in the removal of bacteria from the surface for biocide containing wipes versus water and dry wipes would be anticipated.

Important to consider is the degree of absorption and desorption of the biocide liquid volume to and from the wipe before and during use. The biocide is an aqueous medium, the bulk of which is absorbed and retained within the void volume of the wipe, depending on the surface energy of the constituent fibres.

However, in a hygroscopic substrate such as that made of lyocell, a proportion of the water in the liquid formulation may be chemically combined with the cellulose, restricting its subsequent availability. Therefore, as the biocide is largely

aqueous, the concentration of the benzalkonium chloride (see Figure 2-10 and section 3.7.1.1), the “biocidal” component of the biocide, may be greater outside the lyocell fibre, in the interstitial spaces in the lyocell wipe, as it only has one Hydrogen-bond acceptor and zero Hydrogen-bond donors (329). A hydrogen bond is the electrostatic attraction between two polar groups that occurs when a hydrogen atom covalently bound to a highly electronegative atom experiences the electrostatic field of another highly electronegative atom nearby. Therefore, the availability of benzalkonium chloride may be greater in the lyocell wipes, however it lacks the necessary liquid phase to deliver it to the contaminated surface and the bacterial cells on it.

During use, compression of the wipe structure reduces its volume and a proportion of interstitially retained liquid held within the pore structure will therefore be released. This effect was most pronounced in the PP wipe, which was inherently hydrophobic. In the PP wipe, the optimum wiping pressure for *E. coli* and *E. faecalis* was very low, only 4.68 kN.m⁻², compared to 13.80 kN.m⁻² for the lyocell wipes. In the lyocell wipe, a proportion of the aqueous biocide will chemically interact with –OH groups of the lyocell material, such that it will be more effectively retained within the fabric, according to the fibre’s crystal structure. This means that although the fraction of liquid impregnated in to each wipe was identical, a greater proportion of the biocide may be released from the PP wipe, at a lower wiping pressure, which will assist in bacterial removal. Thus, increasing the wiping pressure beyond the lowest value when using PP wipes did not result in significantly better removal of *E. coli* and *E. faecalis*. In contrast,

greater wiping pressure of 13.80 kN.m^{-2} is required using lycell wipes to release sufficient liquid to provide optimal surface bacterial removal.

Table 5-3. Optimised results for fabric basis weight, liquid addition and wiping pressure.

Optimum Process Results				
	Fabric weight	Liquid addition	Wiping pressure	
<i>E. coli</i>	Σ1	171.96	189.70	
	Σ2	201.41	201.18	
	Σ3	211.03	222.86	
	OPP	150 g.m ⁻²	With Biocide	4.68 kN.m ⁻²
	Difference	39.07	60.54	7.66
PP Nonwoven wipe	Fabric weight	Liquid addition	Wiping pressure	
	Σ1	167.8	173.09	
	Σ2	175.07	175.59	
	Σ3	192.75	213.53	186.94
	OPP*	150 g.m ⁻²	With Biocide	13.80 kN.m ⁻²
Difference	24.95	81.93	13.85	
<i>E. faecalis</i>	Fabric weight	Liquid addition	Wiping pressure	
	Σ1	145.6	176.08	
	Σ2	187.99	180.29	
	Σ3	201.91	213.81	179.13
	OPP	150 g.m ⁻²	With Biocide	4.68 kN.m ⁻²
Difference	56.31	85.31	4.21	
<i>E. coli</i>	Fabric weight	Liquid addition	Wiping pressure	
	Σ1	168.35	192.05	
	Σ2	177.88	175.97	
	Σ3	233.97	251.54	212.17
	OPP*	150 g m ⁻²	With Biocide	13.80 kN m ⁻²
Difference	65.62	111.11	36.19	
Lyocell nonwoven wipe	Fabric weight	Liquid addition	Wiping pressure	
	Σ1	153.67	196.21	
	Σ2	185.50	161.58	
	Σ3	231.51	237.37	212.89
	OPP*	150 g.m ⁻²	With Biocide	13.80 kN.m ⁻²
Difference	77.84	94.73	51.31	
<i>E. faecalis</i>	Fabric weight	Liquid addition	Wiping pressure	
	Σ1	149.69	188.56	
	Σ2	192.91	166.47	
	Σ3	229.75	241.78	217.32
	OPP*	150 g.m ⁻²	With Biocide	13.80 kN.m ⁻²
Difference	80.06	98.78	50.85	

5.3.3 Fibre composition of the wipe substrate

During wiping, fibres in the surface of the wipe will directly interface with the bacterially contaminated surface. It may therefore be postulated that a greater fibre contract area will lead to a greater probability of fibre-bacterium interaction, and therefore a greater removal efficiency. The solid area (fibre) volume fraction, measured using the method reported in section 3.17.3, increased with increasing basis weight. The porosity values of the fabrics were also measured, although there was not found to be a significant difference between basis weights or wipe types. SEM images of wipes of different basis weights were analysed using FIJI (298), then output values were subject to ANOVA with a post hoc Tukey's test ($p < 0.05$).

Table 5-4. Solid (fibre) area fraction at the wipe surface interface. Means that do not share a grouping letter are significantly different from each other. S.D. is standard deviation. $n=5$.

	Wipe basis weight (g.m ⁻²)	Mean solid (fibre) area fraction at wipe: surface interface (%)	S.D.	Grouping
Iyocell	50	70.43	2.11	A
	100	81.81	0.97	B
	150	91.25	1.10	C
PP	50	77.23	4.10	B
	100	79.30	1.85	B
	150	94.25	1.46	C

This is predictable given that both the PP and Iyocell 150 g.m⁻² fabric structures contained more fibres and therefore more solid surface than the 50 g.m⁻² and 100 g.m⁻² samples ($p < 0.05$). Accordingly, the heaviest wipes considered in this study of 150 g.m⁻² consistently yielded greater bacterial removal efficiency than the 50

g.m^{-2} and 100 g.m^{-2} wipes. This is relevant to the removal efficiencies in both wet and dry wiping. However, in an impregnated wipe substrate, increasing the wipe basis weight also enables a greater volume of biocide liquid to be retained by the structure, as there is greater absolute absorptive capacity in a heavier-weight wipe, even if the liquid loading in terms of weight fraction was consistent for all wipes. In absolute terms, heavier-weight wipes will have a higher liquid carrying capacity for in terms of volume than those of lighter-weight. Note that in addition, during wiping, the pressure applied to the substrate is likely to reduce the pore volume as a result of compression, leading to a reduction in effective absorbent capacity. Collectively, this points to benefits of selecting heavier weight ($>100 \text{ g.m}^{-2}$), regenerated cellulose wipe substrates or PP wipe substrates loaded with biocide liquid. In the case of lyocell wipes, it is observed that this should be accompanied by use of greater hand wiping pressure, where possible to maximise bacterial removal efficiency. It is interesting to note that the role of hand wiping pressure varies depending on the fibre composition of the wipe substrate. To the author's knowledge this has not been previously reported.

5.4 Summary

Removal of pathogenic bacteria from abiotic surfaces using nonwoven wipes in combination with a biocidal liquid is a stratagem commonly used by healthcare providers (28). Production of wipes with optimal bacterial removal efficiency is therefore crucial. Using an orthogonal array Taguchi strategy, it was determined for the first time, to the best of the author's knowledge, that the optimum basis weight for both lyocell and PP wipes should be the highest possible within the experimental range, i.e. 150 g.m^{-2} . This is substantially higher than the basis

weight of many existing surface wipes currently used in healthcare environments, which are more typically in the range 45-90 g.m⁻². Bacterial removal efficiencies could therefore be practically improved in a healthcare setting by the simple step of specifying wipes of higher basis weights.

The optimum parameters for lyocell nonwoven wipes for effective removal of *E. coli*, *S. aureus* and *E. faecalis* were, 150 g.m⁻² basis weight; 13.80 kN.m⁻² wiping pressure, with the addition of the biocidal liquid. In the case of a PP nonwoven wipe, the same conditions were also optimal for the removal of *S. aureus*; while for *E. coli* and *S. aureus* removal a lower wiping pressure of 4.68 kN.m⁻² was advantageous in the presence of a biocide liquid. This is thought to be due to the availability of the biocidal liquid, i.e. the degree to which it is physically and chemically retained by the fibres and internal structure of the fabric.

The addition of biocidal liquid was found to be the factor in the OATS analysis that had the most influence on bacterial removal efficiency, as was also confirmed by statistical analysis ($p < 0.05$). Collectively, these findings provide a new insight into disinfection and decontamination using nonwoven wipes, allowing greater understanding of the fundamental process underlying bacterial removal during wiping from healthcare surfaces (24, 330).

The benefit of increasing the substrate basis weight is also likely to hold true for dry wipes, as it has also been shown in this work that bacteria will adhere to fibres in the dry state (section 4.3.6). As reported in these experiments, greater fibre surface area is provided at the interface between the wipe and contaminated surface as the wipe basis weight increases, such that there will be more surface provided for bacterial adhesion.

It is therefore suggested that best practice for infection control should involve selection of heavier weight wipes both in the dry and biocide-impregnated states and that regenerated cellulosic wipes (lyocell) impregnated with biocide, with as heavier wiping pressure as possible, are likely to provide the highest overall bacterial wiping efficiency. One advantage of PP wipes impregnated with biocide liquid is that lower wiping pressure can still lead to good bacterial removal efficiency.

Chapter 6

Recontamination of Solid Surfaces and Residual Activity following Wiping with Biocidal Liquid-Impregnated Nonwoven Wipes

6.1 Introduction

It may be argued that an ideal healthcare wipe will provide maximal removal of bacterial contamination and then prevent transfer of this bacterial load between surfaces by resisting its detachment. Furthermore, it could also be argued that the presence of a biocide will confer some degree of residual antimicrobial activity to the surface. The extent to which these hypotheses are correct is not fully understood. Practically these are important questions because, a wipe contaminated with bacteria will commonly come in to contact with other parts of the surface that may not be contaminated as a result of how wiping is typically performed. Also, surfaces may not necessarily be 'wiped dry' during normal wiping procedures.

It has been shown in this work that addition of an aqueous liquid including a biocide to a nonwoven wipe can substantially improve the removal of bacterial contaminants, dependent on the absorptive capacity of the nonwoven structure and the same is true for solid contaminants (176). During dynamic wiping, forces are applied to particles such as bacteria residing on the surface which facilitate their transfer to the wipe by overcoming adhesive forces between the bacterium and the surface (182).

However, in real wiping scenarios, a wipe may be moved across a new surface having just been used to decontaminate another. In other words, only in simplified circumstances will a wipe cycle last one wipe. This raises a number of important questions. An already used wipe is likely to be contaminated with a number of pathogenic bacteria and re-use may cause bacteria to be dislodged from the fibres and be returned to the healthcare surface mediated by the same applied forces operating during wiping. This is far from ideal, as instead of maintaining bacterial removal efficiency, such a mechanism could lead to the recontamination of a clean surface, risking the spread of pathogenic bacteria over a wider area, increasing prospects of HCAI transmission.

The recontamination of surfaces resulting from wiping with already contaminated wipes has previously been investigated (28, 189). However, in this existing work, there was little consideration of the effect of the surface itself, and its contribution towards any recontamination that may occur. Similarly, it is not known if the behaviour is modulated by wipe design specifications. Previous work also only considered the behaviour of commercially available wipe samples with no control of structural or chemical properties in the wipe samples.

Additionally, it is of clinical interest to assess whether wiping with a commercial grade biocidal liquid confers any residual antimicrobial activity to the wiped surface, and whether this influences the potential for further bacterial contamination of the surface.

Sections 4.3.5 and 4.3.6 considered the influence of fibre surface energy and nano-roughness on bacterial wiping efficiency in the dry state (322), as well as optimal wipe design parameters (Chapter 5). The logical progression from dry

wiping, to wet wiping and the effects on bacterial removal now moves on to considering the potential for recontamination of surfaces.

6.2 Experimental Design

The surface structure of different healthcare surfaces composed of PMMA (Perspex), steel and ceramic were examined by means of SEM and EDX as described in section 3.17.2. The incubation of each surface was conducted using the method detailed in section 3.12. Study parameters were carefully selected as there has not yet been an appropriately controlled investigation into:

- a) The extent of recontamination of healthcare surfaces by different fibre polymer compositions in nonwoven wipes impregnated with biocide, and;
- b) The residual antimicrobial activity of a commercial grade biocide remaining on a surface after wiping.

Addressing such questions will allow further elucidation of the underlying interactions governing the removal of bacteria by nonwoven wipes. Accordingly, in this chapter, inherently hydrophilic regenerated cellulose fibre (lyocell) and inherently hydrophobic fibre (PP) wipes substrates were evaluated. Each was produced and with physical characteristics measured using the methods outlined in section 3.1.

Bacterial transfer from the wipe substrates to the model healthcare surface was assessed using three different healthcare surface types, representative of those commonly found in the clinical environment (polymeric, metallic and ceramic). Residual antimicrobial activity was assessed using the same surface types versus common pathogenic bacteria using the method described in section 3.12.

6.3 Results and Discussion

Non-porous low-maintenance solid surfaces are commonplace in the clinical setting (44). High touch material surfaces present in hospitals include stainless steel (45) plastics (46) and ceramics (47). These surfaces are subjected to multiple insults, including cycles of cleaning and disinfection over the course of their lifetime (94, 95). The biocide used in these cleaning regimens can affect surface properties such as the chemistry and topography as a result of prolonged or repeated exposure (61). Any change in these physical properties is likely to alter the way in which bacteria interact and adhere to the surface because of morphological or chemical modifications (24). Although permanent physical modification of the surface is undesirable, if a biocidal residue is left behind after wiping, there is potential for residual antimicrobial activity. Accordingly, in order to analyse the nature of surfaces before and after wiping with biocides, SEM, and EDX analyses were performed.

6.3.1 Analysis of Surfaces

Wiping with a biocidal-loaded wipe (Figure 6-1 C) showed evidence of a degree of surface deposition compared to wiping with water alone (dH₂O), (Fig. 6-1 B) and the control (Fig. 6-1 A). Spherical particles of the order 1 µm were observed, as well as circular marks ranging in size from >1 µm – 5 µm on the biocide loaded surface. The water-wiped and control samples showed no such deposits.

The surface features evident in Figure 6-1 C could be evidence of autophobing. Autophobing is a phenomenon wherein a droplet of a low surface tension solution containing amphiphilic molecules, such as surfactants (331), fails to completely wet a surface, forming “beads” instead (332-334). This, phenomena is typically

found on substrates of higher surface energy than PMMA or Perspex (333, 334). The EDX analysis (Table 6-1) revealed the deposit to be biocide, as the elements that were found were consistent with those that constitute the biocide.

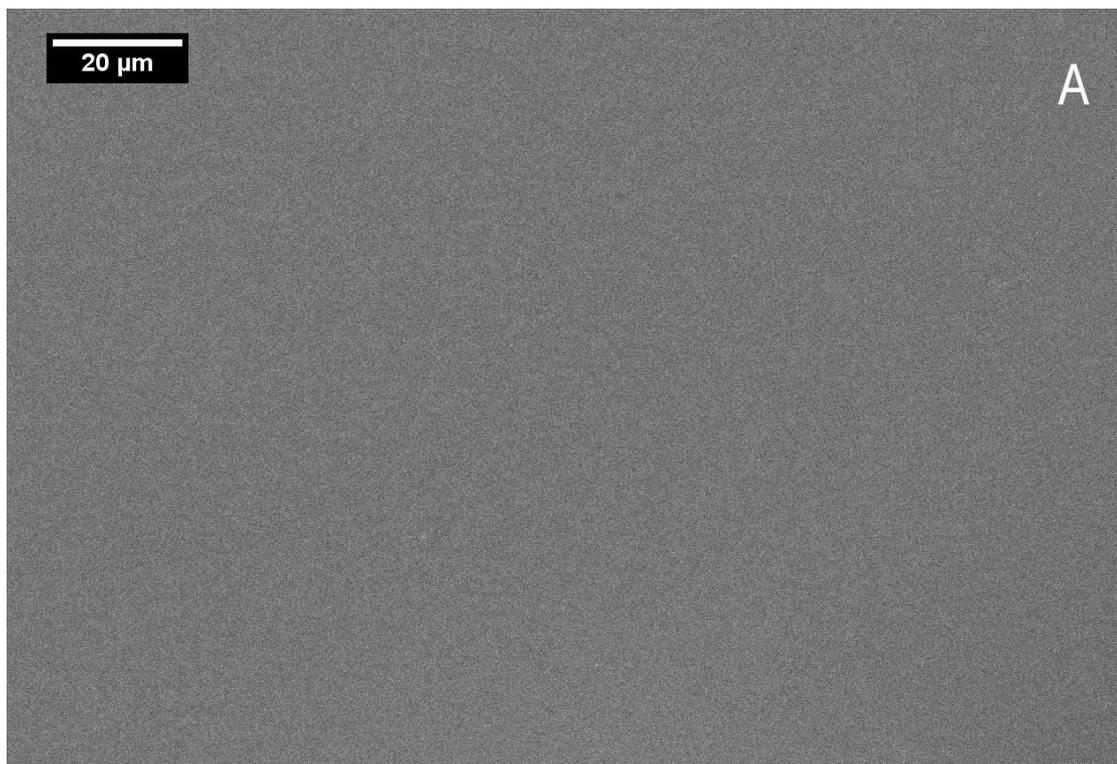


Figure 6 1. (A) SEM of sterile control PMMA healthcare surface sample.

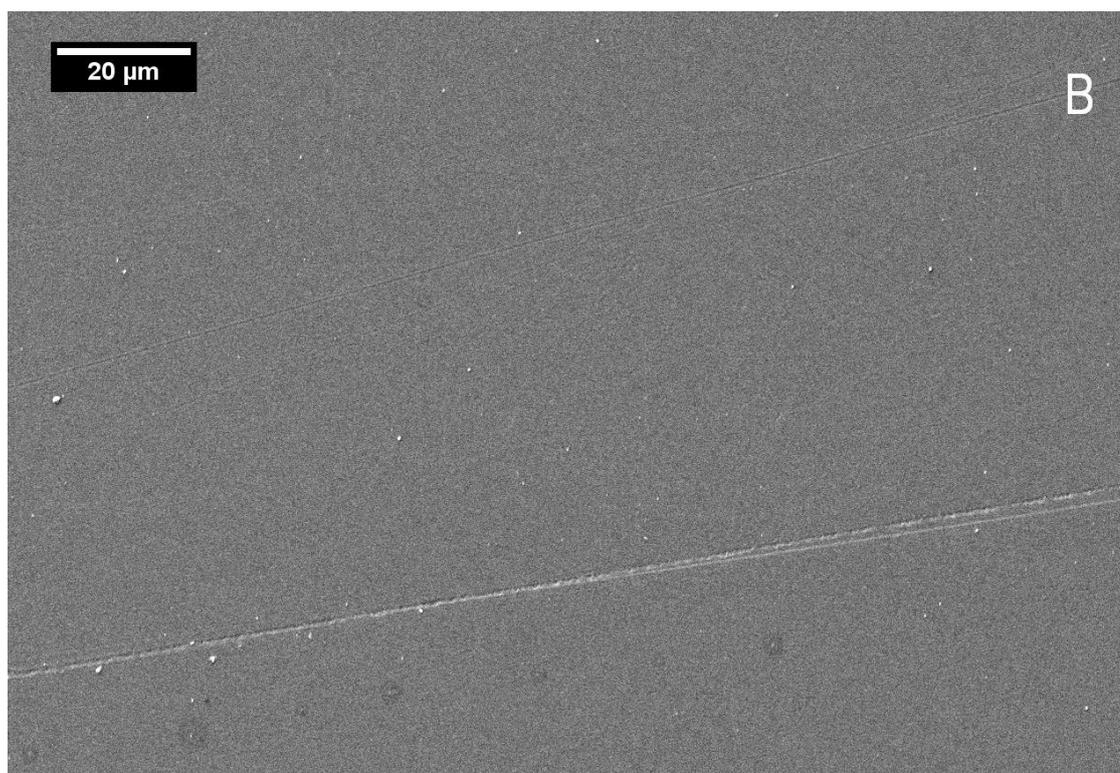


Figure 6 1. (B) SEM of PMMA healthcare surface sample wiped with water (dH₂O).

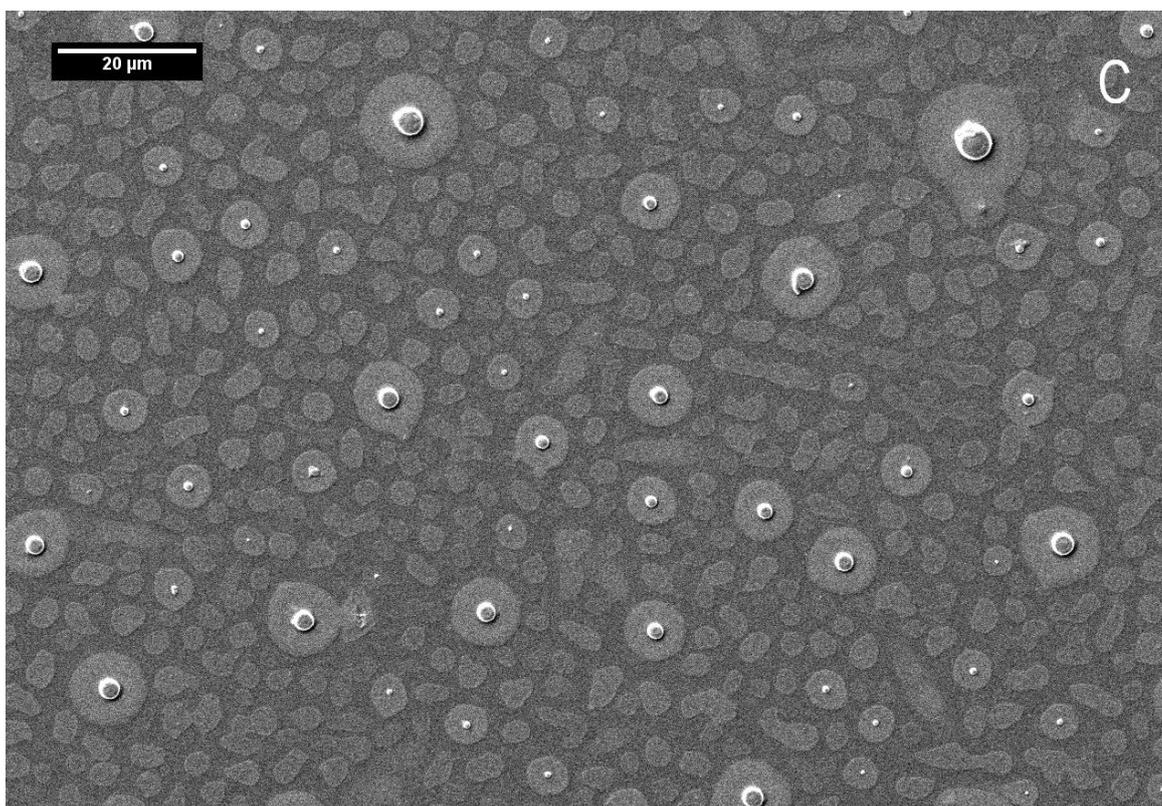


Figure 6-1. (C) SEM of PMMA healthcare surface sample wiped with biocide.

In the EDX analysis (**Table 6-1**), the PMMA control surface indicated evidence of carbon and oxygen, which are the main components of the $(C_5H_2O_8)_n$ repeat unit which comprises PMMA. The biocide sample indicated the presence of carbon and oxygen. The presence of sodium and chlorine were also detected, attributable to the sequesterant or stabiliser component of the biocide, which consists of sodium tripolyphosphate or sodium carbonate and the benzalkonium chloride component of the biocide, respectively (see section 3.7.1.1).

Table 6-1. EDX analysis of biocide and control PMMA (Perspex) samples (Figure 6-1). *n*=3.

PMMA surface Element	Weight %	
	Biocide	Control
C	35.71	61.88
O	42.38	38.12
Na	14.20	-
P	6.34	-
Cl	1.37	-

The satin-finished Grade 304 steel surface exhibited a markedly different surface morphology compared to the PMMA. The unidirectional satin finish is a remnant of the brushing process (335). This linear, ridged structure was particularly evident in the control (

Figure 6-2 A) and water (dH₂O) (Figure 6-2 B) incubations, upon which no deposition could be observed. A glutinous deposit was evident following biocide wiping (

Figure 6-2 C). From the EDX analysis (Table 6-2), it is likely that this is indicative of residual biocide components, as there was no other treatment given to the sample.

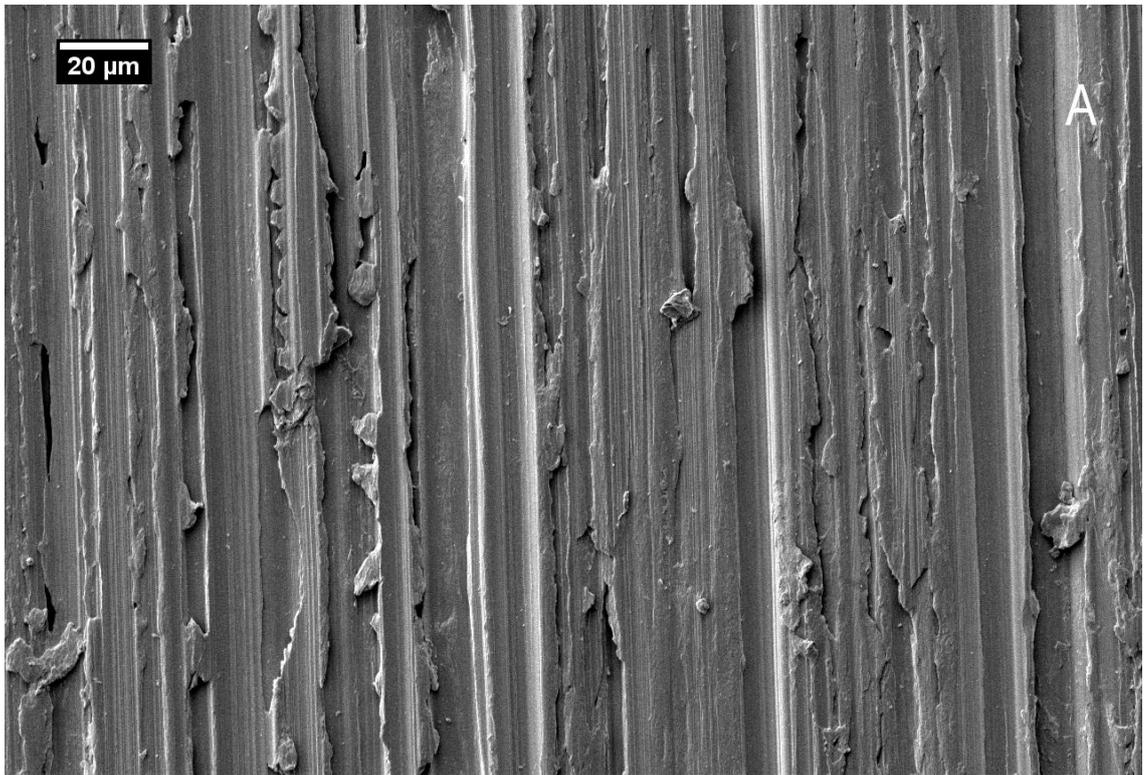


Figure 6-2. (A) SEM of sterile control steel healthcare surface sample.

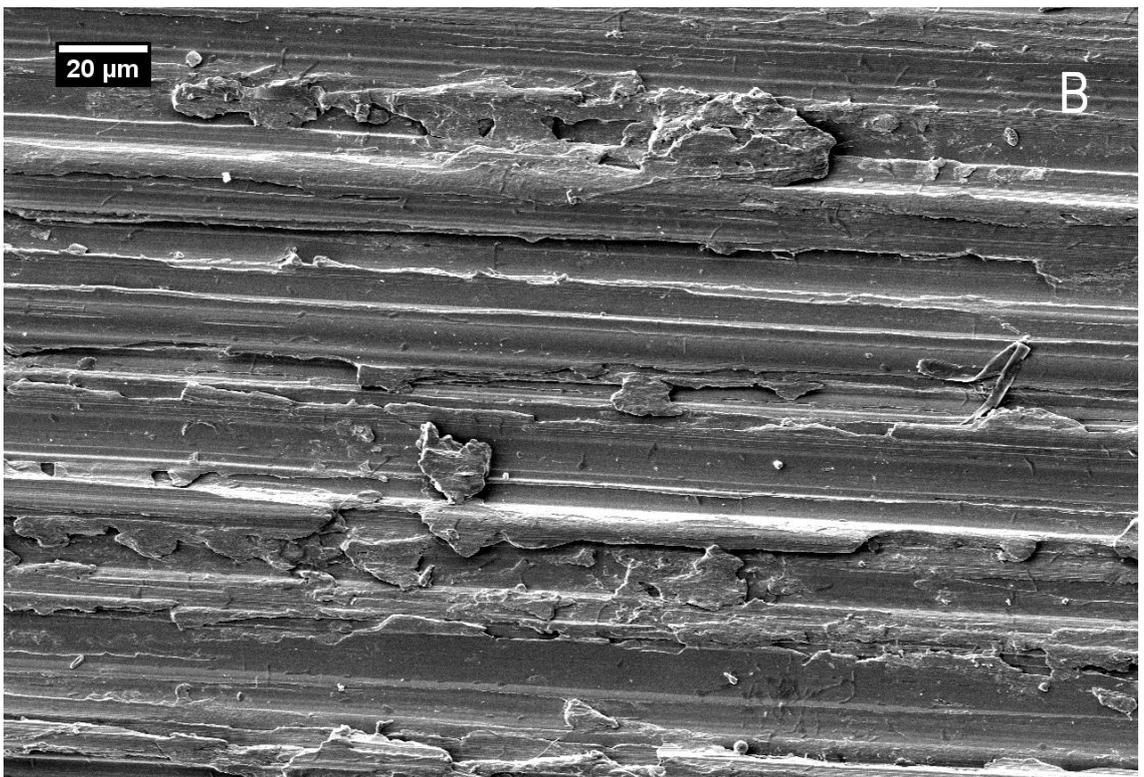


Figure 6-2. (B) SEM of steel healthcare surface sample wiped with water (dH₂O).

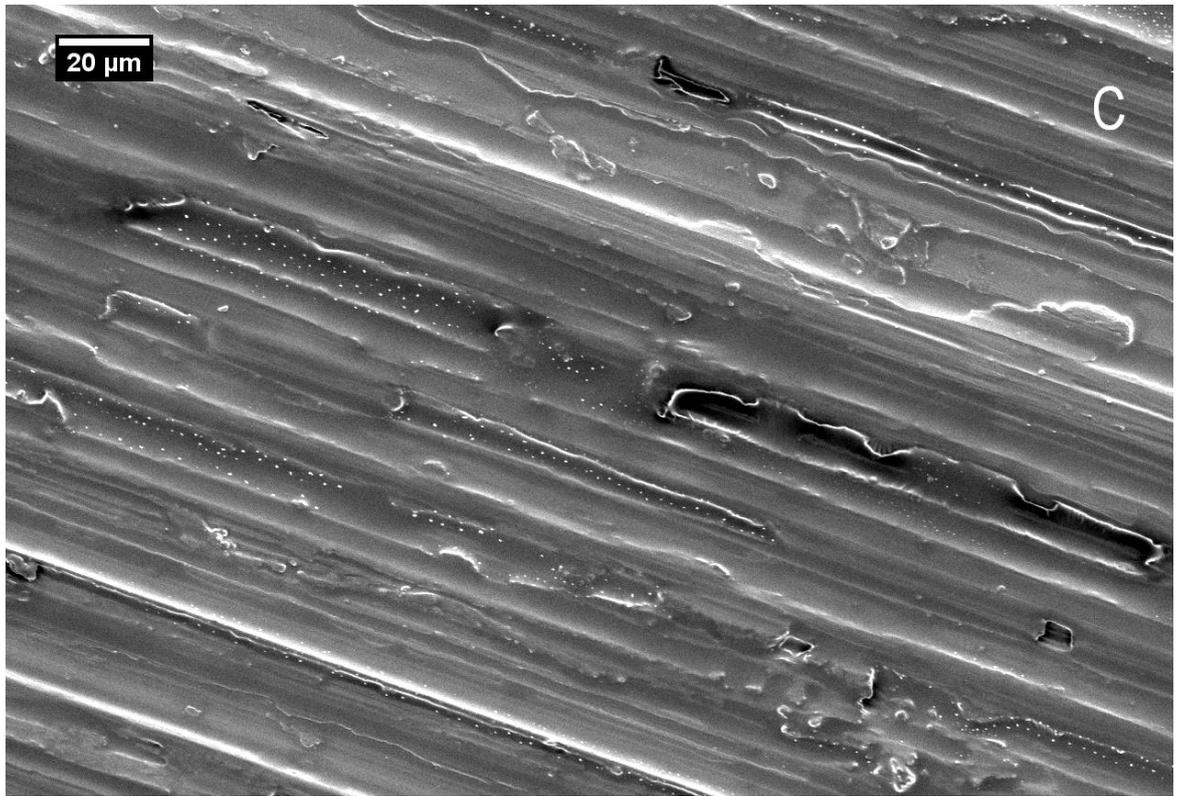


Figure 6-2. (C) SEM of steel healthcare surface sample wiped with biocide.

The steel control revealed the presence of carbon, chromium, manganese, iron and nickel (Table 6-2). Grade 304 brushed stainless steel normally comprises 17.5-20% chromium, 8-11% nickel, <2% manganese, and <1% silicon (336, 337). These values are consistent with the results of the EDX analysis for the steel control surface. The biocide sample showed an increase in carbon, a decrease in chromium and iron, compared to the control sample. Oxygen and sodium was also detected along with trace amounts of phosphorus and chlorine (Table 6-2), attributable to residual biocide.

Table 6-2. EDX analysis of biocide vs control steel samples (Figure 6-2). *n*=3.

STEEL	Weight %	
Element	Biocide	Control
C	32.64	7.48
O	8.05	-
Na	3.07	-
P	0.98	-
Cl	0.39	-
Cr	10.32	18.42
Mn	1.3	1.65
Fe	39.55	65.07
Ni	3.69	7.56

Referring to Figure 6.3-3, the control (A) and water (dH₂O) (B) surface samples revealed some particulate matter present on the substrate, which were found to be artefacts from the sample cutting process. The biocide incubation (C) showed a deposit similar to that observed on the PMMA surface.

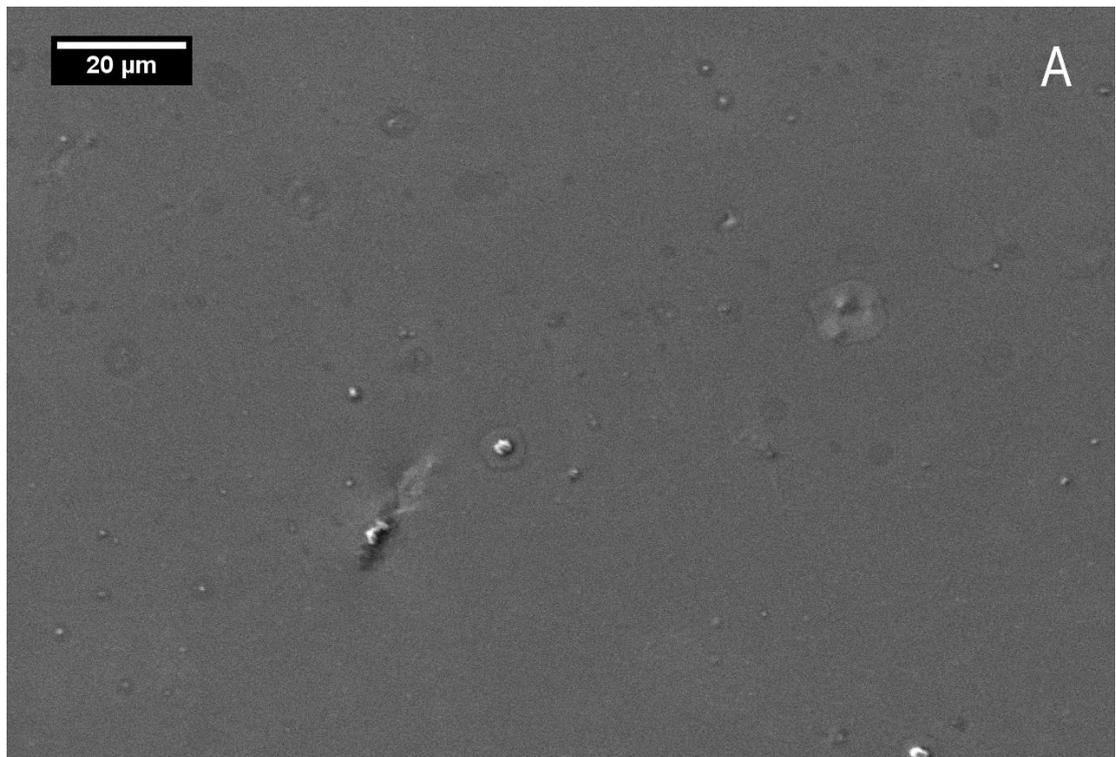


Figure 6-3. (A) SEM of sterile control ceramic healthcare surface sample.

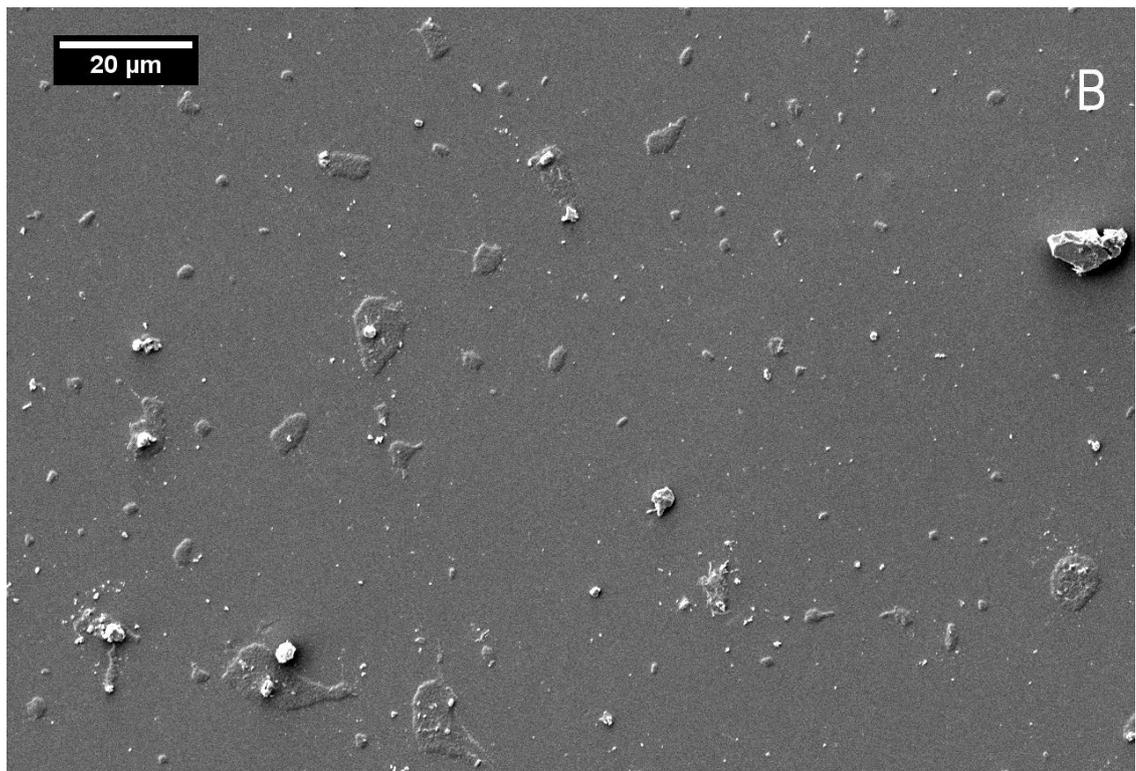


Figure 6-3. (B) SEM of ceramic healthcare surface sample wiped with water (dH₂O).

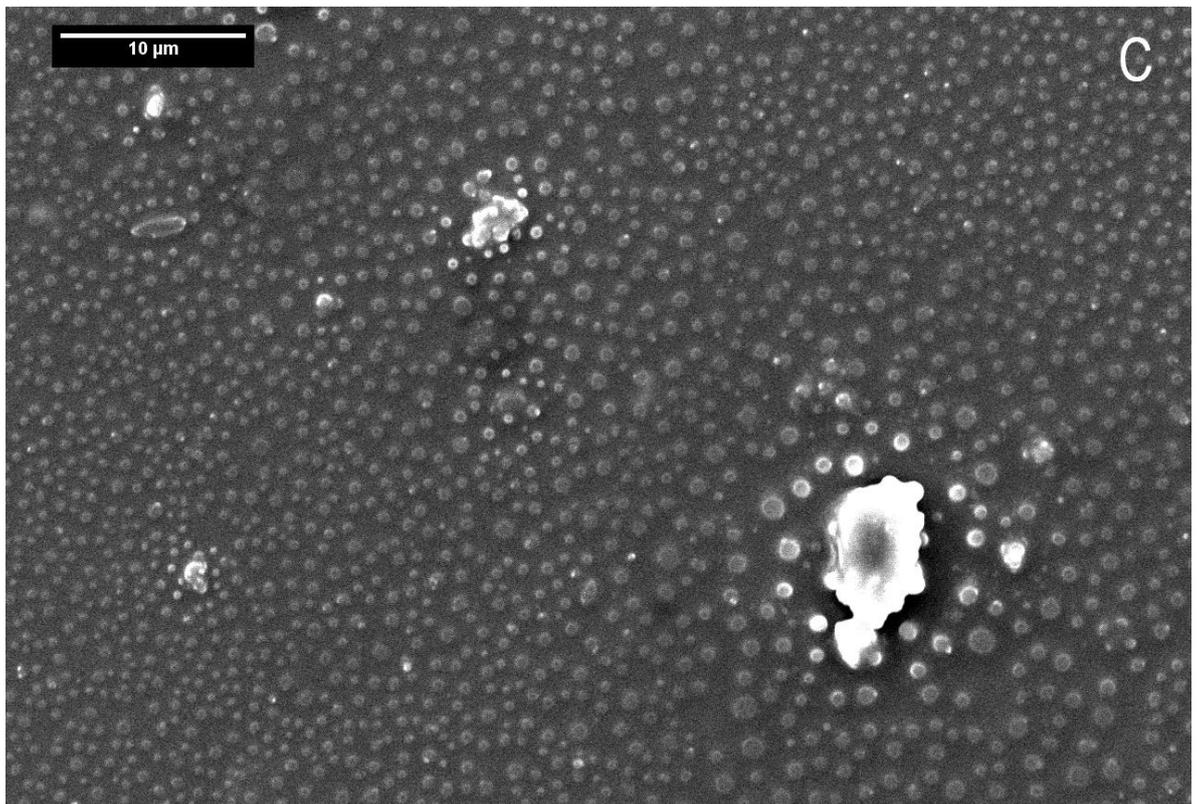


Figure 6-3. (C) SEM of Ceramic healthcare surface sample wiped with biocide.

The EDX analysis of the ceramic control sample indicated the presence of large amounts of oxygen and silicon, with aluminium, potassium, calcium and zinc also present (Table 6-3). Silicon dioxide (SiO_2) (338), aluminium oxide (Al_2O_3) (339), calcium carbonate (CaCO_3) (340), zinc oxide (ZnO) (341) and potassium (342) are present in many ceramics and ceramic glazes, which is consistent with data from the elemental analysis, as well as the large amount of oxygen.

The biocide sample showed a large decrease in oxygen and silicon content, smaller decreases in aluminium, potassium, calcium and zinc content compared with the control. Large amounts of carbon and a trace amount of chlorine were detected in the biocide sample, but not the control sample. This is significant as

these are attributable to the benzalkonium chloride portion of the biocide (see Figure 2-10).

The change in surface characteristics due to deposit from the biocide was most noticeable on steel (see Figure 6.3 C), but was also apparent on the other substrates. One possible reason for the deposit being more evident on steel was that the “brushed” surface finish limits the ability of fluid to bead on the material surface. As a result the biocide liquid will be concentrated into a smaller surface area, allowing a deposit to form. The steel sample’s surface texture, or “finish” is evident in the SEM micrographs (Figure 6.3-3). The steel samples also produced a higher contact angle value with water than the other surfaces (Table 6-5). It is known that surface imperfections such as grooves can accumulate chloride ions due to the biocide, which can potentially break down the chromium oxide passivation layer allowing rust to form (343, 344). This is important because the presence of chlorine is common in biocides, and was also a component of the biocidal formulation used in the present experiments, see EDX data in Table 6-2.

Table 6-3. EDX analysis of biocide vs control ceramic samples (Figure 6-3). n=3.

CERAMIC	Weight %	
	Biocide	Control
C	32.47	-
O	29.53	43.95
Na	4.59	-
Al	2.70	4.26
Si	17.34	31.97
Cl	0.48	-
K	2.48	3.84
Ca	4.36	6.74
Zn	6.05	9.23

The data shown in Table 6-1, Table 6-2 and Table 6-3 give the elemental composition of the surface being analysed and the proportion by weight of each element. There was a persistently large proportion of carbon; and smaller amounts of sodium and chlorine. The phosphorus present on the steel and PMMA biocide samples could be indicative of the sequesterant component of the biocide, as sodium tripolyphosphate is commonly used to provide this function. Grade 304 steel usually contains ~0.45% phosphorus (337), so detection of this element is also consistent with reported values in the literature.

The carbon detected in the biocide relates to the alkyl chains of the Pareth-5 and the benzalkonium chloride content. As expected PMMA was found to comprise only of carbon, oxygen and hydrogen, so any deposits from the biocide could only be practically detected by the presence of other elements.

To reiterate, the chlorine is present in each residual deposit is due to the benzalkonium chloride from the biocide, as this contains chlorine and has been shown to deposit on surfaces (152). The sodium present in all biocide samples is most likely to be from the sequesterant or stabiliser component of the biocide. There was deposition on surfaces from the biocidal solution, as shown by SEM and EDX analysis. A water only (dH₂O) wiping control was not used in this experiment as there was no evidence of deposition onto substrates. This means that any deposition seen on the surfaces treated with the biocide must therefore be from the non-aqueous components of the biocide.

6.3.2 Residual Antimicrobial Activity

The residual biocidal deposits left on the healthcare surfaces detected by the SEM and EDX analysis was subject to further investigation, to assess whether wiping a surface confers residual antimicrobial activity. The methodology is described in section 3.12.

Referring to Table 6-4, no statistically significant difference was observed in the number of bacteria present on the sterile surface and the same surface with a biocidal deposit ($p > 0.05$). This confirms the efficacy of the biocide during and immediately after delivery by the wipe, but once the biocide formulation dries upon the healthcare surface it does not provide a significant residual effect on the bacteria remaining on the surface. Note that this was observed even when the amount of biocide on the surface was artificially high, as in the present experiment.

Table 6-4. Residual antimicrobial activity of biocide. “+” value indicates increase in bacterial recovery versus control, “-“indicates a reduction in the number of bacteria recovered versus control. SEM indicates standard error of the mean. Steel (R) denotes rough steel samples. *n*=3.

Bacteria	Surface	Mean Recovery (CFU %)	SEm
<i>E. coli</i>	Ceramic	+8.17	11.33
<i>S. aureus</i>	Ceramic	-7.69	2.73
<i>E. faecalis</i>	Ceramic	+7.37	12.76
<i>E. coli</i>	Steel (R)	-3.03	7.47
<i>S. aureus</i>	Steel (R)	-5.26	2.90
<i>E. faecalis</i>	Steel (R)	-12.22	9.32
<i>E. coli</i>	PMMA	-2.62	15.25
<i>S. aureus</i>	PMMA	-4.29	14.05
<i>E. faecalis</i>	PMMA	-16.40	27.21

The biocide is an aqueous medium the bulk of which is absorbed and retained within the void volume of the wipe, depending on the surface energy of the constituent fibres. During use, compression of the wipe structure reduces its volume and a proportion of interstitially retained liquid will be released.

Therefore, the availability of benzalkonium chloride may be available when dried onto on the surface tiles, however it lacks the necessary liquid phase (and surfactants) to deliver it to the bacterial cells on the surface(s), unlike when delivered from a wipe.

6.3.3 Bacterial Removal and Surface Recontamination

Recontamination of typical healthcare surfaces by nonwoven wipes composed of either PP or lyocell was studied using three common bacterial pathogens based on the methodology described in section 3.11.

A qualitatively smoother steel surface (Table 6-5), with a roughness value between the ceramic and PMMA of identical chemical composition as the rough steel sample, was studied in order to decouple the effects of roughness and chemical composition on bacterial removal and recontamination of surfaces. Quantification of the differences in roughness were addressed as part of the experiment. The contact angle and wetting tension of surface samples were measured with water in both the sterile state and with the presence of a simulated organic load using the method described in section 3.16. The surface roughness of the sterile samples (R_a) was measured by AFM using the method in section 3.5.

No surface showed hydrophilic wetting behaviour, with all exhibiting hydrophobic contact angles of $>90^\circ$. The water contact angle of the wiping surface can be expected to change according to the bacterial contamination placed upon it and was measured before and after the surface was contaminated with the simulated organic load.

Table 6-5 indicates an increase in contact angle and a decrease in wetting tension as the level of organic load increased, for all surfaces. While this is expected given the chemical nature of BSA (protein), the salts in the PBS will also deposit on the surface, leading to an increase in the contact angle and

decreasing the wetting tension of the surfaces. Similar behaviour has been demonstrated in previous experiments (322).

Table 6-5. Contact angle, wetting tension and roughness of model surfaces. Steel (R) denotes rough steel samples, Steel (S) denotes smooth steel samples. R_a means that do not share a “grouping” letter are significantly different - ANOVA with a post hoc Tukey’s test ($p < 0.05$). $n=3$.

Surface	Organic load	Contact angle	Wetting tension	Roughness (R_a)	Tukey grouping - Roughness
PMMA	Clean	29.22°	63.54 mJ.m ⁻²	3.8 nm	A
	0.015 g.m ⁻² BSA	62.30°	33.84 mJ.m ⁻²	-	
Ceramic	Clean	18.43°	69.06 mJ.m ⁻²	14.8 nm	A
	0.015 g.m ⁻² BSA	38.37°	57.08 mJ.m ⁻²	-	
Steel (S)	Clean	38.61°	65.95 mJ.m ⁻²	128 nm	B
	0.015 g.m ⁻² BSA	64.20°	32.67 mJ.m ⁻²	-	
Steel (R)	Clean	60.49°	72.80 mJ.m ⁻²	583 nm	C
	0.015 g.m ⁻² BSA	63.90°	32.03 mJ.m ⁻²	-	

The R_a mean values were subject to ANOVA with a post hoc Tukey’s test (95% confidence interval). The PMMA and ceramic surfaces had very low surface roughness values, while those of the rough and smooth steel samples were significantly greater. The two steel samples were significantly different from each other in terms of surface roughness (R_a) (all $p < 0.05$).

Wiping experiments using nonwoven substrates manufactured in house (Chapter 3 section 3.1) using industrially applicable nonwoven manufacturing processes

were undertaken to determine the influence of model surface roughness on the bacterial removal and surface recontamination using “low organic load” conditions.

The unmodified, 60 g.m⁻² PP or lyocell wipes combined with biocide used in the part of the experiment displayed bacterial removal efficiencies in the range of 73-89% (Table 6-6 and Table 6-7). When all conditions were compared by a post-hoc Tukey test, no significant difference was observed in the dynamic removal of bacteria from the surface by wiping efficiency between bacterial groups, wipe substrate types or surface types. This suggests that the roughness of the contaminated surface has no significant effect on removal of bacteria by PP and lyocell nonwoven wipes impregnated with a biocidal liquid. The simulated wiping method (detailed in section 3.9) is rotational, so the wiping is across the striations in the sample surface.

This is supported by the work of Lee *et al.* (345) who compared removal by wipe from “smooth” plastic and “rough” metal surfaces, and found there was no significant difference ($p > 0.05$) in the reduction numbers for all microorganisms tested – including *E. coli* and *E. faecalis*.

In dynamic wiping forces are applied, which will assist transfer to the fibre surfaces, overcoming the adhesive forces between bacteria and surface (182). These forces will also be applied when wiping a surface with an already-used contaminated wipe, potentially facilitating the transfer and spread of pathogens over a wider area. Referring to Figure 6-4 and Figure 6-5, both wipe fibre types were found to repeatedly transfer bacteria over three consecutive uncontaminated surfaces.

For both Table 6-6 and Table 6-7 Steel (R) denotes rough steel samples, Steel (S) denotes smooth steel samples. *Average number of colony forming units on the nonwoven following wiping, calculated as the difference between bacteria remaining on the surface before and after wiping (190). SE_m is standard error of the mean. n=3. T1, T2 and T3 refer to the individual bacterial transfer percentages, and “total” is the sum of these 3 values.

Table 6-6. Removal of bacteria and recontamination of surfaces of different material compositions by a lyocell nonwoven wipe.

Bacteria	Surface	Removal (CFU %)	SEm	CFU on wipe*	T 1 (%)	T 2 (%)	T 3 (%)	Total (%)
<i>E. coli</i>	Ceramic	83.05	4.67	4900000	12.24	6.12	6.12	24.49
<i>S. aureus</i>	Ceramic	80.71	3.78	10250000	7.32	1.95	2.44	11.71
<i>E. faecalis</i>	Ceramic	73.33	6.83	2016667	12.40	3.31	1.65	17.36
<i>E. coli</i>	PMMA	84.62	6.65	7700000	8.44	1.08	0.22	9.74
<i>S. aureus</i>	PMMA	82.33	1.45	4116667	2.43	6.07	2.43	10.93
<i>E. faecalis</i>	PMMA	84.15	0.72	5133333	2.92	3.90	0.97	7.79
<i>E. coli</i>	Steel (S)	79.95	5.60	4916667	9.49	4.75	0.00	14.24
<i>S. aureus</i>	Steel (S)	84.34	3.82	8350000	8.18	5.79	1.80	15.77
<i>E. faecalis</i>	Steel (S)	87.30	1.73	3666667	10.91	9.09	0.45	20.45
<i>E. coli</i>	Steel (R)	82.88	4.96	4600000	21.74	2.17	3.26	27.17
<i>S. aureus</i>	Steel (R)	89.09	0.95	9800000	6.63	6.63	2.55	15.82
<i>E. faecalis</i>	Steel (R)	84.44	2.31	3800000	19.74	14.47	2.63	36.84

Table 6-7. Removal of bacteria and recontamination of surfaces by polypropylene nonwoven wipe.

Bacteria	Surface	Removal (CFU %)	SEm	CFU on wipe*	T 1 (%)	T 2 (%)	T 3 (%)	Total (%)
<i>E. coli</i>	Ceramic	84.58	6.22	3383333	11.82	4.43	1.48	17.73
<i>S. aureus</i>	Ceramic	89.17	5.38	12483333	16.42	0.80	2.40	19.63
<i>E. faecalis</i>	Ceramic	80.11	2.34	2483333	10.74	3.36	0.67	14.77
<i>E. coli</i>	PMMA	78.93	3.28	9866667	7.09	1.52	1.01	9.63
<i>S. aureus</i>	PMMA	79.51	3.19	7633333	4.59	3.93	3.28	11.79
<i>E. faecalis</i>	PMMA	78.18	5.96	4300000	3.49	3.49	4.65	11.63
<i>E. coli</i>	Steel (S)	82.73	2.92	4550000	9.16	4.76	2.20	16.12
<i>S. aureus</i>	Steel (S)	83.33	2.67	8250000	5.86	4.24	0.81	10.91
<i>E. faecalis</i>	Steel (S)	83.19	5.89	4700000	9.93	5.67	0.35	15.96
<i>E. coli</i>	Steel (R)	88.00	0.77	6600000	11.36	7.58	7.32	26.26
<i>S. aureus</i>	Steel (R)	69.81	5.52	6283333	15.92	14.32	3.98	34.22
<i>E. faecalis</i>	Steel (R)	79.59	1.77	3900000	16.67	8.97	5.13	30.77

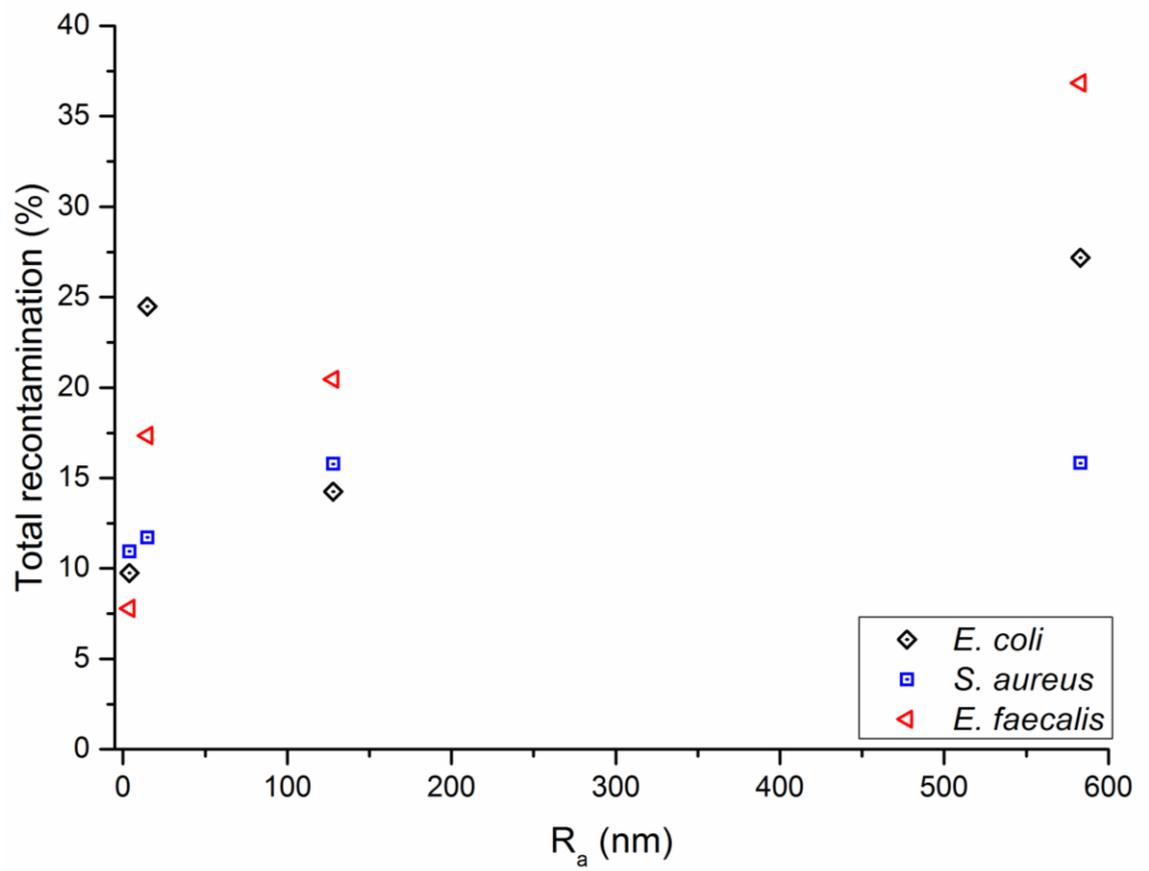


Figure 6-4. Total bacterial recontamination of surfaces of healthcare surfaces of different average roughness (R_a) by nonwoven lyocell wipes, calculated according to Chapter 3 section 3.11. $n=3$.

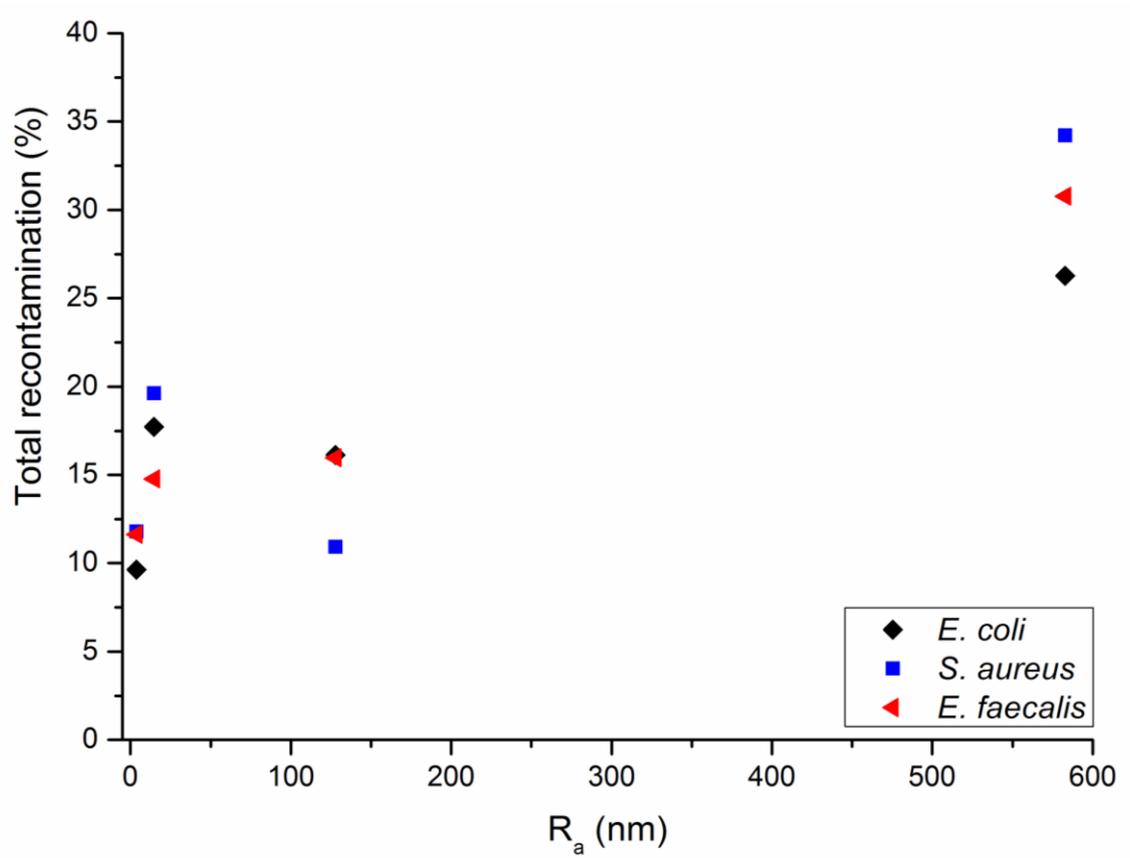


Figure 6-5. Total bacterial recontamination of healthcare surfaces of different average roughness (R_a) by PP nonwoven wipes, calculated according to Chapter 3 section 3.11. $n=3$.

The proportion of the total microorganism loading transferred from the wipes after each wiping cycle increased with surface roughness (R_a) when comparing two chemically identical surfaces of different surface roughness (R_a) - the (S) and (R) steel samples. Thus, it is apparent that an increase in surface roughness is likely to increase surface recontamination from an already bacterially contaminated wipe (Figure 6-4 and Figure 6-5).

More variation was also observed in the recontamination levels between replicates with the 60 g.m^{-2} untreated lyocell wipes used in this experiment (Table 6-6 and Figure 6-4) compared to the 60 g.m^{-2} PP wipes (Table 6-7 and Figure 6-5). The proportion of bacteria transferred was estimated based on the assumption that the difference in the number of colony forming units on the surface before and after wiping ended up either on or in the wipe. Owing to the nature of the recontamination calculation, statistical analysis could not be performed. This is because the total recontamination data is the sum of the three consecutive transfers such that T1, T2 and T3 are themselves the average transfer values for three replicates.

Considering the data obtained together with other reported studies that investigated the performance of commercial wipes (28, 346), it is apparent that the transfer of bacteria from used wipes to sterile surfaces is highly likely. Given that a minimum time period is needed for the biocide to be effective and that a used wipe may be used to wipe an uncontaminated surface within seconds of collecting bacteria from a contaminated surface, there are obvious implications in terms of controlling transmission of HCAs.

The biocide will allow re-contamination of surfaces if it does not have sufficient time to kill pathogens, because the wipe substrate itself cannot prevent re-

transfer to the contact surface. In the present experiment, the contact time was only 30 s at a wiping speed of 60 r.min⁻¹. This is also significant in practice because biocidal product claims are commonly based on suspension tests, where the contact time is of the order of 5 min rather than seconds (96).

In the present study, less bacteria was found to transfer than has been reported in other published work using detergent wipes (189). Given that the biocide used herein did not have sufficient contact time to maximise its effectiveness, the improved performance may be due to the chemical composition of the biocide being optimised for delivery from a wipe and a potentially more rapid kill time than biocides reported in previous studies. This could also explain why no significant residual antimicrobial activity was observed on the surfaces after deposition of the biocide by the wipe. It could also be a function of the wipe design parameters selected to produce the experimental wipes in the present study.

Wiping with a typical wet wipe biocide does not appear to confer residual antimicrobial activity once applied to a target surface. This leads to the recontamination of previously sterile surfaces irrespective of the roughness or chemical composition, therefore hospital hygiene practices should be updated to reflect this. Based on the data in the present experiments, a regular disinfection of surfaces can be recommended with a “one wipe, one surface” policy, which should be implemented and rigorously enforced.

6.4 Summary

Removal of pathogenic bacteria from abiotic surfaces using nonwoven wipes in combination with a biocidal liquid provides a means of both reducing the bacterial contamination on the surface, but also transferring it to another surface, risking transmission of potential HCAs. Previous studies have investigated bacterial removal from and recontamination of surfaces with reference to commercially sourced wipe substrates, but not with respect to the properties of the constituent fibres or surface morphology.

Assessment of residual antimicrobial activity on surfaces wiped by substrates containing a biocidal liquid was also undertaken. It was found that there was no significant difference in removal of *E. coli*, *S. aureus* and *E. faecalis* from PMMA, ceramic or metal surfaces by either 100% cellulose (lyocell) or 100% polypropylene nonwoven wipes ($p < 0.05$).

Transfer of bacteria from an already bacterially contaminated wipe to a sterile surface was found with to occur from both wipe types (i.e. PP and lyocell) across all surfaces. The magnitude of the recontamination is found to be influenced by the roughness of the surface, such that as the surface roughness increases, so too does the propensity for it to be re-contaminated by the wipe.

No significant residual antimicrobial activity was observed following deposition of the biocide on any of the investigated surfaces after wiping ($p < 0.05$), confirming that there is no substantial residual biocidal activity following a wiping cycle with a biocide-loaded nonwoven wipe.

Wiping of surfaces using a wipe that has already been contaminated with bacteria during a prior wipe cycle is highly likely to result in re-contamination of the contact

surface. There is therefore substantial scope to engineer improvements to wipe substrate composition and structure to improve retention and/or adhesion of bacteria residing on the fibre surfaces. It is conceivable that in the same way that a liquid medium assists the transfer of bacteria to fibre surfaces during wiping of a surface, that the reverse occurs when a new surface is wiped, i.e. the biocidal liquid acts as a transport medium.

Although the biocide-impregnated wipes remove some of the bacterial burden from healthcare surfaces of differing composition and physical characteristics, it is clear they should be used with caution since improper use could lead to the spread of pathogen by recontamination of surfaces of otherwise uncontaminated surfaces during wiping. This is particularly important if the efficacy of the biocide is of the order of minutes rather than seconds.

Chapter 7

Conclusions

7.1 General Conclusions

Effective removal of bacteria from healthcare surfaces during wiping is a common strategy to reduce the risk of HCAI transmission. Despite the apparent simplicity of this approach there have been relatively few systematic studies of the underlying mechanisms and important design factors governing the efficacy of bacterial removal. Comparative studies using commercially available wipes have gone some way to elucidating important issues, but interpretation of these studies is complicated by the fact that the structure and properties of the wipe substrates were not controlled. Understandably, there has been a greater focus on the performance of different biocides used in conjunction with wipes.

The purpose of this work was to develop a greater understanding of the factors controlling bacterial removal efficiency as well as to determine the importance of various parameters relating to the design of wipe substrates.

Following discussion of the key challenges in Chapter 1 and a critical review of key technical parameters and considerations in Chapter 2 relevant to the development of the experimental work, Chapter 3 focused on the development of methodology relating to the manufacture, testing and characterisation of the wipe substrates, as well as the healthcare surfaces studied.

The experimental work in Chapter 4 revealed that wiping in the dry state in static conditions of adpression without the presence of a biocidal fluid can still influence bacterial removal efficiency, albeit to a comparatively small extent. The relative surface energies of the fibres and the healthcare surface itself or the contaminants that reside upon it, influence dry wiping efficiency, as well as the surface roughness of the fibre, but the degree depends on the type of bacterium. Reduction of the surface energy of hygroscopic lyocell nonwoven fibres by C₂F₆ plasma treatment was found to increase the bacterial removal efficiency of *E. coli*, but there was no significant effect on the elimination of Gram-positive bacteria (*S. aureus* and *E. faecalis*). This differential response in bacterial removal efficiency suggests that modification of the surface energy of a nonwoven can modulate the removal of specific bacteria, providing that the bacterial cell membrane conformation can adapt to the changes in surface nano-roughness caused by the plasma treatment.

Dynamic wiping of surfaces removes significantly more bacteria than adpressive wiping alone, for all types of bacteria and wipe substrate compositions. Interestingly, under dynamic conditions, substantial bacterial removal can be achieved in dry conditions without a biocidal liquid. The implications of this are important, since overuse of antibacterial chemistry in the healthcare environment is a source of risk in relation to degradation of surfaces as well as building microbial resistance.

Clearly, the design and production of wipes with optimal bacterial removal efficiency is key to reducing spread of HCAs and in addition to the biocide, the design of the wipe substrate and the conditions of its use may be important considerations. To explore these factors, Chapter 5 adopted an orthogonal array

experimental strategy and Taguchi analysis to determine that the optimum wipe basis weight for removal of *E. coli*, *S. aureus* and *E. faecalis* by both lyocell and PP wipes was 150 g.m⁻². In other words, regardless of wipe polymer composition, it was advantageous to select a heavier weight nonwoven substrate. This is substantially higher than the basis weight of many surface wipes currently used in healthcare environments, which are more typically in the range 45-90 g.m⁻². As a practical intervention, cleaning efficiencies could therefore be improved by specifying wipes of higher basis weights, with or without the use of a biocide.

The optimum characteristics of lyocell nonwoven wipes in terms of the absolute quantity of *E. coli*, *S. aureus* and *E. faecalis* removed were a 150 g.m⁻² basis weight, a high wiping pressure of 13.80 kN.m⁻² and the addition of the biocidal liquid. Using a PP nonwoven wipe, the same conditions applied for the removal of *S. aureus* but for *E. coli* and *S. aureus*, optimal bacterial removal necessitates a 150 g.m⁻² basis weight wipe with a low wiping pressure of only 4.68 kN.m⁻² when used with biocidal liquid. The addition of a biocidal liquid was found to be the main influencing bacterial removal, as determined both by the orthogonal array and statistical analysis, ($p < 0.05$).

The data in Chapter 5 suggest that best practice for bacterial removal from surfaces should involve use of heavier basis weight wipes composed of regenerated cellulose fibres impregnated with a biocide. In such cellulosic wipes, as heavier wiping pressure as possible should be applied to maximise the bacterial removal efficiency. Selection of a hydrophobic substrate such as PP, necessitates a lower wiping pressure to achieve optimal bacterial removal efficiency, but the absolute values of removal were found to be greater in the cellulosic wipes studied herein.

Therefore, from a practical viewpoint, recontamination of abiotic surfaces or wiped surfaces during multiple wiping cycles is a concern and the extent to which this may be modulated by the design of the wipe and the nature of the healthcare surface is important to understand. Considering the data reported in Chapter 6, it was found that there was no significant difference in removal of *E. coli*, *S. aureus* and *E. faecalis* from plastic (PMMA), ceramic or metal surfaces by either 100% cellulose (lyocell) or 100% polypropylene nonwoven wipes ($p < 0.05$). This may reflect the fact that in all cases, the fibres in the wipe are interacting directly with the bacterial contaminants supported on each surface. This interface therefore appears to be the major factor governing the chemical and surface energy interactions between the fibres and the contact surface. Furthermore, the roughness of the healthcare surface was found to be important in terms of the degree of bacterial contamination likely to be transferred back to the surface from a pre-contaminated wipe. The rougher the surface, the more likely bacterial recontamination was found to be.

Assessment of the potential residual antimicrobial activity conferred to surfaces by wiping with biocide-loaded wipes was also undertaken. No significant residual antimicrobial activity was identified resulting from prior deposition of the biocide on a healthcare surface after wiping ($p < 0.05$), indicating that unless bacteria are removed by the wipe such that they can be acted upon by the biocide in the wipe, bacteria are unlikely to be killed on the surface itself. In short there was no evidence of residual biocidal activity on the model healthcare surfaces following wiping with a biocide.

Considering the observations regarding recontamination of surfaces that are wiped multiple times with the same wipe and the lack of a residual effect left on

the surface after wiping, although the use of biocidal wipes removes some of the bacterial burden from healthcare surfaces wipes need to be employed with caution as improper use could lead to the spread of pathogens.

Collectively, this work provides a new insight into the decontamination of solid healthcare surfaces. The benefits of selecting higher basis weight wipes, combined with the efficacy of dry wiping under dynamic conditions are particularly valuable findings that could be practically applied to wipe design. It appears that by providing greater solid surface at the wipe-contaminated surface interface, more effective bacterial removal can be achieved.

7.2 Recommendations for Further Work

Logical development of the research presented in this thesis can be envisaged in a number of areas. Many pathogenic bacteria and some of the most common were examined during the work reported herein, specifically, *E. coli*, *S. aureus* and *E. faecalis*. *Pseudomonas aeruginosa* was also examined in preliminary testing, however it exhibited swarming behaviour when inoculated on agar plates, making cell counts impossible. *Clostridium difficile* and *Acinetobacter baumannii* represent two other common organisms used in healthcare settings, which would generate useful data if included in further wiping experiments following the procedures described in this work.

A variety of bonding and consolidation methods for nonwoven wipe substrate production are discussed in section 2.4.3. The effects of methods of manufacture are to modulate the structure of the wipe on a global and local level. Further modifications to substrate structure and their effects on bacterial removal

efficiency would be valuable to study, as the academic literature provides little insight in to the likely results.

Fundamental studies of the molecular interactions between a bacterium and surfaces were also contemplated during the present work, but could not be pursued due to the lengthy methodological development work. Such work would focus on an analysis of binding between a polymer surface and a bacterium. Microscale thermophoresis (MST) is a technique for analysing the interaction of biomolecules, to provide K_d values that characterise these interactions. Bacterial cell models were evaluated with micronized polymers, and the initial results proved promising. However more optimisation is needed. AFM performed with immobilised bacteria on the stylus tip can be used to gain similar interaction data. The results from either of the MST or AFM experiments would then be compared with data gathered from wiping experiments, to explore the possibility of correlation between the micro- and macro-scale results.

References

1. Scotti, M.T. et al. Nosocomial Infections: An Increasing Challenge to Medicinal Chemistry. *Current Topics in Medicinal Chemistry*. 2014, **14**(1), pp.2-3.
2. Kilgore, M.L. et al. The costs of nosocomial infections. *Medical Care*. 2008, **46**(1), pp.101-104.
3. Katsikogianni, M. and Missirlis, Y.F. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. *Eur. Cells Mater*. 2004, **8**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.37-57.
4. Raza, S. *SPICe Briefing - Healthcare Associated Infections*. Scotland: Scottish Parliament, 2011.
5. Inweregbu, K. et al. Nosocomial infections. *Continuing Education in Anaesthesia, Critical Care & Pain*. 2005, **5**(1), pp.14-17.
6. ECDC. *Healthcare-associated infections*. [Online]. 2015. [Accessed 16/03/2016]. Available from:
http://ecdc.europa.eu/en/healthtopics/Healthcare-associated_infections/Pages/index.aspx
7. Allegranzi, B. *Report on the Burden of Endemic Health Care-Associated Infection Worldwide*. WHO, 2011.
8. Joshi, R. et al. Tuberculosis among Health-Care Workers in Low- and Middle-Income Countries: A Systematic Review. *PLoS Med*. 2006, **3**(12), p.e494.
9. Hughes, A.J.F. et al. Prevalence of Nosocomial Infection and Antibiotic Use at a University Medical Center in Malaysia • *Infection Control and Hospital Epidemiology*. 2005, **26**(1), pp.100-104.

10. Duffy, L. Care of immunocompromised patients in hospital. 2009, (0029-6570 (Print)).
11. Hunter, P.R. and Nichols, G. Epidemiology and Clinical Features of Cryptosporidium Infection in Immunocompromised Patients. *Clinical Microbiology Reviews*. 2002, **15**(1), pp.145-154.
12. HPA. *English National Point Prevalence Survey on Healthcare-associated Infections and Antimicrobial Use, 2011*. May 2012 ed. London, 2012.
13. Linden, P.K. Approach to the Immunocompromised Host with Infection in the Intensive Care Unit. *Infectious Disease Clinics of North America*. 2009, **23**(3), pp.535-556.
14. Oakley, M. Central Venous Access Devices: Care and Management. *British Journal of Anaesthetic and Recovery Nursing*. 2006, **7**(1-2), pp.16-16.
15. Russotto, V. et al. Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *Journal of Intensive Care*. 2015, **3**, p.54.
16. Boone, S.A. and Gerba, C.P. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl. Environ. Microbiol.* 2007, **73**(6), pp.1687-1696.
17. Tuladhar, E. et al. Residual viral and bacterial contamination of surfaces after cleaning and disinfection. *Appl. Environ. Microbiol.* 2012, **78**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.7769-7775.
18. Oelberg, D.G. et al. Detection of Pathogen Transmission in Neonatal Nurseries Using DNA Markers as Surrogate Indicators. *Pediatrics*. 2000, **105**(2), pp.311-315.
19. Mary K. Hayden, M.D. et al. Risk of Hand or Glove Contamination After Contact With Patients Colonized With Vancomycin-Resistant

- Enterococcus or the Colonized Patients' Environment • *Infection Control and Hospital Epidemiology*. 2008, **29**(2), pp.149-154.
20. Drees, M. et al. Prior Environmental Contamination Increases the Risk of Acquisition of Vancomycin-Resistant Enterococci. *Clinical Infectious Diseases*. 2008, **46**(5), pp.678-685.
 21. Datta, R. et al. Environmental cleaning intervention and risk of acquiring multidrug-resistant organisms from prior room occupants. *Archives of Internal Medicine*. 2011, **171**(6), pp.491-494.
 22. Rampling, A. et al. Evidence that hospital hygiene is important in the control of methicillin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection*. 2001, **49**(2), pp.109-116.
 23. Dunne, W.M. Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clinical Microbiology Reviews*. 2002, **15**(2), pp.155-166.
 24. Tuson, H.H. and Weibel, D.B. Bacteria-surface interactions. *Soft Matter*. 2013, **9**(17), pp.4368-4380.
 25. Mah, T.-F.C. and O'Toole, G.A. Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology*. 2001, **9**(1), pp.34-39.
 26. Dodds, M.G. et al. Modeling biofilm antimicrobial resistance. *Biotechnol. Bioeng.* 2000, **68**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.456-465.
 27. Jerassy, Z. et al. Prospective hospital-wide studies of 505 patients with nosocomial bacteraemia in 1997 and 2002. *Journal of Hospital Infection*. 2006, **62**(2), pp.230-236.
 28. Williams, G.J. et al. The development of a new three-step protocol to determine the efficacy of disinfectant wipes on surfaces contaminated with *Staphylococcus aureus*. *Journal of Hospital Infection*. 2007, **67**(4), pp.329-335.
 29. Team, I.P.a.C. *Decontamination – Cleaning, Disinfection and Sterilisation* [Online]. 2015. [Accessed 03/04/2017]. Available from:

<http://www.bcpft.nhs.uk/documents/policies/i/881-infection-prevention-and-control-assurance-sop-7-decontamination/file>

30. Dvorak, G. *Disinfection 101* [Online]. 2008. [Accessed 03/04/2017]. Available from: <http://www.cfsph.iastate.edu/Disinfection/Assets/Disinfection101.pdf>
31. Kirk Huslage, R.B.M. et al. A Quantitative Approach to Defining “High-Touch” Surfaces in Hospitals. *Infection Control and Hospital Epidemiology*. 2010, **31**(8), pp.850-853.
32. Sattar, S.A. et al. Disinfectant wipes are appropriate to control microbial bioburden from surfaces: use of a new ASTM standard test protocol to demonstrate efficacy. *Journal of Hospital Infection*. 2015, **91**(4), pp.319-325.
33. Knapp, L. et al. Bacterial resistance to microbicides: Development of a predictive protocol. *Applied and Environmental Microbiology*. 2015.
34. Lim, S.-H. and Hudson, S.M. Application of a fiber-reactive chitosan derivative to cotton fabric as an antimicrobial textile finish. *Carbohydrate Polymers*. 2004, **56**(2), pp.227-234.
35. Finlay, B.J. and Clarke, K.J. Ubiquitous dispersal of microbial species. *Nature*. 1999, **400**(6747), pp.828-828.
36. Sathyabama, S. et al. Friendly pathogens: prevent or provoke autoimmunity. *Critical Reviews in Microbiology*. 2014, **40**(3), pp.273-280.
37. Taur, Y. and Pamer, E.G. The Intestinal Microbiota and Susceptibility to Infection in Immunocompromised Patients. *Current Opinion in Infectious Diseases*. 2013, **26**(4), pp.332-337.
38. Woese, C.R. et al. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*. 1990, **87**(12), pp.4576-4579.

39. Ochman, H. and Wilson, A.C. Evolution in Bacteria - Evidence for a Universal Substitution Rate in Cellular Genomes. *Journal of Molecular Evolution*. 1987, **26**(1-2), pp.74-86.
40. Peterson, J.W. Bacterial Pathogenesis. In: Baron, S. ed. *Medical Microbiology*. 4 ed. Galveston (TX): University of Texas Medical Branch at Galveston, 1996.
41. Sydnor, E.R.M. and Perl, T.M. Hospital Epidemiology and Infection Control in Acute-Care Settings. *Clinical Microbiology Reviews*. 2011, **24**(1), pp.141-173.
42. Edwards, J.R. et al. National Healthcare Safety Network (NHSN) report: Data summary for 2006 through 2008, issued December 2009. *American Journal of Infection Control*. 2009, **37**(10), pp.783-805.
43. Abreu, A.C. et al. Current and emergent strategies for disinfection of hospital environments. *Journal of Antimicrobial Chemotherapy*. 2013, **68**(12), pp.2718-2732.
44. Neely, A.N. and Orloff, M.M. Survival of some medically important fungi on hospital fabrics and plastics. *Journal of Clinical Microbiology*. 2001, **39**(9), pp.3360-3361.
45. Ahmadi, F. et al. Effect of Silver Nanoparticles on Common Bacteria in Hospital Surfaces. *Jundishapur J Microbiol*. 2013, **6**(3), pp.209-14.
46. Rangel-Frausto, M.S. et al. An experimental model for study of *Candida* survival and transmission in human volunteers. *European Journal of Clinical Microbiology and Infectious Diseases*. 1994, **13**(7), pp.590-595.
47. Weber, D.J. et al. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: Norovirus, *Clostridium difficile*, and *Acinetobacter* species. *American Journal of Infection Control*. 2010, **38**(5, Supplement), pp.S25-S33.

48. Kramer, A. et al. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *Bmc Infectious Diseases*. 2006, **6**.
49. Neely, A.N. and Maley, M.P. Survival of enterococci and staphylococci on hospital fabrics and plastic. *Journal of Clinical Microbiology*. 2000, **38**(2), pp.724-726.
50. Stewart, P.S. Mechanisms of antibiotic resistance in bacterial biofilms. *International Journal of Medical Microbiology*. 2002, **292**(2), pp.107-113.
51. Popescu, A. and Doyle, R.J. The Gram stain after more than a century. *Biotechnic & Histochemistry*. 1996, **71**(3), pp.145-151.
52. Denyer, S.P. and Maillard, J.Y. Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria. *Soc. Appl. Microbiol. Symp. Ser.* 2002, **31**(Antibiotic and Biocide Resistance in Bacteria), pp.35S-45S.
53. Gupta, R.S. Protein phylogenies and signature sequences: A reappraisal of evolutionary relationships among archaeobacteria, eubacteria, and eukaryotes. *Microbiology and Molecular Biology Reviews*. 1998, **62**(4), pp.1435-+.
54. Moyes, R.B. et al. Differential Staining of Bacteria: Gram Stain. In: *Current Protocols in Microbiology*. John Wiley & Sons, Inc., 2005.
55. Lopes, S.P. et al. Role of planktonic and sessile extracellular metabolic byproducts on *Pseudomonas aeruginosa* and *Escherichia coli* intra and interspecies relationships. *Journal of Industrial Microbiology & Biotechnology*. 2011, **38**(1), pp.133-140.
56. Garrett, T.R. et al. Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*. 2008, **18**(9), pp.1049-1056.
57. Murray, P.R. *Gram positive and Gram negative bacteria*. [Online]. n/d. [Accessed 01/02].

58. Russell, A.D. Plasmids and bacterial resistance to biocides. *J. Appl. Microbiol.* 1997, **83**(2), pp.155-165.
59. van den Ent, F. et al. Bacterial actin MreB assembles in complex with cell shape protein RodZ. *Embo Journal.* 2010, **29**(6), pp.1081-1090.
60. Madigan, M.T. and Martinko, J.M. *Brock Biology of Microorganisms.* 11 ed. Upper Saddle River, NJ, USA: Prentice Hall, 2006.
61. McDonnell, G. and Russell, A.D. Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.* 1999, **12**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.147-179.
62. Russell, A.D. et al. Possible Link between Bacterial Resistance and Use of Antibiotics and Biocides. *Antimicrobial Agents and Chemotherapy.* 1998, **42**(8), pp.2151-2151.
63. NCBI. Spore-forming bacterium. *European Nucleotide Archive.* 2013.
64. Nicholson, W.L. et al. Resistance of Bacillus Endospores to Extreme Terrestrial and Extraterrestrial Environments. *Microbiology and Molecular Biology Reviews.* 2000, **64**(3), pp.548-572.
65. Setlow, B. and Setlow, P. Role of DNA repair in Bacillus subtilis spore resistance. *Journal of Bacteriology.* 1996, **178**(12), pp.3486-95.
66. Wilson, K.H. Efficiency of various bile salt preparations for stimulation of Clostridium difficile spore germination. *Journal of Clinical Microbiology.* 1983, **18**(4), pp.1017-1019.
67. Grass, G. et al. Metallic Copper as an Antimicrobial Surface. *Applied and Environmental Microbiology.* 2011, **77**(5), pp.1541-1547.
68. Leggett, M.J. et al. Bacterial spore structures and their protective role in biocide resistance. *Journal of Applied Microbiology.* 2012, **113**(3), pp.485-498.
69. Gan, L. et al. Molecular organization of Gram-negative peptidoglycan. *Proceedings of the National Academy of Sciences.* 2008, **105**(48), pp.18953-18957.

70. Slama, T.G. Gram-negative antibiotic resistance: there is a price to pay. *Critical Care*. 2008, **12**.
71. Salton MRJ, K.K. Structure. In: *Medical Microbiology*. 4 ed. University of Texas Medical Branch at Galveston: Galveston (TX), 1996.
72. Takeda, K. and Akira, S. Toll-like receptors in innate immunity. *International Immunology*. 2005, **17**(1), pp.1-14.
73. Hsieh, W.-S. et al. Types and Prevalence of Carbapenem-Resistant *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* Complex in Northern Taiwan. *Antimicrobial Agents and Chemotherapy*. 2013.
74. Eliopoulos, G.M. et al. *Acinetobacter baumannii*: Epidemiology, Antimicrobial Resistance, and Treatment Options. *Clinical Infectious Diseases*. 2008, **46**(8), pp.1254-1263.
75. Rutala, W.A.W., D. J. *Guideline for Disinfection and Sterilization in Healthcare Facilities*. [Online]. 2008. [Accessed 01/02]. Available from: http://www.cdc.gov/hicpac/Disinfection_Sterilization/acknowledg.html
76. Corrigan, K.M. et al. Association of *Acinetobacter* species with contact lens-induced adverse responses. *Cornea*. 2001, **20**(5), pp.463-466.
77. Weinstein, R.A. et al. Overview of Nosocomial Infections Caused by Gram-Negative Bacilli. *Clinical Infectious Diseases*. 2005, **41**(6), pp.848-854.
78. Lister, P.D. et al. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. *Clinical Microbiology Reviews*. 2009, **22**(4), p.582.
79. Hanberger, H. et al. Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. *Jama-Journal of the American Medical Association*. 1999, **281**(1), pp.67-71.
80. Vollmer, W. et al. Peptidoglycan structure and architecture. *Fems Microbiology Reviews*. 2008, **32**(2), pp.149-167.

81. Egan, A.J.F. et al. Outer-membrane lipoprotein LpoB spans the periplasm to stimulate the peptidoglycan synthase PBP1B. *Proc. Natl. Acad. Sci. U. S. A.* 2014, **111**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.8197-8202.
82. Vollmer, W. Structural variation in the glycan strands of bacterial peptidoglycan. *Fems Microbiology Reviews.* 2008, **32**(2), pp.287-306.
83. van Heijenoort, J. Assembly of the monomer unit of bacterial peptidoglycan. *Cellular and Molecular Life Sciences CMLS.* 1998, **54**(4), pp.300-304.
84. Schleifer, K.H. and Kandler, O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* 1972, **36**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.407-77.
85. Raymond, J.B. et al. A method for the enzymatic synthesis and HPLC purification of the peptidoglycan precursor UDP-N-acetylmuramic acid. *FEMS Microbiol. Lett.* 2003, **229**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.83-89.
86. van Heijenoort, J. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology.* 2001, **11**(3), pp.25R-36R.
87. Spiro, R.G. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. *Glycobiology.* 2002, **12**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.43R-56R.
88. Csonka, L.N. and Hanson, A.D. Prokaryotic osmoregulation: genetics and physiology. *Annu. Rev. Microbiol.* 1991, **45**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.569-606.
89. Gumbart, J.C. et al. Escherichia coli peptidoglycan structure and mechanics as predicted by atomic-scale simulations. *PLoS Comput. Biol.*

- 2014, **10**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.e1003475/1-e1003475/10, 10 pp.
90. Sharif, S. et al. Staphylococcus aureus Peptidoglycan Tertiary Structure from Carbon-13 Spin Diffusion. *Journal of the American Chemical Society*. 2009, **131**(20), pp.7023-7030.
 91. Caparros, M. et al. Effect of D-amino acids on structure and synthesis of peptidoglycan in Escherichia coli. *J. Bacteriol.* 1992, **174**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.5549-59.
 92. Lam, H. et al. D-Amino acids govern stationary phase cell wall remodeling in bacteria. *Science (Washington, DC, U. S.)*. 2009, **325**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.1552-1555.
 93. Moody, J. et al. Antimicrobial Stewardship: A Collaborative Partnership between Infection Preventionists and Health Care Epidemiologists. *Infection Control and Hospital Epidemiology*. 2012, **33**(4), pp.328-330.
 94. Dettenkofer, M. and Block, C. Hospital disinfection: efficacy and safety issues. *Current Opinion in Infectious Diseases*. 2005, **18**(4), pp.320-325.
 95. Dettenkofer, M. and Spencer, R.C. Importance of environmental decontamination – a critical view. *Journal of Hospital Infection*. 2007, **65**(0), pp.55-57.
 96. Sattar, S.A. and Maillard, J.Y. The crucial role of wiping in decontamination of high-touch environmental surfaces: Review of current status and directions for the future. *American Journal of Infection Control*. 2013, **41**(5), pp.S97-S104.
 97. Vigeant, M.A.S. et al. Reversible and irreversible adhesion of motile Escherichia coli cells analyzed by total internal reflection aqueous fluorescence microscopy. *Applied and Environmental Microbiology*. 2002, **68**(6), pp.2794-2801.

98. Bos, R. et al. Physico-chemistry of initial microbial adhesive interactions – its mechanisms and methods for study. *Fems Microbiology Reviews*. 1999, **23**(2), pp.179-230.
99. Boks, N.P. et al. Bond-Strengthening in Staphylococcal Adhesion to Hydrophilic and Hydrophobic Surfaces Using Atomic Force Microscopy. *Langmuir*. 2008, **24**(22), pp.12990-12994.
100. Schneider, R.P. and Marshall, K.C. Retention of the Gramnegative marine bacterium SW8 on surfaces — effects of microbial physiology, substratum nature and conditioning films. *Colloids and Surfaces B: Biointerfaces*. 1994, **2**(4), pp.387-396.
101. Palmer, R.J. and Stoodley, P. Biofilms 2007: Broadened Horizons and New Emphases. *Journal of Bacteriology*. 2007, **189**(22), pp.7948-7960.
102. Boks, N.P. et al. Residence time dependent desorption of *Staphylococcus epidermidis* from hydrophobic and hydrophilic substrata. *Colloids and Surfaces B: Biointerfaces*. 2008, **67**(2), pp.276-278.
103. Barnhart, M.M. and Chapman, M.R. Curli biogenesis and function. In: *Annual Review of Microbiology*. Palo Alto: Annual Reviews, 2006, pp.131-147.
104. Vidal, O. et al. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: Involvement of a new *ompR* allele that increases curli expression. *Journal of Bacteriology*. 1998, **180**(9), pp.2442-2449.
105. Telford, J.L. et al. Pili in Gram-positive pathogens. *Nature Reviews Microbiology*. 2006, **4**(7), pp.509-519.
106. Murphy, T.F. and Kirkham, C. Biofilm formation by nontypeable *Haemophilus influenzae*: strain variability, outer membrane antigen expression and role of pili. *Bmc Microbiology*. 2002, **2**.

107. Pratt, L.A. and Kolter, R. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Molecular Microbiology*. 1998, **30**(2), pp.285-293.
108. Kierck, K. and Watnick, P.I. Environmental determinants of *Vibrio cholerae* biofilm development. *Applied and Environmental Microbiology*. 2003, **69**(9), pp.5079-5088.
109. Wang, Q. et al. Sensing wetness: a new role for the bacterial flagellum. *The EMBO Journal*. 2005, **24**(11), pp.2034-2042.
110. Lemon, K.P. et al. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *Journal of Bacteriology*. 2007, **189**(12), pp.4418-4424.
111. Klausen, M. et al. Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. *Molecular Microbiology*. 2003, **48**(6), pp.1511-1524.
112. Misselwitz, B. et al. Near Surface Swimming of *Salmonella* Typhimurium Explains Target-Site Selection and Cooperative Invasion. *PLoS Pathog.* 2012, **8**(7), p.e1002810.
113. Busscher, H.J. et al. Interfacial re-arrangement in initial microbial adhesion to surfaces. *Current Opinion in Colloid & Interface Science*. 2010, **15**(6), pp.510-517.
114. Busscher, H.J. and van der Mei, H.C. How Do Bacteria Know They Are on a Surface and Regulate Their Response to an Adhering State? *PLoS Pathog.* 2012, **8**(1), p.e1002440.
115. Chao, Y.Q. and Zhang, T. Probing Roles of Lipopolysaccharide, Type 1 Fimbria, and Colanic Acid in the Attachment of *Escherichia coli* Strains on Inert Surfaces. *Langmuir*. 2011, **27**(18), pp.11545-11553.
116. Renner, L.D. and Weibel, D.B. Physicochemical regulation of biofilm formation. *Mrs Bulletin*. 2011, **36**(5), pp.347-355.
117. Absolom, D.R. et al. Surface thermodynamics of bacterial adhesion. *Applied and Environmental Microbiology*. 1983, **46**(1), pp.90-97.

118. Li, Y.H. and Tian, X.L. Quorum Sensing and Bacterial Social Interactions in Biofilms. *Sensors*. 2012, **12**(3), pp.2519-2538.
119. Davey, M.E. and O'Toole, G.A. Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews*. 2000, **64**(4), p.847.
120. O'Toole, G. et al. Biofilm formation as microbial development. *Annual Review of Microbiology*. 2000, **54**, pp.49-79.
121. Bouwer, E.J. and Zehnder, A.J.B. Bioremediation of Organic Compounds - Putting Microbial-Metabolism to Work. *Trends in Biotechnology*. 1993, **11**(8), pp.360-367.
122. Laird, K. et al. Reduction of surface contamination and biofilms of *Enterococcus* sp. and *Staphylococcus aureus* using a citrus-based vapour. *Journal of Hospital Infection*. 2012, **80**(1), pp.61-66.
123. Costerton, J.W. et al. Bacterial Biofilms: A Common Cause of Persistent Infections. *Science*. 1999, **284**(5418), pp.1318-1322.
124. Soto, S.M. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. *Virulence*. 2013, **4**(3), pp.223-229.
125. Staudt, C. et al. Volumetric measurements of bacterial cells and extracellular polymeric substance glycoconjugates in biofilms. *Biotechnology and Bioengineering*. 2004, **88**(5), pp.585-592.
126. Evans, L.V. and Editor. *Biofilms: Recent Advances in Their Study and Control*. Harwood, 2000.
127. Costerton, J.W. Overview of Microbial Biofilms. *Journal of Industrial Microbiology*. 1995, **15**(3), pp.137-140.
128. Nadell, C.D. et al. The sociobiology of biofilms. *Fems Microbiology Reviews*. 2009, **33**(1), pp.206-224.
129. Hall-Stoodley, L. et al. Bacterial biofilms: From the natural environment to infectious diseases. *Nature Reviews Microbiology*. 2004, **2**(2), pp.95-108.

130. Donlan, R.M. Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*. 2002, **8**(9), pp.881-890.
131. Kubota, H. et al. Stress resistance of biofilm and planktonic *Lactobacillus plantarum* subsp *plantarum* JCM 1149. *Food Microbiology*. 2009, **26**(6), pp.592-597.
132. Banerjee, I. et al. Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Advanced Materials*. 2011, **23**(6), pp.690-718.
133. Tiller, J.C. et al. Designing surfaces that kill bacteria on contact. *Proceedings of the National Academy of Sciences of the United States of America*. 2001, **98**(11), pp.5981-5985.
134. Wei, J. et al. Stainless steel modified with poly(ethylene glycol) can prevent protein adsorption but not bacterial adhesion. *Colloids and Surfaces B: Biointerfaces*. 2003, **32**(4), pp.275-291.
135. Pringle, J.H. and Fletcher, M. Influence of substratum hydration and adsorbed macromolecules on bacterial attachment to surfaces. *Applied and Environmental Microbiology*. 1986, **51**(6), pp.1321-1325.
136. Dancer, S.J. Controlling Hospital-Acquired Infection: Focus on the Role of the Environment and New Technologies for Decontamination. *Clinical Microbiology Reviews*. 2014, **27**(4), pp.665-690.
137. Leeming, K. et al. The use of immobilised biocides for process water decontamination. *International Biodeterioration & Biodegradation*. 2002, **49**(1), pp.39-43.
138. EC. *Regulation concerning the making available on the market and use of biocidal products*. [Online]. 2013. [Accessed 01/02]. Available from: http://ec.europa.eu/environment/chemicals/biocides/index_en.htm
139. Maillard, J.Y. Bacterial target sites for biocide action. *Journal of Applied Microbiology*. 2002, **92**, pp.16S-27S.

140. Andrews, J.M. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*. 2001, **48**, pp.5-16.
141. Turnidge, J.D. et al. *Susceptibility Test Methods: General Considerations*. 2007.
142. WHO. *WHO Global Strategy for Containment of Antimicrobial Resistance*. WHO, 2000.
143. WHO. *Antimicrobial Resistance*. [Online]. 2013. [Accessed 01/02]. Available from: <http://www.who.int/mediacentre/factsheets/fs194/en/>
144. HMA. *HMA definitions of the terms "Antibiotic" and "Antimicrobial"*. [Online]. 2012. [Accessed 01/02]. Available from: http://www.hma.eu/fileadmin/dateien/Veterinary_medicines/00-HMA_Vet/02-HMA_Task_Force/03_HMA_vet_TF_AMR/2012_11_HMA_agreed_AB_A_M_definitions.pdf
145. Berg, J.C. Chapter V The role of surfactants. In: Chatterjee, P.K. and Gupta, B.S. eds. *Textile Science and Technology*. Elsevier, 2002, pp.149-198.
146. ECO-FORUM. *Surfactants*. [Online]. n/d. [Accessed 07/02]. Available from: http://www.eco-forum.dk/detergents/index_files/Page718.htm
147. Loontjens, J.A. Quaternary Ammonium Compounds. In: Moriarty, T.F. et al. eds. *Biomaterials Associated Infection*. Springer New York, 2013, pp.379-404.
148. Lucassen, J. and Giles, D. Dynamic surface properties of nonionic surfactant solutions. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases*. 1975, **71**(0), pp.217-232.
149. Itrich, N.R. and Federle, T.W. Effect of ethoxylate number and alkyl chain length on the pathway and kinetics of linear alcohol ethoxylate

- biodegradation in activated sludge. *Environmental Toxicology and Chemistry*. 2004, **23**(12), pp.2790-2798.
150. Jaramillo, D.E. et al. Decreased Bacterial Adherence and Biofilm Growth on Surfaces Coated with a Solution of Benzalkonium Chloride. *Journal of Endodontics*. 2012, **38**(6), pp.821-825.
151. De Saint Jean, M. et al. Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells. *Investigative Ophthalmology & Visual Science*. 1999, **40**(3), pp.619-30.
152. Machado, I. et al. Effect of antimicrobial residues on early adhesion and biofilm formation by wild-type and benzalkonium chloride-adapted *Pseudomonas aeruginosa*. *Biofouling*. 2011, **27**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.1151-1159.
153. Kjellin, M. et al. *Surfactants From Renewable Resources*. John Wiley & Sons Ltd., 2010.
154. Shanthy, P. et al. Corrosion inhibition and biocidal activity of a cationic surfactant. *Indian J. Chem. Technol.* 2009, **16**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.328-333.
155. McGowan, J.E. Antimicrobial Resistance in Hospital Organisms and its Relation to Antibiotic Use. *Reviews of Infectious Diseases*. 1983, **5**(6), pp.1033-1048.
156. Levy, S.B. Active efflux, a common mechanism for biocide and antibiotic resistance. *Journal of Applied Microbiology*. 2002, **92**, pp.65S-71S.
157. Hart, C.A. Antibiotic resistance: an increasing problem? It always has been, there are things we can do. *British Medical Journal*. 1998, **316**(7140), pp.1255-1256.
158. Masri, N.M. et al. The Immunomodulatory, Antimicrobial and Bactericidal Efficacy of Commonly Used Commercial Household Disinfectants, Sterilizers and Antiseptics *in vitro*: Putative Anti-Inflammatory Infection Control Mechanisms and Comparative Biochemical Analysis of the

- Microbial Growth of Gram-Positive Bacteria. *American Journal of Medical and Biological Research*. 2013, **1**(4), pp.103-133.
159. Savage, V.J. et al. Staphylococcus aureus Biofilms Promote Horizontal Transfer of Antibiotic Resistance. *Antimicrobial Agents and Chemotherapy*. 2013, **57**(4), pp.1968-1970.
160. Qu, Y. et al. Densely adherent growth mode, rather than extracellular polymer substance matrix build-up ability, contributes to high resistance of Staphylococcus epidermidis biofilms to antibiotics. *Journal of Antimicrobial Chemotherapy*. 2010, **65**(7), pp.1405-1411.
161. Stewart, P.S. et al. Daptomycin Rapidly Penetrates a Staphylococcus epidermidis Biofilm. *Antimicrobial Agents and Chemotherapy*. 2009, **53**(8), pp.3505-3507.
162. John, A.K. et al. Reversible Daptomycin Tolerance of Adherent Staphylococci in an Implant Infection Model. *Antimicrobial Agents and Chemotherapy*. 2011, **55**(7), pp.3510-3516.
163. Eun, Y.-J. et al. DCAP: A Broad-Spectrum Antibiotic That Targets the Cytoplasmic Membrane of Bacteria. *Journal of the American Chemical Society*. 2012, **134**(28), pp.11322-11325.
164. Maira-Litrán, T. et al. Expression of the multiple antibiotic resistance operon (mar) during growth of Escherichia coli as a biofilm. *Journal of Applied Microbiology*. 2000, **88**(2), pp.243-247.
165. Talon, D. The role of the hospital environment in the epidemiology of multi-resistant bacteria. *Journal of Hospital Infection*. 1999, **43**(1), pp.13-17.
166. Dötsch, A. et al. Genomewide Identification of Genetic Determinants of Antimicrobial Drug Resistance in Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy*. 2009, **53**(6), pp.2522-2531.

167. Guz, N.R. et al. Flavonolignan and Flavone Inhibitors of a *Staphylococcus aureus* Multidrug Resistance Pump: Structure–Activity Relationships. *Journal of Medicinal Chemistry*. 2000, **44**(2), pp.261-268.
168. Verkouteren, J.R. et al. A method to determine collection efficiency of particles by swipe sampling. *Measurement Science & Technology*. 2008, **19**(11).
169. Moore, G. and Griffith, C. A laboratory evaluation of the decontamination properties of microfibre cloths. *Journal of Hospital Infection*. 2006, **64**(4), pp.379-385.
170. Li, X. et al. Role of Hydrodynamic Drag on Microsphere Deposition and Re-entrainment in Porous Media under Unfavorable Conditions. *Environmental Science & Technology*. 2005, **39**(11), pp.4012-4020.
171. Hoerner, S.F. *Fluid-dynamic drag : practical information on aerodynamic drag and hydrodynamic resistance*. Brick Town: Hoerner, 1965.
172. Mao, N. and Russell, S.J. Anisotropic liquid absorption in homogeneous two-dimensional nonwoven structures. *Journal of Applied Physics*. 2003, **94**(6), pp.4135-4138.
173. Edwards, V. et al. Electrokinetic analysis of hydroentangled greige cotton-synthetic fiber blends for absorbent technologies. *Textile Research Journal*. 2013, **83**(18), pp.1949-1960.
174. Edwards, J.V. et al. Fluid handling and fabric handle profiles of hydroentangled greige cotton and spunbond polypropylene nonwoven topsheets. *Proceedings of the Institution of Mechanical Engineers, Part L: Journal of Materials: Design and Applications*. 2016, **230**(4), pp.847-859.
175. Hamada, H. et al. Absorption mechanism of aqueous and solvent inks into synthetic nonwoven fabrics. *J. Imaging Sci. Technol.* 2009, **53**(5), pp.050201/1-050201/6.

176. Koh, E. et al. Influence of fabric structure on dynamic dirt removal in hydroentangled wipes. In: *Nonwovens Research Academy (NRA), Chemnitz, Germany*. EDANA, 2008, pp.268-280.
177. Mao, N. and Russell, S.J. Capillary pressure and liquid wicking in three-dimensional nonwoven materials. *Journal of Applied Physics*. 2008, **104**(3), p.034911.
178. Pietsch, K. and Fuchs, H. Nonwoven Semi-finished Products and Nonwoven Production Technology. In: Cherif, C. ed. *Textile Materials for Lightweight Constructions Technologies - Methods - Materials - Properties*. Berlin Heidelberg: Springer-Verlag, 2016, pp.347-360.
179. Gupta, B.S. Chapter III Fluid absorption in high bulk nonwovens. In: Chatterjee, P.K. and Gupta, B.S. eds. *Textile Science and Technology*. Elsevier, 2002, pp.93-127.
180. Rebenfeld, L. Chapter VI Fibers and fibrous materials. In: Chatterjee, P.K. and Gupta, B.S. eds. *Textile Science and Technology*. Elsevier, 2002, pp.199-232.
181. Pourdeyhimi, B. and Maze, B. *Structure and mechanics of nonwovens*. Cambridge: Woodhead Publ Ltd, 2008.
182. Oathout, J.M. Determining the dynamic efficiency of cleanroom wipers for removal of liquids and particles from surfaces. *Journal of the IEST*. 1999, **42**(3), pp.17-26.
183. Oathout, J.M. and IEST; IEST, I. *Determining the dynamic efficiency of cleanroom wipers for removal of liquids and particles from surfaces*. 2000.
184. Nobile, D.P. *Presaturated Wipes Help Ensure Consistent Processes and Higher Product Quality*. [Online]. 2013. [Accessed 01/02]. Available from: <http://www.contecinc.com/literature/presaturated-wipes-help-ensure-consistent-processes-and-higher-product-quality.php>

185. Park, S.J. et al. Melt-spun shaped fibers with enhanced surface effects: Fiber fabrication, characterization and application to woven scaffolds. *Acta Biomaterialia*. 2013, **9**(8), pp.7719-7726.
186. Shanbhag, A.S. et al. Macrophage/particle interactions: Effect of size, composition and surface area. *Journal of Biomedical Materials Research*. 1994, **28**(1), pp.81-90.
187. Keevil, C.W. et al. Biocide treatment of biofilms. *International Biodeterioration*. 1990, **26**(2–4), pp.169-179.
188. Maillard, J.Y. *RCN guidance on the selection and use of disinfectant wipes*. 20 Cavendish Square London W1G 0RN: Royal College of Nursing, 2011. 2011. [Guideline]. Available from: https://www.rcn.org.uk/_data/assets/pdf_file/0011/382538/003873.pdf
189. Ramm, L. et al. Pathogen transfer and high variability in pathogen removal by detergent wipes. *American Journal of Infection Control*. 2015.
190. Goldsmith, M.T. et al. Adsorption of Available Chlorine and Quaternary by Cotton and Wool Fabrics from Disinfecting Solutions. *Applied Microbiology*. 1954, **2**(6), pp.360-364.
191. Sansone, E. Redispersión of Indoor Surface Contamination and Its Implications. In: Mittal, K.L. ed. *Treatise on Clean Surface Technology*. Springer US, 1987, pp.261-290.
192. Levy, S.B. and Marshall, B. Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine*. 2004, **10**(12), pp.S122-S129.
193. Percival, S.L. et al. Bacterial resistance to silver in wound care. *Journal of Hospital Infection*. 2005, **60**(1), pp.1-7.
194. Gupta, B.S. and Smith, D.K. Chapter X Nonwovens in absorbent materials. In: Chatterjee, P.K. and Gupta, B.S. eds. *Textile Science and Technology*. Elsevier, 2002, pp.349-388.

195. Lockington, D. et al. Bacterial contamination of the disposable prism holder during routine tonometry for intraocular pressure. *Eye*. 2009, **23**(6), pp.1474-1475.
196. SmartSafetech. *Steady Growth for the Global Nonwoven Wipes Market Despite Economic Slowdown*. [Online]. 2011. [Accessed 03/10]. Available from: <http://smart-safetech.blogspot.co.uk/2011/06/steady-growth-for-global-nonwoven-wipes.html>
197. Pira, S. *Global Wipes Market Overview*. 2016.
198. *Five key trends in the future of global nonwovens*. [Online]. [Accessed 05/04/2017]. Available from: <http://www.smithe rspira.com/resources/2015/december/five-key-trends-in-the-future-of-global-nonwovens>
199. Rengasamy, R.S. 6 - Composite nonwovens in wipes. In: *Composite Non-Woven Materials*. Woodhead Publishing, 2014, pp.89-119.
200. Sahu, P.B. *Wipes: What, Where, Why & How? The wipes market has been influenced by many factors*. [Online]. 2012. [Accessed 05/11]. Available from: http://www.nonwovens-industry.com/issues/2012-01/view_features/wipes-what-where-why-how/
201. Sifuentes, L.Y. et al. Microbial contamination of hospital reusable cleaning towels. *American Journal of Infection Control*. 2013, **41**(10), pp.912-915.
202. Wahal, S. et al. *Cleaning wipe for use with disinfectants, method of manufacture thereof, and system*. WO2011130258A2. 2011.
203. Mitra, S. and Thompson, S.M. *Improved cleaning wipes*. Google Patents, 2010. Available from: <https://www.google.com/patents/CA2390318C?cl=en>
204. Mitra, S. and Thompson, S.M. *Cleaning wipes*. Google Patents, 2006. Available from: <https://www.google.com/patents/EP1266957B1?cl=en>

205. Dreilinger, L. and Kaser, M.J. *Aqueous acidic hard surface cleaning and disinfecting compositions*. Google Patents, 2015. Available from: <https://www.google.com/patents/CA2704765C?cl=en>
206. Smith, J. *Composite non-woven wiping cloth*. Google Patents, 2014. Available from: <https://www.google.co.uk/patents/CN203438626U?cl=en>
207. Goene, F. et al. *Synthetic nonwoven wiping fabric*. Google Patents, 2006. Available from: <https://www.google.co.uk/patents/US20060068673>
208. Ellis, D. *Nonwoven sanitizing wipe including an anionic binder formulation*. Google Patents, 2006. Available from: <https://www.google.co.uk/patents/US20060128248>
209. Goene, F. et al. *Synthetic nonwoven wiping fabric*. Google Patents, 2006. Available from: <https://www.google.co.uk/patents/WO2006036966A2?cl=en>
210. Oathout, J.M. *Nonwoven fabrics for wiping applications*. Google Patents, 2000. Available from: <https://www.google.co.uk/patents/CA2351723A1?cl=en>
211. Oathout, J. *Nonwoven fabrics for wiping applications*. Google Patents, 2004. Available from: <https://www.google.co.uk/patents/US20040198128>
212. Collins, G.W. et al. *Dispersible non-woven fabrics*. Google Patents, 2014. Available from: <https://www.google.co.uk/patents/EP2776616A1?cl=en>
213. Jones, J.D. et al. *Disposable semi-moist wipes*. Google Patents, 1988. Available from: <https://www.google.com/patents/US4725489>
214. Mitra, S. et al. *Bactericidal cleaning wipe comprising a cationic biocide*. Google Patents, 2004. Available from: <https://www.google.com/patents/US6825158>
215. Bhat, G.S. Nonwovens as Three-Dimensional Textiles for Composites. *Materials and Manufacturing Processes*. 1995, **10**(4), pp.667-688.
216. Yilmaz, N.D. The Technology of Terry Towel production. *Journal of Textile and Apparel Technology and Management*. 2005, **4**(4), pp.1-46.

217. Jones, P. New Range for The Production of Terry Towels. *Melliand Textilberichte International Textile Reports*. 1979, **60**(10), pp.848-849.
218. EDANA. *Bonding*. [Online]. n/d. [Accessed 01/02]. Available from: <http://www.edana.org/discover-nonwovens/how-they're-made/bonding>
219. Larrondo, L. and St. John Manley, R. Electrostatic fiber spinning from polymer melts. I. Experimental observations on fiber formation and properties. *Journal of Polymer Science: Polymer Physics Edition*. 1981, **19**(6), pp.909-920.
220. Bhat, G.S. et al. Development of structure and properties during spunbonding of propylene polymers. *Thermochimica Acta*. 2002, **392–393**(0), pp.323-328.
221. Rosseinsky, D.R. ENCYCLOPEDIA OF POLYMER SCIENCE AND ENGINEERING, 2ND EDITION - KROSCWITZ,JI. *Nature*. 1989, **339**(6222), pp.268-269.
222. Wang, S. Status quo and development trend of polypropylene fiber. *Hecheng Shuzhi Ji Suliao*. 2011, **28**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.73-77.
223. Gehrig, H. et al. *Bonded, continuous nonwoven fabrics*. DE2216283A1. 1973.
224. Hadert, H. Binders for natural and synthetic fibers. *Z. Gesamte Textilind.* 1967, **69**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.151-2.
225. Russell, S. *Handbook of Nonwovens*. CRC Press, 2004.
226. Hoyle, A.G. Thermal bonding of nonwoven fabrics. *Tappi J.* 1990, **73**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.85-8.
227. Trgo, M. Development of the process of thermal bonding of nonwovens. *Tekstil*. 1989, **38**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.657-63.

228. Begenir, A. et al. Effect of Nozzle Geometry on Hydroentangling Water Jets: Experimental Observations. *Textile Research Journal*. 2004, **74**(2), pp.178-184.
229. Scholz, R. and Sigmund, S. Steering of dispersibility of spunlaced nonwovens with fibre properties and spunlacing parameters. *Lenzinger Berichte*. 2012, **90**, pp.72-75.
230. Anantharamaiah, N. et al. A study on hydroentangling waterjets and their impact forces. *Experiments in Fluids*. 2006, **41**(1), pp.103-113.
231. Kamath, M.C. et al. *Spunlace (Hydroentanglement)*. [Online]. 2004. [Accessed 01/02]. Available from: <http://www.engr.utk.edu/mse/Textiles/Spunlace.htm>
232. Ran, J. et al. Characterization of nonwoven poly(ethylene terephthalate) devices functionalized with cationic polymer. *Journal of Applied Polymer Science*. 2012, **124**(5), pp.3583-3590.
233. Ghassemieh, E. et al. Improvement of the efficiency of energy transfer in the hydro-entanglement process. *Composites Science and Technology*. 2001, **61**(12), pp.1681-1694.
234. Midha, V.K. and Mukhopadhyay, A. Bulk and physical properties of needle-punched nonwoven fabrics. *Indian Journal of Fibre & Textile Research*. 2005, **30**(2), pp.218-229.
235. Drelich, A. Nonwoven Classification: A Simple System. *Nonwovens Industry*. 1998, (October 1998), pp.54-55.
236. White, C. Wet-laid web formation. In: Russell, S.J. ed. *Handbook of Nonwovens*. Cambridge, UK: CRC, 2007, pp.112-142.
237. Kamath, M.C. et al. *WET-LAID NONWOVENS*. [Online]. 2004. [Accessed 01/025]. Available from: <http://www.engr.utk.edu/mse/Textiles/Wet%20Laid%20Nonwovens.htm>
238. Matson, R.S. et al. Biopolymer Synthesis on Polypropylene Supports: I. Oligonucleotides. *Analytical Biochemistry*. 1994, **217**(2), pp.306-310.

239. Nelson, G. Application of microencapsulation in textiles. *International Journal of Pharmaceutics*. 2002, **242**(1–2), pp.55-62.
240. Jeurissen, A. et al. Safety of non-woven polypropylene surgical adhesive drapes to prevent wound infection. *BMC Proceedings*. 2011, **5**(Suppl 6), pp.P190-P190.
241. Yerdel, M.A. et al. Effect of Single-Dose Prophylactic Ampicillin and Sulbactam on Wound Infection After Tension-Free Inguinal Hernia Repair With Polypropylene Mesh: The Randomized, Double-Blind, Prospective Trial. *Annals of Surgery*. 2001, **233**(1), pp.26-33.
242. Kroon-Batenburg, L.M.J. and Kroon, J. The crystal and molecular structures of cellulose I and II. *Glycoconjugate Journal*. 1997, **14**(5), pp.677-690.
243. Dent, R.W. Inter-fiber distances in paper and nonwovens. *Journal of the Textile Institute*. 2001, **92**(1), pp.63-74.
244. Dever, M. et al. Lyocell Solutions from Alternative Cellulose Sources. *Clothing and Textiles Research Journal*. 2003, **21**(4), pp.167-173.
245. Leuchs. Chemical processes in the ripening of viscose. *Kunstseide*. 1925, **7**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), p.286.
246. Fink, H.-P. and Ebeling, H. Novel routes to cellulose fibres and nonwovens. In: American Chemical Society, 2011, pp.CELL-120.
247. Pahimanolis, N. et al. Nanofibrillated cellulose/carboxymethyl cellulose composite with improved wet strength. *Cellulose*. 2013, **20**(3), pp.1459-1468.
248. Sazhin, B.S. and Efremov, G.I. Sorption-desorption isotherms for fibres with high hygroscopicity. *Fibre Chemistry*. 1997, **29**(2), pp.130-132.
249. Buyle, G. Nanoscale finishing of textiles via plasma treatment. *Materials Technology*. 2009, **24**(1), pp.46-51.

250. Goswami, P. Finish, Textile. In: Luo, R. ed. *Encyclopedia of Color Science and Technology*. New York, NY: Springer New York, 2014, pp.1-10.
251. Blackburn, R.S. 7. Environmentally Friendly Plasma Technologies for Textiles. In: *Sustainable Textiles - Life Cycle and Environmental Impact*. Woodhead Publishing, 2009.
252. Jelil, R.A. A review of low-temperature plasma treatment of textile materials. *Journal of Materials Science*. 2015, **50**(18), pp.5913-5943.
253. Shishoo, R. *Plasma Technologies for Textiles*. Woodhead Publishing Ltd., 2007.
254. Sparavigna, A. Plasma treatment advantages for textiles. *Los Alamos Natl. Lab., Prepr. Arch., Phys.* 2008, (Copyright (C) 2015 American Chemical Society (ACS). All Rights Reserved.), pp.1-16, arXiv:0801.3727v1 [physics.pop-ph].
255. Kale, K.H. and Desai, A.N. Atmospheric pressure plasma treatment of textiles using non-polymerizing gases. *Indian J. Fibre Text. Res.* 2011, **36**(3), pp.289-299.
256. Thorsen, W.J. Improvement of Cotton Spinnability, Strength, and Abrasion Resistance by Corona Treatment. *Textile Research Journal*. 1971, **41**(5), pp.455-458.
257. Carneiro, N. et al. Dyeability of corona-treated fabrics. *Coloration Technology*. 2001, **117**(5), pp.298-302.
258. Ryu, J. et al. Effect of Corona Discharge on the Surface of Wool and Its Application to Printing. *Textile Research Journal*. 1991, **61**(10), pp.595-601.
259. Roth, J.R. et al. A study of the sterilization of nonwoven webs using one atmosphere glow discharge plasma. In: TAPPI Press, 1996, pp.225-230.

260. Kogelschatz, U. et al. Dielectric-barrier discharges. Principle and applications. *J. Phys. IV*. 1997, **7**(C4, XXIIIrd International Conference on Phenomena in Ionized Gases, 1997), pp.C4/47-C4/66.
261. De Geyter, N. et al. Surface modification of a polyester non-woven with a dielectric barrier discharge in air at medium pressure. *Surface and Coatings Technology*. 2006, **201**(6), pp.2460-2466.
262. Borra, J.P. Charging of aerosol and nucleation in atmospheric pressure electrical discharges. *Plasma Physics and Controlled Fusion*. 2008, **50**(12), p.124036.
263. Schutze, A. et al. The atmospheric-pressure plasma Jet. A review and comparison to other plasma sources. *IEEE Trans. Plasma Sci.* 1998, **26**(6), pp.1685-1694.
264. Hwang, Y.J. et al. Effects of Helium Atmospheric Pressure Plasma Treatment on Low-Stress Mechanical Properties of Polypropylene Nonwoven Fabrics. *Textile Research Journal*. 2005, **75**(11), pp.771-778.
265. Subedi, D.P. et al. Plasma treatment at low pressure for the enhancement of wettability of polycarbonate. *Indian Journal of Pure & Applied Physics*. 2008, **46**(8), pp.540-544.
266. Shin, B.S. et al. Extreme water repellency of nanostructured low-surface-energy non-woven fabrics. *Soft Matter*. 2012, **8**(6), pp.1817-1823.
267. Masaeli, E. et al. Study of the wettability properties of polypropylene nonwoven mats by low-pressure oxygen plasma treatment. *Surface and Interface Analysis*. 2007, **39**(9), pp.770-774.
268. Morent, R. et al. Non-thermal plasma treatment of textiles. *Surface and Coatings Technology*. 2008, **202**(14), pp.3427-3449.
269. Khelifa, F. et al. Free-Radical-Induced Grafting from Plasma Polymer Surfaces. *Chemical Reviews*. 2016, **116**(6), pp.3975-4005.
270. Wei, Q. et al. Functionalization of Textile Materials by Plasma Enhanced Modification. *Journal of Industrial Textiles*. 2007, **36**(4), pp.301-309.

271. De Vietro, N. et al. Plasma treatment for preparing durable water repellent and anti-stain synthetic fabrics for automotive applications. *J. Surf. Eng. Mater. Adv. Technol.* 2015, **5**(3), pp.103-109.
272. Shin, Y. et al. Functional finishing by using atmospheric pressure plasma: Grafting of PET nonwoven fabric. *Journal of Applied Polymer Science.* 2007, **103**(6), pp.3655-3659.
273. Goswami, P. et al. Dyeing behaviour of lyocell fabric: effect of fibrillation. *Color. Technol.* 2007, **123**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.387-393.
274. Lugscheider, E. et al. The effect of PVD layer constitution on surface free energy. *Thin Solid Films.* 1999, **355–356**, pp.367-373.
275. Taylor, J.H. et al. A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa* at 10°C and 20°C. *J. Appl. Microbiol.* 1999, **87**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.718-725.
276. Knapp, L. et al. The effect of cationic microbicide exposure against *Burkholderia cepacia* complex (Bcc); the use of *Burkholderia lata* strain 383 as a model bacterium. *Journal of Applied Microbiology.* 2013, **115**(5), pp.1117-1126.
277. Berendt, A.E. et al. Three swipes and you're out: How many swipes are needed to decontaminate plastic with disposable wipes? *American Journal of Infection Control.* 2011, **39**(5), pp.442-443.
278. Hsu, L.C. et al. Effect of Micro- and Nanoscale Topography on the Adhesion of Bacterial Cells to Solid Surfaces. *Applied and Environmental Microbiology.* 2013, **79**(8), pp.2703-2712.
279. Holinka, J. et al. Effects of selenium coating of orthopaedic implant surfaces on bacterial adherence and osteoblastic cell growth. *Bone & Joint Journal.* 2013, **95-B**(5), pp.678-682.

280. Gallardo-Moreno, A.M. et al. Serum as a factor influencing adhesion of *Enterococcus faecalis* to glass and silicone. *Appl. Environ. Microbiol.* 2002, **68**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.5784-5787.
281. Zhou, C.-E. et al. Plasma treatment applied in the pad-dry-cure process for making rechargeable antimicrobial cotton fabric that inhibits *S. Aureus*. *Textile Research Journal.* 2015.
282. ISO. 20645 (*International Standard*) *Textile fabrics -- Determination of antibacterial activity -- Agar diffusion plate test.* ISO, 2004.
283. Tomasino, S.F. et al. Enumeration procedure for monitoring test microbe populations on inoculated carriers in AOAC use-dilution methods. *J. AOAC Int.* 2006, **89**(6), pp.1629-1634.
284. Coday, B.D. et al. Indirect determination of zeta potential at high ionic strength: Specific application to semipermeable polymeric membranes. *J. Membr. Sci.* 2015, **478**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.58-64.
285. Childress, A.E. and Elimelech, M. Effect of solution chemistry on the surface charge of polymeric reverse osmosis and nanofiltration membranes. *Journal of Membrane Science.* 1996, **119**(2), pp.253-268.
286. Daoud, W.A. et al. Superhydrophobic silica nanocomposite coating by a low-temperature process. *J. Am. Ceram. Soc.* 2004, **87**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.1782-1784.
287. White, L.R. On deviations from Young's equation. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases.* 1977, **73**(0), pp.390-398.
288. Sing, K.S.W. and Williams, R.T. Historical aspects of capillarity and capillary condensation. *Microporous Mesoporous Mater.* 2012,

- 154**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.16-18.
289. Kalin, M. and Polajnar, M. The wetting of steel, DLC coatings, ceramics and polymers with oils and water: The importance and correlations of surface energy, surface tension, contact angle and spreading. *Appl. Surf. Sci.* 2014, **293**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.97-108.
290. Yuan, Y. and Lee, T.R. Contact angle and wetting properties. *Springer Ser. Surf. Sci.* 2013, **51**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.3-34.
291. Bachmann, J. et al. Development and application of a new sessile drop contact angle method to assess soil water repellency. *Journal of Hydrology.* 2000, **231–232**(0), pp.66-75.
292. Skinner, F.K. et al. Contact angle measurements from the contact diameter of sessile drops by means of a modified axisymmetric drop shape analysis. *Journal of Colloid and Interface Science.* 1989, **130**(1), pp.25-34.
293. Hunter, R.J. *Foundations of Colloid Science, 2nd Edition.* Oxford University Press, 2001.
294. Sajadinia, S.H. and Sharif, F. Thermodynamic analysis of the wetting behavior of dual scale patterned hydrophobic surfaces. *J. Colloid Interface Sci.* 2010, **344**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.575-583.
295. Schwartz, L.W. and Garoff, S. Contact angle hysteresis on heterogeneous surfaces. *Langmuir.* 1985, **1**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.219-30.
296. Gao, L. and McCarthy, T.J. Contact Angle Hysteresis Explained. *Langmuir.* 2006, **22**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.6234-6237.

297. Mognetti, B.M. and Yeomans, J.M. Modelling receding contact lines on superhydrophobic surfaces. *arXiv.org, e-Print Arch., Condens. Matter*. 2010, (Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.1-19, arXiv:1009.4660v1 [cond-mat.soft].
298. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods*. 2012, **9**(7_part1), pp.676-682.
299. Yosef, I. et al. Counteracting selection for antibiotic-resistant bacteria. *Bacteriophage*. 2016, **6**(1), p.e1096996.
300. Berne, C. et al. Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiology spectrum*. 2015, **3**(4), pp.10.1128/microbiolspec.MB-0018-2015.
301. Krasowska, A. and Sigler, K. How microorganisms use hydrophobicity and what does this mean for human needs? *Frontiers in Cellular and Infection Microbiology*. 2014, **4**, p.112.
302. Bagherifard, S. et al. The influence of nanostructured features on bacterial adhesion and bone cell functions on severely shot peened 316L stainless steel. *Biomaterials*. 2015, **73**, pp.185-197.
303. Mitik-Dineva, N. et al. Differences in colonisation of five marine bacteria on two types of glass surfaces. *Biofouling*. 2009, **25**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.621-631.
304. Blitshteyn, M. Wetting tension measurements on corona-treated polymer films. *Tappi J.* 1995, **78**(Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), pp.138-43.
305. Bico, J. et al. Wetting of textured surfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2002, **206**(1-3), pp.41-46.
306. Jazbec, K. et al. Functionalization of cellulose fibres with oxygen plasma and ZnO nanoparticles for achieving UV protective properties. *J. Nanomater.* 2015, (Copyright (C) 2016 American Chemical Society (ACS). All Rights Reserved.), p.346739pp.

307. Biffinger, J.C. et al. The Polar Hydrophobicity of Fluorinated Compounds. *ChemBioChem*. 2004, **5**(5), pp.622-627.
308. Zhang, D. and Kushner, M.J. Investigations of surface reactions during C₂F₆ plasma etching of SiO₂ with equipment and feature scale models. *Journal of Vacuum Science & Technology a-Vacuum Surfaces and Films*. 2001, **19**(2), pp.524-538.
309. Tajima, S. et al. Differential regulation of endothelial cell adhesion, spreading, and cytoskeleton on low-density polyethylene by nanotopography and surface chemistry modification induced by argon plasma treatment. *Journal of Biomedical Materials Research Part A*. 2008, **84A**(3), pp.828-836.
310. Agraharam, S. et al. Plasma chemistry in fluorocarbon film deposition from pentafluoroethane/argon mixtures. *J. Vac. Sci. Technol., A*. 1999, **17**(6), pp.3265-3271.
311. Smiley, R.J. and Delgass, W.N. AFM, SEM and XPS characterization of PAN-based carbon fibres etched in oxygen plasmas. *Journal of Materials Science*. 1993, **28**(13), pp.3601-3611.
312. Kou, R.-Q. et al. Surface Modification of Microporous Polypropylene Membranes by Plasma-Induced Graft Polymerization of α -Allyl Glucoside. *Langmuir*. 2003, **19**(17), pp.6869-6875.
313. Kale, K.H. and Desai, A.N. Atmospheric pressure plasma treatment of textiles using non-polymerising gases. *Indian J. Fibre Text. Res.* 2012, **37**(3), pp.289-299.
314. Mandal, J. *Polypropylene Fiber and Its Manufacturing Process, Properties, Advantages, Disadvantages and Applications of Polypropylene Fiber*. [Online]. [Accessed 22/04/2017]. Available from: <http://textilelearner.blogspot.co.uk/2013/01/polypropylene-fiber-and-its.html>

315. Cardinaud, C. et al. Plasma etching: principles, mechanisms, application to micro- and nano-technologies. *Applied Surface Science*. 2000, **164**(1–4), pp.72-83.
316. Li, L. et al. Creation of Superhydrophobic Stainless Steel Surfaces by Acid Treatments and Hydrophobic Film Deposition. *Acs Applied Materials & Interfaces*. 2012, **4**(9), pp.4549-4556.
317. Silhavy, T.J. et al. The Bacterial Cell Envelope. *Cold Spring Harbor Perspectives in Biology*. 2010, **2**(5), p.a000414.
318. Wren, M.W.D. et al. Removing bacteria from hospital surfaces: a laboratory comparison of ultramicrofibre and standard cloths. *Journal of Hospital Infection*. 2008, **70**(3), pp.265-271.
319. Truong, V.K. et al. The influence of nano-scale surface roughness on bacterial adhesion to ultrafine-grained titanium. *Biomaterials*. 2010, **31**(13), pp.3674-3683.
320. Pogodin, S. et al. Biophysical Model of Bacterial Cell Interactions with Nanopatterned Cicada Wing Surfaces. *Biophysical Journal*. 2013, **104**(4), pp.835-840.
321. Dancer, S.J. Missing a trick? Response to: ‘Disinfectant wipes are appropriate to control microbial bioburden from surfaces’.
- Journal of Hospital Infection*. 2016, **92**(2), pp.208-209.
322. Edwards, N.W.M. et al. Role of surface energy and nano-roughness in the removal efficiency of bacterial contamination by nonwoven wipes from frequently touched surfaces. *Science and Technology of Advanced Materials*. 2017, **18**(1), pp.197-209.
323. French, G.L. et al. Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. *Journal of Hospital Infection*. 2004, **57**(1), pp.31-37.

324. Wiemken, T.L. et al. The value of ready-to-use disinfectant wipes: Compliance, employee time, and costs. *American Journal of Infection Control*. 2014, **42**(3), pp.329-330.
325. Zhou, C.-E. et al. Plasma treatment applied in the pad-dry-cure process for making rechargeable antimicrobial cotton fabric that inhibits *S. Aureus*. *Textile Research Journal*. 2016, **86**(20), pp.2202-2215.
326. Beerse, P.W. et al. *Antimicrobial wipes which provide improved residual benefit versus gram negative bacteria*. Google Patents, 2001. Available from: <https://www.google.com/patents/US6197315>
327. Chesters, A.K. et al. The influence of surfactants on the hydrodynamics of surface wetting. *J. Pet. Sci. Eng.* 1998, **20**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.217-222.
328. Vargaftik, N.B. et al. International tables of the surface tension of water. *J. Phys. Chem. Ref. Data*. 1983, **12**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.817-20.
329. N/A. *Compound - Benzalkonium Chloride*. [Online]. 2012. [Accessed 22/04/2017]. Available from: <https://www.phenomenex.com/Compound?id=Benzalkonium+chloride-C14>
330. Koo, O.-K. et al. Comparison of cleaning fabrics for bacterial removal from food-contact surfaces. *Food Control*. 2013, **30**(1), pp.292-297.
331. Bruni, R. et al. Surfactant protein B: lipid interactions of synthetic peptides representing the amino-terminal amphipathic domain. *Proc. Natl. Acad. Sci. U. S. A.* 1991, **88**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.7451-5.
332. Sharma, R. et al. Autophobic On Liquid Subphases Via Interfacial Transport of Amphiphilic Molecules. In: *AIChE, 29/10/2012, Pittsburg*. 2012.

333. Sharma, R. et al. Autophobic on Liquid Subphases Driven by the Interfacial Transport of Amphiphilic Molecules. *Langmuir*. 2012, **28**(43), pp.15212-15221.
334. Craster, R.V. and Matar, O.K. On Autophobic in Surfactant-Driven Thin Films. *Langmuir*. 2007, **23**(5), pp.2588-2601.
335. Cook, D.C. Strain induced martensite formation in stainless steel. *Metallurgical Transactions A*. 1987, **18**(2), pp.201-210.
336. AZOM. *Stainless Steel - Grade 304 (UNS S30400)*. [Online]. n/d. [Accessed 01/02]. Available from:
<http://www.azom.com/article.aspx?ArticleID=965>
337. ASSDA. *Stainless Steel Fasteners*. [Online]. 2004. [Accessed 06/02]. Available from:
<http://web.archive.org/web/20070929001157/http://www.assda.asn.au/asp/index.asp?pgid=18732>
338. Atsu, S.S. et al. Effect of zirconium-oxide ceramic surface treatments on the bond strength to adhesive resin. *The Journal of Prosthetic Dentistry*. 2006, **95**(6), pp.430-436.
339. Howie, D.W. and Vernon-Roberts, B. Synovial macrophage response to aluminium oxide ceramic and cobalt-chrome alloy wear particles in rats. *Biomaterials*. 1988, **9**(5), pp.442-448.
340. Ohgushi, H. et al. Bone formation process in porous calcium carbonate and hydroxyapatite. *Journal of Biomedical Materials Research*. 1992, **26**(7), pp.885-895.
341. Rao, B.B. Zinc oxide ceramic semi-conductor gas sensor for ethanol vapour. *Materials Chemistry and Physics*. 2000, **64**(1), pp.62-65.
342. Motolese, A. et al. Contact dermatitis and contact sensitization among enamellers and decorators in the ceramics industry. *Contact Dermatitis*. 1993, **28**(2), pp.59-62.

343. Wessling, B. Passivation of metals by coating with polyaniline: corrosion potential shift and morphological changes. *Adv. Mater. (Weinheim, Ger.)*. 1994, **6**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.), pp.226-8.
344. Honess, C. *Importance of Surface Finish in the Design of Stainless Steel*. [Online]. 2006. [Accessed 06/02]. Available from: <http://www.bssa.org.uk/cms/File/surfacefinishbssaVer2.pdf>
345. Lee, J. et al. Development of a Sanitizing Fabric Wipe for Use on Food Contact Surfaces. *Journal of Food Science*. 2007, **72**(9), pp.M375-M381.
346. Williams, G.J. et al. Limitations of the Efficacy of Surface Disinfection in the Healthcare Setting. *Infection Control and Hospital Epidemiology*. 2009, **30**(6), pp.570-573.