

# **DEVELOPING WOUND DRESSING FROM BACTERIAL CELLULOSE**

Nipunika Shashikala Gamage

(09/8096)



University of Moratuwa, Sri Lanka.  
Electronic Theses & Dissertations  
[www.lib.mrt.ac.lk](http://www.lib.mrt.ac.lk)

Degree Master of Science

Department of Chemical and Process Engineering

University of Moratuwa

Sri Lanka

July 2013

# **DEVELOPING WOUND DRESSING FROM BACTERIAL CELLULOSE**

Nipunika Shashikala Gamage

(09/8096)



University of Moratuwa, Sri Lanka.  
Electronic Theses & Dissertations  
[www.lib.mrt.ac.lk](http://www.lib.mrt.ac.lk)

Thesis submitted in fulfillment of the requirements for the degree Master of Science

Department of Chemical and Process Engineering

University of Moratuwa

Sri Lanka

July 2013

**Abstract****Developing Wound Dressing from Bacterial Cellulose**

Cellulose produced by bacterium *Acetobacter xylinum* has unique properties including high mechanical strength, high water absorption capacity and highly pure fiber network structure. These properties have enabled bacterial cellulose to be used in applications such as Nata de Coco, enzyme immobilization, artificial skin and wound dressings.

The objective of this project was to investigate the production and properties of bacterial cellulose as appropriate for wound dressings using coconut water as the main substrate medium. Preliminary focus of the research was to identify and isolate *Acetobacter xylinum* from a kombucha mixed culture. Bacterial cellulose pellicles were prepared by static fermentation of *Acetobacter xylinum* containing culture in coconut water while supplementing with glucose and  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$  at an initial pH of 4.3.

Properties of resulting bacterial cellulose pellicles were investigated for its strength, structure and permeability characteristics. The average thickness of each pellicle was 3 mm in wet form and 0.25 mm after oven drying. Young's modulus was in the range of 1 - 3 G Pa up to 15% moisture content. Fourier Transform Infrared spectroscopy (FTIR) on oven dried bacterial cellulose were very much similar to commercial products of bacterial cellulose wound dressings. Water vapor transmission rate (WVTR) through the dressing was in the range of 5-15 ( $\text{g/hr}^{-1} \text{m}^{-2}$ ) while increasing the moisture content decreased the WVTR. These investigations proved that there is an optimum moisture content of 15% that gives the most appropriate properties for a wound dressing.

Further the wound dressings that were prepared and packed in sterile conditions were applied on selected patients. The results showed that dry dressings were more appropriate than wet dressings. However, dry dressings lose their strength when reabsorbing moisture.

In conclusion, it could be said preliminary research showed coconut water can be used in preparation of bacterial cellulose as wound dressing since it has suitable characteristics. However, further research is required to find the variation of properties with moisture content and re-absorption characteristics of bacterial cellulose.

Key words: bacterial cellulose, *Acetobacter xylinum*, coconut water, wound dressing

## **Acknowledgement**

I am very much grateful to Dr. Marliya Ismail, Senior Lecturer, Department of Chemical and Process Engineering, University of Moratuwa for being the main supervisor of this research project.

I am also grateful to Dr Terrence Rohan Chinniah, Senior Lecturer, Department of Microbiology, Faculty of Medicine, University of Colombo, Prof. Mandika Wijeyerathne, Professor, Department of Surgery, Faculty of Medicine, University of Colombo and Prof. Ajith de Alwis, Professor, Department of Chemical and Process Engineering, University of Moratuwa for being co supervisors of this project.

I am thankful to Dr. Marliya Ismail for giving me the moral support and guidance throughout this research work and Dr Terrence Rohan Chinniah for his invaluable advice and training in the microbiological aspects of this project. I am also grateful to Prof. Mandika Wijeyeratne for giving me the opportunity to get clinical exposure without any hesitation while Prof. Ajith de Alwis for giving me words of encouragements along the way.

I owe a word of thanks to Staff Technical Officers, Mrs. Jayanthi Maskorala, Mrs. Renuka Jayalatharachchi and Technical Officers, Mrs. D.L.C. Priyadarshanee, Mr. Priyantha Senevirathne and all the staff members of the Microbiology Lab of Medical Faculty of Colombo University for their fullest support in conducting microbial isolation part of the project.

I wish to express my gratitude to Technical Officer, Mrs. I.K Athukorala and Senior Staff Technical Officer Ms. A.S Wahalathanthri and all the other Technical officers and lab assistants of Department of Chemical and Process Engineering, University of Moratuwa for their assistance in carrying out experiments for developing the wound dressing.

I wish to thank to Mr. Bandusena Samarasekera, Senior Lecturer, Department of Material Science and Engineering, University of Moratuwa for facilitating me in obtaining FTIR spectroscopy measurements.

My heartiest thanks will also go to my parents, husband, siblings, all my family members and friends who always gave courage and made persistent confidence in me throughout the completion of this project.

This M.Sc. thesis was supported by University of Moratuwa Senate Research Grant Number SRC/LT/2009/38.

Finally, I would appreciate everybody, who helped me in numerous ways in different stages of the project, which was of utmost importance in bringing out this effort a success.



University of Moratuwa, Sri Lanka.  
Electronic Theses & Dissertations  
[www.lib.mrt.ac.lk](http://www.lib.mrt.ac.lk)

**TABLE OF CONTENTS**

Declaration of the candidate & Supervisors	i
Abstract	ii
Acknowledgement	iii
Table of Content	v
List of Figures	ix
List of Tables	xi
List of Abbreviations	xii
1 Introduction and Objectives	1
1.1 Introduction	1
1.2 Objectives	2
2 Literature Survey	3
2.1 Cellulose	3
2.2 <i>Acetobacter xylinum</i>	4
2.3 Bacterial Cellulose	5
2.4 Biosynthesis of Bacterial Cellulose	8
2.5 Fermentation techniques	9
2.6 Culture Media	11
2.6.1 Coconut water culture media	11
2.6.2 Distilled water culture medium	14
2.7 Bacterial Cellulose properties	16
2.7.1 Ultra fine structure	16
2.7.2 High water holding capacity	17
2.7.3 High mechanical strength	19
2.7.4 High mouldability	20
2.7.5 High crystallinity	20
2.7.6 High purity	21
2.7.7 High elasticity	21
2.7.8 High thermal stability	21
2.7.9 High transparency	21
2.7.10 Non- toxicity	22

2.8 Purification of Bacterial Cellulose	22
2.9 Drying of Bacterial Cellulose	23
2.10 Bacterial Cellulose Applications	24
2.10.1 Food industry	25
2.10.2 Textile Industry	25
2.10.3 Paper Industry	25
2.11 Medical Applications	26
2.11.1 Blood vessels	26
2.11.2 Scaffold	26
2.11.3 Skin grafts	27
2.11.4 Wound Dressing	28
2.12 Commercially Available Bacterial Cellulose Wound Dressings	30
2.13 Wound Dressing Packaging	31
2.14 Bacterial cellulose composites	32
3 Materials and Methods	35
3.1 Isolation of Bacterial Cellulose	35
3.1.1 <i>Acetobacter xylinum</i> culture	35
3.1.2 Preparation of solid culture medium	36
3.1.3 Indirect Confirmation of isolated <i>Acetobacter xylinum</i>	39
3.1.4 Investigation of sterility by autoclaving	40
3.2 Application of Wound Dressing	40
3.2.1 Preparation of bacterial cellulose pellicles	40
3.2.2 Drying of bacterial cellulose pellicles	42
3.2.3 Packing of bacterial cellulose wound dressings	42
3.2.4 Application of bacterial cellulose wound dressing	43
3.3 Property Analysis	44
3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)	
Measurement	44
3.3.2 Tensile test Measurement	44
3.3.3 Measurement of Water vapor transmission rate (WVTR)	46
3.3.4 Measurement of Water re-absorption	46
3.3.5 Test for Shelf life analysis of dressing packs	47

4 Results and Discussion	49
4.1 Isolation of <i>Acetobacter xylinum</i>	49
4.1.1 Isolation of <i>Acetobacter xylinum</i> in solid culture medium	49
4.1.2 Verification of <i>Acetobacter xylinum</i> through liquid culture medium	53
4.1.3 Verification of sterility by autoclaving	55
4.2 Application of Wound Dressing	55
4.2.1 Bacterial cellulose pellicles as wound dressings	55
4.2.2 The effect of drying bacterial cellulose pellicles	57
4.2.3 The importance of sterile packaging of bacterial cellulose wound dressings	59
4.2.4 The results of Application of bacterial cellulose wound dressing	60
4.3 Property analysis of Bacterial cellulose wound dressing	62
4.3.1 FTIR test comparison on bacterial cellulose Wound Dressing	62
4.3.2 Tensile strength of bacterial cellulose wound dressing	64
4.3.3 Effect of Water Vapor Transmission Rate (WVTR) through BC Wound Dressing	67
4.3.4 Water re-absorption characteristics of BC	71
4.3.5 Shelf life analysis of packaged dressings	73
5 Conclusions and Future Works	74
5.1 Conclusions	74
5.1.1 Identification and isolation of <i>Acetobacter xylinum</i> from Kombucha culture	74
5.1.2 Production of bacterial cellulose as appropriate for wound dressing application	74
5.1.3 Investigating the properties of biosynthesized wound dressings	74
5.1.4 Study the effectiveness of bacterial cellulose with clinical assessment	75
5.2 Future Works	75



Reference List	75
Appendix A: Agar plate inoculation techniques	81
Appendix B: Gram Staining	86
Appendix C : Agar Types	88
Appendix D: FTIR (Fourier Transform Infrared Spectrometry)	92
Appendix E : Tensile Test Results	94
Appendix F :Tensile Test	96



University of Moratuwa, Sri Lanka.  
Electronic Theses & Dissertations  
[www.lib.mrt.ac.lk](http://www.lib.mrt.ac.lk)

**LIST OF FIGURES**

	Page
Figure 2.1 Pathways to Cellulose	4
Figure 2.2 <i>Acetobacter xylinum</i> with secreted cellulose fibrils	4
Figure 2.3 Formation of bacterial cellulose fiber bundles	7
Figure 2.4 Pathways of Carbon metabolism in <i>Acetobacter xylinum</i>	8
Figure 2.5 Static fermentation of Bacterial Cellulose	9
Figure 2.6 RBC fermentation of Bacterial Cellulose	10
Figure 2.7 Agitated fermentation of Bacterial Cellulose	10
Figure 2.8 Microfibrill organization on bacterial cellulose and plant	16
Figure 2.9 Range of structure design of polysaccharaides in comparison with synthetic and living materials	17
Figure 2.10 SEM images of surface morphology of BC film in dry form and in re-swollen form	18
Figure 2.11 Water retention value comparison	18
Figure 2.12 Transparency of bacterial cellulose	22
Figure 2.13 Freeze dried bacterial and evaporated dried bacterial cellulose	24
Figure 2.14 Blood vessels made out of bacterial cellulose for micro surgeries	26
Figure 2.15 Commercially available bacterial cellulose wound dressings	31
Figure 3.1- Summary of Materials and Methods	35
Figure 3.2 Directions taken to compare the strength	44
Figure 3.3 Dumbbell shape specimen	45
Figure 3.4 WVTR test apparatus	46
Figure 3.5 Apparatus for moisture re-absorption test	47
Figure 4.1 Pathways of Carbon metabolism in <i>Acetobacter xylinum</i>	50
Figure 4.2 Modified agar plates with CaCO <sub>3</sub>	51
Figure 4.3 Comparison of nutrient agar mediums	52
Figure 4.4 Isolated bacterial fermentation in coconut water medium	53
Figure 4.5 Dried bacterial cellulose wound dressings	57
Figure 4.6 Packaging of bacterial cellulose wound dressing	58

Figure 4.7 FTIR absorption spectroscopy for bacterial cellulose	61
Figure 4.8 Tensile test results of bacterial cellulose wound dressing with 8% moisture content	64
Figure 4.9 SEM diagrams of Bacterial cellulose of bacterial cellulose	66
Figure 4.10 Time vs. weight loss in WVTR analysis	67
Figure 4.11 Moisture re-absorption characteristics of Bacterial Cellulose	71



University of Moratuwa, Sri Lanka.  
Electronic Theses & Dissertations  
[www.lib.mrt.ac.lk](http://www.lib.mrt.ac.lk)

**LIST OF TABLES**

	Page
Table 1.1 Objectives	2
Table 2.1 Strains of <i>Acetobacter</i> used in various studies	5
Table 2.2 Cellulose producing bacterial species	6
Table 2.3 Basic composition of coconut water	12
Table 2.4 Coconut water enrichment media for <i>Acetobacter xylinum</i> Fermentation	12
Table 2.5 Distilled water culture media for <i>Acetobacter xylinum</i> fermentation	14
Table 2.6 Young's modulus of Bacterial Cellulose in static media	19
Table 2.7 Young's Modulus of other wound dressing	20
Table 2.8 Commercially available wound dressing packaging materials	32
Table 2.9 Bacterial cellulose composites	33
Table 3.1 Solid medium selection	36
Table 3.2 Composition of modified nutrient agar plates	38
Table 3.3 Composition of re-modified agar medium	38
Table 3.4 Composition of liquid medium for confirmation of <i>Acetobacter xylinum</i>	39
Table 3.5 Composition of BHI	40
Table 3.6 Medium for preparation of bacterial cellulose pellicles	41
Table 3.7 Bacterial Cellulose Pellicle purification	42
Table 3.8 Details of the clinical trial	43
Table 3.9 Tensile Strength test specimen details	45
Table 4.1 Observation on verification of <i>Acetobacter xylinum</i>	53
Table 4.2 Bacterial cellulose wound dressing preparation studies	55
Table 4.3 Moisture content of wound dressings	57
Table 4.4 Summary of the trial wound dressing application	59
Table 4.5 Details of Specimens for FTIR analysis	62
Table 4.6 FTIR absorption wavenumbers of bond in bacterial cellulose	62
Table 4.7 Tensile test calculation of wound dressings	65
Table 4.8 WVTR values	69

**LIST OF ABBREVIATIONS**

Abbreviation	Description
BC	Bacterial cellulose
BHI	Brain Heart Infusion
CS	citrate synthase
FTIR	Fourier Transform Infrared Spectroscopy
GK	Glucokinase
G Pa	Giga Pascal
IR	infrared
M Pa	Mega Pascal
PGA	phosphoglycerate
PGM	phosphoglucomutase
RBC	Rotating Biological Contactor
SEM	Scanning Electron microscopy
UDPGlc	uridine diphosphoglucose
WVTR	Water Vapor Transmission Rate



University of Moratuwa, Sri Lanka  
 Electronic Theses & Dissertations  
[www.lib.mrt.ac.lk](http://www.lib.mrt.ac.lk)