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## Conclusions and Future Prospects

*“There is no substitute for hard work”*

*- Thomas. A. Edison*

Evolution has brought humankind a long way from nomadic living to the current world of microprocessors, laptops, email and Internet. But, this huge expansion has come at the expense of the environment and the human quality of life. Realization about the environmental concerns that can have a negative impact on human beings and animals has prompted researchers across the globe to find a suitable green material as an alternative to the existing toxic and non-degradable materials.

This chapter recapitulates the summary of individual chapters in this thesis work. It highlights the relevance of BC in bionanotechnology and introduction of BCS as a novel biomaterial for future biomedical and nanotechnological applications, including therapy, diagnostics, material research, pharmaceutical research, green electronics.

## Conclusions and Future Outlooks

Ever since the advent of nanotechnology, it has conquered the center stage of research. Tremendous attention is being given to the nanomaterials and its related applications owing to their immense potential. On a parallel road, there is been a significant interest developing in the microbial polysaccharides due to their unique structural and mechanical properties. With an increasing awareness about environmental issues caused by synthetic polymers and other toxic non-degradable materials, the quest for a performing, renewable alternative is at its peak. The abundance, tailorable properties and biocompatibility are the key factors that have propelled the use of polysaccharides in biomedical applications. Among all the bacterial polysaccharides that have been extensively studied, bacterial cellulose is a most promising candidate that has been utilized in various fields that includes biomedical sciences, electronics and optoelectronics, food and packaging.

Unique features of BC that can be applied for diverse applications suited our interests. Hence, we produced BC through large-scale fermentation process using *Komagataeibacter sucrofermentans*, under controlled conditions in a jar fermenter. Subsequently, we functionalized BC with sulfate groups and prepared a highly transparent. This novel biocompatible film has good mechanical properties as well as biodegradable. Also, we have evaluated the antioxidant properties, hemocompatibility and anti-bacterial efficacy of BCS film.

## Large-Scale Production of Bacterial Cellulose

*Komagataeibacter sucrofermentans* bacterial strain procured from Riken Bioresource Center was used for the production of BC. Bacterial cells were revived using Acetobacter medium and pre-culture was prepared. Pre-culture of bacterial cells was inoculated into the jar fermenter under controlled conditions to initiate the bacterial fermentation process. BC sphere-like pellicles were harvested after 10 days post inoculation. BC pellicles were purified by the alkali treatment

method. The physical and chemical characterization was carried out using electron microscopy, XPS, FTIR. The results showed almost complete removal of contaminants after alkali treatment. The method used here is simple and reliable with a low concentration of NaOH treatment. Consequently, we acquired pure BC, which was further tested for various characteristics based on the requirement of applications.

### **Evaluation of Antioxidant Properties of BCS**

To use BCS for biomedical applications, evaluation of its bioactive properties is vital. In this chapter, various assays were conducted to evaluate the antioxidant potential, cell viability and hemocompatibility of BCS samples. The antioxidant results infer that no significant difference in the levels of antioxidants was observed with different concentrations of BCS used in L929 cell line or liver homogenate. The cytotoxicity results showed that the cell viability of L929 and KUSA cells was >85% that deduces that BCS is highly biocompatible and non-toxic. The hemolytic study confirms that BCS is highly blood compatible. The *in vitro* and *ex vivo* studies infer that BCS concentrations do not contribute to cells or tissue homogenate damage and is deemed safe for biomedical applications. However, further *in vivo* evaluation is required to study its dose dependent effects with different concentrations of BCS.

### **Bacterial Cellulose Sulfate Transparent Film**

As discussed in the earlier sections, BC has unique features and applications. Though it has tremendous properties and can find multiple applications, its insolubility in most solvents has a severe toll on its reputation. To solve this issue, functionalization of cellulose has been done over the years. However, negligible data is available on acetosulfation of BC. In this chapter, we have discussed about the functionalization of BC with sulfate groups. Acetosulfation of BC yields, BCS that has tremendous potential in biomedicine and bionanotechnology. A highly transparent film was synthesized using BCS, which had good biocompatibility and mechanical properties.

### **Synthesis of BCS/AgNP Composite Films**

The development of antibiotics revolutionized human health by providing a simple cure for dreaded diseases. However, the emergence of multiple drug-resistant (MDR) infectious organisms owing to the widespread production, use, and misuse of antibiotics has contributed to the next-generation concern for global public health. Lately, the use of AgNPs with antimicrobial activity has been presented as a new defense against MDR infectious organisms. The preliminary anti-microbial tests conducted with BCS/AgNP have shown little microbicidal activity. Further investigation in this detail would promote the use of this novel film in biomedicine and food packaging industries.

### **Future Prospects**

Global awareness about the toxic effects of plastics and petroleum products is demanding an effective replacement. Bacterial polysaccharides find themselves as potential contenders in that list as an effective substitute in packaging industries and biomedicine. Their superior mechanical properties and unique features offer immense applications. However, a lot of focus has to be given to large-scale production of BC from simple sources to counter the cost of production that has not received much attention.

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## Publications

1. **Vivekanandan Palaninathan**, Neha Chauhan, Aby Cheruvathoor Poulouse, Sreejith Raveendran, Toru Mizuki, Takashi Hasumura, Takahiro Fukuda, Hisao Morimoto, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. Acetosulfation of bacterial cellulose: An unexplored promising incipient candidate for highly transparent thin film. *Mater. Express* (Revised Manuscript Submitted).
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3. Sreejith Raveendran, Neha Chauhan, **Vivekanandan Palaninathan**, Yutaka Nagaoka, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. Extremophilic polysaccharide for biosynthesis and passivation of gold nanoparticles and photothermal ablation of cancer cells. *Part. Part. Syst. Charact.* **2014**.
4. **Vivekanandan Palaninathan**, Sreejith Raveendran, Neha Chauhan, Toru Mizuki, Takashi Uchida, Yoshikata Nakajima, Tomofumi Ukai, Yasushi Sakamoto, Toru Maekawa, D. Sakthi Kumar. *Ex vivo and in vitro* evaluation of antioxidant property, hemocompatibility and cytotoxicity of bacterial cellulose sulfate. (Communicated)
5. Neha Chauhan, **Vivekanandan Palaninathan**, Sreejith Raveendran, Aby Cheruvathoor Poulouse, Yoshikata Nakajima, Takashi Hasumura, Takashi Uchida, Tatsuro Hanajiri, Toru Maekawa, D. Sakthi Kumar. N<sub>2</sub>-Plasma Assisted One-step Alignment and Patterning of Graphene Oxide on SiO<sub>2</sub>/Si substrate via Langmuir Blodgett Technique. (Communicated)
6. **Vivekanandan Palaninathan**, Neha Chauhan, Sreejith Raveendran, Yasushi Sakamoto, Toru Maekawa, D. Sakthi Kumar. Versatile bacterial cellulose sulfate–chitosan film incorporated with silver nanoparticles

for food packaging and biomedical applications. (Communicated)

7. Sreejith Raveendran, **Vivekanandan Palaninathan**, Yutaka Nagaoka, Takahiro Fukuda, Seiki Iwai, Toru Mizuki, Yasushi Sakamoto, Toru Maekawa, P.V. Mohanan, D. Sakthi Kumar. Extremophilic Polysaccharide Nanoparticles: A Potential Incipient for Defending Cancer Proliferation and Oxidative stress. (Communicated)

### **Book Chapter**

1. **Vivekanandan Palaninathan**, Sivakumar Balasubramanian, D. Sakthi Kumar. Molecular imaging and neurodegenerative disorders: Imaging and neurodegenerative disorders. 2013, Oxford University Press (UK) (Communicated).

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2. **Vivekanandan Palaninathan**, Sreejith Raveendran, Neha Chauhan, Toru Mizuki, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (Poster). *Mass cultivation of Komagataeibacter sucrofermentans and cellulose production through fermentation for wound healing applications*. The 11<sup>th</sup> International Symposium on Bioscience and Nanotechnology. 15 Nov, 2013, Hakusan Campus, Toyo University, Japan.
3. Neha Chauhan, **P. Vivekanandan**, R. Sreejith, Yoshikata Nakajima, Takashi Hasumura, Tatsuro Hanajiri, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (Poster). *Large Area Graphene Oxide for High Performance ‘Smart’ Electronics*. The 11<sup>th</sup> International Symposium on Bioscience and Nanotechnology. 15 Nov, 2013, Hakusan Campus, Toyo University, Japan.
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5. Sreejith Raveendran, Neha Chauhan, **Vivekanandan Palaninathan**, Yutaka Nagaoka, Yasuhiko Yoshida, Toru Maekawa, D. Sakthi Kumar. (Poster). *Bioreduction and stabilization of gold nanoparticles using extremophilic exopolysaccharide, mauran and photothermal ablation of cancer cells*. BNERC and IJAA Joint International Symposium on

Advanced Science and Technology, 7–8 Dec, 2012, Hakusan Campus, Toyo University, Japan.

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# Glossary

2 D	Two Dimensional
3 D	Three Dimensional
$\varepsilon$	Strain
$\sigma$	Stress

## A

AAc	Acrylic Acid
AFM	Atomic Force Microscopy
Ag	Silver
AgNO <sub>3</sub>	Silver Nitrate
AgNP	Silver Nanoparticle
Al	Aluminum
Ar	Argon

## B

BC	Bacterial Cellulose
BCS	Bacterial Cellulose Sulfate
BCSF	Bacterial Cellulose Sulfate Film
BNC	Bacterial Nanocellulose
BRGO	Bacterially Reduced Graphene Oxide
BSA	Bovine Serum Albumin

**C**

C	Carbon
CaCl <sub>2</sub>	Calcium Chloride
CdS	Cadmium Sulfide
CdSe	Cadmium Selenide
ClSO <sub>3</sub> H	Chlorosulfuric Acid
cm	Centimeter
CMC	Carboxymethyl Cellulose
CNF	Carbon Nanofiber
CNT	Carbon Nanotube
CO <sub>2</sub>	Carbon Dioxide
CONH <sub>2</sub>	Amide
COOH	Carboxyl
CPS	Capsular Polysaccharides
CS	Cellulose Sulfate
CTE	Co-efficient of Thermal Expansion
Cu	Copper

**D**

D	Dalton
DLS	Dynamic Light Scattering
DMEM	Dubelcco's Modified Eagle's Medium
DMF	Dimethyl Formamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxy Ribonucleic Acid
DTNB	5 - 5' Dithiobis(2-nitrobenzoic acid)
DTPA	Diethylenetriamine Penta Acetic Acid

**E**

EDS	Electron Dispersive X-ray Spectroscopy
EDX	Electron Dispersive X-ray Spectroscopy
EM	Electron Microscopy
EPS	Exopolysaccharide
EDTA	Ethylene Diamine Tetra Acetic Acid
ESCA	Electron Spectroscopy for Chemical Analysis
ET	Electron Tomography
eV	Electron Volt

**F**

FDA	Food and Drug Administration
FOLED	Flexible Organic Light Emitting Diode
FTIR	Fourier Transmission Infrared Spectroscopy

**G**

g	Gram
GO	Graphene Oxide
GRAS	Generally Recognized as Safe
GSH	Reduced Glutathione
GSSG	Glutathione Oxidized

**H**

HCl	Hydrochloric Acid
HPMC	Hydroxypropyl Methyl Cellulose
HS	Hestrin-Schramm
KOH	Potassium Hydroxide

**L**

LPO	Lipid Peroxide
LPS	Lipopolysaccharide
LSCM	Laser Scanning Confocal Microscopy

**M**

MC	Methyl Cellulose
MDA	Malondialdehyde
MWCNT	Multi-walled Carbon Nanotube

**N**

NaOH	Sodium Hydroxide
NIBS	Non-invasive Back Scatter Technology
nm	Nanometer
NMR	Nuclear magnetic Resonance Spectroscopy
NP	Nanoparticle

**O**

OLED	Organic Light Emitting Diode
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**P**

PALS	Phase Analysis Light Scattering
PAni	Polyaniline
PBS	Phosphate Buffer Solution
Pd	Palladium
Pt	Platinum



**R**

RBC	Red Blood Cells
ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute

**S**

S	Sulphur
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
SO <sub>3</sub> <sup>-</sup>	Sulfate
SOD	Superoxide Dismutase
SPM	Scanning Probe Microscope
SPR	Surface Plasmon Resonance
SPS	Sulfated Polysaccharides

**T**

TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid
TEM	Transmission Electron Microscopy
TG	Thermogravimetric
TGA	Thermogravimetric Analysis

**U**

UV-VIS	UV-Visible Spectroscopy
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**X**

XPS      X-ray Photoelectron Spectroscopy

XRD      X-ray Diffraction

**Z**

ZS      Zeta Sizer