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【論文審査】Review of the thesis

Polysaccharides are relatively complex carbohydrates composed of monosaccharide units bound together by glycosidic linkages. The abundance, tailorable properties and biocompatibility are the key factors that have propelled the use of polysaccharides in biomedical applications. These biomolecules can be categorized based on their source, function, structure, chemical composition and charge. Based on source they can be grouped into plant, animal and microbial polysaccharides. From these available natural resources, microbial polysaccharides have gained a lot of attention in recent years. Microbial polysaccharides have found applications in food, pharmaceutical and medical fields, which could be attributed to their diverse functional and structural properties. Microbial polysaccharides or biopolymers are used as gelling agents and to alter the flow characteristics of liquids. Bacterial polysaccharides have drawn a lot of interest recently and a thorough study on their composition, structure, function and biosynthesis have been reported. Bacterial polysaccharides can be Lipopolysaccharide (LPS), which is confined to the outer membrane of the bacterial cell or capsular polysaccharides (CPS), which forms a capsule (discrete surface layer) or exopolysaccharides (EPS), which is loosely bound to the cell surface. CPS has functions related to the pathogenicity and adherence of bacterial cell, whereas EPS extends support in multiple functions like adhesion, biofilm formation, cell-cell interaction and protection from extreme environment. The function of CPS as virulence factors has encouraged research on these polymers and commercial applications have not been reported yet. On the contrary EPS have tremendous commercial interest due their material properties.

Among all the bacterial polysaccharides that have been extensively studied, bacterial cellulose is a promising candidate that has been utilized in various fields that includes biomedical sciences, electronics and optoelectronics, food and packaging etc. Based on the availability and properties, it is evident that bacterial cellulose hold tremendous potential for a sustainable future. The heightened awareness about environment friendly materials now demands a replacement for non-biodegradable films and other films produced through the use of toxic chemicals. Luckily cellulose, which are developed from plants and bacteria are an inexhaustible organic material for our disposal.

Here Mr. Vivek has performed large-scale production of bacterial cellulose (BC) through fermentation using Komagataeibacter sucrofermentans. Through fermentation, he could harvest BC under controlled conditions. Thereafter, he functionalized of BC and prepared highly transparent film that is biocompatible. This novel film has good mechanical properties and is biodegradable as well. The evaluated the antioxidant properties and hemocompatibility to demonstrate the non-toxic nature of the material. BCS was blood compatible and no significant changes in cells or tissue homogenate were observed in antioxidant levels when treated with it. The film was used with silver nanoparticles as a versatile nanocomposite material for food packaging and biomedical applications owing to its antimicrobial activities.

The thesis comprises 7 chapters that explore the applications of bacterial cellulose in various fields like medicine, tissue engineering, bionanoelectronics. Following sections overviews the highlights of each individual chapters of the thesis entitled “Bacterial Exopolysaccharides: A Potential Candidate For Biomedical Applications And Green Electronics”.

Chapter 1 is an introduction chapter and details about the polysaccharides and its applications. Polysaccharides are relatively complex carbohydrates composed of monosaccharide units bound together by glycosidic linkages. The abundance, tailorable properties and biocompatibility are the key factors that have propelled the use of polysaccharides in biomedical applications. Polysaccharides, otherwise known as

'Glycans' are an integral part of any living system, as a structural unit or as an energy storage unit. Based on the source they can be grouped into plant, animal and microbial polysaccharides and all living organisms contain several forms of polysaccharides. Polysaccharides are one of the indispensible biomolecules on earth. Researchers have minutely observed and utilized several sources of natural polysaccharides for human welfare. Here, Vivek has studied and presented a comprehensive review on Microbial polysaccharides for various biomedical and nanotechnological applications. As an introduction to this research thesis, the first chapter discusses various types of polysaccharides available in nature and importance of bacterial polysaccharides among them. Further more, it emphasizes the important applications of BC in biomedical science and bionanotechnology.

Chapter 2 comprises of the experimental design to finish the research work and the provides the detailed information of the equipment he has used. Modern experimental research requires the combination of many traditional disciplines including biology, optics, mechanics, mathematics, electronics and chemistry. The field of bioinstrumentation has seemingly endless possibilities because of its fusion of different fields for the common purpose of developing new and exciting ways of managing and treating disease and disabilities. This chapter covers the bioinstrumentation employed for this work; it includes the list of all sophisticated instruments exploited for the characterization and analysis of bacteria, their polysaccharides and BCS film. It also elaborates the principles and procedures of different colorimetric assays used for the chemical characterization polysaccharides and in vitro cell studies.

In chapter 3 Vivek provides the details of the development of bacterial cellulose. Owing to its biocompatibility and biodegradability, BC gives plenty of room for researchers across the globe to use it for diverse applications such as in biomedicine, bionanoelectronics, food and packaging. Furthermore, BC has a distinct advantage over plant cellulose in terms of purity. This chapter is focused mainly on the cultivation, collection and purification of BC through fermentation of Komagataeibacter sucrofermentans. The entire process starts with the microbial cultivation. A pre-culture is prepared and is transferred to the jar fermenter, through which controlled agitated conditions are provided for bacterial growth and BC production. Thereafter, BC pellicles are harvested and purified through alkali treatment. Post purification, BC is subjected to various physical and chemical characterizations like electron microscopy and XPS, respectively.

In chapter 4 he provides the details about the biocompatibility results. Modified cellulose is being used for various biomedical applications, food and packaging industries, thus the evaluation about environmental friendly nature is must after modification. Present study was attempted to synthesize bacterial cellulose sulfate and develop a highly transparent film. Thereafter, evaluate its cytotoxicity, hemocompatibility, antioxidant property that has received lesser attention compared to the other chemically modified cellulose. Bacterial cellulose was produced by Komagataeibacter sucrofermentans by controlled agitated method and later was purified by alkali treatment method. Acetosulfation of bacterial cellulose was carried out to functionalize it with sulfate groups. Confirmation of successful sulfation was done through XPS, FTIR and FT Raman spectroscopy. The degree of sulfation was found to be 0.28. The cytotoxicity results infer that bacterial cellulose sulfate is non- toxic, blood compatible and biocompatible. Also, no significant difference in the antioxidant levels was observed in L929 cells or liver homogenate. Thus, the ex vivo and in vitro studies show that varying concentrations of BCS used here is deemed safe for biomedical applications, edible films, food and packaging.

Chapter 5 gives the detailed characterization of the BCS film, which can be used as transparent flexible substrate and as the food package covering material. The rising consciousness about the benefits of environment friendly and biodegradable materials demands a substitute for the prevailing non-degradable and toxic materials. Towards this aspect bacterial cellulose has numerous potential applications owing to their important properties like high biocompatibility, biodegradability, and ecofriendly nature. In the present study, we have synthesized bacterial cellulose using Komagataeibacter sucrofermentans strain through batch fermentation and functionalized it with sulfate groups to form bacterial cellulose sulfate (BCS) that has negligible reports so far. Using this BCS, we synthesized a highly transparent film by drop cast method, which exhibited high optical transmittance of 90-92% in the visible wavelength range. BCS was thoroughly characterized, using SEM and AFM for its surface morphology; XPS, FTIR, TGA and XRD to confirm the successful functionalization of bacterial cellulose. The results inferred the successful sulfation of bacterial cellulose and the film has a smooth surface with good integrity and mechanical properties. Furthermore, cell viability studies confirmed the biocompatible nature of the sample. These properties of the film hold immense promise for applications in biomedicine, optoelectronics and food packaging

Chapter 6 discusses the application of functionalized bacterial nanocellulose in food packaging and biomedical applications. Recent progress of modern healthcare and food packaging technologies initiates searching novel materials with superior safety properties for their applications. Antimicrobial polymer composites with highly active antimicrobial agents are a class of materials that are in demand for such applications. With the advent of nanotechnology, the ability to manipulate a material at nanoscale has imparted huge merit that directly affects the material of choice. Through acetosulfation, BC has been functionalized to BCS and thereafter, BCS film is incorporated with AgNPs to get the broad-spectrum antimicrobial properties. Various characterizations like FTIR, Zeta potential, UV-vis spectroscopy, TEM and SEM analysis have been performed to assess the physical and chemical features. This film holds tremendous potential in food packaging and modern healthcare owing to the broad-spectrum applications it has to offer.

Chapter 7 provides the summary of individual chapters in this thesis work. 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.

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.

【審査結果】Summary and decision

The thesis entitled “Bacterial Exopolysaccharides: A Potential Candidate for Biomedical Applications and Green Electronics” presents the synthesis, characterization and application of bacterial exopolysaccharides (bacterial cellulose) as biocompatible polymer materials which can be used in food packaging industry and as flexible substrates in electronic industry. The results shown in the thesis are outstanding and the significant points in the present study are summarized below;

(1) A new methodology for synthesising bacterial exopolysaccharides has been developed.
(2) By using the acetosulphation method, the exoploysaccharides (bacterial cellulose), which are insoluble in water and most of the solvents, turned into highly water soluble and soluble in other solvents too.
(3) The developed Bacterial cellulose sulphate is highly transparent even without adding resins (a toxic element normally used to add along with the cellulose to make it transparent) and in this way he developed a non-toxic highly transparent polymer. BCS is highly edible and also water soluble (within 8 seconds), which suggests its use in food packaging industry.
(4) The developed BCS found to be highly biocompatible which can be used in biomedical industry as wound dressing materials.
(5) High transparency (92%) indicates its use in display industry as flexible transparent substrates in the near future with some surface modifications.

1 first-authoring paper has been accepted by an international journal and 1 co-authoring paper has been accepted. The results obtained in this study have been presented at 6 international conferences and symposia.

Judging by the results shown in the thesis, the number of international papers published so far and the number of presentations at international conferences and symposia, the level of the research results is definitely high by international standards and the present results may well make a great contribution to the development of new methodologies for developing bacterial cellulose as well as highly transparent polymer films from bacterial cellulose sulphate which can be utilized in food packaging industry as well as flexible transparent substrate in electronic industry in the near future. In conclusion, the thesis is considered to be a high quality, high standard one by international standards.