Report of Thesis evaluation

Submitted by Mr. Thomasz Witko, Thesis titled " Biophysical characteristics and cellular studies of polyhydroxyoctanoate (PHO)- biodegradable and biocompatible polymer for biomedical applications.

Plastics are becoming indispensable in our day-to-day life, primarily due to their extensive applications in different fields. The extensive production and consumption of plastic materials is a growing concern. This work timely in addressing the pressing global problem of plastics.

The present thesis mainly focus on evaluation of polyhdroxyoctanoate (PHO) bio based and biodegradable polymer for biomedical applications. Films of PHO was prepared in various solvents and characterized for their mechanical and cytotoxicity properties. The cytotoxicity data reveled good biocompatibility and cell adhesion to PHO polymer matrix with fibroblast cells. This study shows the potential use of PHO polymer for biomedical applications. The present dissertation work comprises of 4 main parts apart from abstract and abbreviations.

***Part 1: Introduction plastics and Bioplastics*:** This chapter provides background information about traditional fossil-based plastic and problems associated with plastic waste. In addition, this chapter emphasizes importance of bio-based polymers, types of bio-based polymers, production and their applications in various fields. An extensive information about PHO synthesis, accumulation, properties and applications of PHO was described. Various properties of substrates that influences morphology of the cells, structures of cytoskeleton and cell migration was discussed with reference to properties of the substrates.

Throughout the chapter the term PHO and mcl-PHA is interchanged to represent the substrate polymer, but there was no clear definition provided to make sure these two polymers are same. The literature review could have broaden to problems associated with various other polymers and their properties to use in biomedical applications would have been helpful to bench mark the contribution of knowledge form this work.

Part 2: Subject of Dissertation: I understands this is structure of thesis. This could have been better structured to explain what each chapter in the thesis describes about. Towards the end of the chapter it was mentioned about single used, plastic waste, plastic

waste, etc. However, in reality the waste generated from medical devices are well controlled and disposed in appropriate methods. Some information/literature on current disposal methods of plastic waste generated from medical devices could have been helpful to strengthen claims about plastics waste generated by medical devices. The context of the problem should be clear.

Part 3: Materials and Methods: This chapter clearly describes the synthesis of PHO polymer, preparation of substrates and different characterization methods. Mouse embryonic fibroblast cell line was used to assess the cytotoxicity of PHO and other polymer and methods are well explained. Analytical methods such Fluorescence microscopy, confocal microscopy and nanoidentiation was utilized to characterize the substrates after exposing fibroblast cell line. Over this chapter clearly describes the various analytical methods used during the course of the work.

Part 4: Experimental results: This chapter presents experimental data obtained from various characterization methods of PHO substrate. The mechanical, chemical, molecular properties were characterized. The mechanical properties such as hardness and modulus was measured using micro indentation and atomic force microscopy. The young's modulus calculated for PHO polymer depend on type of solvent used. From the data presented the mechanical properties of PLA and PHO is quite different and there need to be rationale why PLA was chosen for comparable study with PHO.

In order to understand the cytotoxicity of PHO films, MEFT 3T3 cells was cultured on PHO and PLA substrates prepared with different solvents. After 96 hours of culture, the substrates did not show much difference in their cell viability indicating that PHO films are non-toxic. Extensive microscopic analysis was carried out to understand the cell morphology, cell height and cell migration of MEF3T3 cells grown both PLA and PHO substrates and compared with glass substrates. The softer substrates seems slow down the migration of cells with small step size. The cytoskeleton morphology of cells was monitored by observing the actin and microtublar filaments. The PHO substrates showed thicker and dense network of microtubalar filaments and occupying the most of the cell volume.

There was lot of data presented in this chapter; however, there was no explanation or rationale provided why the presented results are different for various substrates (PLA, PHO and glass): in terms of cytotoxicity, mechanical and other properties. At the end of this chapter, it is expected to have clear summary and conclusions of results. I suggest including conclusions at the end of this chapter.

Part 5: Importance of research: Towards end of the thesis, an overview of market and production of fossil polymers and biopolymers was presented. The importance of and future prospectus for PHAs in general was discussed.

Overall, this work developed an understanding of PHO polymer to be use in biomedical applications. The cytotoxicity indicates that PHO polymer is a potential candidate for biomedical application e.g. for wound dressings. A co-relation was developed between the polymer substrate type, mechanical properties (hardness and modulus) and cytotoxicity of different polymer substrates suitable for biomedical applications.

Particular Strengths and weaknesses:

The major strength of current research work is use of biopolymer produced from renewable sources and its evaluation for biomedical application such as wound dressings. The weakness in the thesis are listed below

I) There is no consistency in representing the polymer substrates.

II) In number of places poor grammar, incoherent and long sentences and contradictory statements.

III) Chapter 6: No conclusions was provided.

IV) There was lot emphasis on fossil plastics and plastic waste problems.

Details of questions and corrections required:

Minor corrections are required and questions are mentioned below:

1. Why there was lot of emphasis on plastic waste and fossil polymers? Can you explain why?

2. Why PLA was chosen for the study? Why not other polymers such as PLLA or PLGA??

3. Was the intended application of this study was for wound applications? Why the films of PHA studied rather than scaffolds? Is there any rationale?

4. Why cell height and cell migration is important for biomedical applications. Why cell migration is higher in harder substrates like glass?

5. Why the cytoskeleton structures are different for PHO substrates compared to PLA? Because of the mechanical properties?

6. What is circular economy? Why this is important for biomedical applications?

Page 24: what is the difference between bio-based and biodegradable polymers?

Page 38: Check the spelling for tensile strength

Page 50: Check the chapter number just above the table. There is no chapter 12 in the thesis.

Page 59: Page number is missing.

Page 61: Was PHO polymer used for entire is the same or different batches of PHO was used? These details should be provided in the section 5.1.1

Page 88 and 89: There is no table numbers and descriptions for the table.

Page 89: Why different solvent cast films showing different youngs modulus? Can you explain?

Page 91: Table 11. The data provided is all measured from AFM analysis?. Why AFM is preferred method for measuring the mechanical properties? There are other methods to measure the mechanical properties.

Page 96: Figure 51 why there is no cell vitality data for PLA was not presented for comparison?

Page 105: Line 1: to ensure both the polymerization.....
Polymerization of what? Not clear. Can you correct it.

Recommendation:

The work described in the thesis meets the standards of an PhD thesis requirements is conducted in systematic manner and all the procedures adopted and the results obtained were well presented. I recommend that the thesis is accepted for award of the PhD degree subject to minor corrections being made.

Place : **Dublin**

Date : **06th April, 2019**

Signature :

Name : **Prof. Ramesh Babu**