MICROWAVE ASSISTED ALOE VERA COATING ON METALLOCENE POLYETHYLENE FOR IMPROVING BIOCOMPATIBILITY

ARUNPANDIAN BALAJI

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy (Biomedical Engineering)

Faculty of Biosciences & Medical Engineering
Universiti Teknologi Malaysia

MAY 2016

I dedicate this thesis to my lovely family:

My dearest parents, Mr. Balaji Baskaran, Mrs. Bharathi Balaji

&

In memory of my grandfather Mr. Baskaran

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor; Dr. Saravana Kumar Jaganathan, (Faculty of Biosciences and Medical Engineering, UTM), under whose supervision this research was conducted. Thank you very much for the phenomenal encouragement, assistance and advice during the work. I would also like to thank my co-supervisors Prof. Ida Idayu Muhammad (Faculty of Chemical Engineering, UTM) and Dr. Ahmad Zahran Md. Khudzari (Faculty of Biosciences and Medical Engineering, UTM) for their guidance as well.

I would like to acknowledge Mr. Yong Lee Ming and Miss. Farah Nadiya binti Muhamad Sobri, MSI Technologies, Malaysia for their support to use Hirox 3D digital microscope KH-8700. My sincere thanks and appreciation to UTM laboratory technicians Pn. Mas Linda Yunos, Pn. Junaidah and En. Jefri for their assistance in performing characterization studies. A special thanks to my senior Ms. Aziurah mohd shah for the suggestions and thoughts in carrying out contact angle studies. And this thesis would have been impossible without the unconditional love and support from my parents, my lab mates, my friends and all my well-wishers.

Ultimately, my sincere appreciation goes to dean of the Faculty of Biosciences & Medical Engineering, Prof. Dr. Jasmy bin Yunus and the IJN-UTM cardiovascular engineering center director Prof. Dr. -Ing. Eko Supriyanto for their constant support in helping me to complete my research.

ABSTRACT

Metallocene polyethylene (mPE) is known for its commendable physical and mechanical properties, but the problem of hemocompatibility hampers its clinical application. Therefore, an *Aloe vera* (AV) extract was coated on mPE assisted by microwave to rectify this problem. Initially, the duration of microwave treatment was optimized to 60 s by considering the weight degradation of the samples. Similarly, the coating time of fibrous AV extract was optimized to 12 h (A-12 h-mPE) and 24 h (A-24 h-mPE) based on wettability increment. Fourier transform infrared (FTIR) spectra showed the addition of OH^- groups and the vibration characteristic of several active constituents available in the AV coating. The decrease in mean contact angle of pristine mPE (P-mPE) from 88.43° to 32.93° in the A-24 h-mPE sample, depicts an increase in the wettability. Meanwhile, scanning electron microscopy (SEM) images displayed the presence of AV extract. The influence of microwave in enhancing the coating characteristics was investigated through Hirox 3D images, peel test, and degradation studies. In addition, an improvement in average surface roughness (Ra) of P-mPE from 2.069 nm to 7.796 nm for the A-24 h-mPE was interpreted through atomic force microscopy (AFM) analysis. Finally, the *in vitro* coagulation studies indicated a reasonable delay in blood clotting time on the AV coated mPE samples, which was presented by activated partial thromboplastin time (170 s) and prothrombin time (39 s) assay. The coated mPE samples also reduced hemolysis and platelet adhesion insinuating the potential usage of AV coated mPE in permanent and temporary blood contacting devices.

ABSTRAK

Metallocene polietilena (mPE) dikenali dengan sifat-sifat fizikal dan mekanikal yang mengagumkan, namun masalah keserasian dengan darah menghadkan aplikasi klinikal bahan ini. Oleh itu, ekstrak lidah buaya (AV). Disalut ke atas mPE dengan bantuan gelombang mikro untuk mengatasi maseilah ini. Pada permulaanya, tempoh rawatan gelombang mikro telah dioptimumkan kepada 60 saat dengan merujuk kepada kadar degradasi berat sampel. Begitu juga dengan masa penyalutan ekstrak gentian AV telah dioptimumkan kepada 12 jam (A-12 h-mPE) dan 24 jam (A-24 h-mPE) berdasarkan peningkatan ciri kebolehbasahan. Spektrum daripada Fourier inframerah (FTIR) menunjukkan pertambahan kumpulan OH^- dan ciri getaran bagi beberapa komponen aktif yang terdapat di dalam salutan AV. Purata sudut bersentuhan MPE tulen (P-mPE) menurun secara daripada 88.43° kepada 32.93° untuk sampel A-24 h-mPE menggambarkan peningkatan kebolehbasahan. Sementara itu, imej mikroskop elektron pengimbas (SEM) menunjukkan kehadiran ekstrak AV. Pengaruh gelombang mikro dalam peningkatan ciri-ciri penyalutan disiasat melalui imej-imej 3D Hirox, ujian pengupasan, dan kajian degradasi. Tambahan, peningkatan purata kekasaran permukaan (Ra) P-mPE daripada 2.069 nm kepada 7.796 nm untuk A-24 h-mPE telah ditafsirkan melalui analisis mikroskop tenaga atom (AFM). Akhir sekali, kajian koagulasi darah secara *in vitro* menunjukkan kelengahan masa yang wajar bagi pembekuan darah pada sampel mPE yang disaluti AV, yang ditunjukkan oleh masa pengaktifan esei tromboplastin separa (170 s) dan protrombin (39 s). Sampel mPE yang bersalut juga mengurangkan hemolisis dan lekatan platelet menggambarkan potensi penggunaan mPE yang disaluti AV dalam peranti perhubungan darah yang kekal dan sementara.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscopy
AMPs	-	Antimicrobial peptides
APTT	-	Activated Partial Thromboplastin Time
ATR	-	Attenuated total reflectance
AV	-	Aloe vera
BSM	-	Bovine submaxillary gland mucin
CT	-	Computed Tomography
DS	-	Dextran sulfate (DS)
ECM	-	Extracellular matrix
EM	-	Electromagnetic
EVA	-	Ethylene-vinyl acetate
FTIR	-	Fourier transform infrared
Hep	-	Heparin
HUVEC	-	Human umbilical vein endothelial cell
LDPE	-	Low density polyethylene
mPE	-	Metallocene polyethylene
NH_3	-	Ammonia
PBS	-	Phosphate Buffer solution
PCL	-	Polycaprolactone
PDMS	-	Polydimethylsiloxane

PE	-	Polyethylene
PEEK	-	Polyether ether ketone
PEG	-	Polyethylene glycol
PET	-	Polyethyleneterephthalate
PETG	-	Glycol-modified polyethylene terephthalate
PLACL	-	Poly (L-lactic acid)-co-poly (ε- caprolactone)
PMMA	-	Polymethylmethacrylate
PP	-	Polypropylene
PPP	-	Platelet poor plasma
PRP	-	Platelet Rich Plasma
PS	-	Polystyrene
PSM	-	Plasma-surface modification
PT	-	Prothrombin Time
PTFE	-	Polytetrafluoroethylene
PU	-	Polyurethane
PVC	-	Polyvinylchloride
RBCs	-	Red Blood Cells
ROS	-	Reactive oxygen species
SEM	-	Scanning Electron Microscope
SPS	-	Segmented polystyrene
Ti	-	Titanium
TPU	-	Thermoplastic polyurethane
USSCs	-	Unrestricted somatic stem cells
UV	-	Ultraviolet
vWF	-	von Willebrand

WBC	-	White blood cells
WHO	-	World Health Organization
WSC	-	Water-soluble chitosan
Z-N	-	Ziegler-Natta

LIST OF SYMBOLS

˚C	-	Degree Celsius
µm	-	Micrometer
cm	-	Centimeter
CO_2	-	Carbon-di-oxide
GHz	-	Gigahertz
He	-	Helium
mg	-	Milligram
MHz	-	Megahertz
ml	-	Milliliter
mm	-	Millimeter
mol%	-	Mole fraction
nm	-	Nanometer
O_2	-	Oxygen
Si	-	Silicone
µmol	-	Micro moles

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Overview

Biomaterials are promising materials for an extensive range of utilizations in both diagnostic and therapeutic industries. Scientists have defined it in different perspective based on its rapidly changing outlook [1-3]. Typically, biomaterials can be defined as materials which can provide an environment to assist the rehabilitation of biological systems or replace the entire system itself. Biomaterials have a well-established reputation in the field of tissue engineering, clinical devices, drug delivery, medical implants, biosensors, cosmetics and food industries [4, 5]. Hence, the total market value of biomaterial-based industries is anticipated to exceed $88.4 billion by 2017 from the current value of $58.1 billion. Every year, USA alone spend 7-8% of its total global healthcare outgoings exclusively for biomaterial-related usages [6]. Meanwhile, in coming years the demand for promising biomaterials is anticipated to surge radically due to an increasing number of diseased population. It insinuates the need for more research toward improving the properties of existing materials using simple and feasible modification techniques. So, biomaterials have a significant future in both research and commercial fields.

In general, biomaterials can be classified into three groups based on their origin and applications as (1) synthetic materials, (2) naturally derived, and (3) semisynthetic or hybrid materials. Among the above, synthetic materials like metals, ceramics, polymers and composites are most commonly used for various biomedical applications. The exceptional mechanical properties of metals and their alloys such as

tensile strength, elasticity coefficient and fatigue life makes them attractive materials for many load-bearing biomedical systems. Some of the examples include wires, screws, etc., to fracture fixation plates and artificial joints. Nevertheless, metallic materials are highly prone to corrosion and tend to release harmful side products in the form of ions, chemical compounds and insoluble components which will cause adverse biological reactions. In the meantime, ceramics emerged as desirable biomaterials because of its captivating bioactive, bioinert and biodegradable properties. They have been used in several applications in the dental field; though the poor mechanical characteristics like brittleness and low strength, made them unsuitable for wide exploitation. Later, polymers gained greater attention than other materials because of their versatility and easy to tailor nature. Presently, polymers are reported to be the most promising type of biomaterials.

Common biological substances fall under the second category like collagen, heparin, proteins, peptides, carbohydrates, bio-ceramics, etc., are utilized for both surface coating and material synthesis. Though materials completely made of natural substances possess fascinating biocompatible properties they fail in several aspects because of poor physicochemical and mechanical properties. To avoid that complication, natural materials are coupled with synthetic substances and it falls under the third category [5].

The longevity of an implant/biomaterial inside the human body is dependent on its ability to avoid any adverse reaction or damage to the surrounding environment which chiefly relies on the biocompatibility of materials used. But this crucial property is greatly influenced by its physical, chemical, mechanical and biological characteristics [7]. If analyzed deeply, the existence of interconnections between all these essential properties and the durability of a biomaterial can be inferred. In general, the physicochemical properties such as roughness, hardness, temperature, wettability, surface chemistry, surface reactivity (inert or active) and surface charge, play a crucial role in determining the hemocompatibility of a material by delaying the activation of coagulation pathways, resisting platelets adhesion and avoiding red blood cells (RBCs) damage On the other hand, mechanical properties such as elasticity, yield stress, ductility, toughness, deformation, fatigue, hardness, and wear resistance will

determine the ability of a material to withstand the dynamism of the internal environment. So, the presence of appropriate surface, mechanical and biological properties will ensure desired function and longevity of an implant [8-13].

Therefore, in this study, the hemocompatibility of mPE is improved by microwave assisted coating of AV extract. This approach not only eliminates the usage of harsh chemicals but also encourage researchers to utilize various natural products, which will ultimately help us to produce cost-effective multifaceted biomaterials.

1.2 Research Background

Polymers have gained a fascinating reputation in the field of biomaterials because of excellent physicochemical and mechanical properties. Basically, a polymer is a large molecule built up by the repetition of small and simple chemical units called monomers. The repetition is either linear, much like a chain or branched. Unlike many products whose structure and reactions were well known before their industrial application, some polymers were produced on an industrial scale long before their chemistry or physics was studied. Traditionally, polymers are synthesized by either simple condensation/step-reaction polymerization methods or addition/chain-reaction polymerization methods. In the biomedical field, polymers like polyurethane (PU), polyethylene (PE), polypropylene (PP), silicone, polytetrafluoroethylene (PTFE) etc., have sealed vital reputations for usage as surgical devices, implants, drug delivery systems, biosensors, bio-adhesives, ocular devices, dental materials, tissue adhesives, cardiac valves, artificial hearts, vascular grafts, breast prosthesis, facial prostheses, kidney and liver parts, tracheal tubes, food preservation, etc., as illustrated in Fig. 1.1 [14-22].

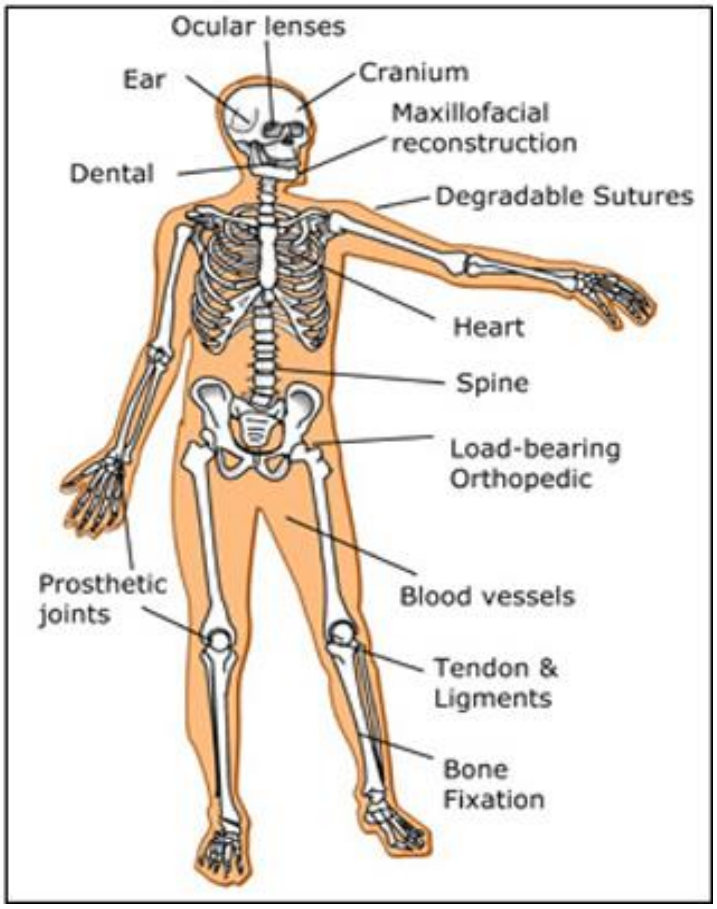

Figure 1.1 Applications of polymeric implants in the human body [2]

The prime advantage of polymers compared to other biomaterials is their ease of manufacture to yield intended shapes like membranes, fibers, gels, capsules, etc., at minimum cost. At present, a variety of biodegradable, bio-adhesive and bio-responsive polymers are mass produced for commercial purposes. Meanwhile, the advent of innovative technologies has created new platforms for exploitation of polymers in the form of hydrogels, nanofibers, nanoparticles, nanocomposites, nanosponges, nanocapsules, etc., [16]. Besides having commendable mechanical and physical properties, polymers fail in numerous cases because of their poor surface characteristics, which ultimately activate undesired host-mediated reactions. Hence, the research on exploring feasible approaches to improve the biocompatibility of polymers is still actively progressing.

The polymer mPE used in this research is one of the rising biomaterials belonging to polyolefin family of polymers synthesized using “metallocene catalyst” [23]. Because of its low density it has been suggested as an effective alternative to commonly used polymers like polyvinylchloride (PVC), for applications including blood bags, syringe tubes, and packaging bottles [12, 23]. In addition, the mPE sheet also offers a number of attractive features such as superior tensile strength, elasticity, toughness, excellent resistance to puncture, impact, blocking and bursting. On top of this, it also inferred to have a better permeability to oxygen when compared with PVC and can also provide an effective barrier against the attack of ammonia [23, 24]. The property of oxygen permeability is not only important for ocular implants, but also for tissue engineering materials since it is reported to facilitate the release of desired biomolecules [11]. Further, the existence of O_2 permeability may also ensure better gas exchange to tissues in contact. However, mPE elicit undesirable reactions when exposed to a biological environment because of poor biocompatibility. Recent efforts have expressed the possibility of enhancing the bioactivity of mPE by subjecting it to various surface modifications [12, 13].

1.3 Problem statement

Though mPE has excellent physical and mechanical properties, they often lead to clot formation because of poor blood compatibility [12]. As mentioned, blood compatibility is a crucial factor which determines the quality of a polymer and its performance in various applications. It precisely reflects the ability of a material to function in the desired region without triggering an appropriate host reaction. In general, a series of events will be triggered when blood comes in contact with the polymer after implementation, which is collectively called blood mediated reactions [21]. Based on those subsequent reactions, a material is said to possess good or poor blood compatibility. In general, if the blood contacts the poorly compatible material it will lead to the complications such as: (1) Adsorption of plasma proteins and platelets on the material surface, (2) Release of clotting factors from activated platelets and initiation of coagulation cascades, (3) Interaction of RBCs with poorly compatible surface will be followed by the upset in cell integrity and leads to lysis [22]. To solve

these issues, several surface modification techniques have been explored but most of them are complicated and limited to a certain family of polymers. Nowadays, millions of investment have been directed towards advanced biomaterial research which involves in the exploration of new alternatives [25]. But to cater the future demand, more research need to be encouraged to improve the properties of existing medical materials using a feasible, eco-friendly and affordable modification technique [26, 27]. So in this study, the problem of poor blood compatibility reported in the medical usage of mPE is rectified by microwave assisted coating of AV extract.

1.4 Research objectives

1. To prepare AV extract and coat them on mPE samples pre-treated with microwaves.
2. To assess the physicochemical changes induced on the surface of mPE after coating with AV extract.
3. To determine the hemocompatibility of pristine and AV extract coated mPE samples.

1.5 Scope of the study

Initially, the semi-transparent gel was separated from fresh, succulent leaves of AV and was blended into a thick fibrous extract. Then, the mPE sheet was cut into square samples of dimension 2 x 2 cm^2 and treated with microwaves for an optimized period of 60 s. Later, the microwave treated mPE samples were coated with the prepared AV extract for selected periods of 12 h and 24 h respectively. The coating process was carried out using a rocking shaker. After coating, the samples were dried and utilized for physicochemical studies includes FTIR, contact angle assay, SEM, Hirox 3D microscopy analysis and AFM to determine the alterations in surface chemistry, wettability and surface roughness of AV coated mPE samples. Moreover, the coating properties like thickness and strength were also determined, using Hirox

3D microscopy, peel test, and degradability test. Finally, the influence of the physicochemical changes in delaying the clotting time, resisting platelet adhesion and avoiding RBC's damage was displayed through *in vitro* blood compatibility assays like activated partial thromboplastin time (APTT), prothrombin time (PT), platelet adhesion studies and hemolysis assay respectively.

1.6 Significance of the study

Usage of plant extract for improving the biocompatibility of polymers not only opens the gate for a spectrum of medical utilization but also offer a nurturing environment for cells to proliferate. Most of the available approaches are complicated, expensive, and not eco-friendly because of usage of harsh chemicals and limited to a particular material or application. Therefore, this research is anticipated to encourage more studies on developing surface modification tools for multifaceted biomaterials.

1.7 Thesis outline

This thesis is divided into five main chapters. In Chapter 1, a brief explanation about the biomaterials and the research background of this study is elaborated. Further, the objectives of this study have been presented in the context of rectifying clinical complications caused by implants. Finally, the importance of the proposed method and its influence in encouraging future research is also projected.

In Chapter 2, key characteristic features of mPE and the problem of biocompatibility associated with polymers is explained in the context of blood interaction and related responses. In addition, the importance of surface modification techniques in improving the physicochemical properties of materials and its ability to act as a coating tool was described in detail. Lastly, a brief discussion of the medical history of AV, its chemical constituents, and biomaterial usages is also framed.

In Chapter 3, the research methodology and characterization studies followed in this thesis are given in detail. The discussions mainly cover the details of materials used, procedures followed in the optimization of parameters and the need for the reported characterization studies.

In Chapter 4, the results obtained from proposed characterization studies have been elaborated and compared with previously reported work. This section is the heart of the thesis since it reflects the achievement and the effectiveness of the study.

In Chapter 5, a short summary of the whole work and its efficacy in eliminating a number of existing problems are projected. Moreover, some suggestion for future research is also presented.

REFERENCES

1. Williams. D.F. Definitions in Biomaterials. *Proceedings of a Consensus Conference of the European Society for Biomaterials*. March 3-5 1986. England Elsevier. 1986. 1-72.
2. Hollinger. J.O. *An Introduction to Biomaterials* Second Edition CRC Press. 2011. 644-917.
3. Sumrita. B. Kumar A. Biomaterials and bioengineering tomorrow's healthcare. Biomatter. 2013. 3(3): e24717-24751.
4. Poncin. E.F. Legeay G. Surface Treatment of Polymeric Materials Controlling the Adhesion of Biomolecules. *Journal of Biomaterials Science Polymer Edition* 2003. 1410: 1005-1028.
5. Binnaz. H.Y.A Cem. S.B. Biomaterials *A Roadmap of Biomedical Engineers and Milestones*. Intech publications. 2012.
6. Markets and Markets: Global Biomaterials Market Statistics (http//www.marketsandmarkets.com/PressReleases/global-biomaterials-market-worth-US58.1-Billion-by-2014.asp). Retrieved date: 4, Jan, 2015.
7. Davis. J.R. Overview of biomaterials and their use in medical devices. In Davis JR ed. *Handbook of materials for medical devices*. Illustrated edition Ohio ASM International. 1-11; 2003.
8. Ratner. B.D. Surface modification of polymers: chemical biological and surface analytical challenges. *Biosensor and Bioelectron*.1995. 10: 797-804.
9. Kummerlowe C Hans WK. *The Journal of Adhesion* 1997. 64: 131-144.
10. Anderson. J.M. Biological response to materials. *Annual Review of Materials Research.* 2001. 318: 1–110.
11. Silvio. L.D. *Cellular Response to Biomaterials*. Cambridge. Woodhead Publishing Elsevier. 2008.
12. Mohandas. H. Sivakumar. G. Kasi. P. Jaganathan. S.K. Supriyanto E. Microwave-Assisted Surface Modification of Metallocene Polyethylene for

Improving Blood Compatibility. *Bio Med Research International*. 2013. 2013: 1-7.

13. Jaganathan. S.K. Mohandas. H. Sivakumar. G. Kasi. P. Theertha S. Sruthi. A.V. Selvakumar. M. and Supriyanto. E. Enhanced Blood Compatibility of Metallocene Polyethylene Subjected to Hydrochloric Acid Treatment for Cardiovascular Implants. *Bio Med Research International*. 2014. 2014: 1-7.
14. Chang. H.I. Wang. Y. Regenerative Medicine and Tissue Engineering - *Cells and Biomaterials Cell Responses to Surface and Architecture of Tissue Engineering Scaffolds* Intech publications. 1-21; 2011.
15. Jaganathan SK, Balaji AP, Vellayappan. M.V. Subramanian.A. Agnes Aruna. J. Asokan. M.K. Review: Radiation-induced surface modification of polymers for biomaterial application. *Journal of Materials Science.* 2014. 50(5): 1-12.
16. Isabel. C. Polymer Biocompatibility Polymerization *InTech Publications*. 2012
17. Simon. S. Thomas. S. Christof. S. Karla. L. Current Strategies in Cardiovascular Biomaterial Functionalization. *Materials.* 2010. 3: 638-655.
18. Guo. S. Pietro. L.D. Factors Affecting Wound Healing. *Journal of Dental Research.* 2010. 89(3): 219–229.
19. Buddy. D.R. Allan. S.H. Frederick. J.S. Jack. E.L. *Biomaterials Science An Introduction to Materials in Medicine*. Third Edition. Elsevier Inc 2013.
20. Xiaoli. L. Lin. Y. Dan. L. Zengchao. T. Yanwei. W. Gaojian. C. Factors Affecting Wound Healing. *Journal of Materials Chemistry B.* 2014. 2: 5718.
21. Anderson. J.M. Rodriguez. A. Chang. D.T. Foreign body reaction to biomaterials. *Seminars in Immunology*. 2008. 20(2): 86-100.
22. Stevens. K.N.J. *Blood-contacting biomaterials for critical clinical applications*. Ph.D. Thesis. Maastricht; 2011.
23. Lipsitt. B. Performance Properties of Metallocene Polyethylene EVA and Flexible PVC Films. Medical Plastics and Biomaterials Magazine 1998.
24. Lipsitt. B. Metallocene polyethylene films as alternatives to flexible PVC film for medical device fabrication. *Proceedings of the Society of Plastics Engineers 55th Annual Technical Conference (ANTEC '97)*. May 1997. SPE Brookfield. 1997. 2854–2858.
25. ReportLinker. (http://www.reportlinker.com/ci02234/Biomaterial.html). Retrieved date: 23, Dec, 2015.

26. Markets and Markets: Bio-Implants Market - by Type. (http://www.marketsandmarkets.com/Market-Reports/bio-implants-market-728.html). Retrieved date: 24, Dec, 2015.
27. Balaji. A. Jaganathan. S. K. Vellayappan M. V. John A. A. Subramanian A. P. SelvaKumar M. Mohandas H. Sundar Raj. M. and Supriyanto. E. Prospects of common biomolecules as coating substances for polymeric biomaterials *RSC Advances.* 2015. 5: 69660–69679.
28. Piyavit. P. Piyasan. P. Comparison of activity of ziegler-natta catalysts prepared by recrystallization and chemical reaction methods towards polymerization of ethylene *Engineering Journal*. 13(1). 2009: 57-63.
29. Global Polyolefin Industry Development. (http://blogs.eci99.com/global-polyolefin-industry-development/). Retrieved date: 27, Dec, 2015.
30. Ahmad. S. M. Chakrabarti. H. Shah. J. M. Walter K. Purushothaman V. A. and Wageeh A. Y. The Influence of Ziegler-Natta and Metallocene Catalysts on Polyolefin Structure Properties and Processing Ability Materials. *Materials*. 2014. 7: 5069-5108.
31. The free Library. Comparison of Ziegler-Natta and metallocene ethylene elastomer-products. (http://www.thefreelibrary.com/Comparison+of+Ziegler-Natta+and+metallocene+ethylene+elastomer...-a0118356600). 5, Jan, 2016.
32. Albert. J.V. Recent advances in metallocene catalyzed polymerization of olefins and other monomers. The 2nd annual UNESCO training. March 29 – 31. 1999.
33. Kaewarsa P. *Polymerization of ethylene over the supported Ziegler-Natta and metallocene catalysts on magnesium hydroxide and magnesiuum hydroxychloriede*. Ph.D. Thesis. Khonkaen University; 2005.
34. Nittaa. K.H. Tanaka. A. Dynamic mechanical properties of metallocene catalysed linear polyethylenes. *Polymer.* 2001. 42: 1219-1226.
35. Razavi-Nouri. M. Hay J.N. Thermal and dynamic mechanical properties of metallocene polyethylene polymer. *Journal of Materials Science*. 2001. 42: 8621-8627.
36. Bubeck. R.A. Structure–property relationships in metallocene polyethylenes. *Materials Science and Engineering.* 2002. 39: 1–28.

37. Sunny M. C. *Studies on Metallocene Polyolefin and Polyvinyl Chloride for Blood and Blood Component Storage Applications*. Ph.D. Cochin University of science and technology; 2006.
38. Jaganathan. S.K. Supriyanto E. Selva Kumar. M. Balaji. A.P. Manjeesh Kumar A. Biomaterials in Cardiovascular Research Applications. *BioMed Research International*. 2014: 1-13.
39. Gorbet. M.B. Sefton. M.V. Biomaterial-associated thrombosis: roles of coagulation Factors complement platelets and leukocytes. *Biomaterials* 2004. 25:5681– 5703.
40. Anderson. J.M. Biological response to materials. *Annual Review of Materials Research.* 2001. 318: 1–110.
41. Allan. S. H. Surface modification of polymers *Chinese journal of polymer science.* 1995. 13:1-9.
42. Gorbet. M.B. Sefton. M.V. Endotoxin: the uninvited guest. *Biomaterials*
    a. 2005. 26(34):6811-7.
43. Pallister. C. Watson. M. Haematology. Scion Publishing. 2010; 334–336.
44. Khorasani. M.T. Mirzadeh. H. Laser surface modification of silicone rubber to reduce platelet adhesion in vitro. *Journal of Biomaterials Science-Polymer Edition.* 2004. 14: 59-72.
45. Allison S. *Kr-F laser surface treatment of poly (methyl methacrylate glycol-modified poly (ethylene terephthalate) and polytetrafluoroethylene for enhanced adhesion of escherichia coli k-12*. Master of Science. Blacksburg; 2002.
46. Weibel. D.E. Michels. A.F. Horowitz. F. Cavalheiro. R.D. Mota G.V. Ultraviolet-induced surface modification of polyurethane films in the presence of oxygen or acrylic acid vapours. *Thin Solid Films* 2009. 517:5489–5495.
47. Rajajeyaganthan R. Kessler F.H. Surface modification of synthetic polymers using UV photochemistry in the presence of reactive vapours. *Macromolecular Symposium*. 2011. 299–300:175–182.
48. Olbrich M. Punshon G. Frischau I. Salacinskid H.J. Alexander. M. Heitz. J. UV surface modification of a new nanocomposite polymer to improve cytocompatibility. *Journal of Biomaterials Science*. 2007. 18:453–468.

49. Siegel. J. Reznickova. A. Chaloupka. A. Slepicka. .P Svorcik. V. Ablation and water etching of plasma-treated polymers. *Radiation Effect and Defects Solids.* 2008. 163:779–788.
50. Reznickova. A. Kolska. Z. Hnatowicz. V. Stopka. P. Svorcik. V. Comparison of glow argon plasma-induced surface changes of thermoplastic polymers. *Nuclear Instruments and Methods in Physics Research Section B.* 2011. 269:83–88.
51. Angshuman P. Sunil S. Surekha D. Microwave-assisted synthesis of silver nanoparticles using ethanol as a reducing agent. *Materials Chemistry and Physics*. 2009. 114: 530–532.
52. Zhang. G. Leparoux. S. Liao. H. Coddet. C. Microwave sintering of poly-ether-ether-ketone (PEEK) based coatings deposited on metallic substrate. *Scripta Materialia.* 2006;55:621–624.
53. Susan D. Surface modification of LDPE film by CO2 pulsed laser irradiation. *European Polymer Journal.* 2002. 38: 2489–2495.
54. Gomathi. N. Sudarsan N. Investigation on Argon–Oxygen Plasma Induced Blood Compatibility of Polycarbonate and Polypropylene. *Journal of Adhesion Science and Technology*. 2009. 23: 1811-1826.
55. Gomathi. N. Debasish M. Tapas K. Sudarsan N. Helium Plasma Treatment to Improve Biocompatibility and Blood Compatibility of Polycarbonate *Journal of Adhesion Science and Technology.* 2010. 24: 2237-2255
56. Heidari. S. Azhdadi. S.N. Asefnezhad. A. Sadraeian. M. Montazeri. M. Biazar. E. The relationship between cellular adhesion and surface roughness for polyurethane modified by microwave plasma radiation. *International Journal of Nanomedicine*. 2011. 6:641–647.
57. Ginn B.T. Steinbock O. Polymer surface modification using microwave-oven-generated plasma. *Langmuir*. 2003. 19:8117–8118
58. Badey. J.P. Urbaczewski. E.E Jugnet. D. Sage. Y. Minh C. B. Surface modification of polytetrafluoroethylene by microwave plasma downstream treatment. *Polymer*. 1994. 35:2472–2479
59. Drinkwater. B. Sounding out good adhesion. *Materials world.* 1998. 6 (3): 149-151

60. Poncin-Epaillard F. Legeay G. Surface engineering of biomaterials with plasma techniques. *Journal of Biomaterials Science Polymer Edition*. 2003. 14:10 1005-1028.

61. Shenton. M. J. Lovell-Hoare. M. C. and Stevens. G. C. Adhesion enhancement of polymer surfaces by atmospheric plasma treatment. *Journal of Physics D: Applied Physics*. 2001. 34: 2754–2760.

62. HA. S.W. Kirch. M. Birchler. F. Eckert. K.-L. Mayer. J. Wintermantel. E. Sittigt C. Spencer I. Guecheva. M. Vonmon. H. Surface activation of polyetheretherketone (PEEK) and formation of calcium phosphate coatings by precipitation. *Journal Of Materials Science: Materials In Medicine.* 1997. 8: 683-690.

63. Chandya T. Gladwin. S. Dasa R. Wilsona .F. Gundu. H.R. Use of plasma glow for surface-engineering biomolecules to enhance blood compatibility of Dacron and PTFE vascular prosthesis. *Biomaterials.* 2000. 21 (7): 699–712.

64. Mojtaba Mirabedinia S. Hamid R. Hamedifara. S. Mohsen Mohsenib S. Microwave irradiation of polypropylene surface: a study on wettability and adhesion. *International Journal of Adhesion & Adhesives*. 2004. 24: 163–170.

65. Alexander I. Aharon G. A microwave assisted process for coating polymer and glass surfaces with semiconducting ZnO submicron particles. *Journal of Applied Polymer Science.* 2009. 113: 1773 – 1780.

66. Schwarz J. Schmidt M. Ohl. A. Synthesis of plasma-polymerized hexamethyldisiloxane (HMDSO) films by microwave discharge. *Surface and Coatings Technology*. 1998. 98: 859–864.

67. Nelson D. L. Lehninger's Principles of Biochemistry. New York W. H. Freeman and Company: 2005.

68. Steinberg J. Neumann A. W. Absolom D. R. and Zingg W. Human erythrocyte adhesion and spreading on protein-coated polymer surfaces. *Journal of Biomedical Materials Research A.* 1989. 23: 591–610.

69. Bahareh H. S. and Ismaila A. Antioxidative peptides from food proteins: A review. *Peptides.* 2010. 31: 1949–1956.

70. Steven R. M. Xiaojuan K. Xin H. Elisabeth B. W. Mark W. G. Daniel J. K. The development of peptide-based interfacial biomaterials for generating biological functionality on the surface of bioinert materials. *Biomaterials* 2009. 30: 277–286.

71. Gao G. Lange D. Hilpert K. Kindrachuk J. Zou Y. Cheng J.T. Kazemzadeh-Narbat M. Yu K. Wang R. Straus S.K. Brooks D.E. Chew B.H. Hancock R.E. Kizhakkedathu J.N. The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. *Biomaterials* 2011. 32: 3899–3909.

72. Steven M. D. and Hotchkiss. J. H. Covalent immobilization of an antimicrobial peptide on poly(ethylene) film. *Journal of Applied Polymer Science*. 2008. 110: 2665–2670.

73. Sagnella S. and Mai-Ngam K. Chitosan based surfactant polymers designed to improve blood compatibility on biomaterials. *Colloids and Surfaces B: Biointerfaces.* 2005: 42 147.

74. Dagne. E. Bisrat. D. Viljoen. A. Van Wyk B.E. Chemistry of Aloe species. *Current Organic Chemistry*. 2000. 4:1055–78.

75. Grindlay. D. Reynolds T. The Aloe vera phenomenon; A review of the properties and modern uses of the leaf parenchyma gel. *J Ethnopharmacol.* 1986: 16:117–51.

76. Davis. R. H. Aloe vera; A Scientific Approach. Vantage Press 1st edition New York; 1997.

77. National Center for Complementary and Alternative Medicine (NCCAM) (http;//nccam.nih.gov/health/aloevera). Retrived date: 10, Feb, 2015.

78. Park. Y. Lee S. New Perspectives on Aloe. Springer Verlag. New York; 2006.

79. Collins. E. Collins. C. Roentgen dermatitis treated with fresh whole leaf of Aloe vera. *Am J Roentgenol.* 1935. 33:396–7.

80. Manderville F. Aloe vera in the treatment of radiation ulcers and mucous membranes. *Radiology* 1939. 32;598–9.

81. Nilanjanan. D. Chattopadhay. R.N. Commercial cultivation of Aloe. *Natural Product Radiance* 2004. 3(2):85-7.

82. The International Aloe Science Council (http;//www.iasc.org/faq.htmlhttp;//www.nutraceuticalsworld.com/issues/2003-05/view_features/aloe-vera-an-international-success-story/). Retrieved date: 15, Feb, 2015.

83. Saji. V.S. Choe. H.C. Yeung. K.W.K. Nanotechnology in biomedical applications; a review. *International Journal of Nano and Biomaterials.* 2010. 3(2):119–139.

84. World Health Organization. *WHO Monographs on Selected Medicinal Plants*. Vol. 1. Geneva; World Health Organization; 1999.

85. Tyler. V. The honest herbal; A sensible guide to the use of herbs and related remedies. 3rd ed. Binghamton New York; *Pharmaceutical Products Press*; 1993.

86. Saccu. D. Bogoni. P. Procida. G. Aloe Exudate; Characterization by Reversed Phase HPLC and Headspace GC-MS. *Journal of Agricultural and Food Chemistry* 2001. 49(10): 4526-4530.

87. Bradley. P.R. British Herbal Compendium. British Herbal Medicine Association Bournemouth; 1992.

88. Shuna. C. Suhara. P. Mohini. S. Abdulah. A. Aloe vera Rind Cellulose Nanofibers-Reinforced Films. *Journal of Applied Polymer Science*. 2014. 131:40592.

89. Vogler. B.K. Ernst. E. Aloe vera; A Systematic Re- view of Its Clinical Effectiveness. *The British Journal of General Practice* 1999. 49:823-828.

90. Townsend J. Aloe vera. T*he UK Reference Guide to Complimentary Medicine*. Chartwell House Publishing London; 1998.

91. Antherton P. Aloe vera; Magic or Medicine? Nursing Standard 1998. 12(41): 49-54.

92. Shelton. M.S. Aloe vera Its Chemical and Therapeutic Properties. International *Journal of Dermatology*. 1991. 30(10): 679-683.

93. Reynolds. T. Dweck. A.C. Aloe vera Leaf Gel; A Review Update. *Journal of Ethnopharmacology* 1999. 68(1-3): 3-37.

94. Pankaj. K.S. Deen. D.G. Ritu. S. Priyanka P. Therapeutic and Medicinal Uses of Aloe vera; A Review. *Pharmacology & Pharmacy* 2013. 4: 599-610.

95. Coats .B.C. *The Silent Healer-A Modern Study of Aloe vera*. Texas Garland; 1979.

96. Seong W. Myung H. A Review On The Relationship Between Aloe vera Components And Their Biologic Effects *Seminars in Integrative Medicine* 2003. 1(1): 53-62.

97. Ni. Y. Turner. D Yates. K.M. Isolation and characterisation of structural components of Aloe vera L. leaf pulp. *International Immunopharmacology*. 2004. 4: 1745-1755.

98. Balaji. A.P. Vellayappan. M.V. Agnes Aruna. J. Aruna Priyadarshini. S. Jaganathan. S.K SelvaKumar. M. Supriyanto. E. Mustafa. Y. Biomaterials based nano-applications of Aloe vera and its perspective: A review. *RSC Advances*. 2015. 5: 86199-86213

99. Josias. H.H. Composition and Applications of Aloe vera Leaf Gel. *Molecules* 2008. 13: 1599-1616.

100. Heggers. J.P. Beneficial Effect of Aloe on Wound Heal- ing in an Excisional Wound Healing Model. *Journal of Alternative and Complementary Medicine* 1996. 2(2): 271-277.

101. Davis. R.H Leitner. MG. Wound Healing. Oral and Topical Activity of Aloe vera. *Journal of the American Paediatric Medical Association* 1989. 79(11): 559-562.

102. Hayes. S. M. Lichen. P. Report of Successful Treat- ment with Aloe vera. *General Dentistry* 1999. 47(3): 268-272.

103. Chithra. P. Sajithal. G. B. Chandrakasan. G. Influence of Aloe vera on Glycosaminoglycans in the Matrix of Healing Dermal Wounds in Rats. *Journal of Ethano-pharmacology* 1998. 59(3): 179-186.

104. Silva. S.S. Popa. E.G. Gomes. M.E. Cerqueira. M. Marquea. A.P. Caridade. S.G. Teixeira. P. Sousa. C. Mano. J.F. Reis. R.L. An investigation of the potential application of chitosan/aloe-based membranes for regenerative medicine. *Acta Biomaterialia*. 2013. 9(6): 6790–6797.

105. Pacifici. E. Bossu. M. Giovannetti. A. La Torre. G. Guerra. F. Polimeni. A. Surface roughness of glass ionomer cements indicated for uncooperative patients according to surface protection treatment. *Annali di Stomatologia*. 2013. 4(3–4): 250–258.

106. Huan. Z. Maryam. N. Sarit. B. B. Microwave assisted apatite coating deposition on Ti6Al4V implants *Materials Science and Engineering C*. 2013. 33: 4435–4443.

107. Kapil D. P. Tae-Hyun K. Eun-Jung L. Cheol-Min H. Ja-Yeon L. Rajendra K. S. and Hae-Won K. Nanostructured Bio interfacing of Metals with Carbon Nanotube/Chitosan Hybrids by Electrodeposition for Cell Stimulation and Therapeutics Delivery. ACS Applied Materials & Interfaces. 2014. 26: 20214-24

108. Leena Pravina Amarnath Arvind Srinivas Anand Ramamurthi. In vitro hemocompatibility testing of UV-modified hyaluronan hydrogels *Biomaterials* 2006. 27: 1416–1424.

109. Fazley M. Elahi G. G. Lu W. Hemocompatibility of surface modified silk fibroin materials: a review. *Reviews on advanced materials science*. 2014. 38: 148-159.

110. Swallowe G.M. Mechanical Properties and Testing of Polymers An A–Z Reference, *Polymer Science and Technology Series*, Springer Science & Business Media, 2013; 1-302.

111. Pan. J. Modelling Degradation of Bioresorbable Polymeric Medical Devices, *Woodhead Publishing.* 2014: 1-260.

112. Geetha R. Torikai A. Nagaya S. Fueki K. Photo-oxidative Degradation of Polyethylene: Effect of Polymer Characteristics on Chemical Changes and Mechanical Properties. Part 1 Quenched Polyethylene. *Polymer Degradation and Stability* 1987. 19: 279-292.

113. Agnes Mary. S Giri Dev. V.R. Electrospun herbal nanofibrous wound dressings for skin tissue engineering. *Journal of the Textile Institute*. 2014. 106(8): 1-14.

114. Balaji. A.P. Jaganathan. S.K. Supriyanto. E. Muhamad. I.I. Zahran M. K. Microwave assisted fibrous decoration of mPE surface utilizing aloe vera extract for tissue engineering applications *International Journal of Nanomedicine* 2015. 10: 1–15.

115. Suganya. S. Venugopal. J. Ramakrishna. S. Lakshmi. B.S. Dev. V.R. Naturally derived biofunctional nanofibrous scaffold for skin tissue regeneration. *International Journal of Biological Macromolecules*. 2014. 68: 135–143.

116. Povnn A.S. The use of infrared spectra for the determination of minerals. *American Mineralogist.* 1978. 63: 956–959.

117. Christian Menno. M. Pejcic. B. Esteban. L. Delle Piane. C. Raven. M. Infrared attenuated total reflectance spectroscopy: an innovative strategy for analyzing mineral components in energy relevant systems. *Scientific Reports*. 2014. 4: 6764.

118. Arima. Y. Iwata. H. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. *Biomaterials*. 2007. 28: 3074–3082.

119. Poncin-Epaillard. F. Legeay. G. Surface engineering of biomaterials with plasma techniques. *Journal of Biomaterials Science Polymer Edition.* 2003. 14: 1005–1028.

120. Justina. V. Kristina. U. Gintaras. K. The effects of surface roughness on adhesion strength of coated ash (Fraxinus excelsior L.) and Birch (Betula L.) Wood. *Materials Science*. 2012. 4: 1-18.

121. Jialong C. Nan H. Quanli L. Chun H. Chuc J. Manfred F. M. The effect of electrostatic heparin/collagen layer-by-layer coating degradation on the biocompatibility. *Applied Surface Science.* 2016. 362: 281–289

122. Youling Y. Betsy M. Chesnutt L. Wright W. Haggard1 O. Joel D. Bumgardner. Mechanical Property Degradation Rate and Bone Cell Growth of Chitosan Coated Titanium Influenced by Degree of Deacetylation of Chitosan. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2008. 86B: 245–252

123. Beachleya. V. Wen. X. Polymer nanofibrous structures: fabrication biofunctionalization and cell interactions. *Progress in Polymer Science*. 2010. 35(7): 868–892.

124. Deligianni. D.D. Katsala. N.D. Koutsoukos. P.G. Missirlis. Y.F. Effect of surface roughness of hydroxyapatite on human bone marrow cell adhesion proliferation differentiation and detachment strength. *Biomaterials*. 2001. 22: 87–96.

125. Li. L. Crosby. K Sawicki. M. Shaw. L.L. Wang. Y. Effects of surface roughness of hydroxyapatite on cell attachment and proliferation. *Journal of Biotechnology & Biomaterials.* 2012. 2: 150.

126. Ruiming L. Yuansen Q. Huijin W. Yong Z. Zuojun H. and Shenming W. The in vivo blood compatibility of bio-inspired small diameter vascular graft: effect of submicron longitudinally aligned topography. *BMC Cardiovascular Disorders*. 2013. 13:79-8.

127. Deligianni. D.D. Katsala. N. Ladas. S. Sotiropoulou. D. Amedee. J. Missirlis. Y.F. Effect of surface roughness of the titanium alloy Ti-6Al-4V on human bone marrow cell response and on protein adsorption. *Biomaterials.* 2001. 22: 1241-1251.

128. Poletti G. Orsini F. Lenardi C. & Barborini E. A comparative study between AFM and SEM imaging on human scalp hair. *Journal of Microscopy.* 2003. 211: 249–255.

129. Josep F.B. Stefan G. G. Notni J.S and Jean M. B. Angela D. Surface characterization techniques for determining the root-mean-square roughness and power spectral densities of optical components. *Applied Optics.* 2002. 41 (1). 1-23.

130. Huanga N. Yanga P. Lenga Y.X. Chena J.Y. Suna H. Wanga J. Wanga G.J. Dingb P.D Xic T.F. Leng Y. Hemocompatibility of titanium oxide films. *Biomaterials.* 2003. 24 (13): 2177–2187.

131. Wen-Ching L. Da-Guang Y. Ming-Chien Y. Blood compatibility of thermoplastic polyurethane membrane immobilized with water-soluble chitosan/dextran sulfate. *Colloids and Surfaces B: Biointerfaces.* 2005. 44: 82–92.

132. Zhengbao Z. Yan Ma X. Yue Meng L. Zhifei D. Self-assembled hemocompatible coating on poly (vinyl chloride) surface. *Applied Surface Science*. 2009. 256: 805–814

133. Wang J. Pan C.J. Huang N. Sun H. Yang P. Leng Y.X. Chen J.Y. Wan G.J. Chu P.K. Surface characterization and blood compatibility of poly(ethylene terephthalate) modified by plasma surface grafting. *Surface & Coatings Technology.* 2005. 196: 307– 311

134. Linneweber J. Maria P. Dohmen U. Kertzscher K. Affeld Y. N. The Effect of Surface Roughness on Activation of the Coagulation System and Platelet Adhesion in Rotary Blood Pumps. *Artificial Organs.* 2007. 31(5):345-51.

135. Singh. S. Fahim. M.A. In vivo antiplatelet aggregator activity of *Aloe vera* juice on mice cerebral micro vessels. *Iranian Journal of Pharmaceutical Research*. 2004. 3: 62-70.