

INVESTIGATING THE BLOOD COMPATIBILITY OF METALLOCENE  
POLYETHYLENE SUBJECTED TO STEAM TREATMENT

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POLYETHYLENE SUBJECTED TO STEAM TREATMENT

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requirements for the award of the degree of  
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*I dedicate this thesis to my beloved family:  
My dearest parents, Mr. A John & Mrs. M Pauline Mary*

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## ABSTRACT

In this study, one of the green surface modification techniques, steam treatment was employed to improve the surface characteristics and haemocompatibility of metallocene polyethylene (mPE). The mean contact angle of untreated mPE (87.4°) decreased sharply for steam exposed mPE (60.25°). The increased surface roughness was demonstrated by atomic force microscopy (AFM), scanning electron microscopy (SEM) and Hirox 3-D microscopy. The mean roughness (Ra) of control mPE (2.757 nm) was increased to 8.753 nm by steam treatment, showed enhanced hydrophilicity. Fourier transform infrared spectroscopy (FTIR) analysis illustrated no chemical changes but the changes in absorbance intensity ensures the morphological changes in the treated samples. The blood compatibility studies were assessed by coagulation assays, haemolysis and platelet adhesion tests. The coagulation assays indicated a delay in clotting time on the steam exposed surface whereas haemolysis and platelet adhesion were significantly reduced. The green surface modification of mPE using steam enhanced its surface properties and haemocompatibility. The improved blood compatibility of mPE may help in efficient designing of haemocompatible biomaterials like cardiovascular implants.

## ABSTRAK

Dalam kajian ini, salah satu teknik pengubahsuaian permukaan semula jadi iaitu rawatan wap telah digunakan untuk meningkatkan ciri-ciri permukaan dan keserasian darah metallocene polyethylene (mPE). Min sudut untuk stim tidak dirawat mPE ( $87.4^\circ$ ) menunjukkan penurunan mendadak selepas rawatan stim untuk mPE ( $60.25^\circ$ ). Peningkatan kekasaran pada permukaan ditunjukkan dengan kekerasan mikroskop atom (AFM), mikroskop imbasan elektron (SEM) dan Hirox 3-D mikroskop. Min kekasaran (Ra) kawalan MPE (2.757 nm) telah meningkat kepada 8.753 nm oleh rawatan stim, menunjukkan hidrofilik dipertingkatkan. Fourier spektroskopi inframerah (FTIR) analisis menunjukkan tiada sebarang perubahan kimia tetapi perubahan keamatan kuantiti menunjukkan perubahan morfologi dalam sampel yang telah dirawat. Kajian keserasian darah dinilai menerusi ujian pembekuan, hemolisis dan ujian lekatan platelet. Masa pembekuan darah di permukaan stim ditangguhkan, hemolisis dan platelet melekat telah berkurang dengan ketara. Pengubahsuaian permukaan melalui kaedah semula jadi untuk mPE menggunakan rawatan stim telah meningkatkan ciri-ciri permukaan dan keserasian darah. Keserasian darah yang bertambah baik dengan mPE boleh membantu dalam mereka bentuk bahan keserasian darah seperti kardiovaskular implan.

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**LIST OF ABBREVIATIONS**

ATR-FTIR	-	Attenuated total reflectance fourier transformed infrared spectroscopy
3T3	-	3-day transfer, inoculum $3 \times 10^5$ cells
AFM	-	Atomic force microscopy
APTT	-	Activated partial thromboplastin time
BEL-7402	-	Hepatoma cells
CAGR	-	Compound annual growth rate
HA	-	Hydroxyapatite
HA	-	Haemolysis assay
HCL	-	Hydrochloric acid
LLDPE	-	Linear low density polyethylene
MC3T3-E1	-	Preosteoblast cell line
mPE	-	Metallocene polyethylene
NaOH	-	Sodium hydroxide
NW-PET	-	Non-woven polyethylene terephthalate
PANi	-	Polyaniline
PANi-AgNp	-	Polyaniline-silver nanoparticle
PC	-	Phosphatidylcholine
PCU	-	Polycarbonateurethane

PDMS	-	Polydimethylsiloxane
PEG	-	Polyethylene glycol
PEGMA <sub>s</sub>	-	Poly(ethylene glycol) monoacrylates
PEO	-	Poly(ethylene oxide)
PGS	-	Poly(glycerol sebacate)
PMMA	-	Poly(methyl methacrylate)
PP	-	Polypropylene
PRP	-	Platelet rich plasma
PT	-	Prothrombin time
PTMC	-	Poly(1,3-trimethylene carbonate)
PU	-	Polyurethane
PVA	-	Poly(vinyl alcohol)
PVC	-	Poly vinyl chloride
RBC <sub>s</sub>	-	Red blood cells
SD	-	Standard deviation
SEM	-	Scanning electron microscopy
VWF	-	Von willebrand factor
WBC <sub>s</sub>	-	White blood cells
XRD	-	X-ray diffraction

**LIST OF SYMBOLS**

$\text{cm}^{-1}$	-	Per centimetre
$\text{cm}^2$	-	Square centimetre
$\text{g/mol}$	-	Grams per mole
Hz	-	Hertz
MPa	-	Megapascal
mL	-	Millilitre
mm	-	millimetre
m Torr	-	Millitorr
M	-	Molar
nm	-	Nanometre
Ra	-	Average roughness
W	-	Watt
w/v	-	Weight per volume
$\mu\text{L}$	-	Microlitre
$\mu\text{m}$	-	Micrometre

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## **CHAPTER 1**

### **INTRODUCTION**

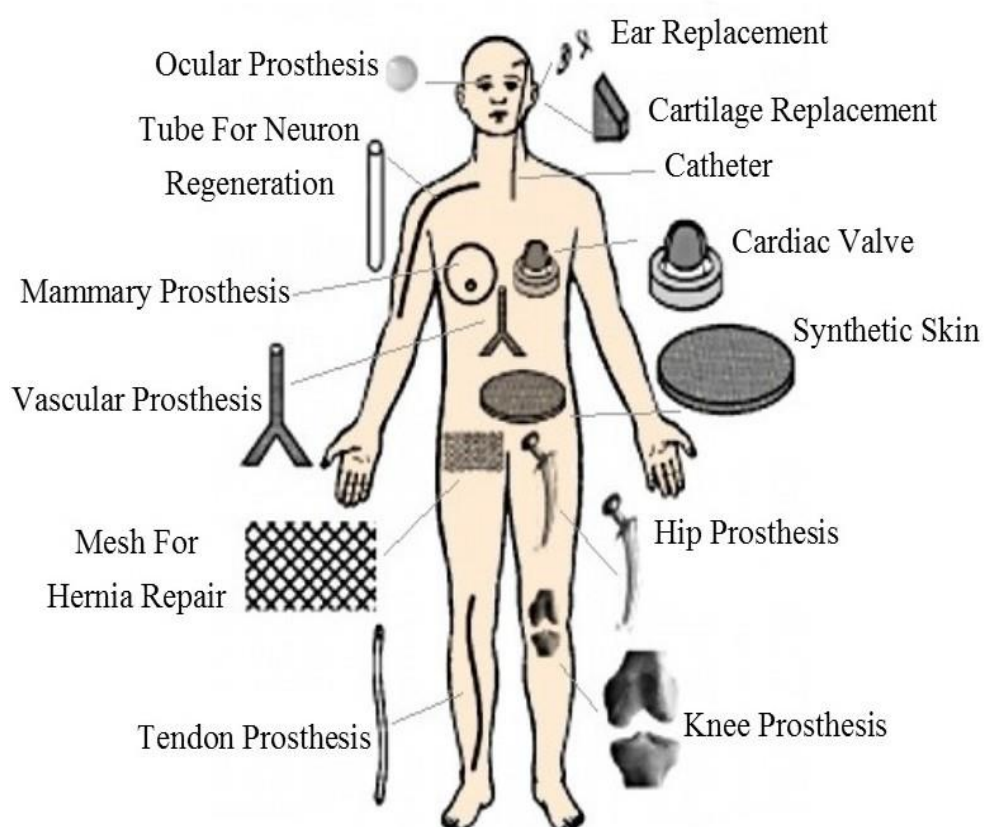
#### **1.1 General Introduction**

A biomaterial is any matter, or construct that interacts with the biological systems. Biomaterials possess biocompatibility which refers to the ability of a material to perform with an appropriate host response in a specific situation (Williams, 1999). Biomaterials can be derived either from nature or synthesized in the laboratory using a variety of chemical approaches utilizing metallic components, polymers, ceramics or composite materials. They are often used and/or adapted for a medical application, and thus comprise whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function (Williams, 2009).

The recent report expresses the reality that by 2017, the estimated global market for biomaterials will be 88.4 billion US\$ with a compound annual growth rate (CAGR) of 15% (Markets and Markets, <http://www.marketsandmarkets.com/PressReleases/global-biomaterials.asp>).

Biomaterials broadly fall into the four main types, namely metals, ceramics, polymers and biological substances. The selection of a biomaterial depends on the surrounding environment where it will be implanted. The implanted material should not cause any adverse effects like allergies, inflammation and toxicity, either immediately after surgery or under post-operative conditions.

The surface modification of biomaterials is defined as the process of changing the surface properties of a biomaterial by altering its physical, chemical or biological properties different from the existing characteristics that found on the surface of a material. The surface modification techniques are classified into three major categories namely 1. Physico-chemical methods, 2. Mechanical methods and 3. Biological methods, with each method having different divisions.



**Figure 1.1:** An example for the application of biomaterials

Among all four types, the polymers have widespread application in the field of biomaterials because of its excellent physico-chemical properties. The total North American market volume of polymers in medical devices totaled 1,370.0 million pounds, corresponding to revenues in excess of \$1 billion. By 2018, revenues are

expected to equal \$1.45 billion, fuelled by a compound annual growth rate of 5.2 percent (<http://www.frost.com/prod/servlet/press-release.pag?docid=266870643>).

**Table 1.1:** Biomedical applications of polymers

<b>Parts of the body</b>	<b>Polymers used</b>
Ear and ear parts	acrylic, polyethylene, silicone, poly vinyl chloride (PVC)
Denture	acrylic, ultrahigh molecular weight polyethylene (UHMWPE), Polymethyl methacrylate
Facial prostheses	acrylic, silicone, nylon, Polyurethane, Polytetrafluoroethylene
Tracheal tubes	acrylic, silicone, nylon
Vascular grafts	Polytetrafluoroethylene, Polyethylene terephthalate.
Breast implants	Polydimethylsiloxane.
Heart valves	polyester, silicone, PVC
Pacemaker	polyethylene, acetal
Lung , Kidney and liver parts	polyester, polyaldehyde, PVC
Oesophagus segments	polyethylene, polypropylene (PP), PVC
Blood vessels	PVC, polyester
Orthopaedic implants	acrylic, nylon, silicone, PP, UHMWPE
Hip and knee joint replacements	Polyethylene, Polydimethylsiloxanes

New advancements in polymer technology to resolve this increasing demand of polymers in medical field inspired us to explore the existing metallocene polyethylene (mPE) that possess a variety of attractive performances like better tensile strength, elongation, toughness with excellent resistance to puncture, impact and bursting (Lipsitt, 1998). The excellent permeability to oxygen and excellent fence to ammonia and water makes metallocene polyethylene as a promising candidate for blood contacting devices and medical implants.

The foremost reason for the limitation of mPE in medical applications is the lacking of its blood compatibility (Mohandas *et al.*, 2013) and so various surface modification techniques are being employed to improve the surface characteristics thereby enhancing the blood compatibility of metallocene polyethylene

The green surface modification using steam is non-toxic, non-corrosive controlled oxidation technique to modify the surface characteristics providing better biocompatibility with improved surface properties. Further, it's safer and eco-friendly which makes steam treatment technology as an attractive choice over the other treatments in surface modification of biomaterials (Feldbauer, 2007).

Steam treatment is interrelated with green chemistry, which does not involve in usage of any chemicals that encourages the design of products and its processes, thereby minimizing the use and production of hazardous substances or wastes. Since, steam is entirely pure it does not produce any harmful effect to the surface and also to the environment and no toxicity to human health (Lee *et al.*, 2013).

Generally, the surface modification of biomaterials can be performed especially for the biocompatibility enhancement, which is the most important feature while selecting a medical implant (Jaganathan *et al.*, 2014b). For the first time, the mPE polymer was treated with steam, a gaseous state of water to enhance its blood compatibility. In this work, the surface characteristic changes along with blood compatibility of steam treated mPE were studied and documented.

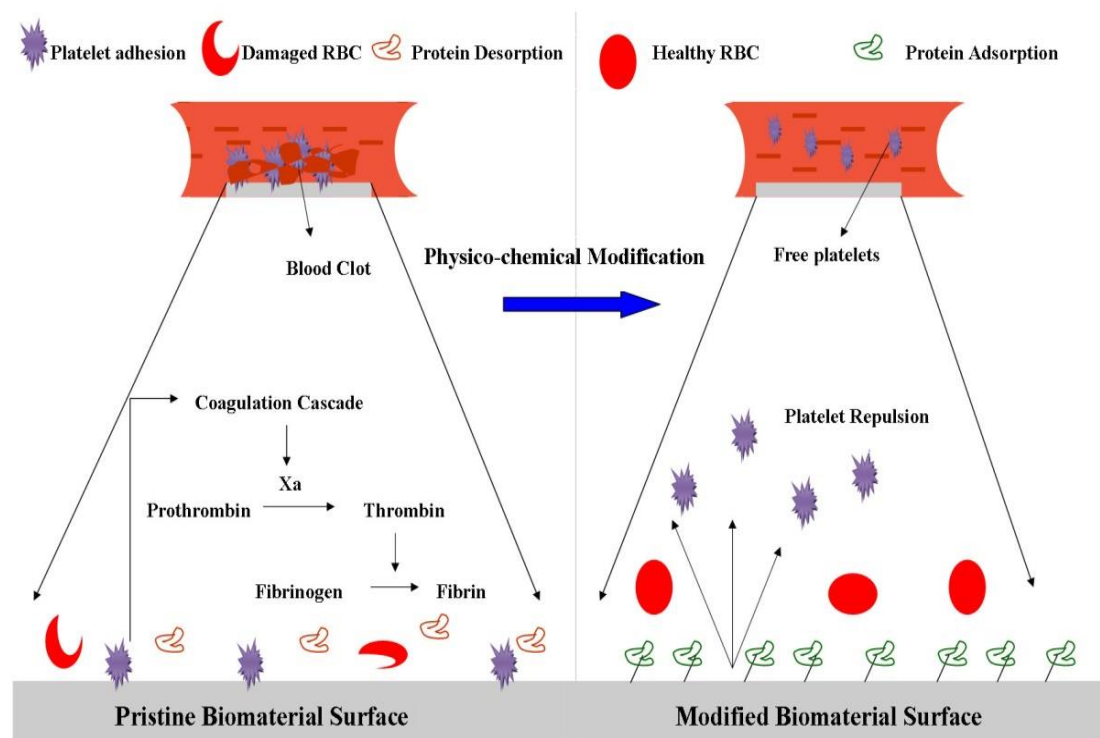
## **1.2 Problem Statement**

Blood compatibility is the foremost consideration for the medical implants. Although mPE has excellent physico-chemical and mechanical properties it fails as a promising biomaterial because of its poor bio and blood compatibility. Biocompatibility is a vital factor which determines the quality of a biomaterial and its application in various arenas. It may be defined as the ability of the material to

perform at a specific region with the appropriate host reaction. The events occur when the blood comes in contact with the implant is collectively called as blood mediated reactions or blood compatibility.

Whenever the blood comes in contact with the implants (biomaterial) it will lead to following complications:

1. Blood component's interaction with surfaces resulting in protein and water absorption
2. Blood cells interfere with the surface of biomaterial and may result in destruction of blood cells and these actions lead to the haemostasis and coagulation.



**Figure 1.2:** Blood compatibility problems resolved through surface modification (John *et al.*, 2015)

A promising biomaterial is one which does not elicit the above reactions. Hence, the biomaterials are subjected to surface modification to enhance its blood compatibility by eliminating the above mentioned complications

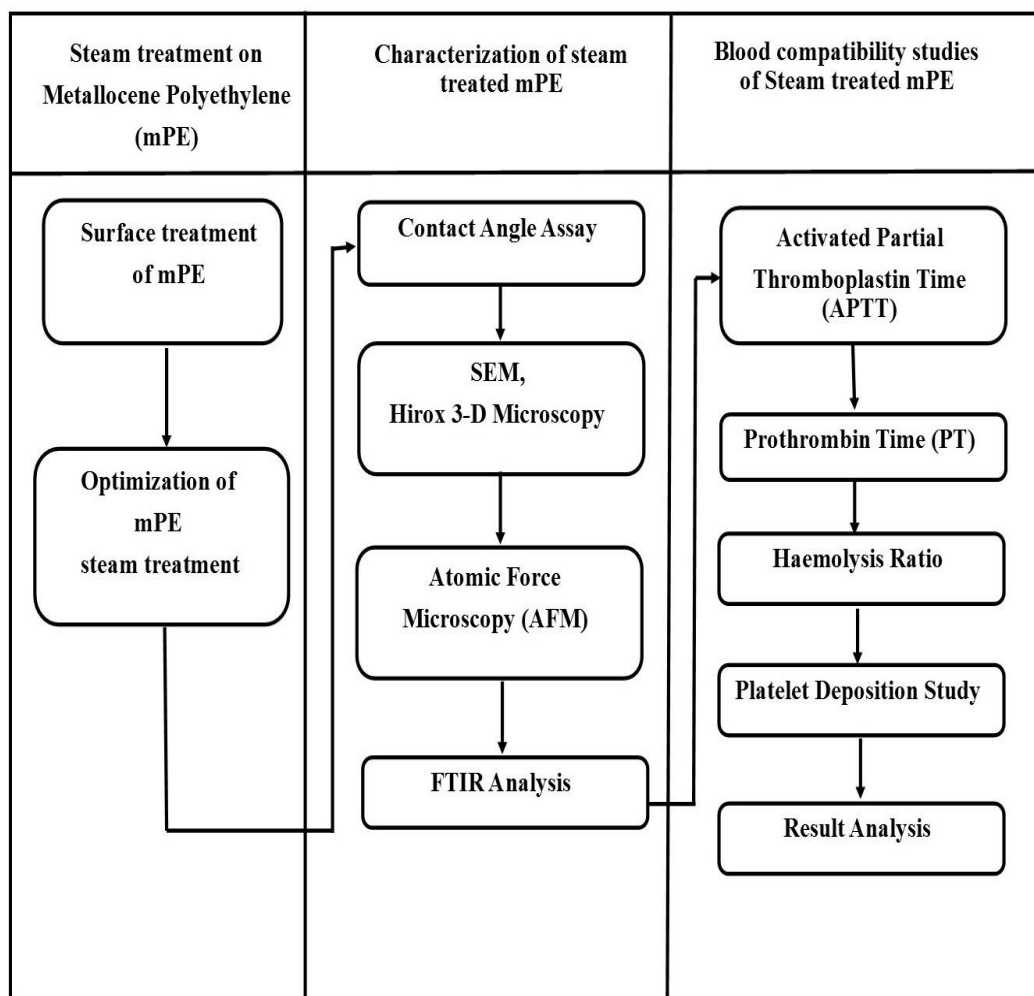
### **1.3 Objectives**

1. To study the physico-chemical modifications of steam treated mPE and comparing it with the physico-chemical characteristics of untreated mPE.
2. To investigate the changes in the blood compatibility of the steam treated mPE surface.

### **1.4 Scope of the Research**

The first part of the research was focused on the surface characterization of the metallocene polyethylene. The hydrophilicity of mPE was evaluated by means of contact angle measurements. The surface roughness were determined using scanning electron microscope (SEM), Hirox 3D microscopy and Atomic force microscopy (AFM). The chemical or functional group changes was investigated through Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR). The purpose of the surface characteristic study was to access the hydrophilicity and surface changes of mPE modified by the steam treatment

The second part of the study involved in blood compatibility studies of steam exposed metallocene polyethylene. The blood clotting time was estimated through Activated partial thromboplastin time (APTT) and Prothrombin time (PT). Haemolysis assay (HA) was performed to evaluate the destruction of red blood cells due to the implant material (mPE). The number of platelets adhered to the material surface was estimated by performing platelet adhesion test. The intention of this study is to evaluate the blood compatibility of steam exposed mPE.



**Figure 1.3:** Scope of the Research

Lastly the steam induced metallocene polyethylene was studied in terms of both surface characteristics and blood compatibility studies.

### 1.5 Significance of the Research

This research provides a scope for the polymer implant manufacturers in producing the steam treated mPE implants possessing enhanced blood compatibility. Evaluating both the physico-chemical and blood compatibility of the steam treated mPE helps in promoting the longevity of biomaterial implants.

Steam treatment is one of the green surface modification techniques that does not involve in usage or production of any chemicals or hazardous wastes. It is the most cost effective surface modification technique and also harmless method. Further, it's safer and eco-friendly which makes steam treatment technology an attractive choice over the other treatments in surface modification for the blood compatibility enhancement.



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