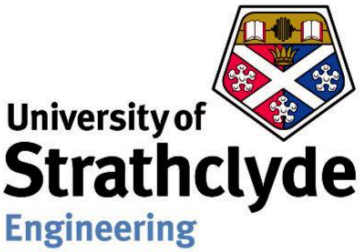
Thesis for the award of Master of Science

A system for impedance characterization of coronary stents

Divya Gopinath

August 2015



Department of Bioengineering

Wolfson Building

106 Rottenrow, Glasgow G40NW

Supervisor:

Dr. Christopher McCormick

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed:

Date:

ABSTRACT

Stents are used to re-open and maintain flow in diseased coronary arteries in patients suffering from coronary heart disease. Drug-eluting stents are now the preferred choice of stent but their performance is still limited by delayed healing of the important endothelial cell layer that lines the artery wall (Capodanno et al, 2011). It is very important that the endothelial cells that are damaged following the implantation of the stent are regenerated.

Much research has been carried out to monitor endothelialisation of the stent-tissue interface using invasive methods, but such methods are commonly only suitable for *in vitro* studies (Prasad et al, 2005), meaning they cannot be practically used clinically and can only provide information at a limited number of time points. The technique of impedance spectroscopy may allow the non-invasive measurement of endothelial cell growth on coronary stents. This project therefore sought to measure the *in vitro* impedance of a range of coronary stents.

Impedance values were recorded from stainless steel wires, bare metal and drug-eluting stents in an in vitro experimental set up, where the stent itself acted as an electrode. The effects of temperature, magnetic agitation, medium, time and various different stent types on impedance were evaluated.

It was found that each of the variables examined had an effect on the system impedance. The highest impedance was observed with the Cypher drug-eluting stent group, followed by a novel polypyrrole coated stent, whilst the bare metal stents displayed relatively low impedance levels. In most stent materials examined, the impedance characteristics varied with time. These findings provide the basis for future investigation of the non-invasive measurement of endothelial cell growth on coronary stents.

Acknowledgements

I would like to extend my gratitude to each individual who has supported and helped in this project. This project work has been a rewarding and long journey with new experiences. This could not have been possible without these individuals.

First of all, I would like to thank my supervisor Dr Chris McCormick, for his guidance and support whenever it was needed. I would also like to thank Brian Cartlidge, Katie Henderson and all staff and students working in the level 4 Laboratories of the Wolfson Building for their advice and support.

Finally, my parents, Dr.Usha Gopinath, and S.Gopinath for supporting me during my studies and for always having been an inspiration in whatever I take up. Also my friends who have all been a fantastic support during my project. Without them I would not have been strong and motivated.

Kind regards,
Divya Gopinath

TABLE OF CONTENTS

List of tables

Chapter 3 - Materials and Methods

Chapter 4 –Results

List of figures

Chapter 1 – Introduction

Chapter 2 –Theory

Chapter 3 - Materials and Methods

Chapter 4 –Results

Chapter 5 – Discussion

CHAPTER 1 – INTRODUCTION

1.1 Coronary artery disease

The leading cause of death in UK and worldwide is coronary heart disease (CHD). It has been estimated that 73,000 deaths occur in the United Kingdom (UK) each year due to CHD. It is estimated that around 2.3 million people are currently living with CHD in the UK. It affects more men than women. However, after 50 years of age the chances of being diagnosed with CHD are similar in both the sexes (NHS, 2014). It is a condition where a waxy substance known as plaque gets deposited inside the coronary arteries. These arteries are responsible for supplying oxygen rich blood to the heart muscle. The condition when the plaque builds up in the arteries is described as atherosclerosis. The plaque takes many years to become fully deposited in the arteries.

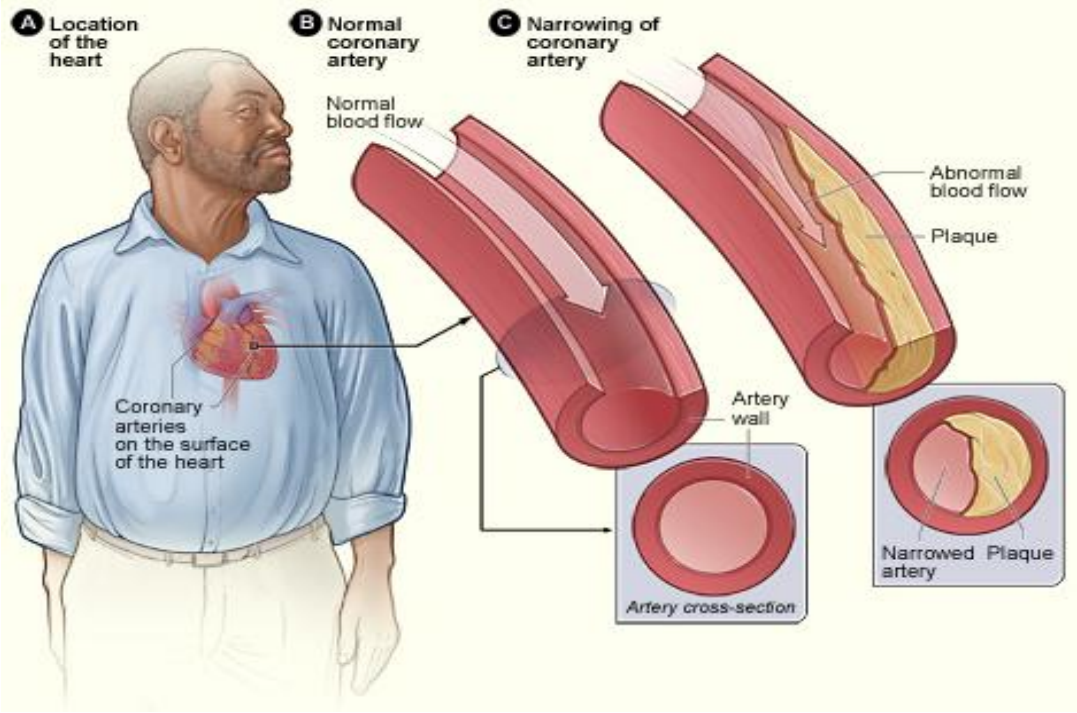


Figure 1-Illulstration of plaque formation in a coronary artery, leading to disturbed blood flow to the heart tissue (Source: Medical Education, INC)

The plaque causes hardening and thickening of the coronary artery walls, reducing the blood flow to the heart (Figure. 1). It may also rupture, resulting in formation of a

blood clot at the plaque surface, completely blocking the flow of blood through the coronary artery, ultimately leading to myocardial infarction (commonly known as a heart attack).

1.2 Percutaneous Coronary Intervention

Coronary angioplasty, also known as percutaneous coronary intervention (PCI), is used to treat CHD. It is a non surgical procedure used to re-open the narrowed lumen of the diseased coronary artery, allowing the flow of blood to circulate to the heart muscle once again.

To perform a PCI procedure, an interventional cardiologist injects local anesthesia and a needle is inserted into the femoral artery. A guide wire is placed through the needle and the access needle is removed. An introducer is placed over the guide wire and then the wire is removed by placing a different sized guide wire in its place. A catheter is inserted through the introducer into the blood vessel, which is guided to the aorta and the guide wire is removed. All of this is performed using X-ray fluoroscopy to guide the procedure. After placing the catheter into the coronary artery a contrast dye is injected and a further X-ray is taken to visualize the extent of the disease.

After finding the blockage, a balloon catheter is passed to the affected site and the balloon is inflated to compress the artery wall and then deflated. This is repeated till the passage of the artery is widened and then a stent is placed in the coronary artery to open the vessel. After stenting, contrast dye is again administered and X-ray is taken to check the condition of the artery after placement of stent.

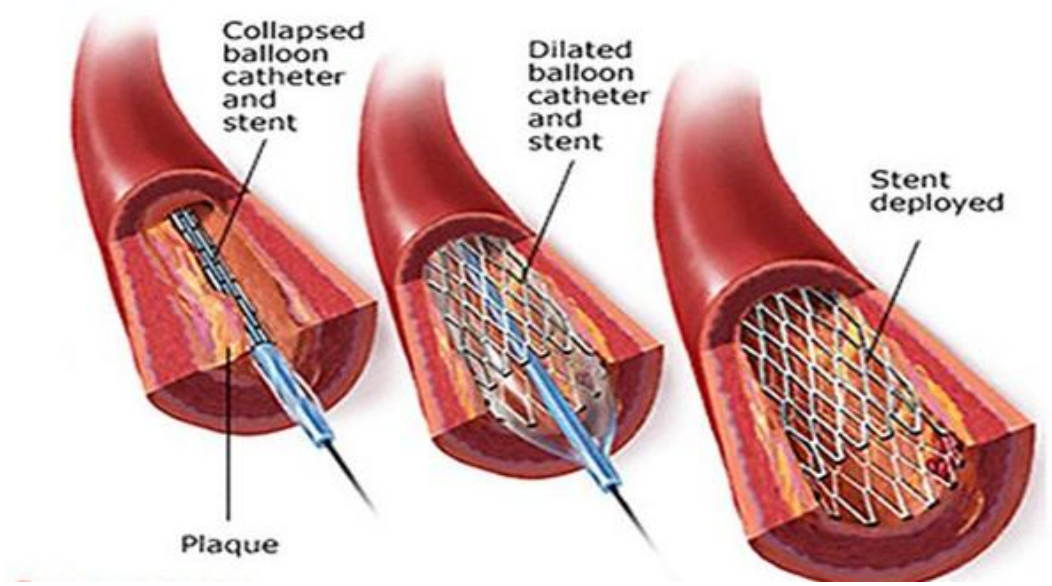


Figure 1.1 – Procedure of coronary angioplasty which is used to widen the narrow coronary arteries to restore the blood flow to heart muscle.

Stents are used to reopen and maintain continuous flow in diseased coronary arteries. They have become the standard method, being used in over two thirds of coronary revascularization procedures carried out in the UK. Coronary stents are mesh tube shaped devices placed in the heart that help provide continued mechanical support to the vessel wall, thus maintaining its diameter following the angioplasty procedure, aiding blood flow in the long term.

1.3 In-stent restensosis

A stent is a foreign object in the body and the body responds to the presence of the stent in a variety of ways. Platelets and inflammatory cells accumulate around the stent and nearby smooth muscle cells proliferate and migrate. These physiological changes lead to restenosis, comprising formation of a neointima which can lead to return of symptoms to the patient. It was found that approximately one third of patients who received a bare metal stent required a repeat revascularization procedure, often within a year or so of the initial intervention (Karin et al, 2013).

There has been a lot of progress in the stent platforms since first generation bare metal stents. The material used was generally stainless steel, although more recent stent platforms are now available that use cobalt chromium or platinum-chromium

metal alloys. Platinum-chromium platforms were found to be stronger and more radio–opaque than stainless steel (Capodanno et al, 2011).

Bare metal stents have 3 different designs – coil, tubular mesh, and slotted tube. Metallic wires are formed into a circular coil shape in case of the coil design. The tubular mesh is described by wires coiled together in a meshwork forming a tube. The slotted tube has metals from a laser cut design. Stents are generally expanded at the diseased vessel site by expansion of a balloon catheter, although some self expanding stents are also used. Stents have different widths, stent diameters, radial strength, thrombogenicity and MRI compatibility.

1.4 Drug-eluting stents

The most advanced present day stents are drug-eluting, which are used for the treatment of long lesions located in the smaller coronary vessels, since the risk of restenosis in these vessels is increased with conventional bare metal stent treatment. Drug-eluting stents (DES) are now an established technology and are now used in more than half of all stent procedures in this country. Clinical data support their use by virtue of demonstrated reductions in in-stent restenosis in many trials (James, 2015). Similarly, their effects on arteries are well described, and it is generally accepted that they act through inhibition of smooth muscle cell proliferation (James, 2015). Drug-eluting stents (DES) are coated with drugs that prevent the process of restenosis and clinical trials have found that rates of restenosis were reduced to below 10% (James, 2015). Also patients with diabetes needed a lower number of repeat procedures compared to bare metal stent treatments (James, 2015). DES consists of mainly three parts – stent platform, coating and drug. The stent is an expandable metal alloy framework as previously described. The coating is normally a polymer which holds and releases the drug over a sustained period of weeks. The drug is responsible for the inhibition of neointimal growth. The advantage of DES is that high drug loads are delivered to the tissue around the stent locally, without causing systemic toxicity.

Many drugs have been investigated, with cell proliferation and migration and platelet activation being the initial therapeutic targets (Waksman, 2002). The most recent

advancement is the use of anti-inflammatory immunomodulators with potent anti-proliferative effects, which include sirolimus, and everolimus. Paclitaxel, another potent anti-proliferative drug, has also been used extensively. Many studies have been carried out and have concluded that both cardiac events and in-stent restenosis are reduced in comparison to bare metal stents (Waksman, 2002; Van der Hoeven , 2004 ; Stone , 2005).

Late stent thrombosis is one disadvantage arising from DES which can lead to blood clots and occlusion of the stented vessel after the implantation. DES release drugs which are thought to stop the growth and migration of cells. This method decreases the proliferation and migration of vascular smooth muscle cells leading to elimination of in-stent restenosis. However, the drugs not only affect the smooth muscle cells but also have an effect on the endothelial cells. After stent implantation, re-endothelialisation is an important factor for healing the damage caused. The drugs cause decrease in the formation of a healthy endothelial layer. The endothelial layer helps in the prevention of platelet aggregation and formation of thrombus. Due to this, patients implanted with DES are thought to have a prolonged risk of thrombus formation. The stent material is a metal which attracts platelets to form clots and has a serious problem if it blocks the arteries and can be fatal (Liistro , 2001 ; Ong et al , 2005).

Anti-platelet drugs such as aspirin and clopidogrel are administered to patients for durations of four to six weeks after the implantation of bare metal stents and for significantly longer following DES implantation (NICE, 2003). They help in the prevention of stent thrombosis by reducing the platelet adhesion at the site of the stent. It has been suggested that the use of anti-platelet therapy for longer durations has some potential side effects, such as risk of haemorrhage and excessive blood loss leading to another surgery (Cowper et al, 2005).

1.5 Stent Thrombosis and the role of the endothelium

Although they have generally been viewed as a significant advance, it is clear that DES do have a number of limitations. Perhaps the most serious one is in-stent thrombosis, which has been described above. This complication is often fatal and

consequently, patients are advised to take antiplatelet drug therapy following stent implantation to lower this risk. Although in-stent thrombosis has also been observed with bare metal stents, generally at similar incidence levels to DES, the phenomenon of late stent thrombosis occurring many months and sometimes years after the implant procedure, lead to prolonged use of dual anti-platelet therapy being the standard recommendation for DES patients. Although the mechanisms responsible for late-stent thrombosis are still not completely understood, post-mortem studies have provided evidence of incomplete healing of the endothelial layer with DES compared to BMS (Figure.1.4). It is therefore worth considering in some detail the components of the healing response following stent placement.

Bare metal stent placement induces a complex healing response, comprising a series of cellular reactions. It involves various processes, including near complete destruction of the endothelial cell layer, thrombosis, inflammation, and smooth muscle cell proliferation and migration, ultimately leading to remodeling of the artery wall and neointima formation and restoration of the endothelium. This healing response is observed to be more rapid in animals, occurring over a period of around one month in the pig coronary artery model, compared to around three months in humans (Karin, 2013).

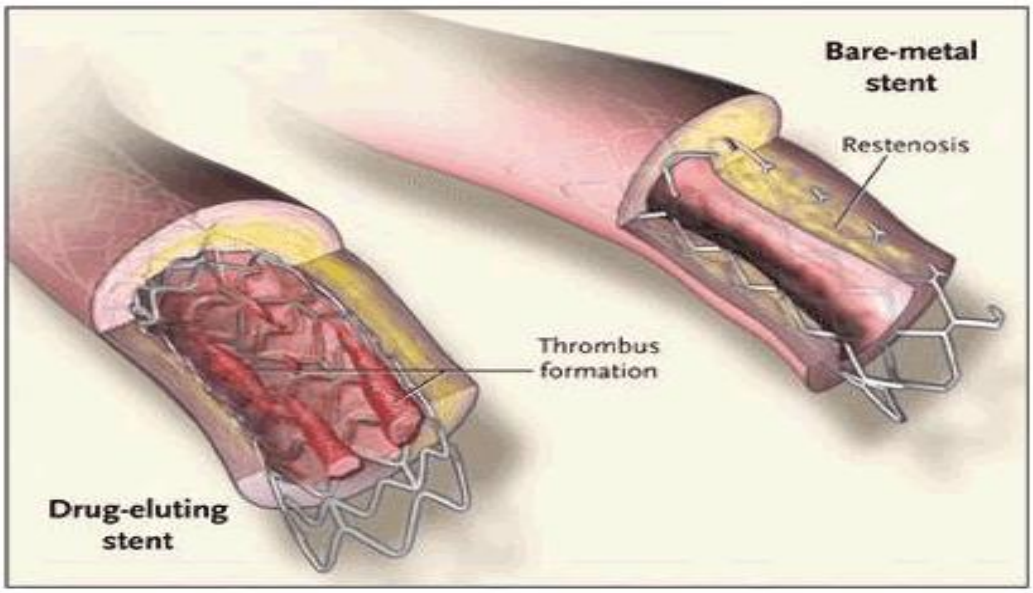


Figure-1.2 Difference between the drug eluting and bare metal stent after being placed in the artery. The problem arising in BMS is restenosis and in DES is the formation of thrombus.

The endothelium plays a crucial role in maintaining normal artery function and therefore its restoration is thought to be a crucial aspect of the healing response following stenting.

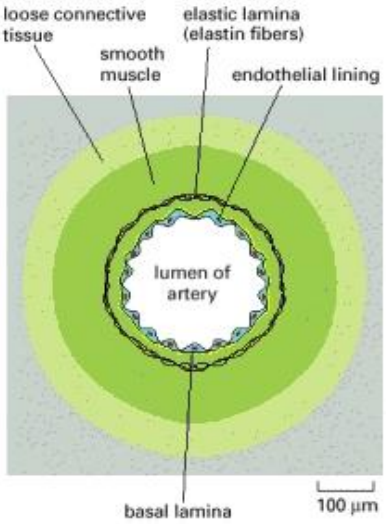


Figure 1.3- The various parts of an artery labeling the innermost part to the various tissues surrounding it.

Endothelial cells have mechanoreceptors for sensing the shear stress occurring from the flow over their surface, allowing these cells to signal the information to the surrounding cells. Consequently, the endothelial cells help the blood vessel to adjust its diameter to variations in shear stress by contracting or relaxing. Over time, such signaling can lead to longer term remodeling of the vessel to accommodate the flow of blood. Prostacyclin and Nitric Oxide (NO) are key molecules that the endothelium release to signal relaxation and to induce other changes within the artery wall. Importantly, release of these molecules prevents thrombus formation.

Post-mortem studies have shown higher rates of delayed healing and incomplete healing following DES compared to BMS (Virmani et al, 2012).

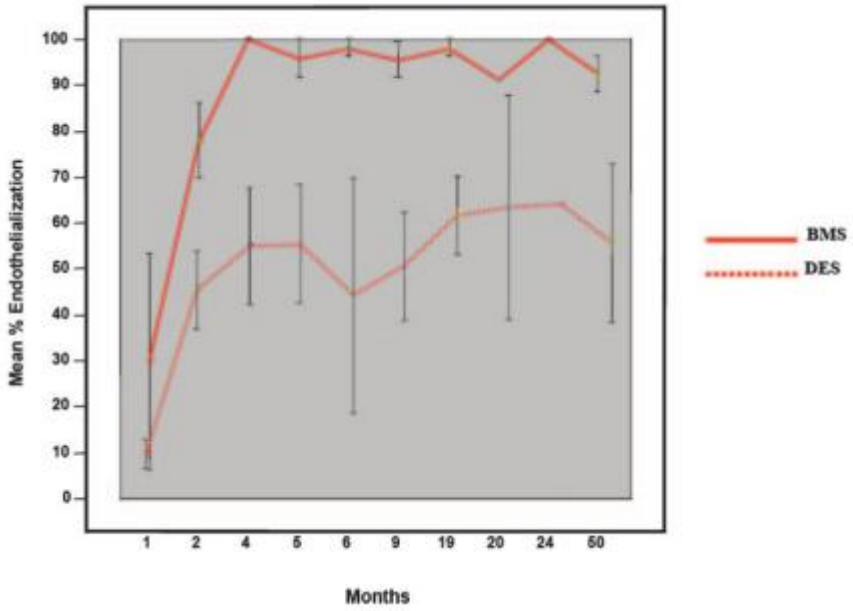


Figure 1.4- Reendothelialsation in BMS and DES considering the time in months and the mean endothelialisation in percentage.

Thomas, et al (2007) observed poor endothelial cell junction formation and micro thrombi of focal platelet aggregation at 16 months after stent with rapamycin was implanted in a patient who died due to a non DES related issue. Rapamycin restrains proliferation, migration and differentiation of human endothelial progenitor cells in vitro and hence may prevent proper endothelial healing.

Prado et al (2011) found that bare metal stents showed complete endothelialisation at 7 days and Finn et al (2012) found similar rate of endothelialisation after 14 days in case of pigs with the use of cobalt chromium stents. A complete endothelialsiation was not found until 28 days after implanting the stent in the iliac artery of rabbit.The polymers used in early generation DES were associated with hypersensitivity responses and late stent thrombosis after implantation. Such polymers have also been associated with impaired recovery of a functional endothelium.

The frequency of in-stent thrombosis was not found to have decreased with DES in comparison to BMS. Many cases have been reported of rapamycin coated stents causing stent thrombosis. Reports suggest that 3 years after implantation late stent thrombosis has been experienced due to DES which was not observed with BMS.

Stents are foreign objects and thus induce platelet adhesion and activates the coagulation cascade. Vascular injury occurs due to high pressure implantation and non compliant balloons. This is due to exposure of thrombogenic molecules of subintima and media to blood stream. Due to this, potent platelet inhibition makes the process feasible and hypo responsiveness of antiplatelet has been linked with an increased risk of stent thrombosis. In case of patients implanted with DES dual antiplatelet therapy is administered for a course of 12 months , although this is being reduced with more advanced generation DES.

The study conducted by Thomas et al(2007) had an in vivo test to check the healing of endothelial cells in various animal models and in healthy pigs. It was found that the endothelialisation is similar for BMS and DES at 28 days. At 48 days there was a clear delay observed in the endothelialization in sirolimus and paclitaxel eluting stents. After implanting BMS, almost complete endothelisation was suggested to take place by 3 to 4 months. A morphological autopsy study compared coronary segments from patients after the implantation of BMS and DES and found a delayed arterial lining and low endothelisation for DES in comparison with BMS of similar duration.

Due to the stent being implanted in the heart, the endothelial cells lining the arteries and blood vessels gets damaged or ruptured. Many factors affect the increase in risk of stent thrombosis which includes the implantation, number of implanted stents, stent length and dissection. Also patient and lesion characteristics, design of stent, premature cessation of anti platelet drugs also play a role. The drugs released from DES have biological effects which include activation of signal transduction pathways and inhibition of cell proliferation. DES damages the development of reendothelialization leading to delay in arterial healing and induces tissue factor expression which in turn leads to prothrombogenic environment (Thomas & Goodyer , 2003).

Although there are many newer generations of stents that may have better performance, in terms of thrombosis, there are still intense research efforts underway to develop stents that promote endothelialisation and these remains an important area. Despite this, there are limited means of measuring endothelialisation.

1.6 Methods of monitoring recovery of the endothelium

It is very important to measure the re-endothelialisation of cells on stent surfaces. However, to date there has not been a non-invasive method found to measure the re-growth of endothelial cells. Existing procedures for visualizing arteries are all invasive, and include Optical Coherence tomography (OCT), Intravascular Ultrasound (IVUS) and contrast angiography.

OCT is a medical imaging technique that uses light to capture very high resolution three Dimensional images from optical scattering media which are the biological tissues. Lasers are employed in this technique.

IVUS involves the use of a specially designed catheter with a miniaturized ultrasound probe attached to distal end of catheter. The proximal end of the catheter is attached to computerized ultrasound equipment. This imaging is helpful for visualizing the blood vessels, surrounding blood column and for endothelium of blood vessels (Prati et al, 2002). The main disadvantages of these techniques are the risk of usage of IVUS catheters, need of trained professionals to operate these techniques, their high cost and it is time consuming.

Due to the above disadvantages there is a need for measuring the endothelialisation of cells by a non-invasive method. In addition OCT , IVUS , angiography can only provide information at a limited number of time points in the healing process.

There are a few non-invasive tests such as magnetic resonance imaging (MRI) and computed tomography (CT) scans that are currently used for measuring in-stent restenois. CT is defined as the imaging technique which uses an injected contrast media to view the coronary arteries. Different CT scanning versions have been investigated for their use in imaging coronary arteries (Schuif et al, 2004; Rist et al, 2006, Achenbach, 2006). Imaging the coronary arteries is a difficult task due to their small dimensions and multi-slice CT has been used in the past for imaging large, stationary vessels (Achenbach , 2006).

There has been much advancement by the advent of 16 and 64 slice CT with improved spatial and temporal resolution and good sensitivity and specificity. Hence they can be used in the detection of coronary artery stenoses. The movement of coronary arteries due to their small dimensions and the artifacts produced by the stent material caused a major concern. The cost of the CT procedure is high and also the availability is an issue. The dosage of radiation the patient is exposed to and the requirement of high spatial resolution led to low and serious morbidity (Hauser, 2006).

1.7 Bioimpedance measurements

There have been studies where the electrical impedance is used to monitor cell proliferation in vivo and in vitro(Shedden et al , 2009). Cells have resistive and capacitive properties which could be measured with different methods. Cell membrane behaves as a capacitor due to its structure, whereas the extracellular fluid and contents are resistive by nature. The resistive property is due to the ion content. Hence the cells and tissues are represented with a combination of resistors and capacitors.

The impedance measurements taken in vivo have been used in the detection of cancer. Based on the different tissues such as malignant, benign and normal , the impedance properties changes based on their cell content and membrane properties (Scholz et al ,2000). A study carried out by Zou and Guo (2003) suggests that higher conductivity and permittivity is exhibited by malignant tissues with lower electrical impedance. The technique where voltage is applied to needle electrodes and inserted into the body is categorized as invasive. Impedance readings are taken when voltage is applied to the electrodes.

Another example relates changes in body mass to changes in impedance. Bioelectrical impedance analysis (BIA) has been used in studies to estimate the composition of the body (Cox-Reijven , 2000 , Nagano et al , 2000). The water in body and cell mass is indicated by the resistance, reactance and phase angle. Resistance is inversely proportional to the water in the body, Reactance is the

capacitance exhibited by the cell membrane. Phase angle is proportional to the ratio of reactance and is a measure of nutritional data. According to Nagano et al (2000) the increase in body weight is linked to increase in reactance and phase angle and inversely proportional to resistance.

Bioimpedance is described as the electrical impedance produced by any material due to a reflex opposition when applied with an alternating current. For this the impedance produced due to the electrodes on application of an alternating current on the cell or tissue needs to be taken into account. Conductivity and permittivity with reference to dielectric theory is expressed with relation to passive electrical properties exhibited by biological cells and tissues.

Shedden ,et al (2008) focused on measuring the electrical impedance of cells and tissues with the neointimal growth that characterizes in-stent restenosis in coronary artery stents. A cell culture with an in vitro model was developed and impedance was measured over a 28-day culture period. However , the impedance measurements of different stent types were not considered in this study. This limitation was the basis of the present study which was to identify the impedance measurements of various stent types.

1.8 PROJECT AIMS

There is a need for a less invasive way to monitor in-stent restenosis and re-endothlialisation to be developed. The methods that are currently used have significant disadvantages, most notably their invasive nature. In addition, they are not suitable for use on a continued basis and so long term follow up of patients is not possible. Electrical impedance may represent a solution to these problems, where the stent itself can act as an electrode. Hence the electrical impedance of stent can be measured when implanted into the body, providing an indication of the extent of healing that has occurred following stent implantation. There has been in vitro research carried out that suggests that neointimal growth on the stent surface gives rise to changes in impedance (Shedden et al, 2008). The changes in the impedance relates to the growth of tissue and the extent of restenosis. However, only bare metal stents were examined.

The overall objective of the project was therefore to measure the basic impedance characteristics of various stents, ranging from those that have been used clinicially through to more novel devices currently in development. In order to achieve this objective, the project aims were to investigate the effect of temperature on the stent impedance in a variety of different media. The study also aimed to examine the effect of different materials and coatings on the impedance characteristics of stents.

CHAPTER 2 – THEORY

Chapter 1 highlights a potential role for non invasive impedance monitoring of endothelial cells using impedance spectroscopy for bare metal stents and drug eluting stents. Physiological properties of biological materials can be characterized by measuring the electrical impedance of cells and tissues. This study focuses on measuring the electrical impedance of coronary stents , which is a necessary first step in a wider research programme at deceloping a method of monitoring endothelialisation.

The theories of electrical impedance are hence needed to understand and model the cell impedance. The frequency of the alternating current has an impact on the cell impedance and the technique of Electrochemical Impedance Spectroscopy is used to measure impedance of cells over a defined frequency range. A helpful way of illustrating the various processes taking place at the electrode interface and in the cells is by electrical circuit. The elements commonly referred to as the resistors and capacitors.

The chapter introduces the theory behind the project. The concept of bioimpedance as an application with respect to biological cells and tissues. The various reactions that take place at the electrode-electrolyte interface will be discussed. The electrical properties exhibited by cells and tissues have been represented with the dielectric theory and expressed as conductivity and permittivity.

The cell membrane exhibits electrical properties and behaves as both insulator and conductor. Current flows due to the accumulation of charges on both sides of the membrane. Ions conduct electric charges through the membrane in and out of cell which is defined by membrane conductance (ϭ).

The ion channels are needed to generate and propagate action potentials in excitable cells. There exists a difference in electrical potential which arises due to the excess positive ions outside the cell membrane and excess negative ions inside the cell. The

channels are maintained by the permeability of membrane to the different ions and the active transport mechanisms.

2.0 Theory of Electrode /Electrolyte Interface and Reactions

Silve-silver chloride and platinum electrodes were employed in this study which acted as a tool in application of electric current to cells or the tissues being tested. The electrodes are immersed in the electrolyte which was Solution A and the endothelial medium and the intrinsic impedance measurements were made. To be able to discriminate the impedance caused by electrolyte and electrodes, a clear acceptance of the various processes occurring at the electrode –electrolyte interface should be known. A boundary occurs when an electrolyte comes in contact with a conductor which is a metal electrode and due to this the reactions taking place at the interface is distinct from that in the electrolyte.

Electrolyte is considered a medium where electrical conduction takes place due to the migration of charges. Endothelial medium is considered as a conducting medium and intra and extra cellular fluids have ions which has free flow carrying charges. The addition and removal of charges is based on the electrovalency of an atom. Sodium has a tendency to lose electron and becomes a positive ion and Chlorine gains electron to become a negative. These act as charge carriers and let the current flow through the electrolyte when a frequency of electric current is applied.

Protein adsorption, change in structure of electrode and changes in potential and electrolyte composition are the reasons to cause the flow of current is passed in the system. The interface between an electrolyte and electrode was found to act as a capacitor. As in case of a capacitor the interface makes way for oppositely charged layers which is drifted apart by a dielectric .Charges are present on the side of the electrolyte and is consisting of molecules and ions that are absorbed. A charged current gets produced at the electrode due to the capacitance of the double layer. The current is negligible and is found at the electrode –electrolyte interface and observed at low concentrations of the electro active reactants.

2.1 Process at electrode

The transfer of charges may occur at the electrode and electrolyte interface, if the electrode being considered is not an ideal polarisable electrode. Chemical energy is formed by the conversion of electrical energy in an electrolytic cell when an external voltage is applied which should be greater than reversible potential of cell. Transfer of electrons occurs from the metal electrodes due to reduction whereas oxidation due to chemical species.

The influence of electrode reactions due to the diffusion in evaluating the total impedance of the system depends on the frequency of the alternating current applied. At low frequency the outcome due to diffusion is more important.

A perfectly polarizable electrode has no passage of charges across the electrode-electrolyte interface on application of current. The electrode acts as a capacitor when current flows through the interface and give rise to a displacement current. Example: Ag/AgCl electrode.

A perfectly non-polarizable electrode has a free passage of current across the electrode-electrolyte interface where no energy is needed for transition. They have no over potentials.

The perfectly electrode / electrolyte interface behaves as a capacitor. The capacitor has two oppositely charged layers separated by a dielectric. The dipoles and charged particles form an electrical double layer and the model has been shown by Bard & Faulkner (1980). The electrode being considered is not an ideal polarisable electrode, tends to have the transfer of charges across the electrode/electrolyte interface. These processes follow Faraday's law and are known as Faradaic process. In case of an electrolytic cell conversion from electrical to chemical energy takes place on application of an external voltage which is greater than reversible potential of cell.

According to Faraday's law the amount of substance produced at an electrode in an electrolytic cell is directly proportional to the amount of electricity which is applied.

Consider current, I in amperes passing through the interface equals rate of change of flow in Coulombs / second.

$I=dQ/dt$ **Equation 2.0**

The number of moles of chemical substance electrolyzed (N) is related to charge (Q) and the number of moles transferred (n).

$\frac{Q}{nF}= N$ **Equation 2.1**

Faraday's constant (F) = 95,484.56 C/mol

The reaction rate of electrode (v) in moles/ second is

$v = \frac{dN}{dt} = \frac{i}{nF}$ **Equation 2.2**

The experiments carried out in this project focuses on control experiments that include two electrodes immersed in Solution A and endothelial medium involving diffusion and rate-limiting reactions having their effects at low ac frequency. The flow of current to electrolyte is an important process which exists due to the electrode concentration gradient in the electro active species. Fick's law of diffusion governs diffusion and states that the flux (J_o) is directly proportional to concentration gradient (C_o).

$-J_o(x,t) = D_o \frac{dCo(x,t)}{dx}$ **Equation 2.3**

Where D_o is the coefficient of diffusion in cm^2/sec.

2.2 Biological tissues exhibiting electrical properties

Biological tissues exhibit electrical conductivity due to their conductive and permeable nature. The electrical property is due to the membrane, tissue structure and composition. On applying electric current the cellular composition of tissues gets affected and a dipolar electric moment occurs. The charges due to the motion of ions and this leads to conduction. Protein and lipids present in the cell membrane constitute a dipolar charge moment. Due to the application of electric field the dipoles get polarized and get linked to electrolyte.

2.3 Dielectric theory

A material that does not consist of free moving ions is termed as dielectric and this is usually a non-conductor and exists between the conducting plates of capacitor. When electric current is applied, the system which polarizes itself can also be termed a dielectric. Water and other biological materials such as proteins and lipids tend to form dipoles. Dipoles are termed as an electric doublet consisting of two equal charges of positive and negative sign and are separated by a small distance. Biological tissues contain many of these dipoles and hence they can be polarized. Polarization is related to permittivity of the material and which in turn is a measure of the dipole moment that is induced by the effect of electric field.

Due to the movement of free charges carrying ions across the material, a current is produced which is associated with static conductivity. The increase in conductivity leads to decrease in permittivity and is maximum at low frequencies.

2.4 Relaxation time

Relaxation time is defined as the time taken for any system to get back to its normal equilibrium after being agitated and this is helpful in determining the measurement of time dependent return to equilibrium.

2.5 Effect of Dielectric in biological tissue

Cell membrane consists of a lipid bilayer having hydrophobic and hydrophilic proteins and the protein molecules are wide spread in the layer. The hydrophilic portions of the lipids align themselves outwards when in contact with water molecules , and in turn provides a conductive surface which can be compared with a plates of a capacitor that are conductive in nature. The hydrophobic region which is present at the ends of the lipids provides selection of ions. Overall due to their orientation the cell membrane acts as a lossy dielectric interface where adequate amount of energy is absorbed .The capacitance of cell membrane is evaluated by the permittivity of dielectric and thickness of cell membrane and was approximated as 1microF/cm^2 (Cole et al, 1968).

On application of electric potential, the charged ions in the cell get attracted on both sides of the membrane, both intracellular and extracellular. This behaves exactly like the charges getting attracted to the capacitor plates on charging. The major role is played by the frequency as the increase in frequency leads to increase in time applicable for charges to accumulate. Decrease in permittivity is linked with increase of conductivity.

At low frequencies the cell membrane exhibits capacitive effect which is at its peak and there is enough time for the charging and discharging process to take place. Due to this high permittivity the current flows through the extracellular conductive medium preferentially.

2.6 Electrical Impedance

All the electrochemical process occurring at the electrodes with the biological tissues can be adapted into electrical counterparts. The electrochemical cell can be expressed in terms of impedance due to a small sinusoidal excitation (Bard & Faulkner, 1980). Hence it is the electrochemical process that is found at the electrode and electrolyte interface and can be represented as a capacitance consisting of dielectric. Based on the physical parameters which is influenced by diffusion at low frequencies and an impedance element is considered to produce a resistance which is termed as Warburg impedance. The biological tissue has its permittivity and conductivity of lossy

dielectric materials which is limitated with the combination of resistors and capacitors.

The preliminary idea of electrical conductivity with resistors and capacitors when introduced with alternating current and their equivalent circuit's to represent the electrodes and biological tissues are discussed further.

Ohms law governs the electrical resistance of a resistor on application of electric current giving rise to impedance,

V=IR

Where V is the voltage across the resistor in volts (V), I is the current flowing through the resistor in amps (A) and R is the resistance in ohms (Ω).

If capacitors and inductors are used and an alternating current I applied the Ohms law is written as

V=IZ

Z is the impedance and is described as the total opposition to electrical current and includes resistive, capacitive and inductive components.

Biological tissues have electrical properties which can be determined by applying alternating current and measuring the output impedance.

Resistance of a cylinder is

$$R = \rho \frac{L}{A} \qquad \textbf{Equation 2.4}$$

Where L is the length and A is cross-sectional area of cylinder and ρ is resistivity of material in ohm.cm. Conductivity (σ) is the inverse of resistivity (Valentinuzzi, 1996).

Capacitance of a parallel plate capacitor which is separated by a dielectric material is described by

$C = \varepsilon A/d$ **Equation 2.5**

2.7 Electrode interface

When a resistor and capacitor are in series, the impedance at any frequency is the vector sum of impedance arising from the resistor and capacitor.

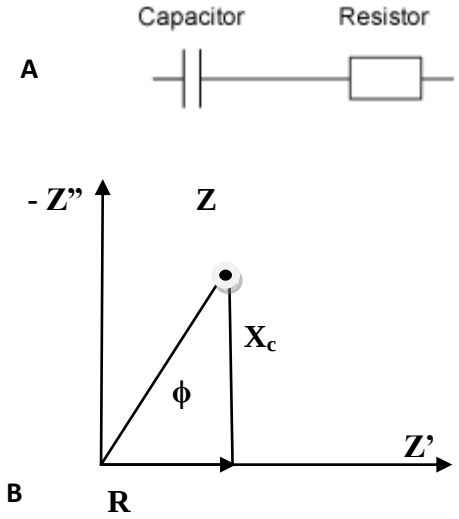


Figure 2.1A and B – Capacitor and resistor in series. Impedance equals the vector sum of resistance and capacitive reactance.

The complex plane of impedance of a resistor and capacitor in parallel is more complex and can be represented by a semi circle of diameter R and center at the real axis traced with the increase in frequency.

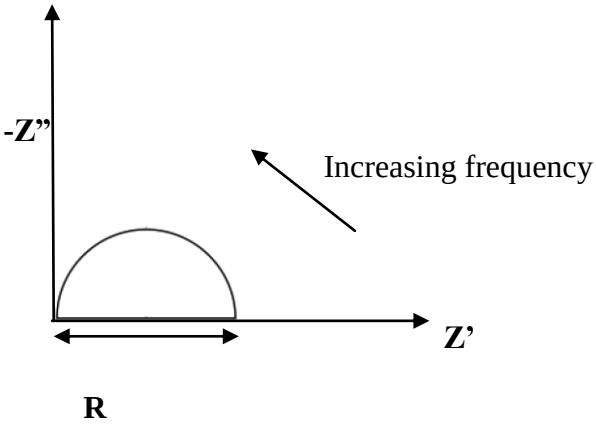


Figure 2.2 – Representation of complex plane of impedance of resistor and capacitor in parallel.

The resistance remains a constant with change in frequency unlike the scenario when the components are in series. Z ’is equivalent to R only at 0 Hz frequency. The capacitor has plenty of time to get charged at low frequency and becomes an open circuit. The resistance leads the current to take the path of low resistance. Hence the impedance is dominated by the resistance. As the frequency increases and tends to infinity the capacitor has less time to charge and behaves as a short circuit. This gives rise to the impedance tending to zero.

The modeling of two electrodes placed inside an electrolyte is as shown below in Figure 2.3 .This is the equivalent circuit of the control experiment carried out in this project. The electrodes are immersed in endothelial media without any cells or tissues. The electrodes are represented by a combination of resistance and capacitance which approximates a condition where the impedance of control electrode is higher than that of the counter electrode.

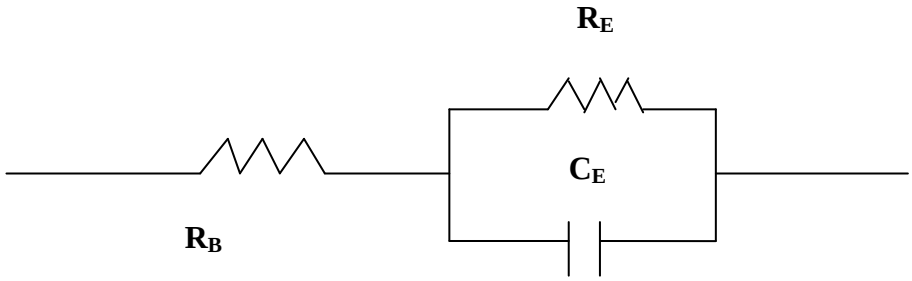


Figure 2.3 – Representation of electrodes immersed in an electrolyte. Resistor (R_E) and capacitor (C_E) in parallel represents electrodes and the electrolyte is represented by resistor (R_B).

The total impedance of circuit is calculated using the relation:

Z= Zelectrolyte + Zelectrode

The capacitive reactance tends to infinity for the double layer at low range of frequencies and hence the impedance of electrodes tends towards the resistive values.

2.8 Equivalent circuit of Biological tissue

Many studies have suggested the use of various models to represent the electrical properties of biological cells and tissues. The cell covered electrode and a cell free electrode was used to measure the impedance rather than using an equivalent circuit model a microscopic method was followed (Giaever & Keese, 1991). Differential equations are constructed to represent the flow of current through the cells and tissues modeled in circular discs.

The impedance of electrode was represented as a capacitor whereas the impedance of cell layer as a capacitor and resistor in parallel and the impedance of cell medium as resistor (Wegener et al, 2000). There were two parameters which were derived to represent the passive electrical properties of cell layer interpreted as transepithelial resistance.

Cell membrane was modeled as a leaky dielectric membrane with a parallel capacitor and resistor and cell nucleus being a resistive element represented a nucleoplasm and nuclear membrane as a shunted capacitance. The values of resistance and capacitance are assumed to create an applied electric field on cell components over a range of frequencies (Ellapan & Sundarrajan, 2005).

2.9 Impedance Spectroscopy

Electrochemical Impedance Spectroscopy (EIS) is the method used to determine electrical properties of biological cells and tissues. For this project Solatron Impedance Analyzer was used. The system consists of cell/tissue as the medium where a working electrode and counter electrode are immersed. An alternating current of small potential is applied to the electrodes and frequency is changed.

The changes in the biological medium are identified by the complex plane plot which is a semi-circle known as Cole plot. The Warburg impedance is helpful for the analysis of equivalent circuit fitting. No information is obtained from the complex plane plot regarding the dependence of frequency of the impedance. The information is provided by Absolute impedance and phase angle versus frequency plots. Absolute impedance plots are suitable for providing the effect of cell /tissue growth on the total impedance of system which is under investigation.

The impedance of electrodes in ionic solutions has been studied previously and to a great extent (Robinson, 1968), (Schwan, 1963), (Simpson, 1963), (Schwan, 1992), (Onaral, 1982) and (Onaral, 1983).

Impedance Spectroscopy is used to represent the electrochemical cell as an electronic model. The passive electrical properties of a system due to its dependence on frequency are studied by this method. In-stent restenosis can be measured by this method (Shedden et al, 2008) and the properties of cells/tissues, cell proliferation and cell/tissue volume can also be measured.

The electrical impedance is the opposition to an alternating current being applied on any material. Bioimpedance is the impedance with respect to biological cells and tissues. Electrochemical theory is used to explain the concept of the various reactions and processes that takes place at the electrode interface. The electrical properties exhibited by cells are passive and can be explained in terms of dielectric theory and expresses as complex permittivity and conductivity. The easiest way of representing the processes that takes place at the electrode interface and the cells is using electrical circuit elements such as resistors and capacitors.

The impedance is dependent on frequency of applied AC voltage and Electrochemical Impedance Spectroscopy (EIS) is a technique which is used to measure the impedance of cells and tissues over a defined range of frequency.

Impedance spectroscopy is a technique used to characterize electrical properties of materials and their interfaces with electronic conducting electrodes. It is used to find the dynamics of bound or mobile charge in bulk or interface regions of a solid or

liquid. The regions can be ionic, semiconducting and insulators. The area of focus is on solid electrolyte materials and solid metallic electrodes where a reference is taken and is fused with salts and aqueous electrolytes and liquid-metal and high molarity aqueous electrodes.

Electrochemical behavior of electrode and/or electrolyte is evaluated by the electrical measurements having cells with identical electrodes being applied to sample which is in a circular cylinder. If living cells are being investigated then simple symmetrical geometry cannot be used. The method followed is application of electrical stimulus which is either voltage or current to electrodes and measure the response which is known as the output voltage or current.

Many small microscopic processes occur throughout the cell when it is stimulated electrically and result in electrical response. The reactions that occur are the movement of electrons through the electronic conductors, transfer of electrons at the electrode electrolyte interface or oxidation and reduction reactions. The rate of flow of the charged particles which is the current is dependent on the ohmic resistance of the electrode and the electrolyte. The flow is also affected by anomalies at grain boundaries and small defects in bulk of the materials.

There are three approaches to measure the impedance and the most common and standard one is measuring impedance by applying single frequency voltage or current to the interface and measuring the amplitude and phase shift and real and imaginary parts due to the current at the frequency with the analog circuit. The commercial impedance analyzers measures impedance as a function of frequency in a given frequency range of about 1mHz to 1 MHz interfaced with computers.

Cells and tissues have resistive and capacitive properties that can be measured using a variety of techniques .Cell membranes, because of their structure are both resistive and capacitive in nature. Extracellular matrix and cell contents are largely resistive due to their own content although proteins and lipids can be capacitive (Shedden et al , 2008).The impedance properties are different for normal, malignant

and benign tissues as they have different cell composition, membrane properties and intracellular relationships.

Non faradic electrode processes were represented by the capacitive element which was the charging and discharging of the electrical double layer. Faradic electrode processes represented by resistive element. The impedance of the stent, the biological tissue of artery and counter electrode were all represented by a combination of resistors and capacitors (Shedden et al, 2008).

Chapter 3 – Materials and Methods

3.1 Test Cell Development

The test cell used in the study was previously developed by Shedden, et al (2008) and is shown in Figure 3.1. The test cell allows impedance to be measured between two electrode wires, or between an electrode wire and a stent. In this study bare metal stents (Cobalt chromium and stainless steel) and polymer coated drug- eluting stents were used as the working electrode and the counter electrode was silver-silver chloride electrode. An 80 ml glass bottle (Fisher brand, Fisher scientific, Leicestershire, UK) was used for experiments. The lid of the bottle was modified for the experimental setup. An insert made of PTFE was custom made and placed in the lid of the bottle. The insert consisted of a large hole to pipette out the media into the bottle. A filter cap lid from a T25 flask was fixed into the opening of the insert for maintaining sterile and gas exchange conditions. Two small holes were drilled into the PTFE insert where the electrodes could be introduced.

The working electrode consisted of stent held in crocodile clip attached to other insulated silver wire .The silver wires were inserted through small holes in PTFE lid insert. The lid was placed on glass bottle and a magnetic stirrer put inside the bottle. Distilled water was poured in small quantity in the bottle and sterilized in autoclave unit. After being sterilized, the bottle and other electrodes and magnets were placed in sterile hood and sprayed with ethanol. The distilled water was removed from the bottles and allowed to cool and dry in the sterilization hood.

Two crocodile clips where used to attach the electrodes and these were then inserted into the cap of the bottle through the cap holes. Solution A and endothelial medium was transferred onto small bottles and kept in the chamber maintained at 37ºC. These were pipetted out into the test bottles inside the sterilization hood. Three test cell setups were built to allow three replicates to be performed for each experimental condition.

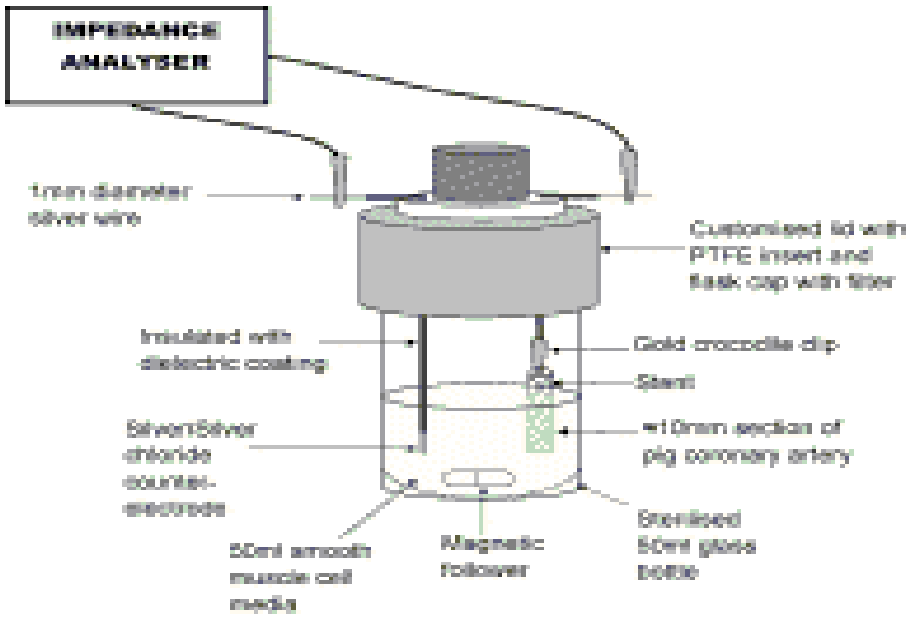


Figure 3.1- Test cell consisting of stent and Ag/AgCl electrode clipped onto the crocodile clip and immersed in electrolyte containing magnetic follower.

3.2 Electrode Materials

3.2.1 Silver-Silver Chloride Electrodes

Silver needle electrodes of 1mm diameter (Good fellow, Huntingdon ,UK) were coated with Chloride making them Silver-Silver chloride electrodes. The Electrochemical Interface (SI1286) (Solartron, Hampshire, UK) was used to coat silver chloride layer on the Silver wire. The wire was immersed in a solution of 0.1M hydrochloric acid (BDH Chemicals, Dorset, UK) and inserted on to crocodile clips connected to the positive side of an electrochemical interface. Before the silver wires are attached to the crocodile clips they are cleaned with sandpaper and sprayed with ethanol to remove the traces of any coating previosuly present on it.

Platinum wires were also cleansed with sandpaper, 70% alcohol and distilled water. They were then attached as control electrodes and immersed in hydrochloric acid. Corrware software (Solartron Analytical, Hampshire, UK) was the software installed on the computer which was connected to electrochemical interface. The selection was turned to Galvanostatic mode with constant current being set at 400microA/cm^2,

based on a previously optimized protocol (Bioengineering Unit, Medical Diagnostics Group, Laboratory procedures, Procedure 4 – Procedure for chloriding silver wire electrodes). A current of 25mA was applied for 15 minutes for an electrode of length 25cm and area 0.785 cm^2. The electrode after coating was suspended in 1 M potassium chloride solution for stabilizing the coating.

Due to the current being passed through the electrodes, the hydrochloric acid causes chloride ions which are negatively charged to move towards the positive electrode due to attractive force and eventually reacts with the Silver forming silver chloride coating on the electrode. The reason for coating chloride onto silver wire is for cutting down the impedance of electrodes and observing the change in impedance due to the tissue.

The chloride coating decreases impedance by promoting electrode transfer across the electrode-electrolyte interface and also helps in giving a rough finish on the surface of the electrodes.

3.3 Test Electrodes

3.3.1 Stainless Steel 316L

Stainless steel wire, 316L grade, 1mm diameter, length 20 cm was used as the counter electrode. Stainless steel wires were cut into the desired length from the stainless steel coil.

3.3.2 Coronary Stents

Four separate groups of stents were tested in the study, comprising two different types of bare metal stent, a polymer coated drug-eluting stent and a novel polypyrrole coated stent. All commercially available stents were balloon-expanded in a sterile environment to a pressure sufficient to achieve their recommended nominal diameter according to manufacturer's instructions. After the expansion of stent the balloon was removed and deflated. Polypyrrole coated stents were coated in their expanded form and no subsequent expansion was therefore required prior to incorporation within the test cell. Details of each stent type are provided immediately below and in the accompanying table (Table 3.1).

Bare Metal Stents-There were 2 BMS used in the experiment namely:

- Bx Velocity(Johnson & Johnson)
- Minivision (Abbott)

Drug-eluting Stent – Sirolimus – coated DES namely:

- Cypher Stent (Cordis-Johnson & Johnson)

Polypyrrole coated stent – Cobalt-Chromium BMS (Bx Veocity –Johnson & Johnson)was coated with a polymer polypyrrole by electropolymerisation.

Name	Type	Material(s)	Dimensions
Multi-Link Mini Vision Coronary Stent system (Abbott)	Bare metal stent	Cobalt-chromium	2.0mm*18mm
Bx Sonic (Johnson & Johnson)	Bare metal stent	Stainless-Steel	4.0mm*13mm
Cypher select – Sirolimus-eluting coronary stent (Cordis-Johnson & Johnson)	Drug eluting stent	Stainless steel	3.50mm*28mm
Polypyrrole coated BMS. Multi-Link Mini Vision Coronary Stent system (Abbott)	Bare metal stent	Cobalt-chromium	2.0mm*18mm

Table 3.1 - Details of coronary stent types and specifications used in impedance measurements.

3.4 Test Solution Preparation

3.4.1 Solution A

Solution A was used as a test electrolyte for the initial studies to characterize the response of the test cell. It consists of a solution of 1M NaCl, 1M CaCl dihydrate and was prepared by addition of the salts to distilled water (900ml). The stock solution was stored at 4 °C in refrigerator between uses. For every experiment the solution was pipetted out into the test setup and heated in a water bath up to 37 ºC and the impedance readings were then taken. For the set of experiments where the readings had to be taken at room temperature, the solution was kept out from the refrigerator for a period of 5 hours to be maintained at room temperature.

3.4.2 Endothelial cell media

Large vessel endothelial cell growth media with low serum growth supplement (Life Technologies) was used as endothelial cell media. It was supplemented with 1% penicillin –streptomycin and was stored at 4ºC in the refrigerator between uses. 180 ml of the media was pipetted out into 4 sterile tubes of volume 45ml each. The bottles were stored in the oven until they had reached a temperature of 37 ºC. For every test cell setup 60ml of the endothelial media was pipetted into the test cell and the electrodes were immersed. These steps were carried out inside the sterilization hood.

3.5 Polypyrrole Coating Production

3.5.1 Materials

Sodium Salicylate (NaSa) and pyrrole monomer (Py) were purchased from Sigma-Aldrich (Poole, UK). Stainless steel sheets (stainless steel, AISI 316L grade, annealed, 150mm x 150mm x 0.5mm) were purchased from Good fellow Cambridge Ltd (Huntingdon, UK). These sheets were cut into 20mm x 20mm x 0.5mm sections for coating. The platinum wire counter electrode (1mm diameter) was also purchased from Good fellow Cambridge Ltd (Huntingdon, UK). A KR5 reference electrode was purchased from Thermo Scientific UK Ltd (Leicestershire, UK)

3.5.2 Equipment

Electropolymerisation was carried out by an electrochemical inter-face (SI 1287, Solartron Analytical, and Hampshire, UK)

3.5.3 Method

The protocols used for the electropolymerisation technique are based on the paper by Arbizzani (2007). The process was conducted at room temperature with aqueous solutions of 0.1M Py , 0.1 NaSa which was prepared in deionised water. The polymerization method was performed with a three-electrode cell with the platinum electrode of 1mm diameter being the counter electrode. KR 5 acted as a reference electrode and the polypyrrole material coating was seen at the stent , which was the working electrode .

Solartron 1287 galvanostat was used to carry out the electropolymerisation by the potentiostatic way. A constant of 0.9V was selected to produce the coating. The voltage levels were based on previous studies (Arbizzani et al, 2007, Okner & Domb , 2007). The potientiostatic electropolymerisation was carried out for duration of 20 minutes to allow deposition of the polymer on to the stent. After coating the stent with polypyrrole it was left to dry at room temperature overnight in the sterilization hood.

3.6 Impedance Measurements

Impedance was measured using a 1260 Impedance Analyzer (Solartron, Hampshire, UK). The electrode pair, consisting of the Ag/AgCl electrode and either a stainless steel wire or coronary stent, were connected to a voltage of 200mV and the impedance recorded over a wide range of frequencies from 0.1 Hz to 1000000Hz. A first set of 'time zero' impedance measurements were performed on each test cell immediately following incubation of the electrode pair in the relevant test solution. Subsequent impedance measurements were recorded periodically over the course of incubation periods up to 6 days. At the end of each measurement, the test cell was

returned to a cell culture incubator maintained at 37 °C (5%CO2:95%air). Each set of impedance measurements comprised graphs of total impedance versus frequency and phase angle versus frequency. Cole plots were also recorded.

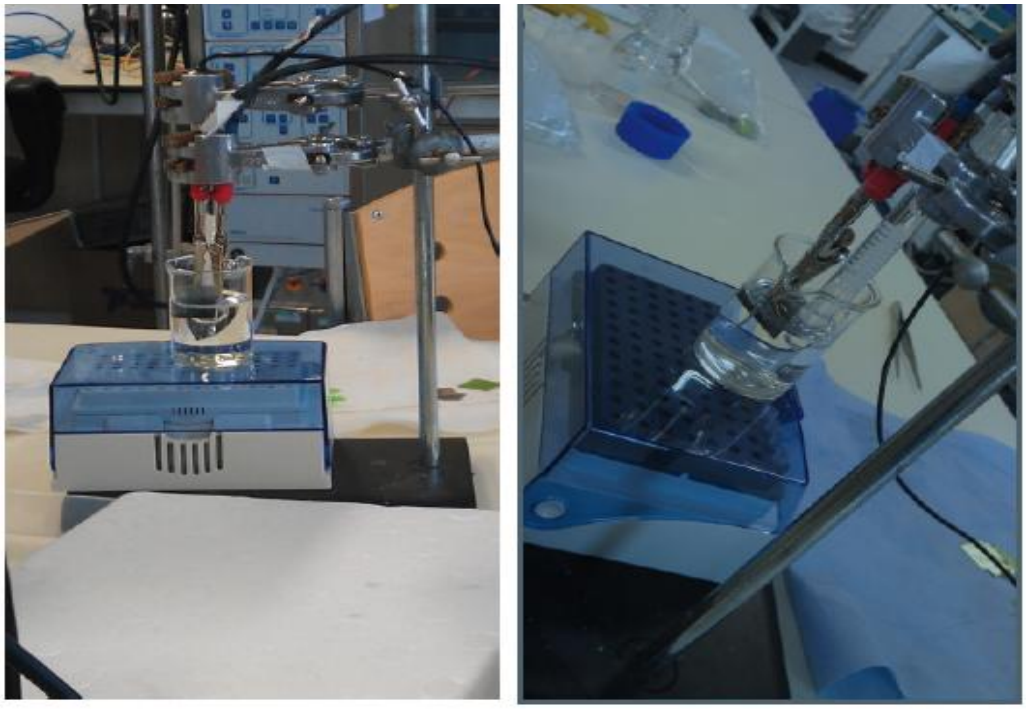


Figure 3.2 – The three electrodes and the stent being immersed polypyrrole solution. The electrochemical cell consisting of the counter electrodes and clamping apparatus to carry out the polymerization technique.

Chapter 4 – Results

4.1 Introduction

Several studies have used impedance techniques for cell behavior where the cells are grown on gold electrodes and the deposition of cells are seen on the surface (Giaever & Keese, 1991; Tiruppathi et al, 1992; Lo et al, 1995; Wegener et al, 1999). In a more recent study, electrical impedance has been proposed as a method for monitoring the neointimal tissue formation around the stent struts (Shedden et al, 2008). However, only a limited number of stent materials and designs were examined. The overall objective of this study was to characterize the impedance profiles of a wide range of different coronary stent types. The experimental results are grouped together around the main aims that were set out to achieve this objective:

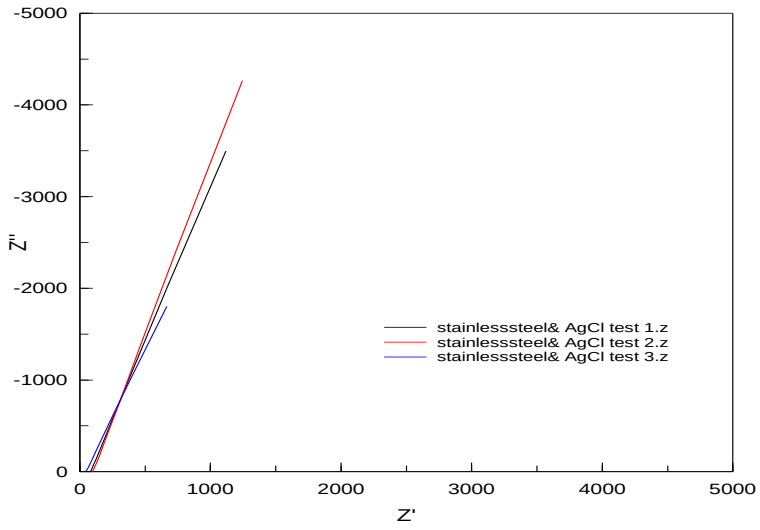
1. Effect of temperature on the impedance using Ag/AgCl electrode and stainless steel wire immersed in Solution A.
2. Effect of magnetic agitation on impedance using Ag/AgCl electrode and stainless steel wire immersed in Solution A.
3. Effect of change in medium on the impedance with the immersion of stainless steel and Ag/AgCl electrodes
4. Comparison between stainless steel wire and stainless steel BMS in endothelial medium
5. Comparison between Co-Cr BMS and stainless steel BMS in endothelial medium
6. Comparison between Co-Cr BMS, stainless steel BMS and Sirolimus coated DES in endothelial medium
7. Comparison between Sirolimus coated DES and polypyrrole coated BMS in endothelial medium
8. Comparison of all stents and stainless steel wire in endothelial medium

The methods used were detailed in chapter 3. Briefly, electrical impedance was measured from the series of electrode pairs of varying materials. Each experiment was performed over periods up to 6 days. Two different media solutions were used. Solution A was used in the first set of experiments to characterize the basic impedance characteristics of the system. Endothelial cell media was used in the

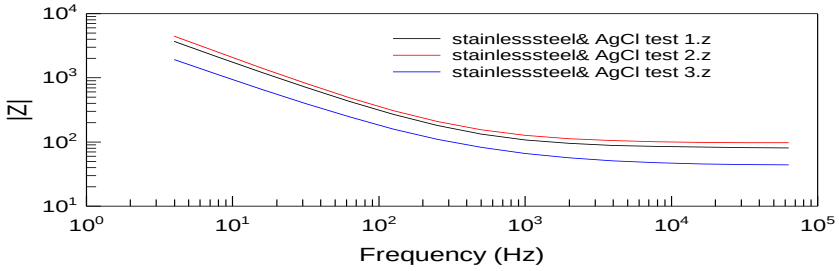
second set of experiments, where the response of the test system in a more physiologically relevant media was assessed. Before using the stents as the measuring electrode, stainless steel wire (316L) was used to characterize the impedance of the system. Three replicates were performed for each experimental condition.

4.2 Silver-Chloride and Stainless steel electrode in Solution A at room temperature.

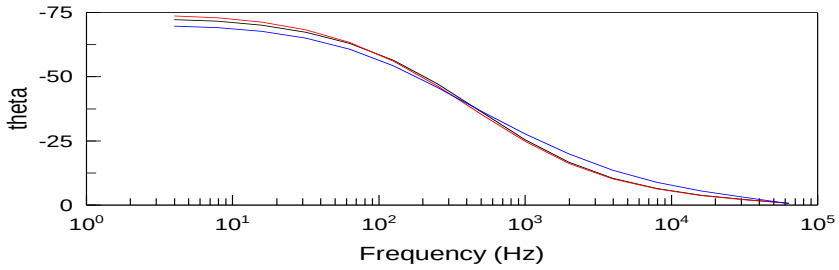
The first 3 sets of experiments were conducted with the electrode pairs immersed in Solution A. The raw data obtained from these experiments are presented in the graphs below. The three graphs presented are the Cole plot, Z'' versus Z', the Bode plot of the modulus of impedance, |Z| versus the frequency, and the phase angle, theta, versus the frequency These graphs are presented to outline the extent and nature of the data collected for each experiment performed. However, the focus of the results and subsequent analyses that will be presented is on the Cole plot and Bode plot.



A



B

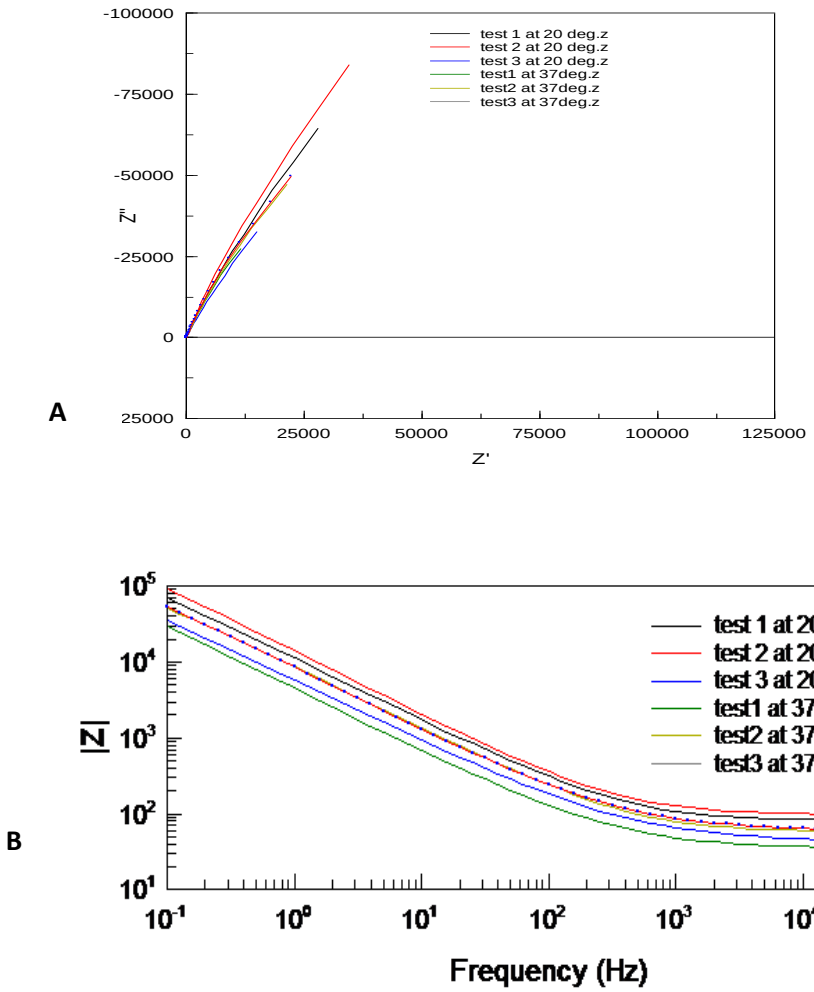


C

Figure 4.1 Changes in impedance on passing electric current through the electrodes immersed in Solution A at room temperature. A - Frequency sweeps from 0.1Hz-1MHz, with impedance shown in the complex plane (Z '' versus Z', both in Ω). A - Frequency sweeps from 0.1Hz-1MHz and impedance is shown in complex plane (Z '' versus Z', both in Ω).B - Bode plot showing impedance (Ω) versus frequency (Hz).C – Phase angle versus frequency. Data represent the results of three replicate experiments.

4.3 Effect of temperature on the impedance using Ag/AgCl electrode and stainless steel wire immersed in Solution A.

One set of impedance readings was taken at a normal room temperature of around 20 ºC and the other set of readings taken at 37 ºC. The data from these experiments is shown in Figure 4.2. It can be seen that the Z'' vs. Z' profile is near linear and this was broadly consistent between the three replicate experiments performed



Figure

Figure 4.2 – Comparison of change in impedance on passing electric current through the electrodes immersed in Solution A at room temperature (20ºC) and 37ºC. Frequency sweeps from 0.1Hz-1MHz. A - Frequency sweeps from 0.1Hz-1MHz and impedance is shown in complex plane (Z '' versus Z', both in Ω). B - Bode plot showing impedance (Ω) versus frequency (Hz). C – Phase angle versus frequency. Data represent the results of three replicate experiments.

The effect of temperature on the overall impedance profile is shown in figure 4.2B. The trends are close together and no clear effect of temperature is observed from this graph. It was therefore decided to investigate this further by calculating the average value of |Z| at two distinct frequencies for the two temperatures examined. The results of this analysis are shown in Tables 4.1a and b. From these tables, it appears that increasing the temperature of the medium from 20 ºC to 37 ºC has reduced the impedance. This effect was observed at both frequencies examined.

Frequency (0.1Hz)	**Impedance at 20ºC(in Ω)**	**Impedance at 37ºC(in Ω)**
1	70393	29674
2	90968	51802
3	35911	54544
Average (Ohms)	65757	45340
StDev	27819	11133

Table 4.1 a – Impedance values at a frequency of 0.1Hz from Ag/AgCl electrode and stainless steel electrode immersed in Solution A at room temperature and 37ºC.

Frequency (1000Hz)	**Impedance at 20ºC(in Ω)**	**Impedance at 37ºC(in Ω)**
1	107.61	48.54
2	127.06	78.96
3	66.029	87.09
Average (Ohms)	100	71
StDev	31	20

Table 4.1 b – Impedance values at a frequency of 1000Hz from Ag/AgCl electrode and stainless steel electrode immersed in Solution A at room temperature and 37 ºC.

4.4 Effect of magnetic agitation on impedance using Ag/AgCl electrode and stainless steel wire immersed in Solution A.

The next set of experiments focused on the effect of magnetic agitation on the test cell maintained at 37 ºC. The electrodes and Solution A remained the same as used previously, whereas a magnetic capsule stirrer was added into the solution and agitated. The impedance profiles obtained under these conditions are presented in figure 4.3A and 4.3B. It can be observed from figure 4.3A that the impedance values are very close for both the set of readings taken with and without magnetic agitation in two out of the three replicates examined. However, in one of the replicates, test1 the introduction of magnetic agitation appears to have had an effect on the impedance (Figures 4.3A)

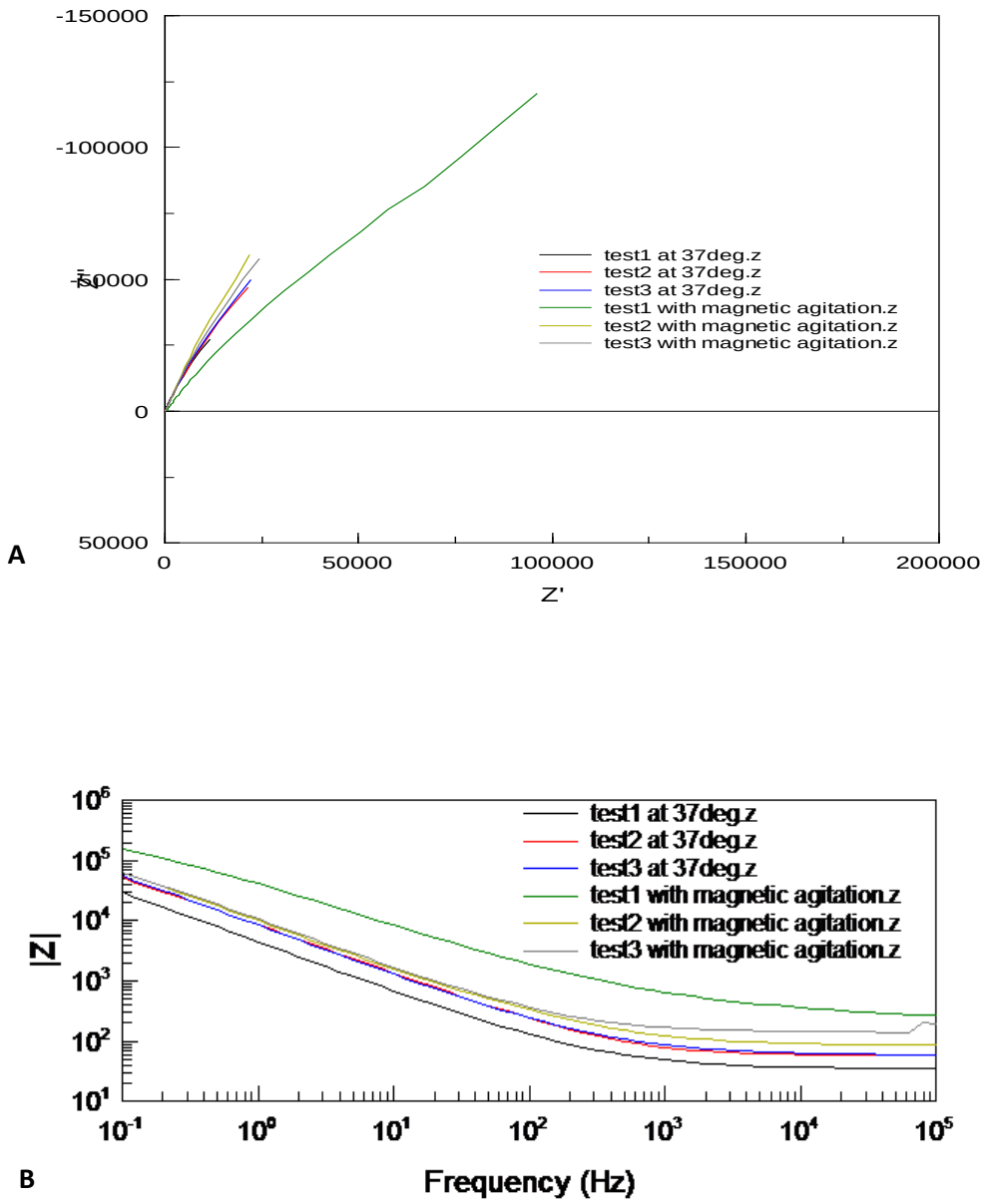


Figure 4.3 – Comparison of change in impedance on passing electric current through the electrodes immersed in Solution A 37ºC and with magnetic agitation. A - Frequency sweeps from 0.1Hz-1MHz and impedance is shown in complex plane (Z '' versus Z', both in Ω*). B - Bode plot showing impedance (*Ω*) versus frequency (Hz).*

The average impedance obtained with magnetic agitation is higher at both 0.1Hz and 1000 Hz. This is presented in table4.2 a and b.

Frequency (0.1Hz)	Impedance without magnetic agitation (in Ω)	Impedance with magnetic agitation(in Ω)
1	29674	154300
2	51802	63197
3	54544	62946
Average (Ohms)	45340	93481
StDev	**13636**	**52670**

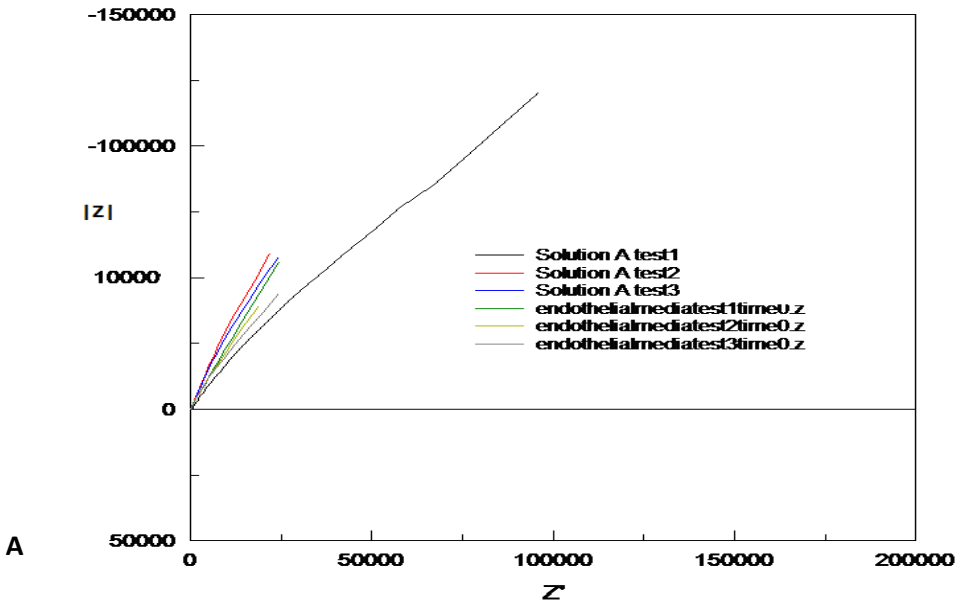
Table 4.2a – Impedance values at a frequency of 0.1Hz from Ag/AgCl electrode and stainless steel electrode immersed in Solution A at 37ºC and with magnetic agitation.

Frequency (1000Hz)	Impedance without magnetic agitation (in Ω)	Impedance with magnetic agitation(in Ω)
1	48.54	645.49
2	78.96	123.39
3	87.09	170.82
Average (Ohms)	71	313
StDev	**20**	**288**

Table 4.2b – Impedance values at a frequency of 1000Hz from Ag/AgCl electrode and stainless steel electrode immersed in Solution A at 37 ºC and with magnetic agitation.

4.5 Effect of change in medium on the impedance with the immersion of stainless steel and Ag/AgCl electrodes.

Stainless steel and Ag/AgCl electrodes were immersed in either Solution A or endothelial cell medium and the impedance profiles were measured as before. Both the media had magnetic agitation .The results presented in figure 4.4a and 4.4b shows the change in impedance due to the medium used. The impedance profiles obtained from endothelial media overlaps with the values obtained from the magnetic agitation and the pattern follows the same path for both Solution A and endothelial medium.



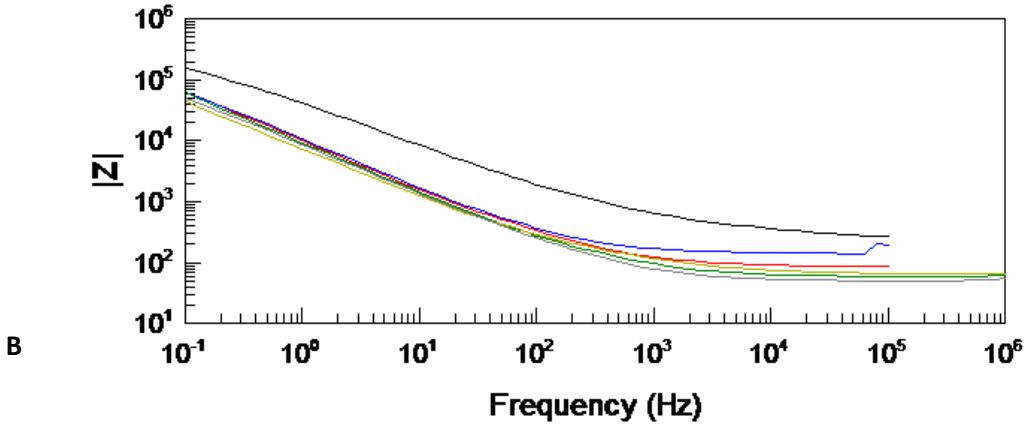


Figure 4.4– Comparison of change in impedance on passing electric current through the electrodes immersed in Solution A and endothelial medium at 37ºC with magnetic agitation. A - Frequency sweeps from 0.1Hz-1MHz and impedance is shown in complex plane (Z '' versus Z', both in Ω). B - Bode plot showing impedance (Ω) versus frequency (Hz).

The average Impedance values have increased for both set of frequencies in Solution A in comparison with endothelial medium.

Frequency (0.1Hz)	**Impedance in Solution A (in Ω)**	**Impedance in endothelial media(in Ω)**
1	154300	9226
2	63197	7286
3	62946	8591
Average (Ohms)	93481	8368
StDev	**52670**	**988**

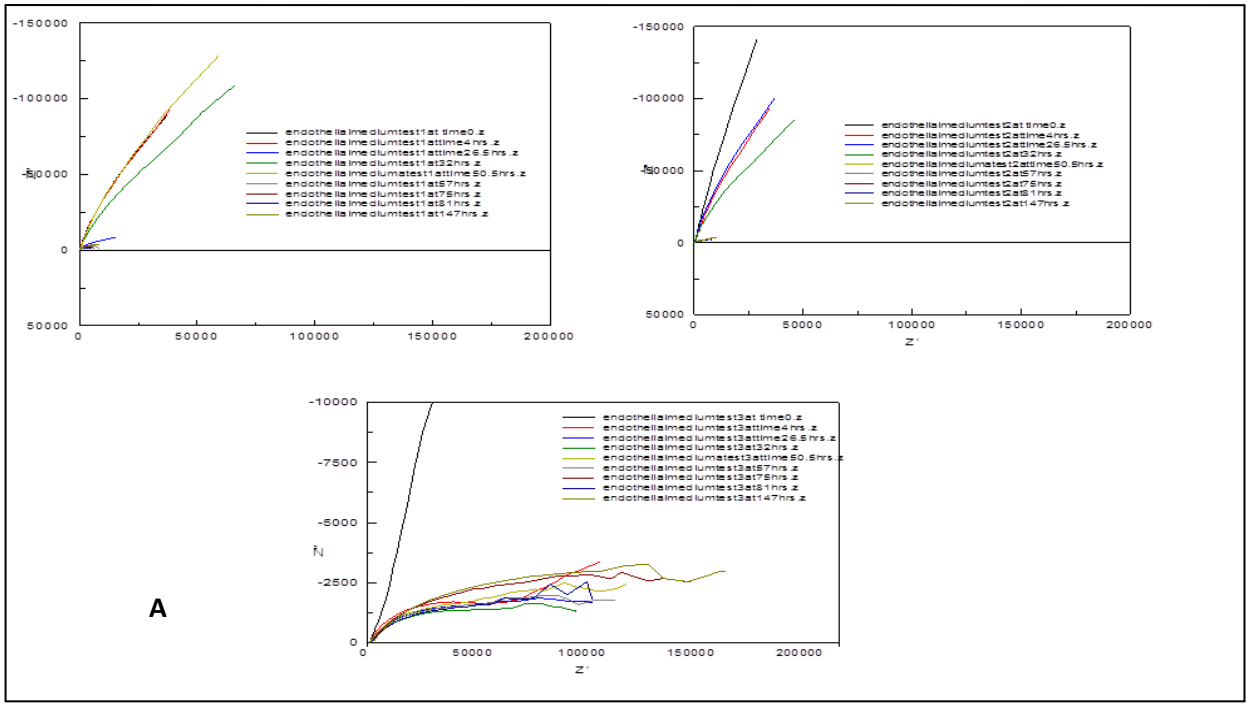
Table 4.3a – Impedance values at a frequency of 0.1Hz from Ag/AgCl electrode and stainless steel electrode immersed in Solution A and endothelial media at 37ºC and with magnetic agitation.

Frequency (1000 Hz)	**Impedance in Solution A (in Ω)**	**Impedance in endothelial media(in Ω)**
1	645.49	64.07
2	123.39	75.33
3	170.82	53.64
Average (Ohms)	313	64
StDev	**288**	**10**

Table 4.3b – Impedance values at a frequency of 1000Hz from Ag/AgCl electrode and stainless steel electrode immersed in Solution A and endothelial media at 37 ºC and with magnetic agitation.

4.6 Impedance readings with Ag/AgCl electrode and Co-Cr BMS immersed in endothelial media.

Cobalt-chromium bare metal stent was used as the working electrode and Ag/AgCl as the counter electrode. The readings were taken at different time intervals from t=0 to t=147 hours. Since there are a great deal of impedance profiles for each test cell setup individual graphs are shown n the figures below (Figure 4.5A and B). These are the raw data and for analysis the average and standard deviation was calculated and presented in the further set of results



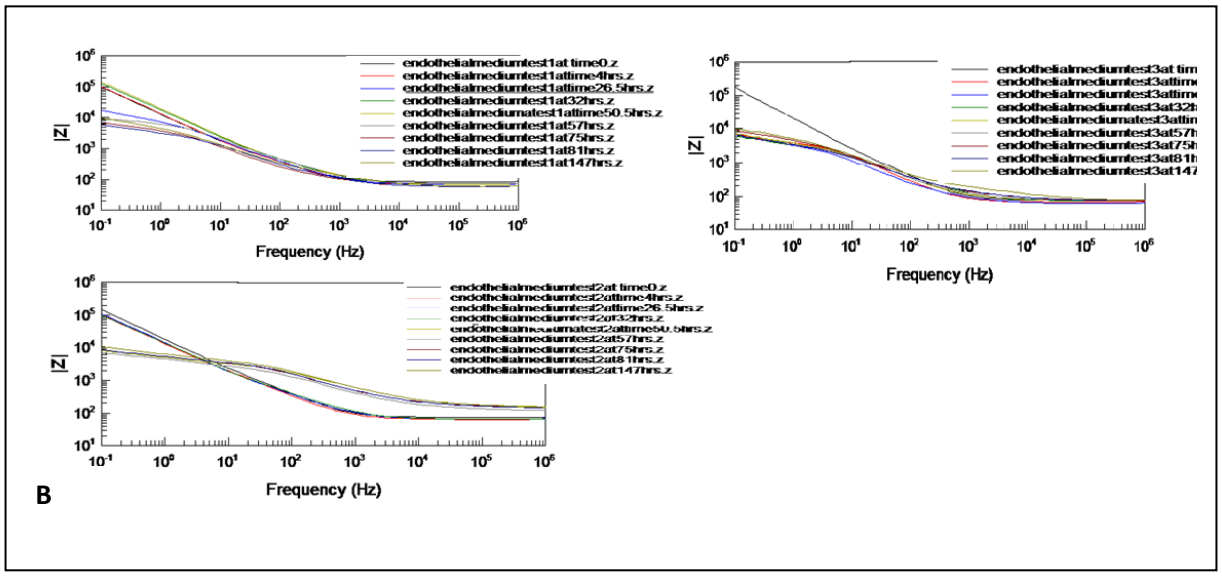


Figure 4.5 A and B – Change in impedance of test1 , 2 and 3 on passing electric current through Ag/AgCl electrode and Co-Cr BMS immersed endothelial medium at 37ºC with magnetic agitation .- Frequency sweeps from 0.1Hz-1MHz and impedance is shown in complex plane (Z '' versus Z', both in Ω). D - Bode plot showing impedance (Ω) versus frequency (Hz).

Frequency at 0.1 Hz – Time in hrs	Impedance of test 1(Ω)	Impedance of test 2(Ω)	Impedance of test 3(Ω)	Average (Ω)	St Dev
0	96355	144260	170590	**137068**	**37636**
4	100950	99803	7038	**69263**	**53891**
26	17263	107470	6209	**43647**	**55547**
32	127340	97161	5624	**76708**	**63382**
50	141960	8173	7313	**52482**	**77491**
57	8865	6906	6813	**7528**	**1158**
75	6623	8516	8927	**8022**	**1228**
81	5716	8406	6212	**6778**	**1431**

Table 4.4a – Impedance values of test 1, test 2 and test 3 at frequency of 0.1Hz from Ag/AgCl electrode and Co-Cr BMS immersed in endothelial media at 37ºC and with magnetic agitation.

Frequency at 1000 Hz-Time in hrs	Impedance of test 1(Ω)	Impedance of test 2(Ω)	Impedance of test 3(Ω)	Average (Ω)	St Dev
0	118.	100	101	**107**	**9**
4	108	91	88	**96**	**10**
26	114	105	94	**105**	**9**
32	129	112	127	**122**	**9**
50	141	607	113	**287**	**277**
57	133	416	120	**223**	**167**
75	102	487	140	**243**	**212**
81	110	490	142	**247**	**210**

Table 4.4b – Impedance values of test 1, test 2 and test 3 at frequency of 1000Hz from Ag/AgCl electrode and Co-Cr BMS immersed in endothelial media at 37ºC and with magnetic agitation.

The average and standard deviation of the 3 tests were performed at time intervals and has been presented as the average over time with standard deviation as the error bars. The figure 4.6 shows the change in average impedance over time for Cobalt-Chromium bare metal stent from 0 hrs to 140hrs considered at 2 different frequencies of 0.1Hz and 1000Hz.

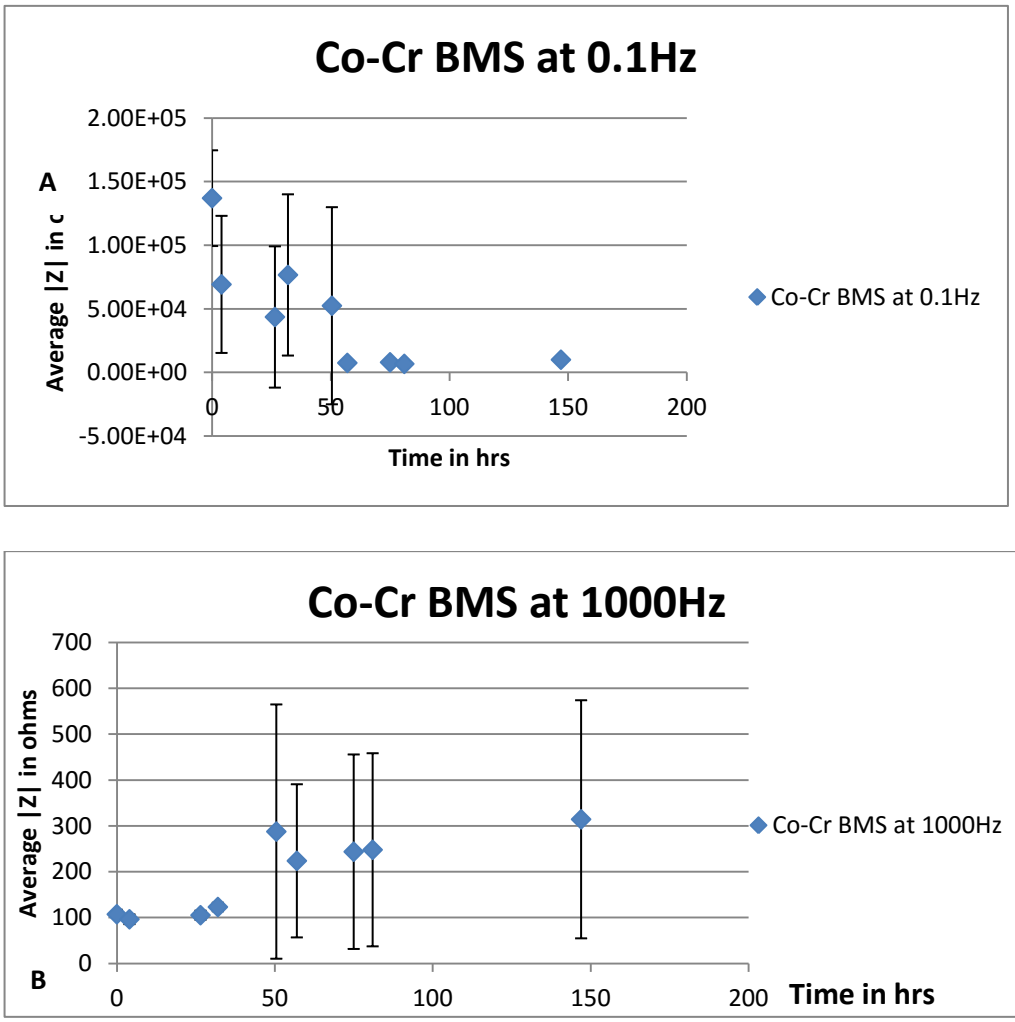
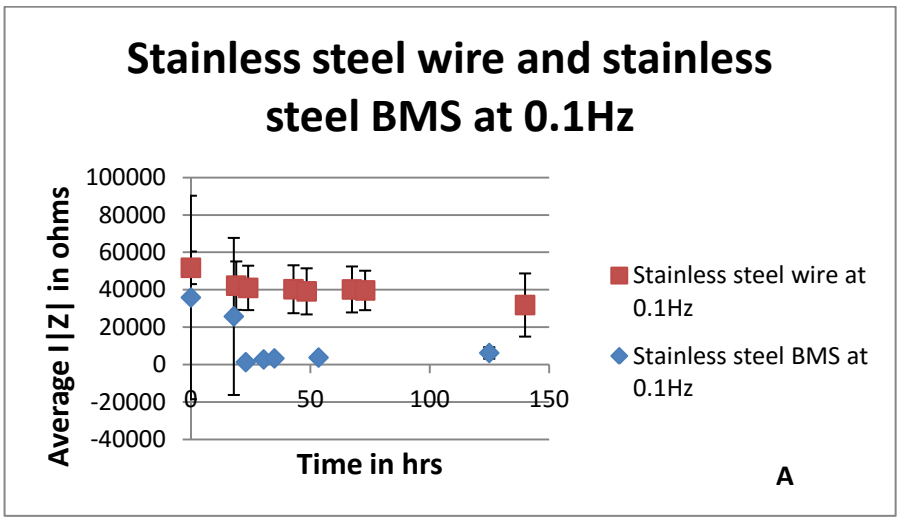


Figure 4.6 A and B – Change in average impedance of test1, test 2 and test 3 (|Z|) over time from 0 hrs to 150 hrs considered at 0.1 Hz and 1000Hz for Co-Cr BMS. The error bars represents the standard deviation and the value points represents the average |Z| of test 1 , test 2 and test 3 at different time having a value of N=3 .

4.7 Comparison between stainless steel wire and stainless steel BMS in endothelial medium

The average values were considered and data presented with standard deviation in figure4.7 A and B. At 0.1 Hz the impedance of stainless steel wire was found to be higher compared to the stainless steel BMS. Also a decreasing impedance profile can be observed for the stainless steel BMS at 0.1 Hz. At 1000 Hz stainless steel BMS has higher impedance compared to stainless steel wire(Fig 4.7 B).



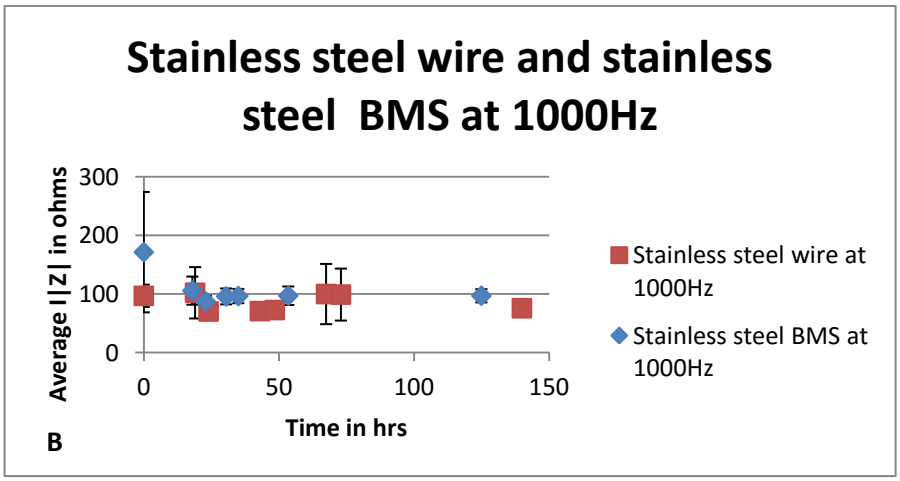


Figure 4.7 A and B – Change in average impedance of test1, test 2 and test 3 (|Z|) over time from 0 hrs to 150 hrs considered at 0.1 Hz and 1000Hz for stainless steel wire and stainless steel BMS. The error bars represents the standard deviation and the value points represents the average |Z| of test 1 , test 2 and test 3 at different time having a value of N=3

4.8 Comparison between Co-Cr BMS and stainless steel BMS in endothelial medium

The study was extended further by replacing the stainless steel wire by stainless steel BMS and comparing the impedance profiles with Co-Cr BMS. The results are presented in Figure 4.8 A and B. From figure 4.8A and B, it can be inferred at 0.1Hz the impedance of Cobalt-chromium BMS is greater than stainless steel BMS. However there are also certain values which collide and have the same impedance for both the BMS at 0.1Hz. At 1000Hz , the stainless steel and Co-Cr BMS have almost same readings and the values collides with each other .But as time increases an increase in Co-Cr is observed.

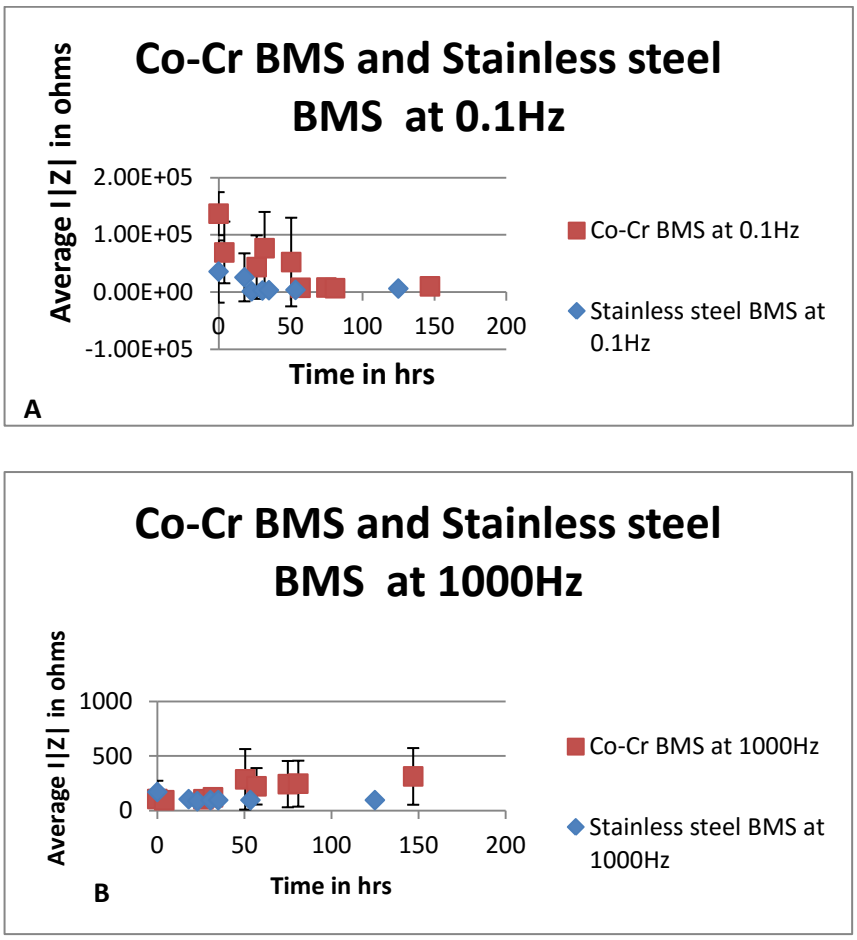


Figure 4.8 A and B – Change in average impedance of test1, test 2 and test 3 (|Z|) of stainless steel BMS and Co-Cr BMS in endothelial media over time from 0 hrs to 140 hrs considered at 0.1 Hz and 100 Hz. The error bars represents the standard deviation and the values points represents the average |Z| of test 1 , test 2 and test 3 at different time having a value of N=3

4.9 Comparison between Co-Cr BMS, stainless steel BMS and Sirolimus coated DES in endothelial medium

The data from section 4.7 and 4.8 are compared with drug eluting stents. Figure 4.9A and B shows the change in impedance with 3 stents being considered. The impedance of DES is higher in comparison with the two bare metal stents at 0.1Hz. At 1000Hz, the impedance of DES is higher than two bare metal stents

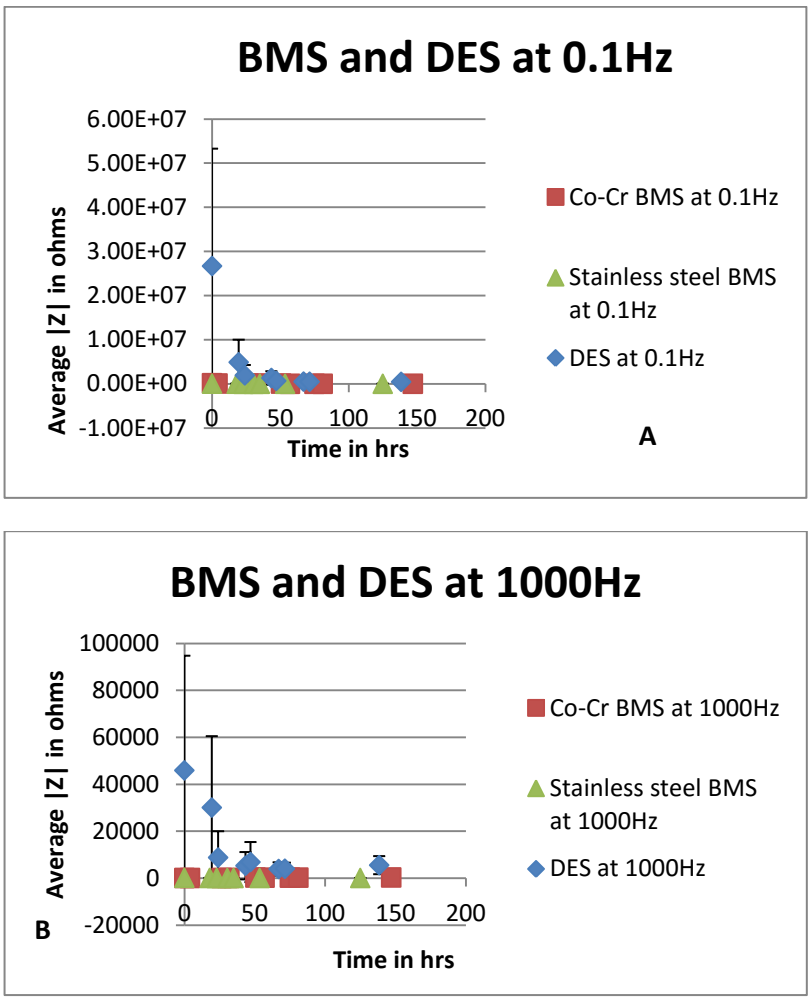
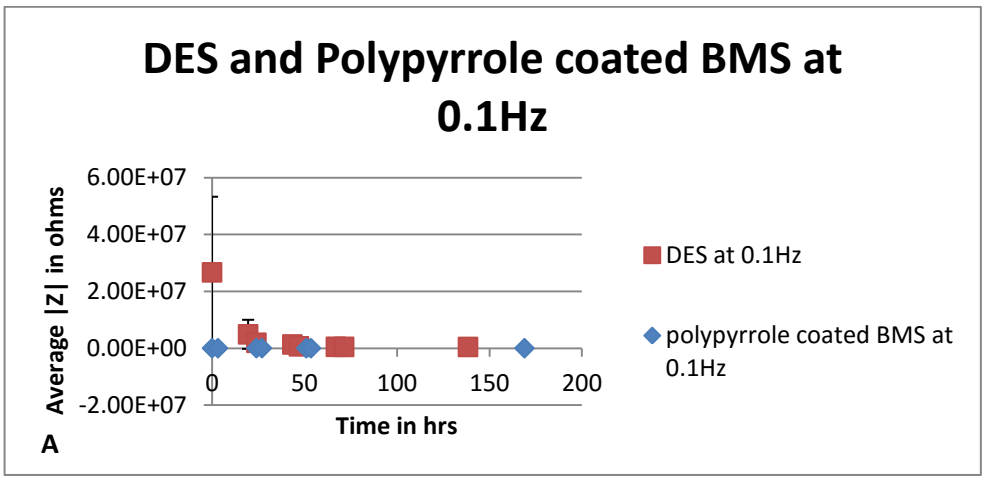


Figure 4.9 A and B – Change in average impedance of test1, test 2 and test 3 (|Z|) of stainless steel BMS, Co-Cr BMS and Sirolimus coated DES in endothelial media over time from 0 hrs to 140 hrs considered at 0.1 Hz and 100 Hz. The error bars represents the standard deviation and the values points represents the average |Z| of test 1 , test 2 and test 3 at different time having a value of N=3

4.10 Comparison between Sirolimus coated DES and polypyrrole coated BMS in endothelial medium

Sirolimus coated DES was compared with the Co-Cr BMS but coated with polypyrrole . The figure 4.10 A and B, shows the change in impedance between the two. At 0.1 Hz the DES has greater impedance compared to polypyrrole coated BMS and at 1000 Hz also the same is observed. In the beginning the DES has higher impedance than polypyrrole coated BMS.



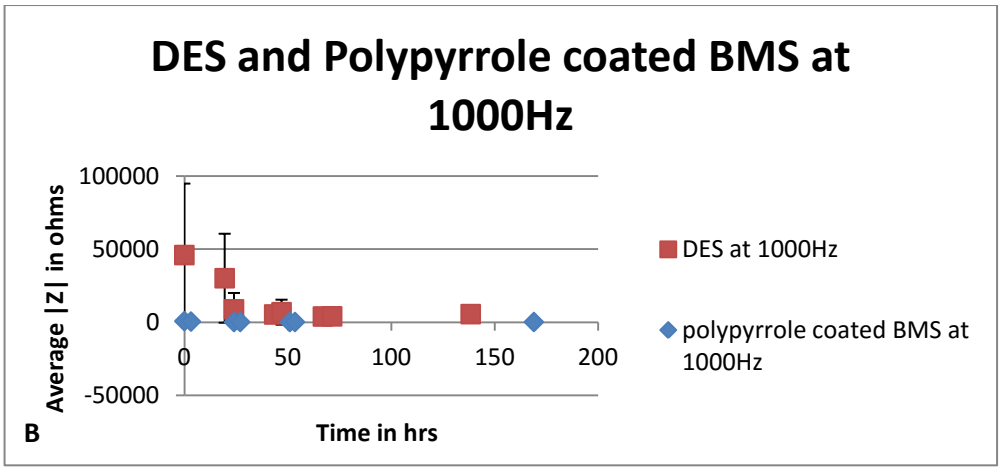
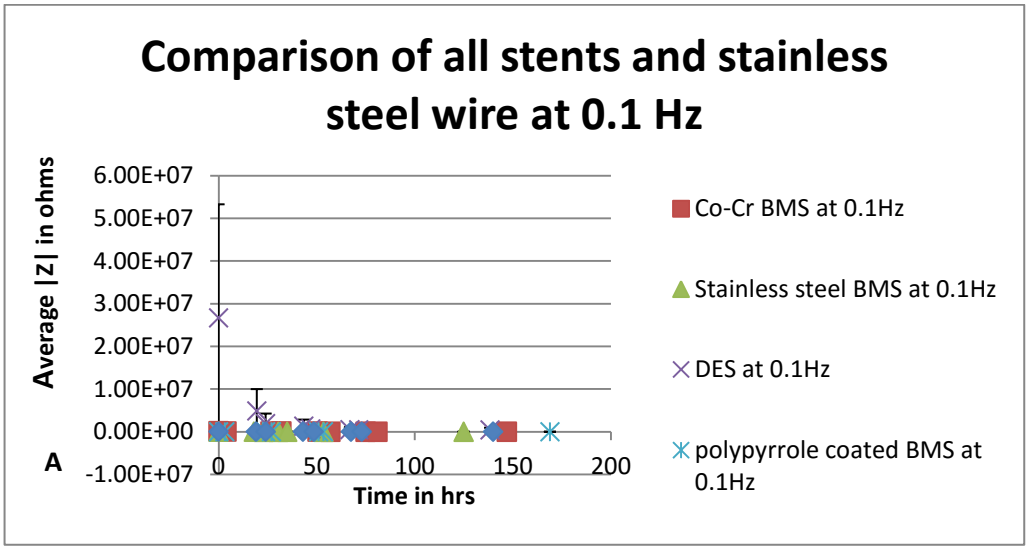


Figure 4.10 A and B – Change in average impedance of test1, test 2 and test 3 (|Z|) of Sirolimus coated DES and polypyrrole coated BMS in endothelial media over time from 0 hrs to 167 hrs considered at 0.1 Hz and 100 Hz.

4.11Comparison of all stents and stainless steel wire in endothelial medium

All the stents discussed above – Co-Cr BMS, Stainless steel BMS, Sirolimus coated DES and polypyrrole coated BMS and stainless steel wire have been compared in figure 4.11 A and B. DES has a higher impedance compared to other stents at 0.1 Hz and 1000 Hz.



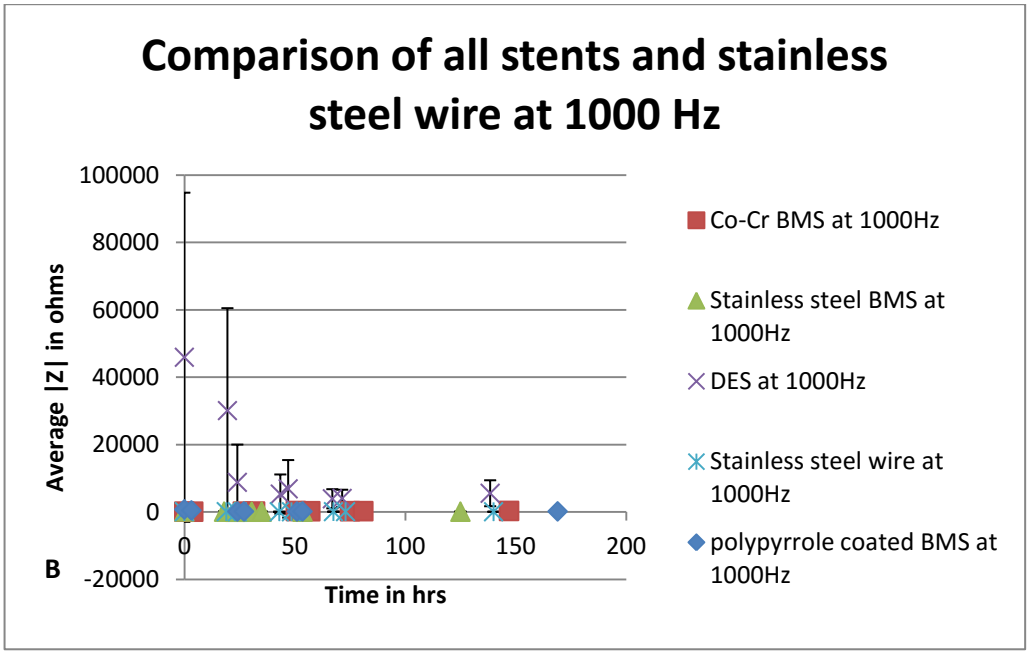


Figure 4.11A and B – Change in average impedance of test1, test 2 and test 3 (|Z|) of all stents and stainless steel wire in endothelial media over time from 0 hrs to 167 hrs considered at 0.1 Hz and 100 Hz.

Type of stent	Average |Z|at 0hrs (Ω)	Average |Z|at 140hrs (Ω)
Stainless steel wire	51747±8722	31847±16887
Co-Cr BMS	137068±37636	10038±542
Stainless steel BMS	35885±54385	6216±3085
DES	26687417±26600274	443540±503145
Polypyrrole coated BMS	11231±4213	7677±533

Table 4.5a –Average impedance |Z| values with standard deviations at 0.1Hz of all the stents and stainless steel wire.

Type of stent	Average |Z|at 0hrs (Ω)	Average |Z|at 140hrs (Ω)
Stainless steel wire	97±19	75±11
Co-Cr BMS	107±9	314±259
Stainless steel BMS	171±102	97±11
DES	45923±48846	5560±3854
Polypyrrole coated BMS	762±972	179±37

Table 4.5b – Average impedance |Z| values with standard deviations at 1000Hz of all the stents and stainless steel wire.

From Table 4.5a and 4.5 b it can be inferred that the impedance of stainless steel wire was the maximum at 0.1Hz at 0hrs compared to stainless steel BMS. At 1000 Hz stainless steel BMS had higher impedance than stainless steel wire .However, while considering among stents -DES had the highest average |Z| at 0.1Hz at 0 hrs. When comparing the |Z| values at 0.1Hz and 140hrs, also had DES with the highest impedance. At 1000Hz, DES has the highest |Z| at 0hrs and 140hrs.

Chapter 5 – Discussion

5.1 Introduction

The physical properties of a tissue can be analyzed by the electrical impedance of the biological sample. The impedance response is dependent on frequency (Schwan, 1957). Commonly , frequency range of single Hz up to thousands of Hz are examined , since impedance changes at different frequencies can provide important information on various aspects of the material under investigation . Variations in impedance measurements can be indicative of a variety of changes, including the shape of cells, the structure of cell membranes and the volume and composition of intra and extracellular solution. An example of the clinical application of measurements has been used in the detection of cancer (Ackmann, 1993), where changes in the shape and size of abnormal cells give rise to significant changes in the impedance signal (Giaever, 1991), (Blady, 1996), (Aberg, 2004).

5.2 System characterization

The first series of experiments sought to characterize the basic impedance characteristics of the test system originally developed by Shedden et al, 2008. Specifically, the aim of these initial experiments was to measure the impedance of a model system, which comprised a Ag/AgCl and stainless steel wire electrode pair, using this system to examine the effect of changes in temperature, media and magnetic agitation, on impedance. It was found that the electrodes used in these experiments were stable and gave rise to consistent initial measurements with low variability in the data obtained. The impedance measurements with the various electrodes were found to be reproducible at high frequencies. When the experiments using the stainless steel wire electrode as the test material were extended , it was found that the overall impedance decreased by around 50% following 6 days immersion in test solution , when measured at 0.1Hz. In contrast, the impedance was found to be more stable across the 6 days when measured at 1000 Hz. Cell adhesion is likely to give rises to both resistive and capacitive elements and so these effects

will need to be controlled for in future experiments involving the use of the test system for the study of cell adhesion on stents.

5.2.1 Impact of temperature on the impedance profiles

Referring to Figure 4.2 A, it can be inferred that the impedance modulus, |Z| was quite linear at both the temperatures 20ºC and 37 ºC in the Cole plot. Also from the table 4.1a and b the values when compared at 0.1Hz shows that the average |Z| at 20ºC was found to be 27819Ω and at 37ºC was 11133Ω. The average |Z| at 1000Hz was found to be 100 Ω at 20ºC and 71Ω at 37ºC. Though from the graphs the impedance profiles looks very close and do not show a great range of variability, these average values shows a clear variation in |Z|. Hence from the results obtained it can be inferred that the temperature does have an impact on the impedance with the increased temperature appearing to reduce the impedance. This effect is consistent with previous studies, which have used impedance measurements to study body mass composition and water content (Hartman, 2005). Other studies have also shown that the temperature has an increase in conductivity and therefore reduction in impedance (Hartman, 2005). As the temperature changes there will be differences observed in resistance, reactance and impedance. If all other variables are kept constant the resistance increases with decrease in temperature (Hartman, 2005). Despite this, a study by Duncan (2008) observed that the bioimpedance analysis does not have a significant change over a 10ºC range. It may therefore be that small changes in media temperature, as would be observed when performing impedance measurements outside a cell culture incubator for short periods as proposed with the present test system, may have sufficiently small effect on impedance as to allow the set up proposed to be used for future experiments involving cells.

5.2.2 Impact of magnetic agitation on impedance profiles.

The effect of magnetic agitation on impedance profiles was studied. From figure 4.3A and B it was found that the impedance profiles for test 1, 2 and 3 without magnetic agitation were very close and did not show any difference in the peaks. The impedance profile of test1 with magnetic agitation showed a high rising impedance profile which was not observed with test 2 and 3 with magnetic agitation. The

magnetic agitation also has increased the impedance values in comparison with the values obtained from the tests carried out without magnetic agitation. The agitation causes magnetic capsule in the solution to spin on being placed on the magnetic agitating device. The solution starts to get agitated at a high speed and the exchange of ions between the electrodes and electrolyte starts at a rapid rate.

5.2.3 Impact of medium type on impedance profiles.

Experiments were carried out with metal wire electrodes immersed in 2 different media – Solution A and endothelial medium. Figure 4.4 B shows that the impedance profile of test 1 in solution A has highest impedance and varies extensively in comparison with test 2 and test 3. However, the impedance profiles of test 1, test 2 and test 3 in endothelial media have similar and close impedance profiles which are overlap between the values of test1 and test 3 of solution A. It can be inferred the impedance of electrodes in solution A is greater when compared with endothelial medium. The electrode surface area decrease when the cells adhere and proliferate on surface of the electrodes and thus changing the total impedance in the cell media. A previous study shows that many biosensors are based on this principle and, monitoring of cells on application of electric field has been reported by Giaver and Keese (1984). The experiments were carried out for measuring the concentration of cell and the state of cells physiologically. The conductivity and permittivity of the medium also plays a role in the impedance spectroscopic responses. Endothelial media contains proteins which makes the media capacitive at higher frequencies (at 1000 Hz) which were observed. The experiments were carried out in two different media types to observe the change in impedance on media which does not contains proteins and hence behaves resistive at low frequency and tends to be capacitive and resistive at higher frequencies.

5.2.4Impedance profiles of stainless steel wire and stainless steel BMS

Stainless steel wires were used to carry out the initial developmental experiments since they are cost effective and readily available. The stents are very expensive and range between £150 – 300. Hence it is not feasible to use stents for the basic experimentation. Stainless steel wires were used in endothelial medium and the

impedance was found to be higher at 0.1Hz and decreased with time in comparison with the stainless steel BMS. Referring to figure 4.7A and B, stainless steel wire has lower impedance at 1000 Hz compared to stainless steel BMS. Stainless steel wire was not found to have similar impedance profile as stainless steel BMS.

5.3 Stents Impedance

Once system characteristics were completed, the study moved to using the set up to measure the impedance of a number of stent types. DES was found to have the highest impedance among the stents compared at 0.1 Hz and1000Hz presented in Table 4.5 a and b. The impedance profile followed the Warburg impedance profile graph which was discussed in Chapter 2 is observed (figure 2.2), which is to be expected in the absence of cell or tissue adhesion.

Since the DES was found to have higher impedance compared to the BMS since DES was covered with an insulator polymer layer. The polypyrrole coated BMS was used which acts as an insulating medium and accordingly should have a robust effect on the impedance profiles. This was the expected behavior since the polymer coating of polypyrrole which is a conducting polymer and hence although it is a polymer, we should still expect it to reduce the impedance in comparison to cypher stent. The impedance values of the polypyrrole coated BMS was expected to be lower at the two frequency ranges in comparison with the DES. This was the result obtained from the experiments.

There were traces of corrosion found on the stent which was connected to the crocodile clips after a period of 24 hrs in all the experiments involving stents. This could be due to humidity and the test cell set up maintained at 37^{o} C and other electrochemical reactions. This could have an effect on the data being inconsistent for the experiments. Figure 5.1 shows the formation of rust observed in the experiment.

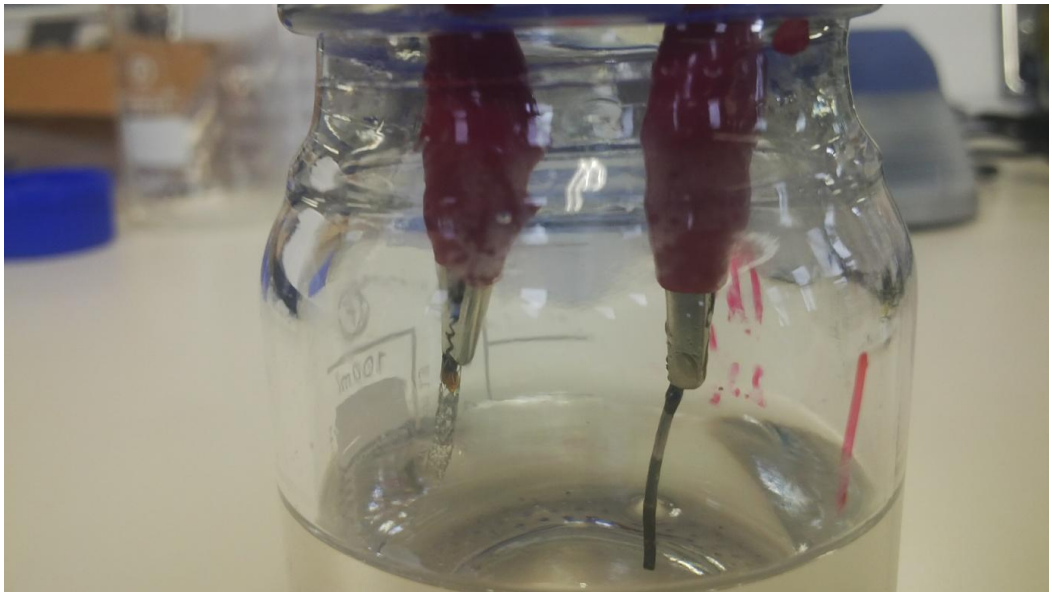


Figure 5.1 – Formation of rust on the ends of the stent connected to crocodile clip immersed in endothelial medium.

5.4 Conclusion

- An in-vitro model of in-stent restenosis has been used to measure impedance profile of a variety of coronary stents.
- The impedance profiles were found to be reliable, with good repeatability and reproducible results.
- The impedance was found to be dependent on temperature, magnetic agitation, media type and stent type.
- It may be possible to use the stent as an electrode to measure the impedance as an implanted device for monitoring the neointimal growth of cells and re-endothelialisation of cells /tissues.
- The impedance was found to be highest at low and high frequencies in Cypher DES , which gives an indication that the polymer coating on the stent surface increases the impedance .
- The various metal wires and stent characteristics based on impedance measurements provided a comprehensive result which could be further used for cell culture based assay experiments.
- There were some unpredicted effects observed in the experiments such as formation of rust on the ends of the electrodes connected to the crocodile clips which could have led to change in the impedance values such effects will need to be minimized and controlled for in future work.

5.5 Future work

- The future development on the monitoring of impedance would be extending the study in cell culture and tissues in animal models to measure the in-stent restenosis in pigs . Investigate the impedance profiles on more drug eluting stents and conclude the best suitable stent which is suitable for the neointimal growth of cells / tissues.
- The future work should also focus on finding alternatives where the rusting of the stent could be avoided and produce more robust and reproducible sets of data from experiments.
- The equivalent circuit models could be constructed from the test cell setup providing specific capacitance and resistance values from the impedance data obtained and could be interpreted from the impedance profile graphs
- Materials that do not form rust could be considered for crocodile clips to get better readings

REFERENCES

Åberg, P., Nicander, I., Hansson, J., Geladi, P., Holmgren, U. and Ollmar, S. (2004). Skin Cancer Identification Using Multi-frequency Electrical Impedance: A Potential Screening Tool, *IEEE Transaction on Biomedical Engineering*. vol. 51, nº 12, pp: 2097-2102

Achenbach S.,(2006) – Computed Tomography Coronary Angiography .Journal of the American College of cardiology 48(10) : 1919-1928 .

Ackmann, J. (1993). Complex Bioelectric Impedance Measurement System for the Frequency

Alberts, B ., Johnson, A ., Lewis, J ., Raff, M ., Roberts, K ., and Peter,W.,*THE CELL*. New York: Garland Science. ,20-350.

Alberts, B ., Johnson, A ., Lewis, J ., Raff, M ., Roberts, K ., and Walter, P . , (2002). Molecular Biology of the Cell. In:

Arbizzani, C., Mastragostino, M., Nevi, L., Rambelli, L., 2007. Polypyr-role: A drug-eluting membrane for coronary stents. Electrochimica Acta 52, 3274–3279.

Bard, AJ.,& Faulkner, LR .,(1980) – Electrochemical methods. Fundamentals and Applications. John Wiley and Sons, Inc.

Barsoukov , E. J., Macdonald , R ., (2005). *Impedance Spectroscopy Theory, Experiment, and Applications*. 2nd ed. New Jersey: John Wiley & Sons. 10-606.

Baumgart ,D ., Haude ,M ., George, G ., Ge ,J. , Vetter ,S. , Dagres, N. , Heusch ,G. , Erbel ,R., (1998) – Improved assessment of coronary stenosis severity using the relative flow velocity reserve . Circulation 98:40-46

Beach, R.D. et al, (2005). Towards a Miniature In Vivo Telemetry Monitoring System Dynamically Configurable as a Potentiostat or Galvanostat for Two- and Three-Electrode Biosensors, *IEEE Transaction on Instrumentation and Measurement*, vol. 54,nº 1, pp: 61-72

Blady, B. ,& Baldetorp, B., (1996). Impedance spectra of tumor tissue in comparison with normal tissue; a possible clinical application for electrical impedance tomography. *Physiol. Meas*., vol. 17, suppl. 4A, pp. A105–A115.

Bockris, J.O'M., & Reddy, A.K.N., (1970). Modern electrochemistry. *Plenum Press*, New York.

Capodanno D, Dipasqua F, Tamburino C. Novel drug-eluting stents in the treatment of de novo coronary lesions (2011). *Vasc Health Risk Manag. 7:103-18*

Chaabane, C ., Otsuka, F ., Virmani, R ., Bochaton-Piallat, M.L., (2013). Biological responses in stented arteries. *European society of cardiology*. 5 (1), 353-363.

Cole KS (1968) – membranes, ions and impulses. University of California Press, Berkeley.

Cowper, PA. , Udayakumar, K., Sketch, MH. , Peterson, ED., (2005) – Economic effects of prolonged clopidogrel therapy after percutaneous coronary intervention. Journal of the American College of Cardiology 45 (3):369-376.

Cox-Reijven, KL. & Soeters, PB. (2000) – Validation of bio-impedance spectroscopy: Effects of degree of obesity and ways of calculating volumes from measured resistance values. International Journal of obesity 24:271-280.

Dugdale, D. C., Michael, A., Chen, Zieve, D ,. (2012). *Percutaneous transluminal coronary angioplasty (PTCA).* Available: http://www.nlm.nih.gov/medlineplus/ency/anatomyvideos/000096.htm. Last accessed 25 Jun 2015.

Duncan, M., Craig, S. R. , Lunger, A. N. , Kunn, D. D ., Salze, G ., and McLean , E ., 2007. Bioimpedance assessment of body composition in cobia *Rachycentron canadum* (L. 1766). Aquaculture 271:432–438.

Ellappan , P ., & Sundararajan ,R ., (2005) – A simulation study of the electrical model of a biological cell . Journal of Elctrostatics 63(3-4) :297-307.

Garcia , E.M ., (2013). The electrochemical behavior of cobalt electrodeposits on 430 stainless steel as solid oxide fuel cell interconnect. *Surface & Coatings Technology*. 235 (1), 10-14.

Giaever ,I. , & Keese , CR ., (1991) – Micro motion of mammalian cells measured electrically . Proceedings of the National Academy of Science USA. Cell Biology. 88: 7896-7900.

Giaever, I., & Keese, C. R. ,(1991). Micromotion of mammalian cells measured electrically,

Grimnes, S. , & Martinsen, O., (2008). Bio-impedance and Bioelectricity Basics, *Second edition.Academic Press*, Elsevier

GUDIVAKA, R ., SCHOELLER,D., AND KUSHNER, R.F., (1996). Effect of skin temperature on multifrequency bioelectrical impedance analysis. *Clinical Nutrition Research*. 81 (2), 838-845.Range from 5Hz to 1MHz. *Annals of Biomedical Engineering*, Vol. 21, pp. 135-146

Hartman , K.J. , Beth ,A. P . , & Rosendale, J.E., (2011). Temperature Effects on Bioelectrical Impedance Analysis (BIA) Used to Estimate Dry Weight as a Condition Proxy in Coastal Bluefish. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*. 3 (1), 307-316.

Hauser , TH .,& Manning, WJ., (2006) – Coronary MRI : More pretty pictures or present –day value ? Journal of the American College of cardiology 48(10) 1951-1952.

Holmes , DR. , Schwartz , .RS , Webster MWI (1991) – Coronary restenosis –What have we learned from angiography .Journal of the American College of Cardiology :17(6) :B14-B22.

James , B ., (2015). *Cardiac Stents Overview.* Available: http://www.webmd.com/heart-disease/guide/stents-types-and-uses?page=2. Last accessed 25 Jun 2015.

Karin ,R. S., (2013). Biological responses in stented arteries .*Cardiovascular research*. 1 (1), 353-363

Kin , F. L. , (2014). Review on Impedance Detection of Cellular Responses in Micro/Nano Environment. *micromachines*. 1 (1), 1-12.

Liistro ,F ., & Colombo ,A. ,(2001) –Late acute thrombosis after paclitaxel eluting stent implantation .Heart86(3):262-264

Lo ,CM . , Keese , CR. , Giaever, I ., (1995) – Impedance analysis of MDCK cells measured by electric cell-substrate impedance sensing . Biophysical Journal 69 :2800-2807.

Nagano ,M. , Suita , S . , Yamanouchi ,T .,(2000) – the validity of bioelectrical impedance phase angle for nutritional assessment in children . Journal of Pediatric Surgery 35(7) :1035-1039.

National heart, lung and blood institute. (2014). *What Is Coronary Heart Disease?.* Available:http://www.nhlbi.nih.gov/health/health-topics/topics/cad. Last accessed 20th Jun 2015.

National Institute for Clinical Excellence (2003) – Technology Appraisal 71. Guidance on the use of coronary artery stents . www.nice.org.uk

NHS. (2014). *Coronary heart disease (CHD) is the leading cause of death both in the UK and worldwide.*. Available: ttp://www.nhs.uk/Conditions/Coronary-heart-disease/Pages/Introduction.aspx. Last accessed 20 Jun 2014.

Okner, R., Domb, A., Mandler, D., 2007. Electrochemical formation and characterization of copolymers based on N-Pyrrole derivatives. Biomacro-molecules 8, 2928–2935.

Olmo, A. & Yúfera, A. (2010). Computer Simulation of Microelectrode Based Bio-Impedance Measurements with COMSOL, *Third International Conference on BiomedicalElectronics and Devices, BIODEVICES 2010*. pp: 178-182. Valencia, Spain

Onaral, B. & Schwan, H.P. (1982). Linear and nonlinear properties of platinum electrode polarization. Part I: Frequency dependence at very low frequencies, *Med. Biol. Eng.Comput.*, 20: 299-306.

Onaral, B. & Schwan, H.P. (1983). Linear and nonlinear properties of platinum electrode polarization. Part II: time domain analysis, *Med. Biol. Eng. Comput*., 21: 210-216.

Ong , ATL ., McFadden ,EP. , Regar, E. , de Jaegere ,PPT. , van Domburg ,RT. , Serruys PW (2005) – Late angiographic stent thrombosis (LAST) events with drug-eluting stents . European Heart Journal 26:640 .

Otsuka , F., Finn, AV., Yazdani, SK., Nakano, .M, Kolodgie, FD., Virmani, R., The importance of the endothelium in atherothrombosis and coronary stenting. Nat Rev Cardiol 2012;9: 439 –453

Pe´rez de Prado, A., Pe´rez-Martı´nez, C., Cuellas-Ramo´,C., Manuel Gonzalo-Orden, J ., Regueiro-Purrin˜os, M., Martı´nez , B., Garcı´a-Iglesias , M. J ., Ajenjo ,J. M., Alto´naga , J. R ,. Diego-Nieto, A . ,(2011). Time Course of Reendothelialization of Stents in a Normal Coronary Swine Model: Characterization and Quantification. *Veterinary Pathology OnlineFirst*. 1 (9),1-9.

Prado,P .D ., Pe´rez-Martı´nez,C., Cuellas-Ramo´n,C., Manuel Gonzalo-Orden ,J., Regueiro-Purrin˜os, M ., Martı´nez, B ., Garcı´a-Iglesias,M.J ., Ajenjo, J.M ., Alto´naga, J.R ., Diego-Nieto, A. , (2011). Time Course of Reendothelialization of Stents in a Normal Coronary Swine Model: Characterization and Quantification. *Veterinary Pathology OnlineFirst*. 1 (1), 1-9.

Prasad , CK., Resmi, KR., Krishnan, K., (2005) Survival of Endothelial Cells in vitro on Paclitaxel-loaded Coronary Stents, J Biomaterial Applications, 19, 271-286

Prati , F . , Pawlowski, T. , Sommavria, L. , Labellarte, A. , Manzoli, A. , Boccaanelli, A. , Motolese, M., (2002) , Intravascular ultrasound and quantitative coronary angiography assessment of late in-stent restenosis :In vivo human correlation and methodological implications . Catheterization and Cardiovascular Interventions 57(2) :155-160 .

Proc. Nail. Acad. Sci. USA. Cell Biology, vol. 88, pp: 7896-7900, Sep. 1991

Radke, S.M .,& Alocilja, E.C., (2004). Design and Fabrication of a Microimpedance Biosensor for Bacterial Detection, *IEEE Sensor Journal*, vol. 4, nº 4, pp: 434-440.

Rist ,C. , Von Ziegler, F. , Nikolaou, K. , Kirchin, MA. , Wintersperger, BJ. , Johnson ,TR. , Knez, A. , LEber, AW ., Reiser, MF. , Becker, CR., (2006) – Assessment of coronary artery stent patency and restenosis using 64-slice computed tomography . Academic Radiology 13(12) : 1465-1473 .

Robinson, D.A., (1968). The electrical properties of metal microelectrodes, *Proceedings of the IEEE*, 56(6): 1065-1071.

Scholz , B., & Anderson, R., (2000) – On electrical impedance scanning –principles and solutions . Electromedica 68:35-44.

Schuijf ,JD. , Bax, JJ. , Jukema, JW. , Lamb, .HJ , Dirksen,. MS , van der Wall ,EE. , de Ross ,A .,(2004) – Coronary stent imaging with multidetector row computed tomography . International Journal of Cardiovascular Imaging . 20(4):341-4.

Schwan, H. P. (1957). Electrical properties of tissue and cell suspensions. *Advances in Biological and Medical Physics. New York, Academic press*, vol. 5, pp. 147–224.

Schwan, H.P. (1963), "Determination of biological impedance", in Physical in Biological Research, W.L. Nastuk, *Ed. New York: Academic press*, 6(ch.6) (1963).

Schwan, H.P. (1992), Linear and Nonlinear electrode polarization and biological materials, *Annal. Biomed. Eng.*, 20: 269-288.

Shedden, L., S. Kennedy, R. Wadsworth and P. Connolly "Towards a self-reporting coronary artery stent--measuring neointimal growth associated with in-stent restenosis using electrical impedance techniques." Biosens Bioelectron 26(2): 661-666.

Simpson, R.W., Berberian, J.G. and Schwan, H.P. (1963). Nonlinear AC and DC polarization of platinum electrodes, *IEEE Transactions on Biomedical Engineering*. BME-27(3): 166-171.

Stone ,GW. , Moses, JW. , Ellis, SG. , Schofer, J. , Dawkins ,KD. , Morice, .MC , Colombo ,.A , Schampaert,. E , Grube, E. , Kirtane, AJ. , Cutlip, DE. , Fahy, M. , Pocock ,SJ. ,Mehran ,R. , Leon, MB., (2007) – Safety and efficacy of sirolimus and paclitaxel-eluting coronary stents . New England Journal of medicine 2007 356:998-1008.

Thomas ,F., Lüscher., Steffel, J., Franz R. E., Joner, M., Nakazawa,G., Felix, C., Tanner, Virmani,R., (2007). Drug-Eluting Stent and Coronary Thrombosis

Biological Mechanisms and Clinical Implications. *Special report*. 1 (115), 1051-1058.

Thomas ,N .,& Goodyer, ID., (2003) –Stealth sensors :real time monitoring of the cell cycle . Targets 2(1) :26-33 .

Tiruppathi, C. , Malik, AB. , Del Vecchio ,PJ. , Keese ,CR. , Giaever, I., (1992) – Electrical method for detection of endothelial cell shape change in real-time : Assessment of endothelial barrier function . Proceeding of the National Academy of Science USA . Medical Sciences 89 :7919-7923.

Valentinuzzi ,ME., (1996) – Bioelectrical impedance techniques in medicine . Part I: Bioimpedance measurement . First Section : General concepts . Critical Reviews in Biomedical Engineering 24 (4-6) :223-255 .

Van der Hoeven ,BL. , Pires, NMM ., Wards,. HM , Oemrawsingh, PV. , van Vlijmen ,BJM .,Quax ,PHA. , Schalij, MJ. , van der Wall, EE ., Jukema, JW., (2004) – Drug eluting stents:results , promises and problems . International Journal of cardiology.

Waksman ,R .,(2002) –Drug eluting stents . from bench to bed . Cardiovascular Radiation Medicine 3:226-241

Wegener ,J. , Keese, CR. , Giaever, I., (2000) – Electric cell –substrate impedance sensing (ECIS) as a non-invasive means to monitor kinetics of cell spreading artificial surfaces . Experimental Cell Research 259 :158-166.

Wegener, J. , Zink, S. , Rosen ,P. , Galla, HJ., (1999) – Use of electrochemical impedance measurements to monitor ß-adrenergic stimulation of bovine aortic endothelial cells . Pflugers Arch –Euopean Journal of Physiology 437:925-934.

Yúfera . A, Olmo . A, Daza . P and Cañete. D (2011). Cell Biometrics Based on Bio-Impedance Measurements. *Advanced Biometric Technologies*. 2 (1), 1-382.

Zou, Y., & Guo ,Z .,(2003) – A review of electrical impedance techniques for breast cancer detection . Medical Engineering and Physics 25 :79-90.