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POLYTECHNIQUE MONTRÉAL
affiliée à l'Université de Montréal

Conducting Polymer Based Flexible and Conductible Heart Patch

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Mémoire présenté en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

Génie biomédical

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POLYTECHNIQUE MONTRÉAL
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Ce mémoire intitulé :

Conducting Polymer Based Flexible and Conductible Heart Patch

présenté par **Erwan SAUVAGE**
en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*
a été dûment accepté par le jury d'examen constitué de :

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DEDICATION

I am dedicating this thesis to my loving family, my supportive girlfriend, and to my colleagues and friends for always being there when I needed them most.

Je dédicace aussi et surtout ce mémoire à mon grand-père, André Brulard (1938-2022).

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Thank you Madison for supporting me throughout my masters, I loved spending this 2 years by your side. Thank you for being my girlfriend, my guide and for helping me discover Canada.

Thank you to my parents, for their love and support. Thank you for being such good parents and for believing in me, I'm proud to be your son. Thank you to my sisters, Océane and Floriane, I miss you and I love you.

And to finish, I would like to thank Oscar, Watson, Loki, Finnick and Figaro for your unconditional love and cuteness.

Thank you all for being there for me, I would not have achieved what I have achieved without you.

RÉSUMÉ

Les maladies coronariennes entraînent souvent des infarctus du myocarde (IM), qui sont les principales causes de décès dans le monde. L'infarctus apparaît lorsque le muscle cardiaque n'est plus vascularisé et commence à se nécroser. Les cellules mortes sont remplacées par un tissu cicatriciel fibreux non contractile, ce qui entraîne une diminution de la capacité cardiaque. Les traitements actuels de l'infarctus du myocarde reposent sur des interventions chirurgicales (pontage coronarien) ou médicamenteuses pour prévenir l'aggravation des lésions et tenter de revasculariser les parties détruites, afin de réduire la morbidité et de limiter les pertes de la fonction de pompage du cœur. Une autre solution consiste à utiliser des dispositifs d'assistance mécanique tels que les cœurs artificiels ou des transplantations cardiaques. Cependant, la technologie et les donneurs disponibles sont limités et ces méthodes n'offrent pas encore de réelles solutions à long terme.

Les patchs cardiaques fabriqués à partir de matériaux synthétiques et conducteurs semblent prometteurs pour aider le cœur à retrouver sa conductivité électrique et promouvoir la régénération du myocarde.

Le PEDOT:PSS est un polymère conducteur qui est considéré comme ayant d'excellentes propriétés pour l'ingénierie tissulaire. Flexibles, étirables, antibactériens, biocompatibles et fonctionnalisables, les hydrogels à base de PEDOT:PSS pourraient être les candidats idéaux pour la fabrication de patchs cardiaques.

Dans ce mémoire, nous avons créé un hydrogel à base de PEDOT:PSS et de PVA. Nous avons mélangé du PVA, du DMSO et du PEDOT:PSS et séché la solution dans un four pour obtenir un film noir, sec et autoportant. Une fois immergé dans l'eau, le film gonfle et devient un hydrogel. Nous avons mesuré le taux de gonflement ainsi que la teneur en eau des hydrogels et découvert qu'ils diminuent avec la quantité de PVA ajoutée dans l'hydrogel. Nous avons effectué des tests de traction sur l'hydrogel et mesuré son module d'Young ainsi que l'élongation à la rupture pour différentes concentrations de PVA. Nous avons constaté que si tous les hydrogels avaient le même module d'Young, l'élongation à la rupture augmentait avec la quantité de PVA ajoutée. Nous avons mesuré la résistance électrique, l'épaisseur et la conductivité de l'hydrogel dans un état de repos et pendant un étirement. Nous avons trouvé que la conductivité électrique de l'hydrogel diminue avec la quantité de PVA ajoutée et que la conductivité de tous les hydrogels est stable pendant leurs élongations. Nous avons également mesuré la conductivité des hydrogels pendant une élongation cyclique et avons trouvé que les hydrogels sont résistants à l'étirement cyclique et que leur conductivité est stable. Nous

avons mesuré l'angle de contact et la topologie de l'hydrogel pour différentes concentrations de PVA. Les hydrogels avec plus de PVA sont plus hydrophiles. Nous avons fonctionnalisé les hydrogels avec un peptide imitant la N-cadhérine et découvert qu'ils présentent des propriétés antibactériennes.

ABSTRACT

Engineering cardiac implants to treat myocardial infarction (MI) has progressively emerged but challenges to mimic structural properties and variability of cardiac tissues with traditional bioconstruct and conventional engineering methods remain. In this work, a synthetic patch with bioactive surface has been prepared to quickly reinstate the functionality of the damaged myocardium. Combination of a composite, soft, and conductive hydrogel based on (3,4- ethylenedioxythiophene):polystyrene-sulfonate (PEDOT:PSS), with polyvinyl alcohol (PVA), a cardiac patch with high electrical conductivity (40 S/cm) and high stretchability (E=5 Mpa), that can stretch until 50 % of its original length has been designed. Our findings demonstrate that the material has also a resilience of 10 % cyclic stretching at 1Hz with no loss of conductivity over time. By adding N-cadherin protein using a Steglich esterification, a bioactive surface has been formed on the cardiac patch, preventing the formation of bacterial biofim (Staphylococcus aureus). This work not only create a structurally approved patch with strong mechanical and conductive properties but also biofunctionality to facilitate biointegration, holding great promise to mitigate the burden from MI.

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LIST OF SYMBOLS AND ACRONYMS

PEDOT	Poly(3,4-ethylenedioxythiophene)
PSS	Sodium Polystyrene Sulfonate
TOS	Tosylate
PVA	Poly(vinyl alcohol)
DMSO	Dimethyl sulfoxide
PEG	Polyethylene glycol
PANI	Polyaniline
PPy	Polypyrrole
PT	polythiophene
PP	Polypropylene
PPy	Polypyrrole
DMF	Dimethylformamide
DCC	N,N'-Dicyclohexylcarbodiimide
DMAP	4-diméthylaminopyridine
SEM	Scanning Electron Microscopy
PLA	Polylactic acid
PHVB	Poly(3-hyroxybutyrate-co-3-hydroxyvalerate
NBR	Nitrile butadiene rubber
PEGDM	Poly(ethylene glycol) dimethacrylate
TCH	Tetracycline hydrochloride
PTFE	Polytetrafluoroethylene

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CHAPTER 1 INTRODUCTION

1.1 Background and problematic

Coronary heart disease often leads to myocardial infarction (MI), which is the leading cause of death worldwide. In the United States alone, it kills more than 360,000 people a year [4]. MI appears when the heart muscle is no longer vascularized and begins to necrotize. The dead cells will be replaced by non-contractile fibrous scar tissue leading to a decrease of heart capacity. Current treatments for myocardial infarction are based on surgical (coronary bypass) or drug interventions to prevent further damage and try to revascularize the destroyed parts, to reduce morbidity and limit losses of the heart's pumping function [5]. Another treatment solution is mechanical assist devices such as artificial hearts or heart transplants, however,technology and available donors are limited and these methods do not yet offer real lon term solutions [6].

Cardiac patches made of synthetic and conductive materials are showing promising potential in promoting cardiac function to help a heart conduct electric signal again and promote myocardium regeneration [7]. PEDOT:PSS is a conductive polymer that is considered to have excellent properties for tissue engineering [8]. Conductive polymer films made of PEDOT:PSS are flexible, stretchable, and known to be biocompatible. PEDOT:PSS have been used in the biomedical fields for drug delivery and tissue engineering applications, especially in neural and cardiovascular applications and could be the perfect candidates for making heart patches [9]. As an extra benefit, PEDOT:PSS also shows antibacterial properties [10]. Hydrogels are polymer based soft and stretchable 3D structures that resemble natural tissues and can support cell adhesion [11]. PEDOT:PSS hydrogels combine both qualities of the hydrogel and the conductive polymer. Further, hydrogels can be functionalized, which consists of adding new functional groups to its polymer base to modify its surface and enhance its biocompatibility property [64].

1.2 Objective

1.2.1 Main objective

The main objective of this project is to find a method of developing a biocompatible, conductive, flexible, stretchable and functionalized PEDOT:PSS based hydrogel that can be used as a cardiac patch.

1.2.2 Specific objectives

1- To make a PEDOT:PSS, DMSO and PVA based hydrogel using the drop casting technique, with varying concentrations of PVA.
2- To evaluate the physicochemical characterization of the hydrogel to screen for the best percentage of PVA to use.
3- To determine the durability of the hydrogel during cycling stretching.
4- To functionalize the hydrogel with N-cadherin mimic proteins.
5- To evaluate the antibacterial properties of the hydrogel.

CHAPTER 2 LITERATURE REVIEW

2.1 Heart and blood flow

According to Merriam-Webster dictionary, the heart is defined as "a hollow muscular organ of vertebrate animals that by its rhythmic contraction acts as a force pump maintaining the circulation of the blood" [12].
The organ consist of a muscle (myocardium) lined with two layers (epicardium, endocardium) and a pericardial sac. Further, it is divided into two distinct parts (left and right) each composed of two communicating cavities (atrium above, ventricle below). The heart has an autonomic nervous network which ensures its automatic functioning, but remains under the influence of the central nervous system [13].
The blood circulates in arteries and veins. Arteries are blood vessels that come from the heart while veins are blood vessels that go towards the heart. The blood coming from muscles is poor in oxygen and full of metabolic waste such as carbon dioxide, therefore it needs to be refueled. It enters the heart through the right atrium via the superior vena cava. Here, it goes through the Tricuspid valve and arrives in the right ventricle. When the heart is compressed, the blood is ejected through the pulmonary artery toward the lungs where it receives oxygen and rids itself of carbon dioxide. The oxygenated blood then returns to the heart via the pulmonary vein and arrive in the left atrium. It passes through the mitral valve to enter the left ventricle where it will be soon ejected via the aorta to continue its journey within the body and to bring nutrient to muscles and other organs before returning to the heart via the veins [14].
During this process, two phases can be distinguished - the systole and the diastole:
- The systole, or contraction, is the part during which a chamber contracts to eject blood.
- The diastole, or expansion, is the part during which a chamber expands to fill with blood.

Both the right and left atrium diastole fill up with blood simultaneously. Then the atrium systole and the ventricular diastole happen, the blood is ejected through the valve to arrive in both ventricles. When the ventricles are full of blood, the ventricular systole contraction pumps the blood outside the heart through the arteries. The process then repeats itself.

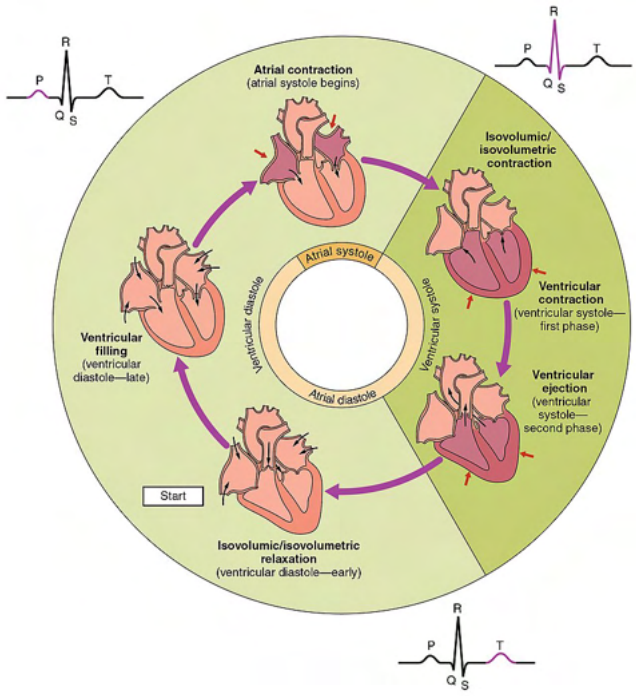


Figure 2.1 Phases of the Cardiac Cycle, [1]

2.1.1 Heart wall

The heart wall is composed of three layers of different thickness, strength, structure, and function: the internal endocardium, the middle myocardium and the external epicardium. These are surrounded by a double-membrane sac called the pericardium [1].

The endocardium is the thin layer lining the inside of the ventricles and is in contact with the blood. Its function is to protect the inner surface of the ventricles. It consists of an endothelium (layer of epithelial cells that lines the inside of the walls of the heart and vessels) facing the lumen of the heart and a subendothelial connective layer, separated by fibrous elements of collagen and elastin.

The epicardium (or visceral serous pericardium) is a thin protective layer largely made up of a mesothelium (biological tissue lining the internal surface of the serous cavities) covering a loose connective tissue rich in elastin. It extends over the surface exterior of the ventricles and covers the myocardium.

Together, these two layers outlined above envelop a third, intermediate layer: The myocardium.

The myocardium is a thick, hollow cardiac muscle, controlled by the autonomic nervous system, surrounded by the endocardium (internal) and the epicardium (external). It is

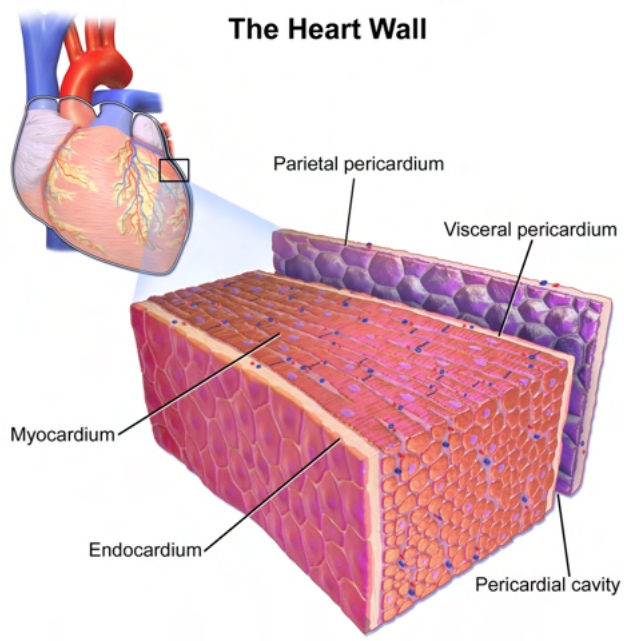


Figure 2.2 Different layers of the human heart wall, [2]

composed largely of specialized cardiac muscle cells: **the Cardiomyocytes**. These cells are tetanizable (incapable of prolonged contraction), excitable (by the autonomic nervous system), endowed with automatism, independence, conductivity (they transmit excitation) and contractility. [15].

The myocardial cells are diversified according to their positions in the heart, the atrial and the myoendocrine cells are close to the atria, and the ventricular cardiomyocytes are placed on the ventricles, ensuring their contractions.

The myocardium binds directly to the two surrounding layers via connections made by mutually shared structural proteins. The extracellular matrix in the myocardium consists mainly of type I and III collagen, mixed with elastin fibers [16].

The last structural layer of the heart is the **pericardial sac**. This is a thin collagen membrane enveloping the ventricles, separated from them by a thin layer of pericardial fluid, facilitating the movements of the heart in the organism [1].

2.1.2 Coronary arteries

The **coronary arteries** are arteries covering the surface of the heart, making it possible to vascularize the myocardium. These arteries come from the aorta and are called "terminal", ie each part of the myocardium is irrigated by a single branch of these arteries. Thus, any

disorder or disease of the coronary arteries has an immediate impact on the myocardium and therefore on the health of the individual, which can lead to heart failure, dysfunction, heart attack and death [1].

2.1.3 Heart Conduction System

The cardiac conduction system is a physiological system by which the myocardium (heart muscle) is stimulated to contract without the need for any external stimulation. The contraction of a cardiomyocyte is triggered by an electrical impulse (the cardiac impulse) which propagates freely through the atrial and ventricular myocardium.

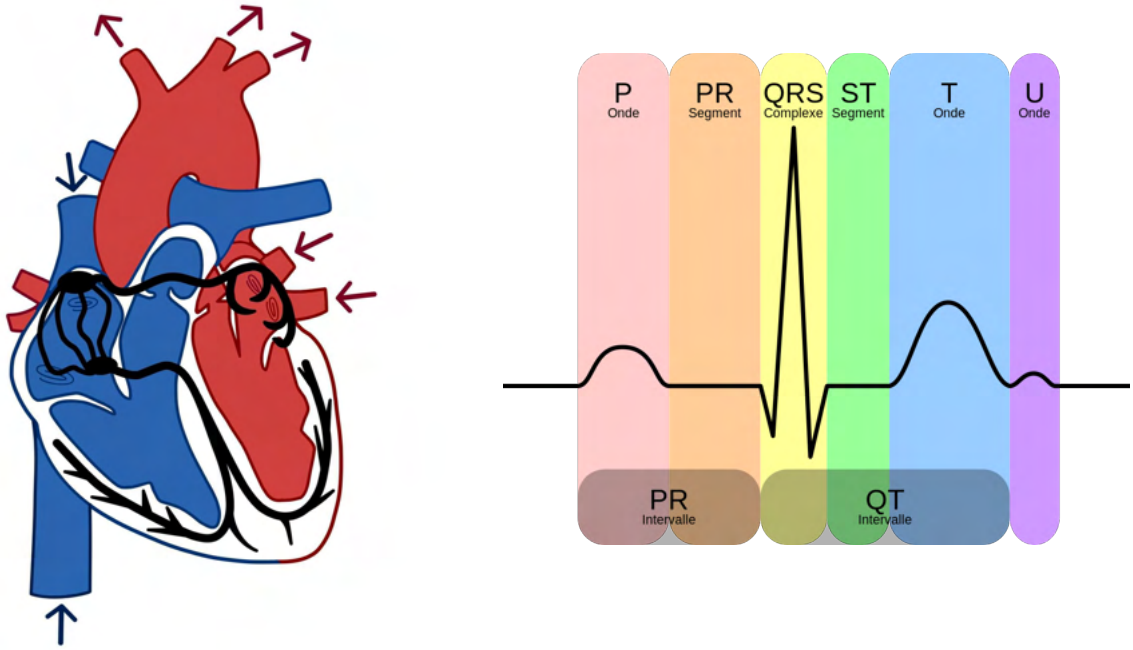


Figure 2.3 Heart Conduction System and ECG of a heart [3]

This phenomenon occurs because cardiomyocytes are electrically coupled via gap junctions. All cardiomyocytes of the heart have the ability to conduct the cardiac impulse; this means that a single stimulation of an atrial or ventricular cardiomyocyte can produce a contraction of the entire myocardium.

During normal activation of the heart, the cardiac impulse originates in the cells of the

sinoatrial node and spreads evenly through the atria. The cardiac impulse is then conducted to the atrioventricular (AV) node, via internodal pathways, where it then propagates along the bundle of His (His Bundle) and throughout the conduction system of the ventricles and ventricular myocardium to cause the heart muscle to contract [17].

The electrical signal from the sinoatrial node first stimulates the right and left atria causing their depolarization and therefore the contraction of the atria (atrial systole) and the filling of the ventricles. This corresponds to the P wave on an electrocardiogram (ECG). The signal then travels from the atria to the myocardial tissue of the ventricles (PQ interval). It then causes depolarization of the right and left ventricles (ventricular systole, QRS wave). Atrial repolarization is hidden by this wave. Then comes the repolarization of the ventricles (ventricular diastole,T wave).

2.2 Cardiac disease and Myocardal Infarction

Coronary diseases represent all the diseases affecting the coronary arteries which supply the heart. It causes myocardial ischemia (insufficient blood supply to the myocardium) leading to myocardial necrosis. Most often, they are due to an obstruction of the heart's arteries by an atheroma plaque (deposit of fat, cholesterol) that will block the blood flow [18]. When a coronary artery is obstructed, the myocardium is no longer vascularized and begins to become necrotic, causing myocardial infarction (MI), or heart attack. The patient will start feeling chest pain, nausea, dizziness, and shortness of breath, leading to an acute myocardial infarction (AMI) also known as heart failure [19].

MI are one of the leading causes of death worldwide, killing 8.9 million people a year. In the United States, they kill more than 360,000 people a year [20]. Cardiovascular disease account for 40 % of deaths worldwide. The American Heart Association estimated that the average annual direct costs of cardiovascular disease in the United States was $216.0 billion in 2016 to 2017, more than double than it was 20 years ago. In total, the average annual direct and indirect cost of cardiovascular disease in the USA was $363.4 billion in 2016 to 2017 [20].

In the adult mammalian heart, damaged cardiomyocytes will be replaced by poorly conductive and non-contractile fibrous scar tissue due to the heart's poor regenerative capacity. This leads to more inflammation, a loss in pumping efficiency and finally causes heart weakness or heart failure [21].

The current treatment to avoid the aggregation of these symptoms are healthy lifestyle changes, drugs to slow down further damage and surgeries [5]. Cardiac surgeries include all surgeries that treat conditions of the heart and large thoracic vessels. After a MI, the first

surgery to perform is an angioplasty to try to unblock the obstructed artery. If the artery can't be cleared, coronary bypass is usually performed to try revascularizing the wounded part and restoring the normal blood flow in the blocked artery. This surgery consists of creating an artery bridge to pass around the blocked coronary and restore an adequate blood flow to vascularize the myocardium [22].

Artificial cardiac pacemakers are used to help the heart maintain a regular contraction and therefore continue pumping blood. In extreme cases, when the heart is too injured, heart transplants are performed [22]. However, available donors are limited and these procedures are complex, dangerous and do not offer real long-term solutions. The patients must take immunosuppressants for the rest of their life to avoid rejection, making the patient more vulnerable to infections [23, 20].

2.3 Cardiac tissue engineering and Cardiac patches

Tissue engineering is a discipline that seeks to use biomaterial, cells, and drugs to repair, replace, or regenerate natural tissues. It's a multi-discipline field that combines, material science, chemistry, and biology to learn more about the interactions between biological tissue and biomaterial, in order to develop new techniques and materials to repair or improve the human body.

Preclinical studies have demonstrated the therapeutic performance of several approaches to treat MI, such as direct injection into the myocardium of stem cells, genes, and growth factors. However, these therapeutic agents still suffer from poor stability and short half-lives. Therefore, the methods and medium used for the administration of these agents are very important to achieve better therapeutic performance. Cardiac patches show promising potential in repairing cardiac function as they provide a suitable environment to better deliver these therapeutic agents [7].

Cardiac patches applied directly to the heart offer a scaffold for cardiomyocyte regeneration which shows encouraging potential in promoting cardiac function to help a heart that lost its contractile and conductive properties. [7, 24]. They promote myocardium regeneration by boosting electrical stimulation in the damaged part of the heart.

Creating a heart patch depends on three main requirements:
(a) a physiologically accurate scaffold microstructure,
(b) a mechanical and conductive structure that supports the dynamics of a beating heart,
(c) appropriate biocompatibility

Further, two categories can be made among cardiac patches to treat myocardial infarction,

one using natural material and the other using synthesized material.

2.3.1 Cells patches

Cardiac patches based on cells have been developed with the use of cardiac stem/stromal cells, mesenchymal stem cells (MSCs) and human pluripotent stem cells. These cells notably release paracrine signals (such as growth factors) which improves cardiac repair and some of them can differentiate to form a new cardiac wall [7]. Other natural materials such as collagen, fibrin, alginate, hyaluronic acid, gelatin, and decellularized extracellular matrix (ECM) shows biocompatibility and are used for manufacturing heart patches. The main issue with this kind of cell patch, like heart transplants, is the immune response of the host body against the foreign cells. A possible solution to avoid this response is to encapsulate these cells in gels, nanogels or other synthetic materials. Another limitation of the use of stem cells alone in heart disease is the low retention rate of cell survival. Therefore, the use of biomaterials seems essential to improve the efficiency of cells based cardiac patches by offering a scaffold for cell regeneration.

Beyond cells based cardiac patches, biological active substances can be added or encapsulated in cardiac patches to enhance cellular integration such as growth factors. The combined use of patches and growth factors makes it possible to protect them from rapid degradation and improve their local applications. Extracellular vesicles from stem cells or microRNAs can also be used, although more detailed studies are needed to better understand how they work.

2.3.2 How cardiac patches work

Heart patches are designed to be attached to the surface of the heart; more specifically, they are intended to "patch" the dying regions of the heart after an MI. To create a therapeutic scaffold that will support cardiac tissue regeneration, the structure and characteristics of healthy native myocardium must be mimicked as closely as possible, in terms of biocompatibility, conductivity, porosity, surface area, and other mechanical properties. The patch must also integrate into the surrounding environment as well as possible, thus ensuring its integration into the native tissue.

One of the many challenges when designing heart patches is considering the important structural components needed to make a material that will fit into the complex anatomy of the heart. The success of these patches is directly linked to their integration into the host tissue and their ability to guide tissue development and maintain biological function. Synthetic polymers are known for their biocompatibility, durability, porosity, and microstructure that

is tailored to meet the specifications of natural heart tissues. Many recently developped cardiac patches incorporate a combination of polymers to achieve these properties. In addition to other considerations, implant success also depends on the signaling pathways that must be activated to bring out the appropriate inflammatory responses, inhibit tissue breakdown, and reduce unwanted remodeling that leads to scar formation. There are currently no conductive synthetic polymer heart patches on the market.

2.3.3 Synthetic support

Due to the unstable bioactivity of cells or biological molecules, a synthetic scaffold is often necessary to provide both a suitable cellular microenvironment and a good mechanical support. The key points to consider when selecting suitable materials are the biocompatibility, biodegradability (if required), stability over time and mechanical strength of these materials. Conductivity is also a property useful regarding cardiac patches [24].

Synthetic biomaterials are already used in cardiac surgeries for the treatment of ventricular septal defect. This congenital disease is a heart defect corresponding to the presence of an orifice in the interventricular septum, allowing the direct passage of blood from one ventricle to the other. The surgery performed consists of closing the communication with a cardiac patch. Several patch materials are available: native pericardium, bovine pericardium, Polytetrafluoroethylene (PTFE) (Gore-Tex or Impra) [25] or Dacron (polyethylene terephthalate) [26]. However, these materials are not conductive and are not intended to replace a conductive part of the heart.

Among the synthetic materials being investigated for heart patches, synthetic polymeric materials have the advantage of being adaptable at the molecular level to meet all the requirements that would need to be met to function as an integral part of a beating heart. Many synthetic polymers for tissue engineering have been investigated, such as poly(vinyl alcohol) (PVA) polymer, Poly(glycerol sebacate), poly(lacticco-glycolic) acid (PLGA), poly-(L-lactic acid) (PLLA), Poly(ethylene glycol), Chitosan, polyurethanes (PU) and Poly(3,4-ethylenedioxythiophene) (PEDOT), polyaniline (PANI) [28, 79, 27, 8, 101]. These materials offer the possibility of creating a link between the added cells, growth factors or drugs and the host myocardium thanks to their strong mechanical properties. Surface modification of these materials can greatly reduce poor tissue adhesion, enhancing their biocompatibility. Another advantage of polymers is their low cost, ease of production and ease of modification.

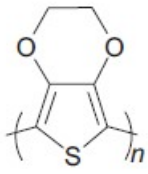
2.3.4 Conductive heart patches

The first use of conducting polymers in the medical fields was in the mid-1970s, they were chosen due to their ability to conduct electricity like metal while also presenting other interesting properties such as their flexibility, durability, and ease of production [27]. Since then, conductive polymers or other conductive biomaterials have been shown to help promote the maturation of Cardiomyocytes [8].

Since the myocardium conducts electricity, an electroconductive biomaterial could be useful to enhance electrical signal propagation among the damaged fibrotic tissue that can no longer be conductive. That is why, in the past decade, conductive polymers have been privileged materials to be studied to try to cure the injured heart as the conductivity quality of a material is now a property added to the list of requirements to make proper cardiac patches [28].

Among the materials used to make patches conductive are gold nanoparticles, carbon-based nanomaterials, metal nanomaterial, silicon-derived nanomaterials, and electroconductive polymers [28] [8]. To daten, the most used polymers to make conductive patches have been Polyaniline (PANI), Polypyrrole (PPy), polythiophene (PT), and poly(3,4-ethylene dioxythiophene) polystyrene sulfonate (PEDOT:PSS) [28, 8, 27].

2.4 Poly (3,4-ethylenedioxythiophene) (PEDOT)



Poly(3,4-ethylene dioxythiophene) (PEDOT)

Figure 2.4 Poly (3,4-ethylenedioxythiophene)

Poly (3,4-ethylenedioxythiophene) (PEDOT) is a conductive polymer from the polythiophenes family based on 3,4-ethylenedioxythiophene (EDOT). It was first developed in 1989 by the Bayer AG laboratory, in Germany [29, 30]. It can be prepared by chemical polymerization or by electrochemical polymerization.

PEDOT is insoluble but has very interesting properties. Subjected to very high conductivity

(about 300 S/cm), PEDOT was found to be almost transparent in the form of oxidized thin films and showed very high stability in the oxidized state. The solubility problem can be circumvented by using a water-soluble polyelectrolyte, poly(styrene sulfonic acid) (PSS), as a charge-balancing dopant during the polymerization to yield PEDOT:PSS. This combination resulted in a water-soluble polyelectrolyte system with good film-forming properties, high conductivity (about 10 S/cm), and excellent stability. PEDOT:PSS is biocompatible and permeable to ions [31]. PEDOT film surface properties are easy to modulate such as conductivity, biocompatibility or mechanical properties [27].

PEDOT and PEDOT:PSS have great potential as a regenerative material toward the heart due to their biocompatibility, conductivity and their successful application that has led to the development of a neurotransmitter delivery system consisting of electronic ion pumps in animal models. PEDOT is also used to synthesize organic electrochemical transistors that can be applied to biosensing [32]. PEDOT:PSS shows encouraging properties for the field of tissue engineering [8, 33, 66, 81].

2.5 Use of PEDOT and PEDOT:PSS in the biomedical fields

The application of PEDOT:PSS in the biomedical fields starts in 1995 where it was first studied for biosensor application. PEDOT:PSS shows a greater conductivity (1 to 100 S cm-1), better thermal stability [33] and is 55 times more stable under constant potential in phosphate buffer solutions than polypyrrole doped with PSS (PPy:PSS), another similar conductive polymer. [34]. PEDOT:PSS is one of the few organic conducting polymers to have a very vast range of use due to its properties. It is used for printed circuit boards, an environmentally friendly antistatic treatment, for biosensors and other biomedical applications [35]. For the past two decades, PEDOT:PSS PEDOT has been used for bioengineering applications and biosensing [36]. Studies show the biocompatibility, the non cytotoxicity and the electrocompatibility of PEDOT with epithelial cells Hep-2 [37], the biocompatibility and low cytotoxicity on NIH3T3 fibroblasts [38], the biocompatibility on L929 murine fibroblasts [39], the biocompatibility and electrocompatibility on neuronal cells [40], biocompatibility and no cytotoxicity on L929 fibroblast and human neuroblastoma SH-SY5Y cell lines [41] and the biocompatibility and enhancement of PC-12 cell differentiation with Nerve Growth Factor modified PEDOT [42].

PEDOT is used for peripheral nerve repair, for nerf grafts and for application in skeletal muscle, smooth muscle and heart muscle [33]. PEDOT have been used in the biomedical fields for drug delivery, tissue engineering applications and especially for neural applications, and cardiovascular applications [9]. PEDOT:PSS is also used to make electrically conduc-

tive bioceramic scaffolds for bone tissue engineering. These scaffolds are biocompatible and can support hMSC growth and could enhance bone healing. [43]. PEDOT:PSS is used for electronic skin and artificial muscles [44] and for epidermal patch electrodes. These skin hydrogels are conductive, stretchable, self-healing and highly adhesive on pig skin [45]. Among its applications, PEDOT is also used to coat implantable electrodes to enhance their conductivity and biocompatibility [41]. PEDOT:PSS is used to coat neural and electromyography electrodes for invasive stimulation and recording. The coating improves biocompatibility and enhances the electrochemical property of these implants [46].

2.6 Use of PEDOT:PSS in the cardiac field

Currently there is plenty of research done on the use of PEDOT for neural implants, however there is less research done for cardiovascular application seeing as the first study that attempted to examine the role of PEDOT for cardiovascular implants dates from 2013 [39]. The study focuses on the ability of poly(3,4-ethylenedioxythiophene) (PEDOT) thin films to promote cell adhesion and proliferation. It is found that PEDOT dopped with PSS and TOS are cytocompatible with L929 murine fibroblasts. These films both promote human serum albumin adsorption, showing their biocompatibility with blood. PEDOT:PSS is used with a chitosan scaffolds to create a biocompatible material that show no cytotoxicity and that can be used to create a cardiac patch [47]. Biocompatibility of PEDOT:PSS can be improved by a collagen IV coating to create a scaffold that can support cultures of healthy spontaneous beating cardiomyocytes [48]. PEDOT:PSS organic electrochemical transistor sensors are made, with performance close to established sensors, to measure the electrophysiological activity (action potential) of the cardiac cell line HL-1 [49], table 2.1.

2.7 PVA and PEDOT:PSS Hydrogel

Hydrogels are polymer based multidimensional structure that contain between 60% and 99% water (similar to natural tissues) [50]. They are used in the biomedical field because they mimic extracellular matrix, thus supporting cell adhesion and growth and they are also cheap, easy to pattern and easy to tune or functionalize [51]. Compared to thin films, hydrogels are considered more ideal interfacing alternatives to biological tissues due to their water-rich nature and tissue-like mechanical properties. Hydrogels have many advantages over simple polymer film, as they can support cell adhesion by providing a suitable microenvironment for cell growth and differentiation. Hydrogels can also be easily patterned, provide mechanical support for cardiac cells to deposit extracellular matrix (ECM), form a newly synthesized

tissue, and they are easy to tune. Hydrogels have swelling properties and are made to work in a wet environment such as inside a body. Hydrogels also show desirable properties for cardiac patch application like extensibility, conductivity, and stability.

Hydrogels made with conductive polymers conduct electricity and can be used as a scaffold for neural tissues, skeletal muscle tissues and cardiac tissue. In tissue engineering, conductive hydrogels are essential due to their great interface with natural tissues because of their 3D microstructure and they help the propagation of ions because of their aqueous structure [51]. They are soft and bendable material and many can be molded in any shape. Some of them have self-healing properties and self-adhesion properties [52] [45]. Hydrogels and conductive hydrogels can be shaped and made to match natural tissues such as skin or cardiac muscle [45], although few can mimic the dynamics of natural tissues yet [53].

Some conductive hydrogels use carbon, graphene or metal as conducting materials, but their stability and biocompatibility is limited. Conductible polymers like Polypyrrole, Polyaniline and PEDOT are preferable because they are more biocompatible, stable, cheaper, and easier to tune. The use of PEDOT:PSS hydrogels are promising to be used in energy storage, bioelectronics and regenerative medicine. They can be patterned by being shaped with a mold or using electrochemical gelation [54].

Poly(vinyl alcohol) PVA have been used to make hydrogel for biomedical application since the early 1970s [55] and are commonly used for biomedical applications today [45] thanks to its mechanical properties, good biocompatibility and low cost. To give PVA hydrogels more interesting properties, other polymers such as conductive polymers are mixed with them, including PEDOT:PSS. The first use of PEDOT:PSS in a PVA hydrogel date from 2017 with the making of an antifreezing conductive hydrogel prepared by mixing PVA and PEDOT: PSS with ethylene glycol [56]. PVA and PEDOT:PSS can be used together in a sandwich structure of PVA–PEDOT:PSS/ acid–PEDOT:PSS/PDMS to make a type of sensors that show a high resistance to stretching [57], to make stretchable and conductible hydrogels for bio-related electronic device such as supercapacitor [58] or to make hydrogel with good flexibility, stretchability and conductivity for wearable electronics and sensitive strain sensors [59]. However, this last method uses hydrochloric acid, and its biocompatibility hasn't been tested on biological tissues. These hydrogels like many are made with the addition of substances that can cause cytotoxicity problems (sulphuric acid, metallic nanoparticles), hence the interest of having a method for making pure PEDOT:PSS hydrogels.

Other hydrogels have been studied in the field of cardiac tissue engineering to patch heart failure. Biocompatible hydrogel, made of collagen, alginate and PEDOT:PSS, presents a fibrous microstructure close to extra cellular matrix [60]. Furthermore, this hydrogel stimulates the

beating rate of neonatal rat cardiomyocyte but its physical properties haven't been studied. Lu et al., 2019 [61] propose a simple method to make a pure PEDOT:PSS hydrogel that consists of mixing a volatile additive, dimethyl sulfoxide (DMSO) in a solution of PEDOT:PSS followed by controlled dry cooking and rehydration, but no study of biocompatibility have been done yet. PEDOT:PSS hydrogels do seems very promising and continue to be studied more and more over the years [62], table 2.1.

2.7.1 PEDOT:PSS based materials used in the cardiac field

Table 2.1 PEDOT:PSS used in the cardiac field

Material	Properties	Limitation
CS/PVA/PEDOT:PSS [47] (2019)	Conductivity : 215-912 $\mu S/cm$, Elongation at break : 5.3-6.4 %, biocompatible on rat bone marrow mesenchymal stem	Low elongation at break, not tested on cardiac cells
PEDOT:PSS [48] (2018)	Wetting angle : 60°	Viability of Mouse 3T3 fibroblasts is low on PEDOT:PSS, produced leachates
PEDOT:PSS sensor [49] (2017)	PEDOT:PSS can be used to create a sensor to record cardiac HL-1 cell	
collagen/alginate/ PEDOT:PSS (Hydrogel) [60] (2018)	Conductivity : $< 10^{-3} S/cm$, Swelling ratio : 20, cytocompatible on neonatal rat cardiomyocytes	No mechanical properties have been tested yet
PEDOT:PSS/PVA (Hydrogel) [61] (2019)	Conductivity : 40 S/cm, Young Modulus : 2Mpa, Elongation at break : >35 %	No biocompatibility have been tested yet

2.8 Biointegration

Biocompatibility has been defined by David F. Williams in 1999 by "The ability of a material to perform with an appropriate host response in a specific application" [63]. Biocompatibility and biointegration of a synthetic polymer are crucial for its use in contact with natural tissues. Its surface properties must be adequate to allow a good integration of the biomaterial with the body. The main issue with biomaterials is the immune response from the body leading to

the rejection of the foreign material. Every biomaterial in contact with the inside of a body will experience the foreign body reaction. Protein will attach to it; macrophage will respond to it. The goal of every biomaterial is to minimize inflammation and maximize surrounding cells integration. In the case of a cardiac patch, the cells in contact with the biomaterial will be cardiomyocytes.

Hydrogels have a very hydrophilic nature that make it difficult to express biocompatibility properties such as adhesion, viability, and growth. Among the methods used to enhance synthetic biomaterials biocompatibility, functionalizing has been reported to be useful [64]. According to the Oxford reference, polymer functionalization refers to "The introduction of chemical groups into a polymer to exert specific chemical, physical, biological, pharmacological, or other functions. See functional polymer." [65]. Quite often, in the biomedical fields, polymer functionalization refers to attaching proteins to a polymer to enhance its biocompatibility.

Functionalization of polymers can be done in many different way, by modifying the monomer itself before the polymerization, by adding molecules in the polymer solution or by functionalizing the polymer film [66].

2.8.1 RGD and protein

Many studies have been made on the functionalization of polymers film or polymer-based hydrogel with RGD peptides (arginylglycylaspartic acid). RGD is used because it's a mimic of the most common peptide motif responsible for cell-surface adhesion throughout the organism [67]. A polymer functionalized with RGD peptide will allow cell anchoring. PEDOT:PSS have been modified with RGD [68] to improve the biocompatibility and hydrophilicity of the polymer film. Among other proteins, cadherin mimic peptides and especially E-cadherin and N-cadherin mimics are used to improve interface of biomaterials with stem cells, neurons, and cardiomyocytes [69, 52, 70].

2.8.2 N-cadherin

N-cadherin (Cadherin-2 or CDH2) named after "neural cadherin" is a transmembrane protein that play a role in conductive cells such as neurone or cardiac cells. In the heart, found in the intercalated disc, the role of the N-cadherin is critical to maintain mechanical and electrical coupling adhesion between cardiomyocytes [70]. N-cadherin is necessary for the cardiovascular development and is the main protein responsible for adhesion in the cardiac muscle, the protein is crucial for the anatomical integrity of the adult mouse myocardium. Loss of

N-cadherin induce arrhythmias and loss of conductivity in the heart [70]. Functionalization of a cardiac patch with N-cadherin is therefore believed to enhance conductivity in the heart and biocompatibility with the foreign biomaterial. Functionalization of PEDOT:PSS with N-Cadherin have been reported improving the performance of carbon microfibers neuron electrodes coated with PEDOT:PSS-co-MA [83]. A method used to functionalize polymer and particularly PVA is the Steglich esterification.

2.8.3 Steglich esterification

Esterification is a chemical reaction in which an alcohol (R-OH) and a carboxylic acid (R-COOH) form an ester (R-COO-R) as a final product.

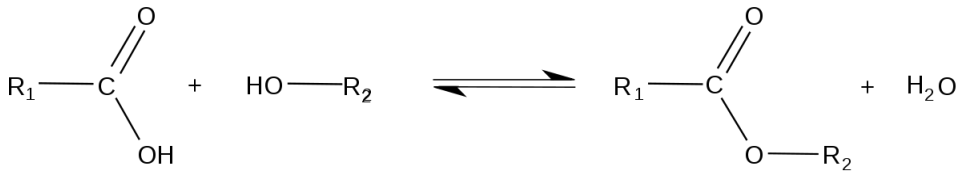


Figure 2.5 Esterification, (Pinpin, 2007)

The Steglich esterification was invented in 1978 by the Dr. Wolfgang Steglich [71] and use 4-dimethylaminopyridine (DMAP) as a catalyst and dicyclohexylcarbo-diimide (DCC) as a coupling reagent. The reaction take place at room temperature.

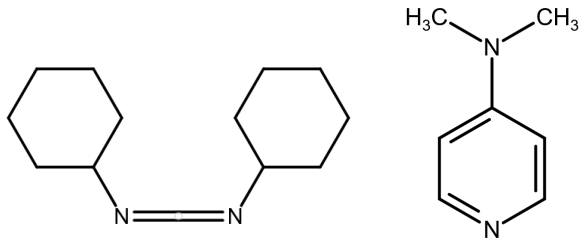


Figure 2.6 dicyclohexylcarbo-diimide (DCC) and 4-dimethylaminopyridine (DMAP)

PVA hydrogels are submerged in Dimethylformamide (DMF) at 37C for 72h, then a poly(amic acid) solution is added with N,N'-Dicyclohexylcarbodiimide (DCC) and 4-diméthylaminopyridine (DMAP). The hydrogels are mixed in the solution for 48h at 37C under a nitrogen blanket then rinsed with water and PBS. Primary porcine vascular ECs cultivated on the hydrogels

show that the functionalization enhance biocompatibility without modifying the hydrogels physical properties [64].

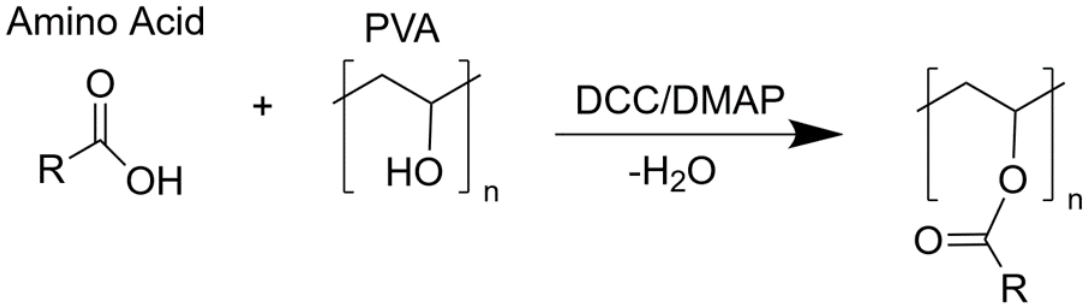


Figure 2.7 Steglich esterification of PVA with Amino Acid with DCC and DMAP.

2.9 AntiBacterial properties

PEDOT:PSS shows interesting properties for antibacterial applications against gram-negative and gram-positive bacteria [10]. PEDOT:PSS mixed with others polymers can exhibit antibacterial against E.Coli and A.Sareus. A fibroblast biocompatible hydrogel based on agarose and PEDOT:PSS shows antibacterial behaviour against these bacteria under near-infrared light [84]. Resistant bacteria becoming a dangerous threat due to antibiotic resistance, the antibacterial properties of conductive polymer PEDOT:PSS could be useful for developing new bendable electronics devices used in a sterile room.

2.10 Summary

The heart muscle also know as the myocardium is made of cardiomyocytes, excitable and contractile cells. By transferring electrical impulses from cell to cell, they are responsible for the beating of the heart. Cardiomyocytes are vascularized by the coronary which are terminal arteries. When a coronary is blocked, the blood flow can't bring oxygen to the heart muscle which begin to necrotize and die, causing a heart attack. If the patient survives, the damaged muscle will be replaced by poorly conductive and stretchable tissue, causing heart failure, a loss of quality of life and a loss of life expectancy. The only current solution to treat such heart damage is heart transplantation, with all the scarcity and rejection it implies. That's why the use of a cardiac patch to create scaffold for cell regeneration and to promote electric conductivity is currently under study. Conductive synthetic polymers offer many properties suitable to the making of cardiac patches such as high conductivity,

flexibility, durability, stability, ease of production and modification. Among them, PEDOT and its derivative PEDOT:PSS is the conductive polymer that show the most potential. This polymer has been studied for bioengineering applications and biosensing and shows antibacterial properties. Versatile, it is used mainly for its application on the neural field, but it has shown biocompatibility among cardiac cells and is now studied for its application in the cardiac field. Hydrogels are 3D hydrated polymeric material that contain between 60% and 99% of water. They share many similarities with extracellular matrix and can easily support cell adhesion. Hydrogel made of polymer are cheap, easily tunable, soft, flexible and, because of their water content, they have tissue-like mechanical properties. Hydrogels made with conductive polymer like PEDOT:PSS make biocompatible biomaterials that can mimic closely the heart muscle and it's properties regarding conductivity, stretchability, and offer a scaffold for cardiomyocyte regeneration. Furthermore, functionalization of the hydrogel surface with peptide that mimic the transmembrane protein that allow mechanical and electrical coupling between cardiomyocytes, the N-cadherin, can boost its biocompatibility, and help regenerate the damage myocardium.

CHAPTER 3 Methodology

3.1 Fabrication of PEDOT:PSS/PVA based hydrogel

The preparation of the conductive hydrogel is based on the preparation of pure PEDOT:PSS hydrogel by lu et al [61]. Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS, PH1000) aqueous solution is poured in a beaker with DMSO and mixed on a magnetic stirrer for 20h at 400rmp at room temperature, covered. In the same time, a solution of Polyvinhyle alcool(PVA, Mw 89,000-98,000, 99+% hydrolyzed) pellets dissolved in 5mL water are mixed in a covered beaker on a magnetic stirrer heated at 95°C at 400rmp. 4h before the casting, both solutions are blended together and mixed on the magnetic stirrer at room temperature. The final solution is solvent-casted on a substrate of Polypropylene (PP) previously washed with acetone, isopropanol, water and dried. The solution is casted on the substrate directly in the oven to avoid dripping. The substrate is a simple borderless piece of Polypropylene, the solution is staying on its surface because of surface tension. The casted solution is dried-annealed for 24h at 60°C (fig 3.2). After the drying process, the result is in the form of a thin black film on the substrate (fig 3.1). The look of the film depend on the concentration of PVA in the mixed. Hydrogels with no or few (<2%) PVA will be flat films the size of the substrate. Hydrogels with small concentration (between 2% and 4%) of PVA will be more shrinked and present curvatures but will flattened when submerged in water. Hydrogels with higher (>4%) concentration of PVA will be very shrinked and they will conserve that form even when submerged in water. The films are easy to peel from the substrate for all concentration of PVA.

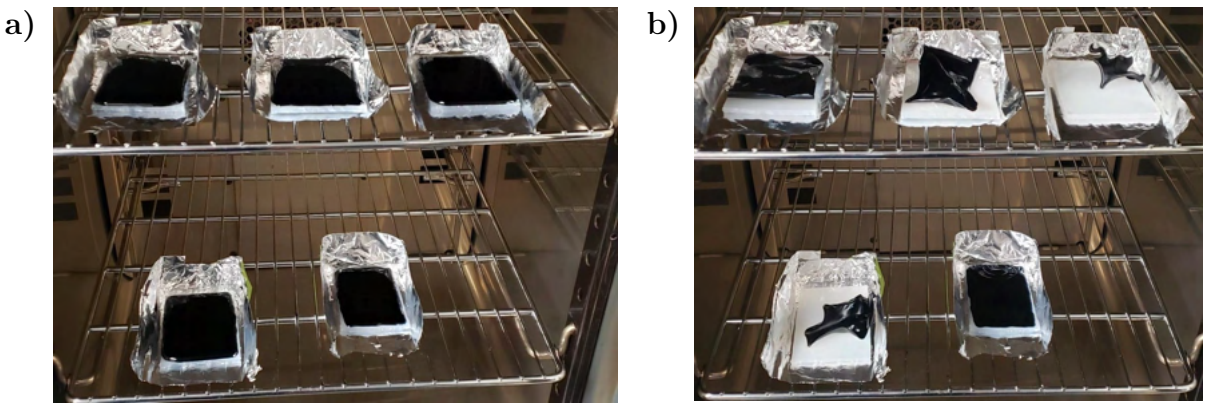


Figure 3.1 PEDOT:PSS and PVA based hydrogel before (a) and after (b) the drying process in the oven

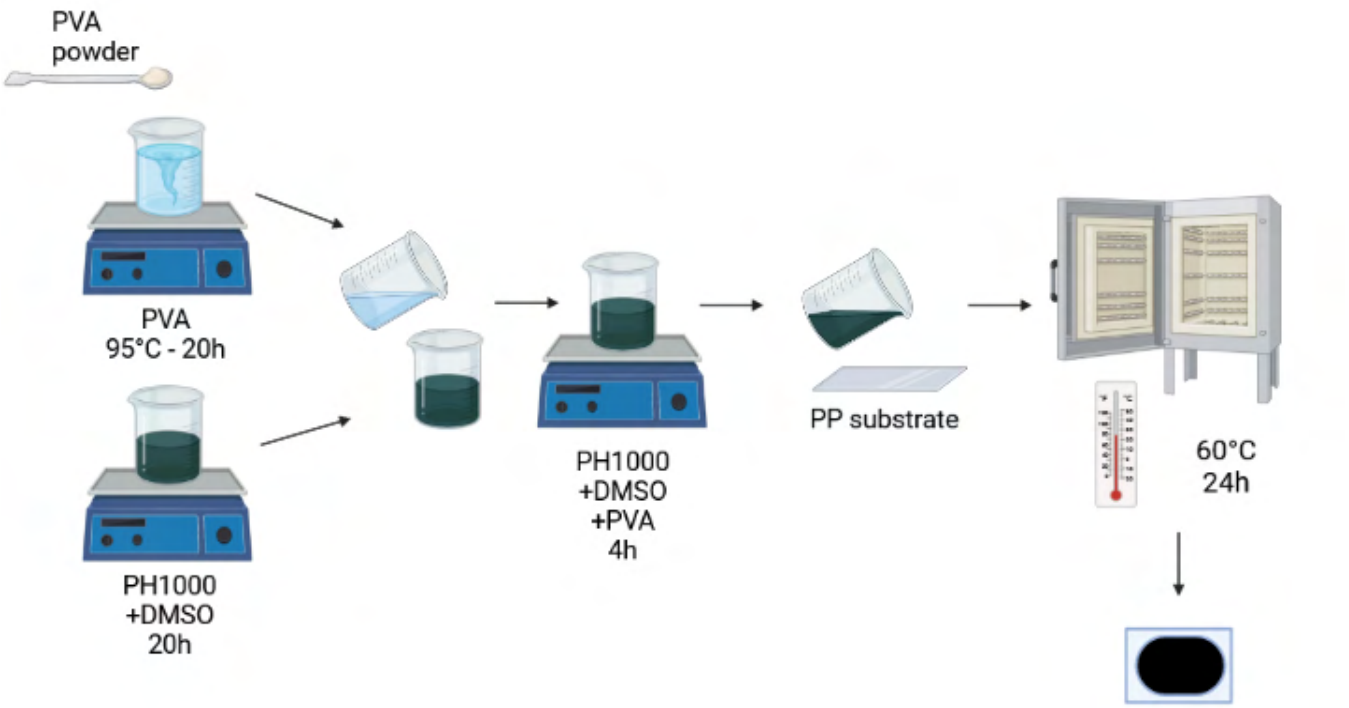


Figure 3.2 Fabrication of PEDOT:PSS/PVA based hydrogel

3.2 Characterization

Mechanical and conductivity tests were made using the Mechanical Tester Model Mach-1 from Biomomentum Inc.

3.2.1 Four Point Probe

A four point probe (or Four-terminal sensing) is a device for measuring the resistivity and/or conductivity of a sample that use two pairs of electrodes. It is more accurate than a two points probes (simple ohmmeter). In this device, current and voltage electrodes are separated, eliminating the contact resistance to interfere with the measure.

The current is generated by a current generator between electrodes 1 and 4 and the voltage is measured between electrodes 2 and 3 (fig 3.3).

The ratio of the measured voltage to the generated current going through the sample gives

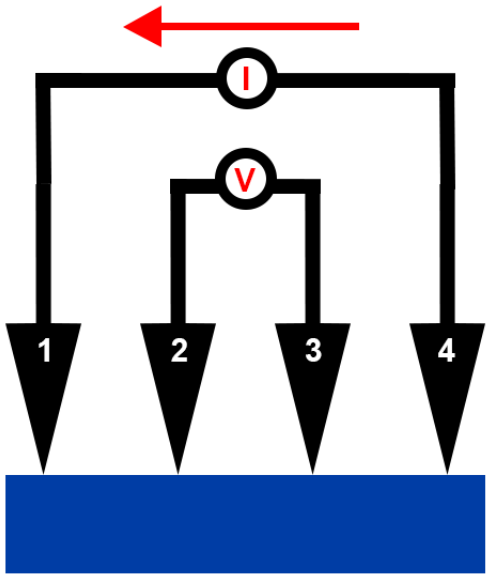


Figure 3.3 Schematic of a four-point probe. Resistance is measured between electrodes 2 and 3 (sense). Current is supplied via electrodes 1 and 4 (force).

the resistance of the section between electrodes 2 and 3 (Eq. 3.1).

$$R = \frac{U}{I} \tag{3.1}$$

Where R is resistance (Ohm, Ω), U is the voltage (Volt, V) and I in the current (Ampere, A)

From the resistance, resistivity can be calculated if the dimension (length and cross-sectional area) of the sample are known, using Eq. 3.2:

$$\rho = R\frac{S}{L} \tag{3.2}$$

Where ρ is the resistivity Ωm, L is length of the of the sample (m) and S is the cross-sectional area of the sample. Conductivity (S/m) is then defined from Eq. 3.3:

$$\sigma_c = \frac{1}{\rho} = \frac{L}{S \times R} \tag{3.3}$$

Resistivity and conductivity measurement were made by using a tailored tensile grip and four point probe made by Biomomentum Inc. This device is made of two separated part allowing them to move freely. Each part have two rows of 5 pins-electrodes, one row is allowing the generated current to flow in the sample and the other one (closest to the edge) is measuring the voltage (fig 3.4). The free-standing hydrogel is placed on the electrodes to make contact, then to secure the sample, a plaque of plexiglas is screwed into the equipment, applying pressure on the hydrogel (fig 3.5). This mechanism secures and clamps the sample, allowing it to be stretched and to measure its resistance and conductivity during its elongation (fig 3.6). The versatility of this device allows to measure the conductivity, the young modulus and the resilience of the hydrogel during cyclic stretching.

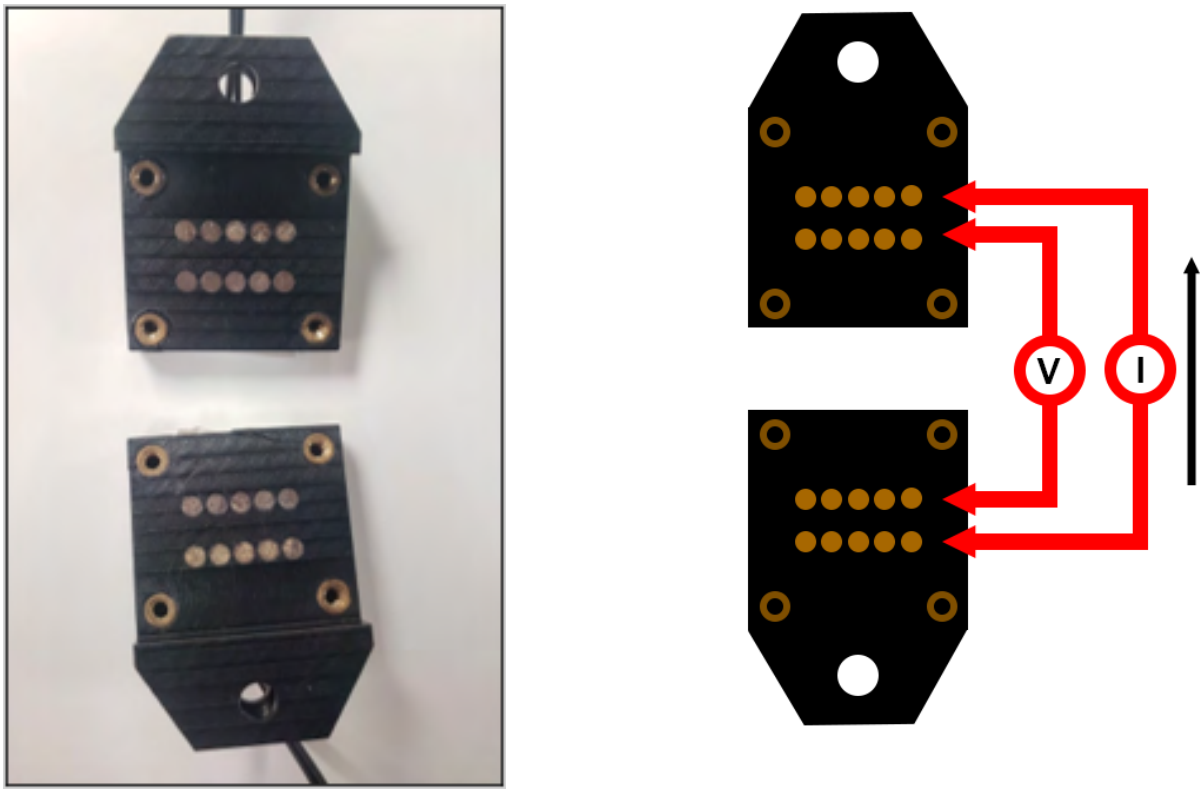


Figure 3.4 Tensile grip and four point probe made by Biomomentum Inc and its schematic

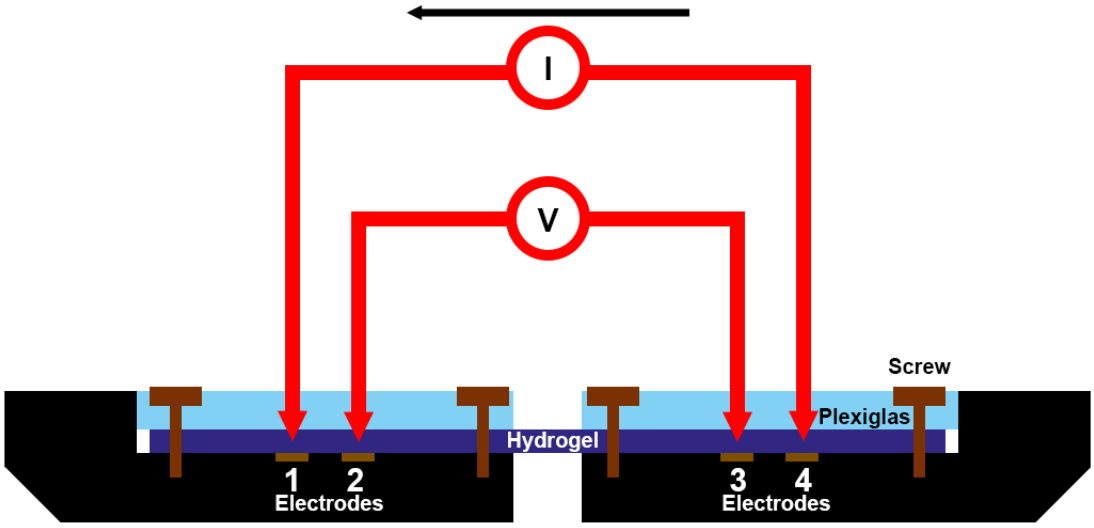


Figure 3.5 Profile schematic of the tensile grip and four point probe made by Biomomentum Inc. Resistance is measured between electrodes 2 and 3. Current is supplied via electrodes 1 and 4. A plaque of Plexiglas keep the sample in place

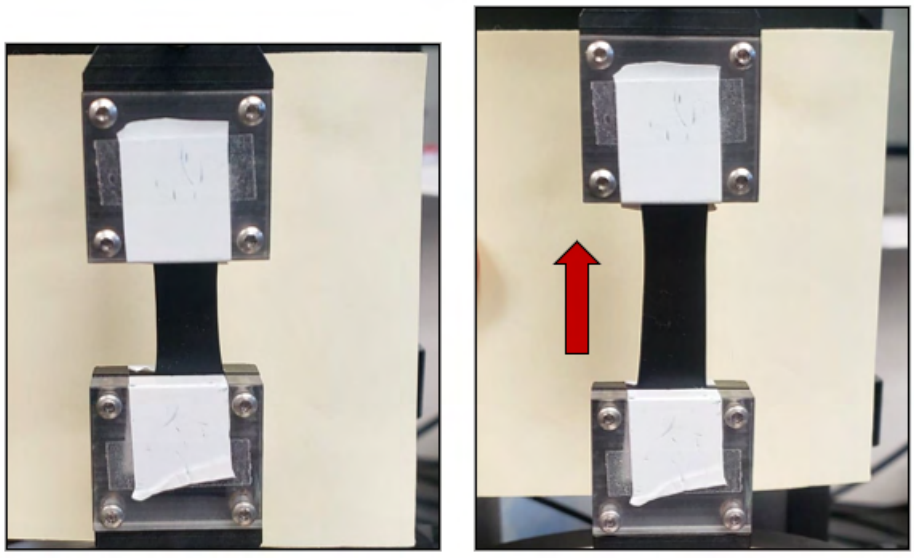


Figure 3.6 Stretching of the hydrogel sample with the tensile grip with electrodes. Resistance can be measured directly during the stretching

3.2.2 Young modulus

The Young modulus is defined as the constant that quantify the relationship between stress and strain in the linear elastic region during a stretch. According to the Hook law (Eq. 3.4):

$$\sigma = E\epsilon \tag{3.4}$$

Where E is the Young modulus (Pa), σ is the stress (Pa) and ϵ is the strain (adimensional) The Stress σ is defined from Eq. 3.5:

$$\sigma = \frac{F}{A} \tag{3.5}$$

Where F is the force applied to the material and A is its cross sectional area. The strain ϵ is defined from Eq. 3.6:

$$\epsilon = \frac{\Delta L}{L} \tag{3.6}$$

Where ΔL is the change in length during the stretching and L is the original length of the sample. During a stretching the machine measure the Force F and the change in length ΔL, thus, the young modulus of a material can be found experimentally using the Eq. 3.7:

$$E = \frac{F}{\Delta L} \times \frac{L}{A} \tag{3.7}$$

3.2.3 Elongation at break

The elongation at break is the elongation ϵ used in percentage before the material break when subjected to tensile stress. The more important it is, the more the material can stretch before breaking.

3.2.4 Scanning electron microscopy (SEM)

Scanning Electron Microscopy is a microscopy technique that use a beam of electrons to create of high-resolution images of a sample. The SEM use the beam of electrons to scan the surface of the sample which, in return, re-emits specific particles carrying information about the topography of the surface, that allow to construct a 3D image of the object, placed in a vacuum. This technique allows a fine observation of the details on the sample that couldn't be seen with an optical microscope because of the limitation of the light wave length [72].

3.2.5 Esterification

The Steglich esterification is a method to functionalize (to attach a functional group) polymer. Here, the reaction aims to join the ester of the amino acid to the PVA hydroxyl group 3.7. The reaction is made in N,N-diméthylformamide (DMF) at room temperature.

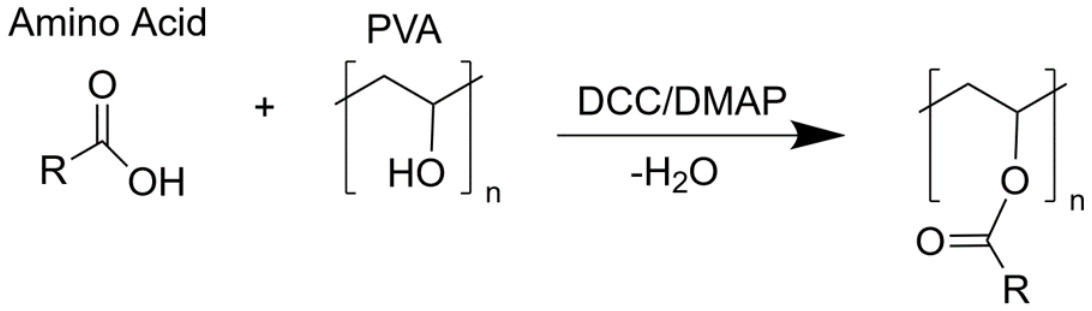


Figure 3.7 Steglich esterification of PVA with Amino Acid with DCC and DMAP

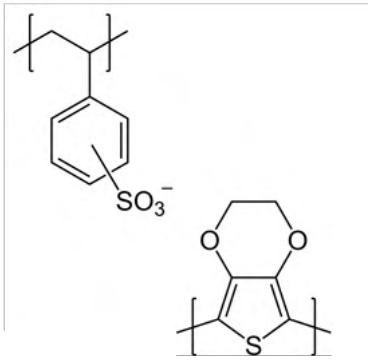
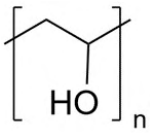
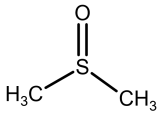
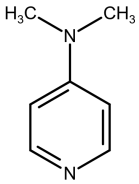
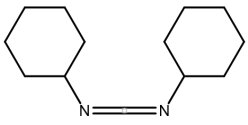
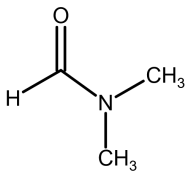
3.2.6 Bacterial Culture

A culture (2–3 ml) of bacteria (Staphylococcus aureus) is prepared in nutrient-rich media (Lysogeny broth) in a tube and placed in a shaker at 37 °C for 20 h. OD600 (optical density of the sample measured at a wavelength of 600 nm) of the tube is measured using a spectrophotometer. The bacterial suspension is then diluted to a final OD600 of 0.01. The samples (circle of 5mm diameter) are disposed in a 48 wells microplate and 200 μl of OD = 0.01 (10^5 CFU/mL) is added on each well. The microplate is incubated in a shaker incubator (200 rpm) at 37 °C for 24 h.

In the microplate, line A to C include hydrogel sample, line D includes styrofoam (same shape as the hydrogel) and line E include bacteria only. Each well is made in triplicate.

3.2.7 Chemicals

Table 3.1 Chemical structure of used materials

Material	Chemical structure	References
PEDOT:PSS		[44]
PVA		[73]
DMSO		[74]
DMAP		[71]
DCC		[71]
DMF		[75]

CHAPTER 4 Conducting polymer based flexible and conductible heart patch

4.1 Introduction

MYOCARDIAL infarctions (MI) – or heart attacks – are among the leading causes of death worldwide, killing around 8.9 million people each year [20]. MI are mainly caused by obstructions of the coronary arteries that surround the heart causing loss of oxygen perfusion and tissue necrosis. After a MI, damaged cardiomyocytes are replaced by non-contractile and poorly conductive scar tissue due to heart's poor regenerative capacity [21]. In return the poor contractility and conductivity of the myocardium lead to more inflammation, a loss in pumping efficiency and subsequently causing heart weakness or heart failure [76]. Current treatments are based on invasive surgery including surgical bypass to restore the revascularization of the wounded part or peacemaker, and if the damages are too pronounced, it will require a heart transplant. The latter suffers from a limited donor pool, use of immunosuppressants with associated infection concerns [23, 20]. There has been extensive progress in engineering synthetic conductive cardiac patches to restore the electrical and mechanical functions of the damaged myocardium after an MI [8, 7, 77].
Biomaterials used to patch the heart must have the following properties: biocompatibility with the heart muscle (myocardium), electrical conductivity to help conduct the beating signal in the muscle, stability over time and proper mechanical properties (elasticity) to mimic the organ [77]. Polymer hydrogels – i.e., multidimensional structures that contain between 60% and 99% of water [11] – are good candidates to achieve these properties [78]. Hydrogels 3D microstructure are similar to natural tissues, they can support cell adhesion and provide mechanical support for cardiac cells to deposit extracellular matrix and form newly synthesized tissue. They can be used in a wet environment (such as an organ), and can be made conductive by addition of conductive materials [11, 78, 79]. Hydrogels are soft, stretchable, offers high elasticity, high mechanical strength and most can be molded in any shape and be easily patterned. Different materials are used to make hydrogels conductive, such as graphite materials, carbon-based nanomaterials and electroconductive polymers [79]. Conductive polymers show better advantages compared to traditional conductive materials [80, 33]. The most used polymers to make conductive hydrogels are Polyaniline (PANI), Polypyrrole (PPy), polythiophene (PT), and poly(3,4-ethylene dioxythiophene) polystyrene sulfonate (PEDOT:PSS) [28, 79, 27, 8]. Among them, PEDOT:PSS is the most encouraging polymer to be used because of its high conductivity, stability and good biocompatibility that can be enhanced through functionalization [8, 33, 66, 81]. It has been already used in biosen-

sors and tissue engineering [35, 36, 38, 39, 40, 41, 32]. Given its electrical properties it makes an excellent candidate for implantable and epidermal electrodes REFS, artificial muscles, peripheral nerve repair REFS, nerf grafts, smooth muscle and more recently for cardiovascular applications [9, 39, 41, 44, 46, 47]. It has been therefore established that conductive polymers such as (3,4-ethylenedioxythiophene):polystyrene-sulfonate (PEDOT:PSS), could be applied to cardiac patches in order to reestablish the electrical conduction of heart tissues while providing a suitable environment for cardiomyocytes [28, 27, 8, 82]. For example, PEDOT:PSS/Chitosan/PVA based scaffolds has been developed for cardiac tissue engineering. They demonstrated that conductivity and biocompatiblility of the scaffold offers a great support fore on rat bone marrow mesenchymal stem cells proliferation. However, the material has low strain at rupture and has not been tested on cardiac cells nor tested on cyclic stretching [47]. Biocompatibility toward cardiac cells is critical and, research has been focusing on improving biointegration of these implants for example via the use of peptide functionalization [66]. Given the importance of cell-cell interactions in tissue engineering, a strategy is to use N-cadherin at the surface of our patch to improve cellular adhesions, and to facilitate the biointegration of our implant in the native tissues. N-Cadherin is a transmembrane protein that play an important role in preserving mechanical adhesion and electrical coupling between neurons, cardiac fibroblast and cardiomyocytes. Successful functionalization of PEDOT:PSS with N-Cadherin have been reported improving the performance of carbon microfibers neuron electrodes coated with PEDOT:PSS-co-MA [83]. Here we hypothesized that modifying the surface of our implant with N-cadherin will improve the biointerface between the material and the cardiac muscle and stimulate cardiac regeneration.

Among its qualities regarding cardiac repair, recent advancements showed that PEDOT:PSS also offers promising properties for antibacterial applications against gram-negative and gram-positive bacteria [10]. Films based of PEDOT:PSS and others polymers can exhibit antibacterial against E.Coli and A.Sareus and fibroblast biocompatible hydrogel based on PEDOT:PSS and agarose can become antibacterial against these bacteria under near-infrared light [84]. PEDOT:PSS antibacterial properties is interesting regarding the constant need of new solution against resistant bacteria.

In this work we developed stretchable and conductive hydrogels based on PVA and PEDOT:PSS for potential cardiac patch use, using biocompatible polymers and a simple drying technique. We showed that adding PVA to a PEDOT:PSS based hydrogel can form a material with high stretchability, high conductivity, a great resilience to cyclic stretching and anti-bacterial properties.

4.2 Experimental

4.2.1 Material

Polyvinyl alcool (PVA, Mw 89,000-98,000, 99+% hydrolyzed), dimethyl sulfoxide (DMSO), N,N-dimethyl-formamide (DMF, anhydrous, 99.8%), 4-(dimethylamino)pyridine (DMAP), N,N'-Dicyclohexylcarbodiimide (DCC, 99%) and crystal violet were all purchased from Sigma Aldrich. PEDOT:PSS (Clevios PH1000) was purchased from Heraeus. The N-cadherin mimic peptides (HAVDIGGGC) were ordered from Genscript. Polypropylene sheet (3.18mm thick, 300mm × 300mm) was purchased from Fisher Scientific. Staphylococcus aureus were purchased from ATCC.

4.2.2 Methods

Preparation of conducting polymer hydrogels

The preparation of the conductive hydrogel is inspired from a procedure reported in the literature [61]. PEDOT:PSS (PH1000) is stirred with dimethylsulfoxide (DMSO, 13% v/v) for 20h at room temperature. Polyvinyl alcohol (PVA) pellets are dissolved in 5mL water overnight at $95°C$. PVA is added to the mixture in the range of 0.5–8 % v/v (0.625-10% m/v). The final solution is mixed for 4h at room temperature before being casted on a $40mm$ by $70mm$ substrate of Polypropylene (PP) and dried for 24h in an oven (Thermo Scientific Heratherm) at $60°C$ (fig **??**). The result is a thin black dry film that can be easily peeled off from the substrate. The hydrogel was stored in water.

Functionalization of hydrogels

PEDOT:PSS and PVA based hydrogels were cut in 4 squares of $20 \times 20mm$. N-cadherin mimic peptides (HAVDIGGGC) ($25\mu mol$), DCC ($27.5\mu mol$), DMAP ($25\mu mol$) and hydrogel squares were mixed in DMF ($30ml$) at $37°C$ for 48h under a nitrogen blanket, according to a pre-existent protocol [64]. The hydrogel squares were rinsed with and stored in distilled water.

4.2.3 Characterization

Water content and swelling ratio measurement

The water content of a hydrogel is defined as the fractional part of the weight of water inside the hydrogel when wet. It represents the weight of the non-polymer part of the hydrogel on the total weight. The swelling ratio of the hydrogel is defined as the fractional part of the weight of water in the hydrogel on the weight of the dried hydrogel. The hydrogels were weighted dry right after the dry-annealing process then submerged in water for 15min and weighted when it is completely swollen. The water content and the swelling ratio of the hydrogel can be calculated using the following formulae:

$$Water\ content = \frac{W_s - W_d}{W_s}$$

$$Swelling\ ratio = \frac{W_s - W_d}{W_d}$$

Where W_s is the weight of the hydrogel when swollen and W_d is the weight of the dry hydrogel.

Mechanical and conductivity test

Tensile tests were made using the Mechanical Tester Mach-1 from Biomomentum Inc. Resistivity and conductivity measurement were made by using a tailored tensile grip with electrodes (MA068) made by Biomomentum Inc. The four point probe-sample holder is made of 2 grips, each with two rows of 5 Ag/AgCl electrodes with a 2 mm diameter. One row (source) is allowing the current to flow in the sample and the other one (sensing) is measuring the voltage. The free-standing hydrogel is deposed on the electrodes to make contact and secured by a plaque of Plexiglas. This mechanism secures and grip the sample, allowing it to be stretched and to measure its resistance during its elongation. Conductivity during the elongation was derived from the measured electrical resistance, assuming constant volume, following the equation :

$$\rho = \frac{L^2}{L_0 A_0 R} = \frac{L^2}{V_0 R}$$

Where R is the electrical resistance of the sample, L_0 is the length before elongation, L is the length during the elongation, A_0 is the cross-sectional area of the sample before elongation and V_0 is the volume $L_0 \times A_0$ of the stretched sample.

The versatility of the point probe-sample holder allows to measure the electrical resistance, the Young modulus and the performance of the hydrogel during cyclic stretching. The PEDOT:PSS and PVA based hydrogel sample, swollen in deionized water were cut into 10mm by 40mm rectangles. Tensile tests were made at room temperature at a speed of 0.1 mm/s. For the cyclic stretching, the hydrogel rectangles were stretched at a 10 % length elongation for 1000 cycles at a frequency of 1Hz. To prevent drying during the cycles, the hydrogels were kept wet by spraying water on them every 30s. The thickness of the sample was measured with spherical indenters (D = 1mm) using the Mechanical Tester Model Mach-1 from Biomomentum Inc. All data were recorded with the Mach1-motion software and Mach1-analysis software.

Scanning electron microscopy (SEM)

SEM pictures were acquired by Scanning Electron Microscopy (Hitachi TM3030).

Contact angle

Static contact angles were measured with the sessile drop technique using an optical tensiometer OCA-20, by Dataphysics. Contact angles were mesured by deposing a $2\mu L$ DI water droplet with an automatic dosing syringe on the hydrogels samples. Contact angle were measured on hydrogel with different concentration of PVA (0.5%, 2% and 4%) and on functionalized Hydrogel with 2% of PVA. Using the camera, the drop was recorded and the angle measurements were made 5s after droplet deposition using the software SCA20 U.

Biofilm Assay

A biofilm Assay was performed on the hydrogels samples (2% PVA and 2% PVA functionalized) and on styrofoam as a control. The day before setting up the assay, 1 colony of Staphylococcus aureus was prepared in 5 mL LB broth and placed in a shaker for 20 h at $37°C$. The absorbance of the overnight culture at 600nm was determined and the bacteria culture was diluted to obtain a concentration of 1×10^5 CFUs/mL. The hydrogels samples (2% PVA and 2% PVA functionalized) were cut in 1cm diameter circles and the Styrofoam samples were cut in 0.8cm circles, 0.5cm thick. Samples were placed in a 48-well plate with 500 µL of bacteria dilution and incubated in a shaker at $37°C$, 200 rpm, for 24h. Bacteria medium was then gently discarded to not damage the biofilm and the samples were washed twice with PBS and let dry. 350 µL of 0.1% crystal violet in water was added to each well for 15min at room temperature. All the samples were repeatedly washed with DI water until

clear and let completely dry at room temperature. 350 µL of 33% acetic acid were added in each well for 15min. 100µL of each well were plated in triplicate in a 96-well plate to read the absorbance at 570 nm. Because the materials by themself tend to absorb crystal violet, samples (hydrogels and Styrofoam) were cultivated without bacteria and the absorbance of the material without bacteria was subtracted to the absorbance of the material cultivated with bacteria.

Statistic

Data are expressed as means ± SD. Tukey test was used for comparisons of different means. Values of $P < 0.05$ were considered significant.

4.3 Results and Discussion

The young modulus of the natural heart muscle varies between 0.001 MPa and 1 Mpa [85, 86, 87] and its electrical conductivity is between 0.05×10^{-3} to 3×10^{-3} S/cm [88, 89, 90]. In this work, we aimed to develop an electroconductive bioactive hydrogel which can enhance reinstate the physiological properties of damaged cardiac tissues. To this end, we exploited both the electroconductive properties of PEDOT:PSS and the functionality and elasticity of PVA. Different hydrogels were prepared with increased concentration of PVA (0.5%, 1%, 4%, 8% and 12%). All the dried hydrogels were easily peeled of the substrate. For each patch, a fixed concentration of DMSO of 13 % v/v was selected. At this concentration, DMSO favorizes PEDOT chain formation arrangement inside the hydrogel leading to the maximum conductivity for a PEDOT:PSS hydrogels [61]. The visual appearance of the dried patch with different PVA concentrations is shown in fig 4.1. It can be clearly seen that the shape of gel varies strongly with the concentration of PVA. At low concentration of PVA (0.5% and 1%), PEDOT:PSS gels remains flat and maintain the shape of the polypropylene mold whereas at higher concentration (above 4%), the gel loses its structural integrity and shrinks on itself. In order to assess the effect of PVA on the structural properties of the hydrogels, we studied the surface topography of the film with scanning electron microscopy (SEM).

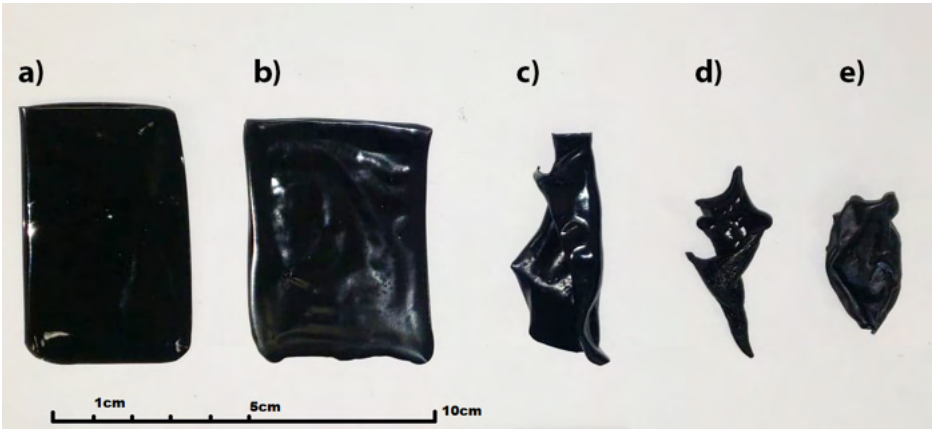


Figure 4.1 Shapes of the dried hydrogels made with 0.5% (a), 1% (b), 4% (c), 8% (d), 12% (e) PVA.

4.3.1 Swelling ratio and water content

To reveal further the effect of PVA on the deformation of the PEDOT:PSS gel, we measured the swelling ratio and water content of the hydrogels for different concentration of PVA (0.5%, 1%, 4%, 8% and 12%). Usually for hydrogels, the water content is between 60 %

and 99 % [50, 79]. The swelling ratios and water content of PEDOT:PSS with various PVA concentrations are significantly different (fig 4.2). The water content and swelling ratio decrease with an increased concentration of PVA in the hydrogel. Hydrogel with 0.5% v/v PVA has a water content close to the the natural heart muscle (respectively 80.8 ± 1.0 and 78.8 ± 1.2 [91]). All hydrogels exhibit a swelling ratio between 1 and 4.5. In previous report, PVA/PEDOT:PSS film prepared by SIPN strategy showed a low swelling ratio of 0.35 ([59] and collagen/alginate/PEDOT:PSS hydrogel showed a hight swelling ratio of 40 [60]. To mimic as close as possible the cardiac muscle properties, the swelling ratio must be closer as possible to 4 [91]. Above 4 % v/v PVA, the water content is below 50% and the PEDOT:PSS film cannot be rehydrated again. We focused our next experiment on the hydrogels with a percentage of PVA between 0,5% and 2%.

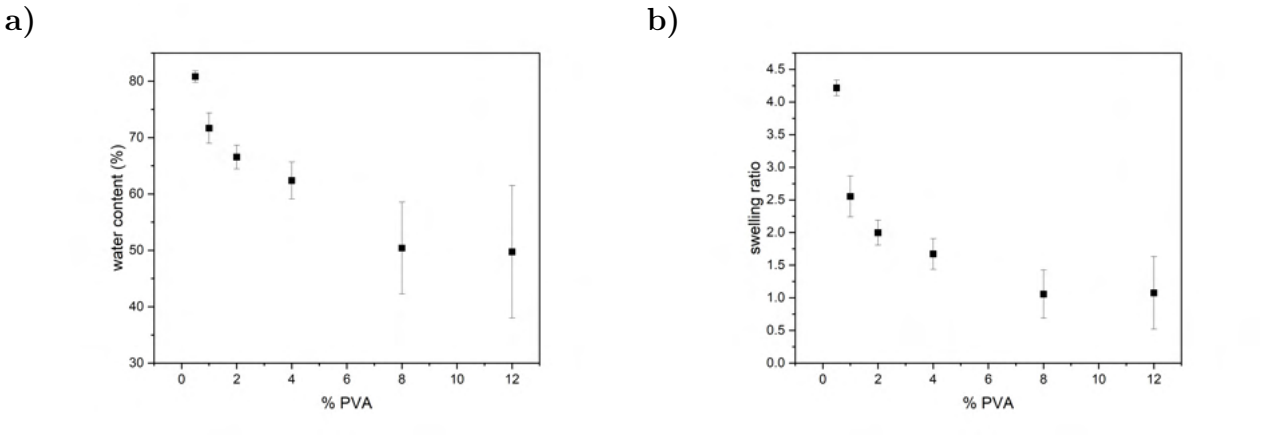


Figure 4.2 Water content (a) and swelling ratio (b) in the hydrogel versus % PVA, n = 3

4.3.2 Young modulus and elongation at break

In order to study if the hydrogels reveal the physical features of native cardiac tissue, the stress-strain curve of the hydrogels was determined using a tensile test, the Young modulus and the elongation at break were measured (fig 4.3). All the hydrogels exhibit an elastic part where the stress/strain relationship is linear, and a plastic part. The behavior of each hydrogel with a different concentration of PVA under uniaxial tension is similar, except for the elongation at break. On the elastic part of the stress/strain curve, each hydrogel has a Young modulus in the range of $4 - 5MPa \pm 1$. Young modulus of the hydrogels with 0.5% PVA ($4.82 \pm 1.09MPa$) is similar that of the hydrogels with 2% PVA ($4.25 \pm 1.10MPa$). The young modulus of the hydrogels are comparable to the young modulus of previous similar cardiac patch such as NBR/PEGDM/PEDOT electrospun membranes ($3.8 \pm 1.8MPa$) [92], PANI based cardiac patch ($6.73 \pm 1.14MPa$) [77] and polyaniline/chitosan bioelectronic patch

$(5.62 \pm 0.93 MPa)$ [24] but it's still more than the young modulus of a natural heart (inferior to 1 Mpa) [85, 86, 87].

Furthermore, the Young modulus of the swollen PVA and PEDOT:PSS based hydrogel is comparable to the young modulus of a PEDOT:PSS hydrogel without PVA with the same amount of DMSO [61], meaning that PVA doesn't have any influence on the elastic deformation of the hydrogel. However, the elongation at break is considerably enhanced when the % of PVA increased. Hydrogels with 0.5 % of PVA break on average at $20 \pm 7\%$ strain while hydrogel with 2% of PVA break at $52 \pm 22\%$ of strain (fig 4.4) which is higher than previously described PEDOT:PSS/Chitosan/PVA scaffold that can only be stretched 6% before breaking [47]. Adding PVA here make the hydrogels more resistant to stretching and more resilient to strain, the hydrogels made of PVA and PEDOT:PSS were more flexible than control PEDOT:PSS [61]. Here, we anticipated that our PVA combined PEDOT:PSS patches would match the mechanical properties of native cardiac tissue. We found that PVA combined PEDOT:PSS patch possessed elasticity for efficient contraction/relaxation cycle and enhanced handling to facilitate insertion and implantation during surgery.

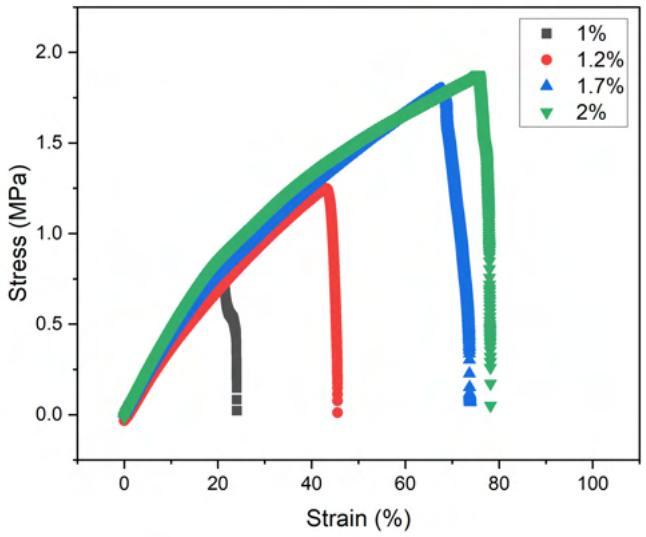


Figure 4.3 Tensile stress–strain curve of the wet hydrogels prepared with different percentages of PVA, using the tensile grip

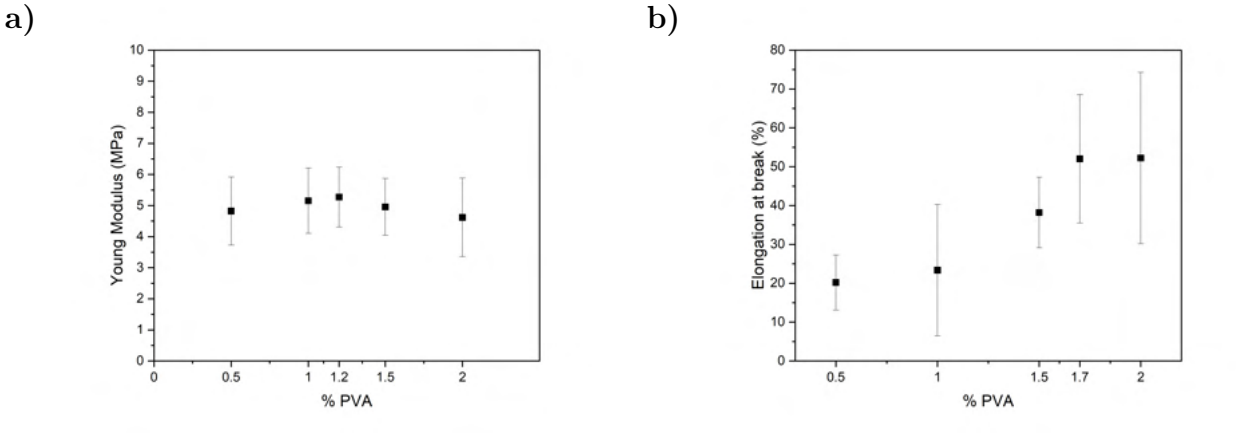


Figure 4.4 Young modulus (a) and elongation at break (b) of the wet hydrogels for different percentages of PVA during a stretching at a speed of 0.1 mm/s, n = 5

4.3.3 Electrical conductivity

To assess the conductivity of the PEDOT:PSS hydrogel during relaxation and contraction, a tailored tensile grip with electrodes was used. All the hydrogels exhibit very high conductivity (more than 20S/cm) compared to other PEDOT:PSS based hydrogels [53, 59, 60, 93, 82].

Figure 4.5 shows that the electrical conductivity decreases with PVA concentration. At rest, the highest conductivity was measured for a concentration of 0.5% v/v PVA (75 S/cm) whereas the 4% v/v PVA exhibits the lowest (23 S/cm) (fig 4.5). Above 4% of PVA, the resulting film is too rigid making the measurement hard to achieve. These results confirm the feasibility of electroconductive hydrogel fabrication combining PEDOT:PSS with PVA.

Conductivity of the hydrogel was then measured during the mechanical stretching at a speed of 0.1 mm/s using the tailored grip/four-points probe.

The hydrogels conductivity, for each concentration of PVA was stable during stretching. Usually in the first 20% strain the conductivity increases slightly (+7% increase at 10% strain, +12% increase at 20% strain) (Fig 4.6). Increase of conductivity of PEDOT:PSS film during stretching has been previously reported [94]. When the deformation reaches the plastic domain, the conductivity stabilizes and decreases when the hydrogel start to break (fig 4.7).

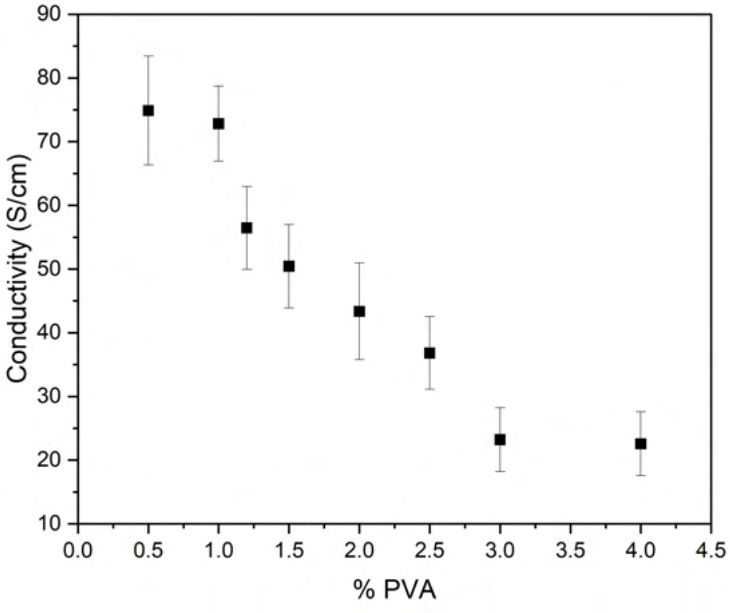


Figure 4.5 Conductivity of the hydrogels with increasing percentage of PVA, n = 6

a) **b)**

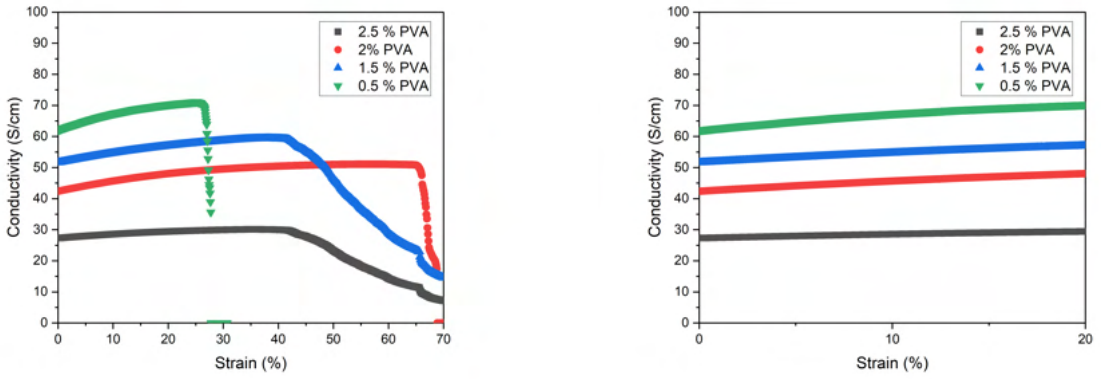
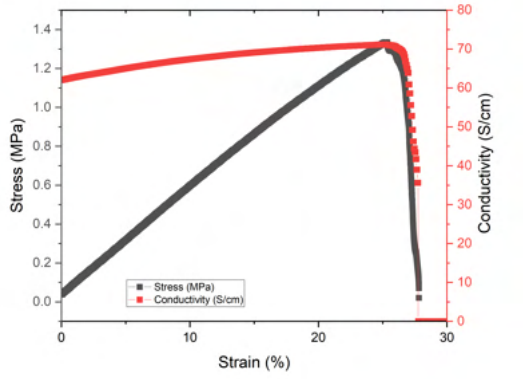


Figure 4.6 Evolution of the conductivity in the hydrogels during a stretching at a speed of 0.1mm/s for a 0.5%, 1.5%, 2% and 2.5% PVA content until break (a) and zoom in for the first 20% strain (b)

a)



b)

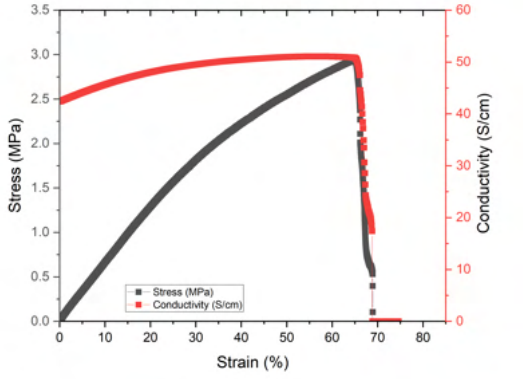


Figure 4.7 Stress-strain curve (black) and conductivity (red) curve of the hydrogel for 0.5% PVA (a) and 2% PVA (b) during a stretching until failure

4.3.4 Stretching and cyclic strain

The heart being a beating organ, a cardiac patch placed directly on it has to be stretched cyclically. The conductivity of the hydrogel for different concentration of PVA was measured under a cyclic traction of 1000 stretching, the amplitude of the cycle was a 10 % strain (1mm) stretching at room temperature while the hydrogel being keep wet to avoid it to dry. The frequency of the stretching was 1Hz to mimic the heartbeat. During the cyclic stretching, the conductivity was stable for every concentration of PVA (±7%) (fig 4.8). The hydrogels showed a reversible increase in conductivity during the cyclic stretching, conductivity after the 1000 cycle was the same as before the test (fig 4.8). This reversible increase of conductivity has been documented previously for small cyclic elongation on PEDOT/PU–hydrogel hybrids, contrary to stretchable polymers with metallic nanoparticle where conductivity irretrievably decrease with stretching [95] .

The resistance and conductivity of the stretched sample follow the traction movement by slightly increasing during stretching and by decreasing during relaxing (fig 4.9). The variation in the resistance caused by the stretching is very limited with 0.5 % on average. The variation in the conductivity during cyclic stretching is on average 7% which is considered good [95]. Hydrogels are therefore very resilient to cyclic stretching and can hold their conductivity for a long time under strain, making them suitable for cardiac patch.

a) b)

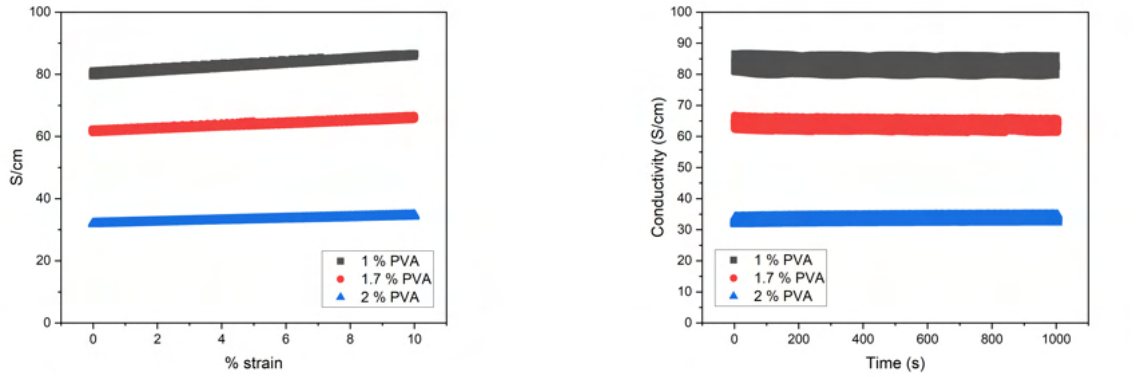


Figure 4.8 Conductivity of hydrogels with different percentages of PVA during a 10% cyclic stretching at 1Hz for 1000s versus strain (a) and time (b)

a) b)

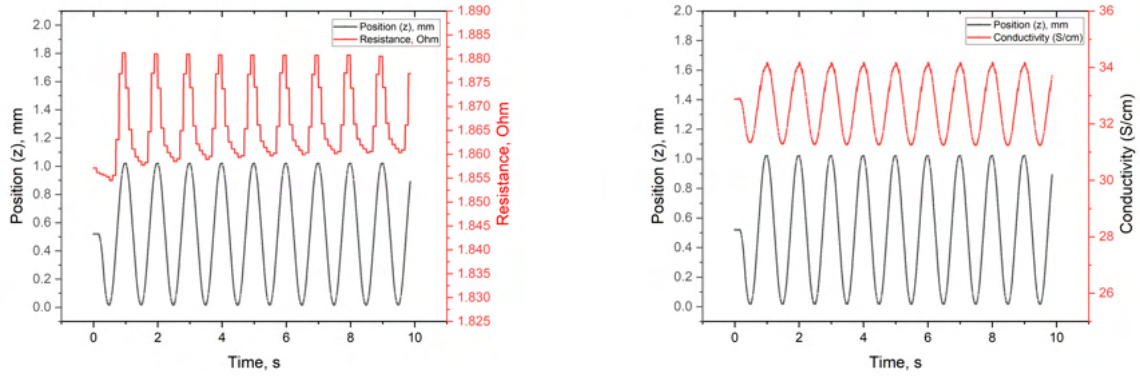


Figure 4.9 Sinusoid position-time (black) and electrical resistance-time (red) curves for a hydrogel with 2% PVA during a 10% cyclic stretching at 1Hz (a). Sinusoid position-time (black) and electrical conductivity-time (red) cruves for a hydrogel with 2% PVA during a 10% cyclic stretching at 1Hz (a)

4.3.5 Biofunctionalization

To enhance the biocompatibility of the hydrogels towards cardiac cells, the hydrogels with 2 % PVA were functionalized in DMF with a peptides sequence (HAVDIGGGC) of N-cadherin responsible for cell-cell adhesion using the Steglich esterification. To confirm the functionalization of PVA on the patch, the contact angles were measured. There is a clear change in wettability on the hydrogels due to the functionalization, with a contact angle of $97.1° \pm 9.3$ for functionalized hydrogels with 2 % of PVA and $81.5° \pm 8.9$ for non-functionalized hydrogels (fig 4.10). However, functionalized hydrogels with 2 % PVA have a wettability comparable to non functionalized hydrogels with 0.5 % PVA, both hydrogels exhibit similar surfaces which

is confirmed by the SEM images. The concentration of PVA in the hydrogel has an impact on the wettability (fig A.1), PEDOT:PSS being hydrophobic and PVA being hydrophilic, the more PVA is added in the hydrogel, the more it will become hydrophilic [96]. Hydrogels with 0.5 % PVA have a contact angle of $96.1° \pm 11.2$, while hydrogels with 4 % PVA are very hydrophilic with a contact angle of $21.6° \pm 5.6$ (fig 4.10).

a) **b)**

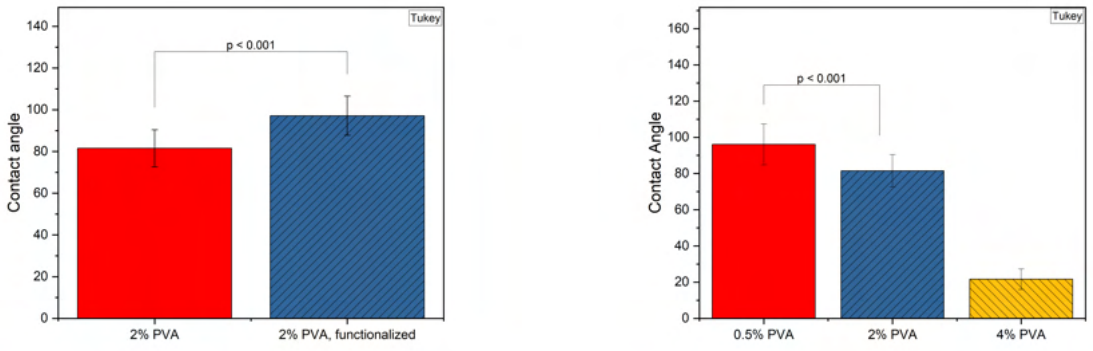


Figure 4.10 Contact angle of non functionalized and functionalized 2 %PVA hydrogel (a), Contact angle of 0.5%, 2%, 4% PVA non functionalized hydrogels (b)

4.3.6 Scanning electron microscopy

The surface of the hydrogels was characterized by scanning electron microscopy (SEM) (fig 4.11). Functionalized hydrogels with 2% PVA have a smoother surface than non functionalized hydrogels with 2% PVA that show more roughness and few large grains. Similarly to the wettability, functionalized hydrogels with 2% PVA have a smooth surface more similar to unfonctionalized hydrogels with 0.5% PVA. Hydrogels with 4% PVA present a surface with deeper rift that hydrogels with 2% of PVA.

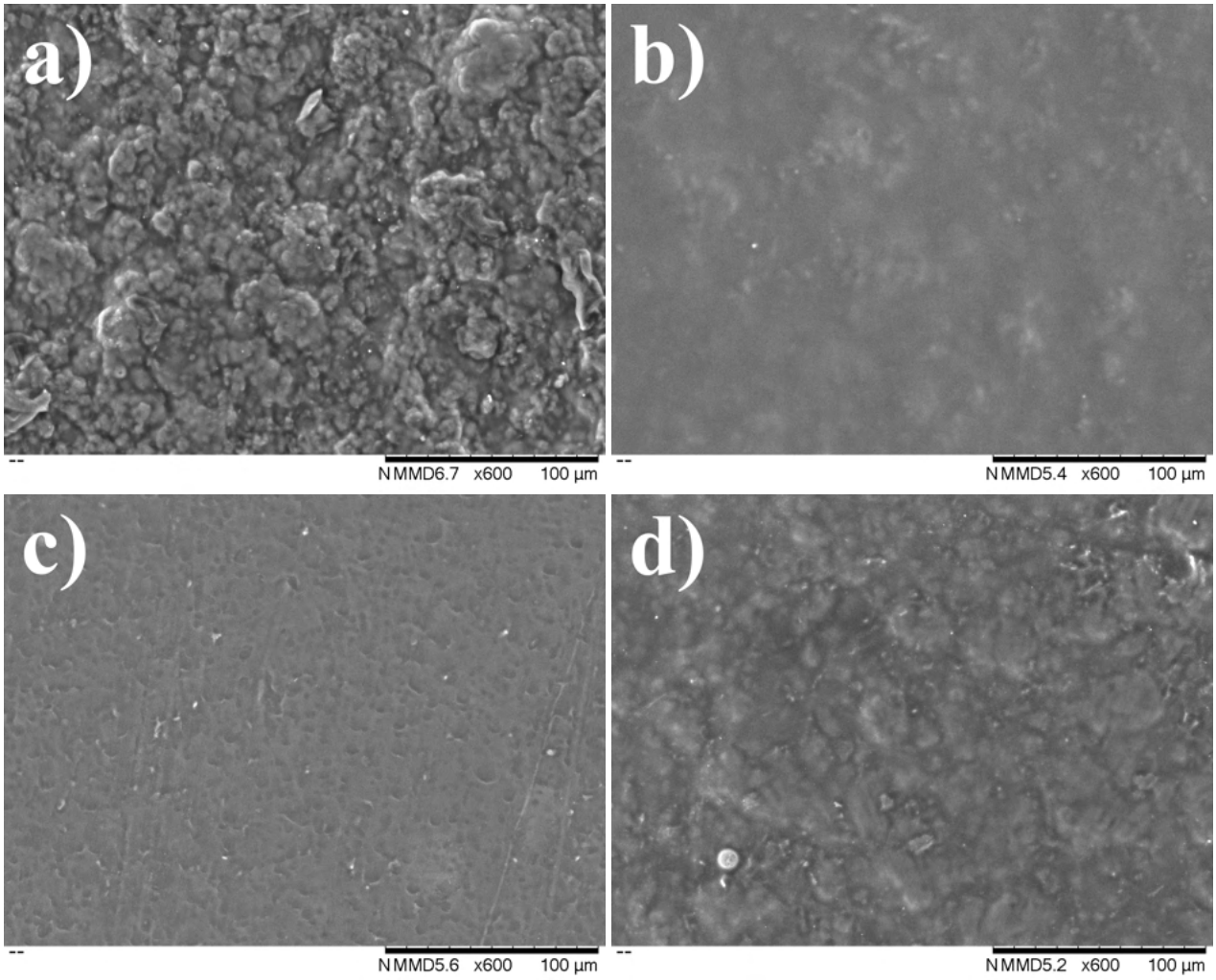


Figure 4.11 Scanning electron microscopy images of the hydrogels with 2% PVA (a), 2% PVA functionalized with HAVDIGGGC (b), 0.5%PVA (c), 4% PVA (d), scale bar is 100 μ m.

4.3.7 Bacteria

Styrofoam and hydrogels absorbing crystal violet even without biofilm formation (negative control), their absorbance (respectively 0.4 ± 0.1 and 1.3 ± 0.1) were subtracted from the absorbance of the sample with bacteria to normalize.,Biofilm formation was high 1.7 ± 0.3 on the Styrofoam as expected and the absorbance of crystal violet on 2%PVA hydrogels is relatively low (0.5 ± 0.1) compared to the positive control showing that S.aureus didnt produce a lot of biofilm on the sample. Biofilm formation was suppressed on the 2% PVA functionalized (0.1 ± 0.3) compared to non functionalized hydrogels (Pvalue <0.05) showing antibacterial properties due to the functionalization with peptide. Previous work reported that pure PEDOT:PSS shows no antibacterial property but films of PEDOT: PSS with

chitosan [97] or PEDOT:PSS coated PLA/PHBV membrane with TCH [98] show effect against S.Aureus bacteria. We showed that highly conductive and stretchable PEDOT:PSS and PVA based hydrogel can have antibacterial properties, leading the way in making new antibacterial, soft, and stretchable electronics that could be used in operating rooms or sterile environments.

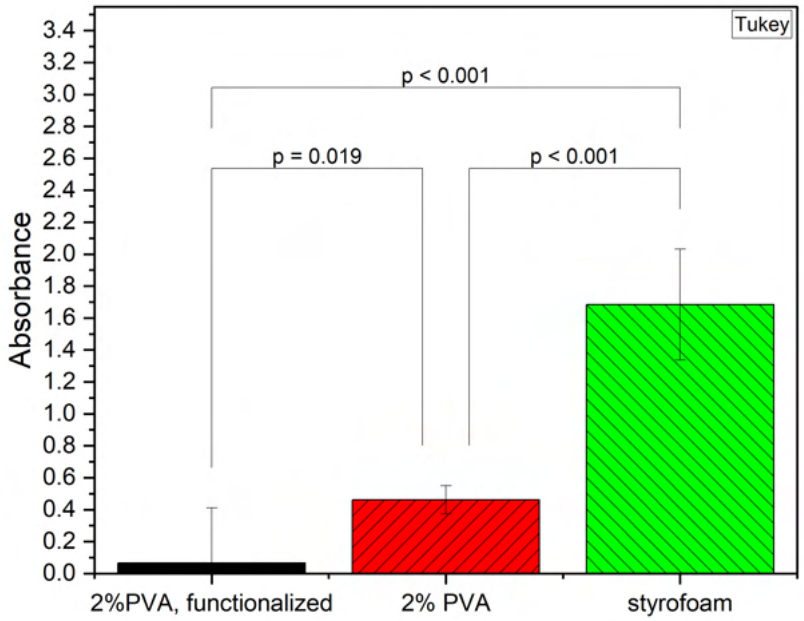


Figure 4.12 Absorbance OD562 of non functionalized and functionalized 2%PVA hydrogels and syrofoam (control) after a Staphylococcus aureus biofilm assay

CHAPTER 5 CONCLUSION

5.1 Conclusion and summary of Works

In this study, we have designed a stretchable and conductive PEDOT:PSS and PVA based hydrogel. We mixed PVA, DMSO and PEDOT:PSS and dried the solution in an oven to make a dry self-standing black polymer film. Once submerged in water, the film swell and become an hydrogel. We measured the water content of the hydrogels and discovered that it decreases with the amount of PVA added in the hydrogel (from 80% to 50%). We conducted tensile test on the hydrogel and measured the young modulus and elongation at break for different concentration of PVA added in the hydrogel. We showed that adding PVA to a PEDOT:PSS hydrogel can considerably enhance its elongation before break (from 20% to 50%) without modifying its young modulus ($4-5MPa\pm1$). Made hydrogel with 2% v/v PVA can be stretched 50% of their total length before breaking. We measured the resistance, thickness and conductivity of the hydrogel in a rest state and during stretching. We found that the conductivity decreased with the amount of PVA added (from 80 to 20S/cm) but the conductivity of every hydrogels is stable during the elongation. We also measured the conductivity of the hydrogels during a cyclic elongation and revealed that these hydrogels can be stretched periodically at 10% strain at 1Hz without losing any conductivity and are thus adapted to cardiac tissue engineering. We measured the contact angle and topology of the hydrogel for different concentration of PVA. The hydrogels with more PVA are more hydrophilic. We functionalized the hydrogels with HAVDIGGGC N-cadherin mimic peptide and discovered that functionalized hydrogels are more hydrophobic and have high antibacterial properties.

5.2 Recommendations

"The history of mankind has always been associated with materials typical of era: stone, bronze, iron, pottery, up to the recent age of plastics. Many scientists expect that the future trend in materials science will shift to soft materials, such as hydrogels. Especially functional types represented by conducting and electroactive hydrogels rank among the most promising objects." Jaroslav Stejskal, Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic [80].

Because of their 3D matrices structures, versatility, high water content, soft and stretchable properties, and their ability to be modified into being both biocompatible and conductive [99, 79], we believe that hydrogels are the future of cardiac tissue engineering. In this Thesis, we

showed that it is possible to make a PEDOT:PSS and PVA based hydrogel that is stretchable, conductive, stable, functionalizable, and with antibacterial properties. This work paves the way for further research in the field of conducting polymer hydrogels applied to cardiology [100].

Future research will focus on tailoring the hydrogel to make it as close to the natural myocardium muscle as possible in terms of physical and electrical properties. For example, one way to enhance its elasticity would be to make the hydrogel in an auxetic design; this would give the structure a negative Poisson's ratio, creating a cardiac patch that can truly follow the mechanics of the heart [101, 61].

In order to ensure the total safety of the biomaterial on the heart, the hydrogels will have to be tested on cardiomyocyte or fibroblast in the future [81]. The hydrogels could also be micropatterned to improve biocompatibility on cardiac cells [82].

To further the development of a conductive cardiac patch that can repair the injured myocardium, hydrogels must also be tested on a rat model of acute myocardial infarction [5].

Outside cardiological fields, the high antibacterial properties of functionalized PEDOT:PSS and PVA based hydrogels could lead the way in making new antibacterial, soft, and stretchable electronics that could be used in operating rooms or sterile environments. However, more diverse bacterial assays must be done to confirm that property.

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APPENDIX A SUPPLEMENTARY INFORMATION

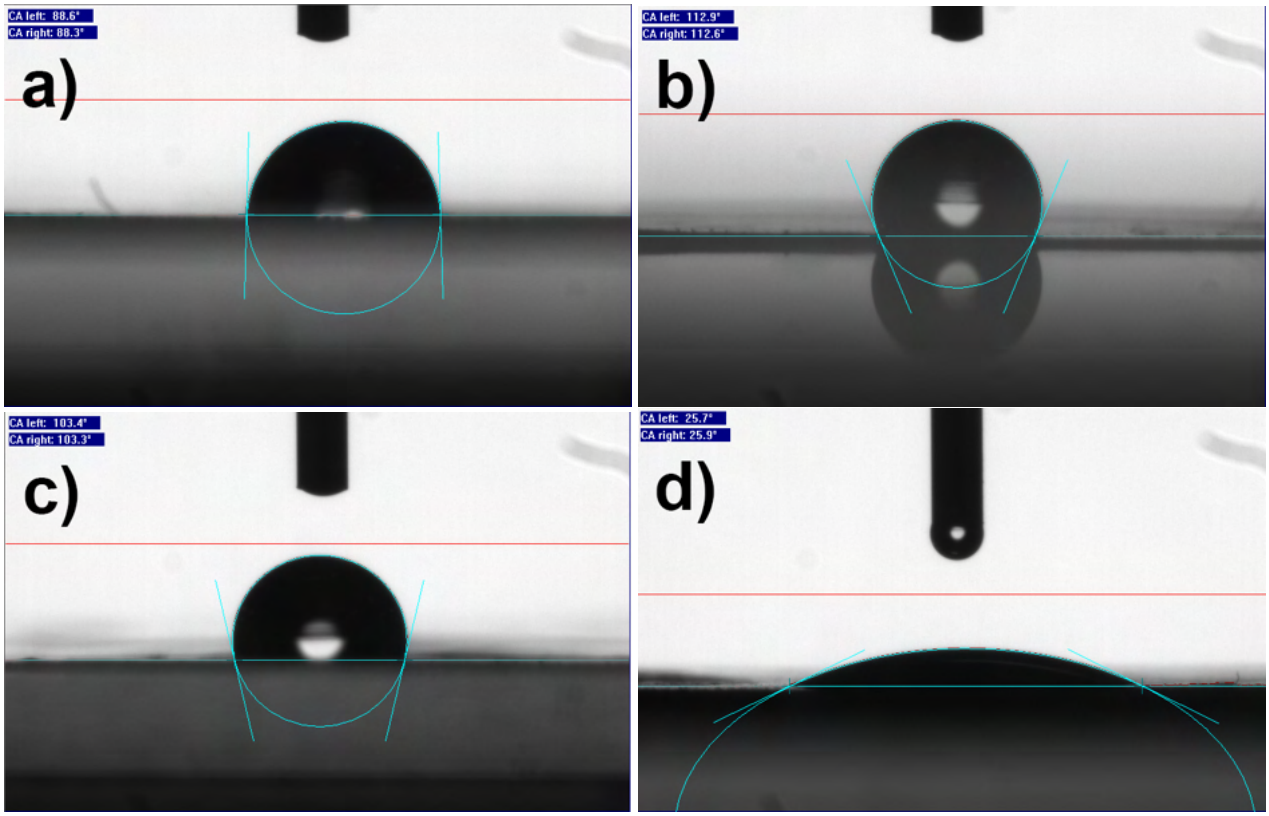

Figure A.1 Contact angle of the hydrogels, 2% PVA (a), 2% PVA functionalized with HAVDIGGGC (b), 0.5%PVA (c), 4% PVA (d)