A NANO-COMPOSITE FOR CARDIOVASCULAR TISSUE ENGINEERING

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ABSTRACT

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Cardiovascular disease (CVD) is one of the largest epidemics in the world causing 800,000 annual deaths in the United States alone and 15 million deaths worldwide. After a myocardial infarction, commonly known as a heart attack, the cells around the infarct area get deprived of oxygen and die resulting in scar tissue formation and subsequent arrhythmic beating of the heart. Due to the inability of cardiomyocytes to differentiate, the chances of recurrence of an infarction are tremendous. Research has shown that recurrence leads to death within 2 years in 10% of the cases and within 10 years in 50% of the cases. Therefore, an external structure is needed to support cardiomyocyte growth and bring the heart back to proper functioning. Current research shows that composite materials coupled with nanotechnology, a material where one of its dimension is less than or equal to 100nm, has very high potential in becoming a successful alternative treatment for end stage heart failure. The main goal of this research is to develop a composite material that will act as a scaffold to help externally cultured cardiomyocytes grow in the infarct area of the heart. The composite will consist of a poly(lactic-co-glycolic acid) (PLGA) matrix, reinforced with carbon nanotubes. Prior research has been conducted with this same composite, however the significance of the composite developed in this research is that the nanotubes will be aligned with the help of an electro-magnetic field. This alignment is proposed to promote mechanical strength and significantly enhance proliferation and adhesion of the cardiomyocytes.

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LIST OF ABBREVIATIONS

CVD	Cardiovascular Disease
tPA	Tissue Plasminogen Activator
CABG	Coronary Artery Bypass Grafting
LVAD	Left Ventricle Assisting Device
ECM	Extra Cellular Matrix
PEG	Poly(ethylene) glycol
PCA	Polycaprolactone
PGA	Polyglycolic Acid
PLGA	Poly(lactic-co-glycolic acid)
PPy	Polypyrol
OPF	Oliog[poly(ethylene-glycol)fumarate]
SEM	Scanning Electron Microscope
EMF	Electromagnetic Field
MPA	Mega Pascals
LCP	Liquid Crystal Polymer
CNTs	Carbon Nanotubes
SWCNTs	Single Walled Carbon Nanotubes
MWCNTs	Multi Walled Carbon Nanotubes
PVA	Polyvinyl Alcohol

THF	Tetrahydrofuran
A.C	Alternating Current
D.C	Direct Current
FDA	Food and Drug Administration

CHAPTER 1

INTRODUCTION

Cardiovascular Disease

Cardiovascular disease (CVD) is one of the largest epidemics causing 800,000 annual deaths in the United States alone and 15 million deaths worldwide [1]. The most common reason for the occurrence of a cardiovascular disease is atherosclerosis [2]. The build-up of plaque on the inner walls of the blood vessels is known as atherosclerosis and this plaque is mainly due to the deposition of fat cells on the inner walls of the blood vessels. The deposition constricts the blood vessels further leading to a blood clot. This blood clot, restricts the flow of blood from and into the heart, therefore depriving the surrounding cardiomyocyte of oxygen and essential nutrients. This results in a myocardial infarction commonly known as a heart attack, which is the main constituent of a CVD. Although there are many known definitions of CVD [2], the above mentioned is most accepted by the general public.

Cardiomyocytes, the cells that make up the muscle of the heart, are deprived of oxygen as a result of insufficient blood supply. The area around the infarction forms scar tissue due to to the lack of ability of the cardiomyocytes to differentiate and repair themselves [3]. This further increases the chances of another myocardial infarction, mainly due to the loss of muscle, resulting in ventricular remodeling. Studies have shown that the probability that the patient will die due to a second heart attack is 10% for the first 2 years and then jumps up to 50% in the next 10 years [4]. Ventricular remodeling is not fully complete due to the inability of the cardiomyocytes to differentiate, resulting in CVD becoming a chronic disease ultimately resulting in death.

Due to the infarcted area, the heart loses its ability to beat at its nominal state, therefore, applying additional stress on the non-infarcted myocytes to pump blood to the extremities of the body, which then further reduces the overall life expectancy of the heart, resulting in a condition known as cardiomyopathy [2].

Modern Methods Used to Cure CVD

Although CVD is known to be a chronic heart condition, several methods have been developed to help the patient lead a normal healthy life after the occurrence of an infarction.

Thrombolytic Drugs

One of the main causes of CVD is the blockage of the arteries called atherosclerosis. Thrombolytic drugs aim at dissolving the blood clot caused by the plaque formation [5]. Some of the most commonly used thrombolytic drugs are: Eminase, Retavase, and Streptase. Thrombolytic drugs function via the mechanism of Tissue Plasminogen Activator (tPA). Plasminogen activators catalyze the activation of plasmin, which is responsible for the breakdown of fibrin polymers, an integral part of the blood clot.

Although this does not guarantee a complete recovery, this is the first drug that is administered to a patient who has arrived at the hospital after a myocardial infarction. One of the main advantages of thrombolytic drugs is that they are easy to administer to the patient and clear blockages in as fast as 3 hours. One of the biggest drawbacks is that it does not help stop another blockage from occurring and studies have shown that the effectiveness of the drug is only about 54% [6].

Antithrombin Drugs

Re-occlusion, the recurrence of the blockage to the artery, is one of the biggest concerns when it comes to the application of thrombolytic drugs. To help solve this problem, scientists have discovered a protein called antithrombin. Antithrombin, whose structure is shown in Figure 1, is a glycoprotein that is secreted by the liver. Antithrombin functions via inactivating several enzymes that are responsible for the clotting to occur. Along with antithrombin, a supplementary drug known as heparin is also prescribed. Heparin further inactivates the coagulation system and helps in the binding of antithrombin to the factors that inhibit coagulation [6].

However, studies have shown that the occurrence of another infarction is highly dependent

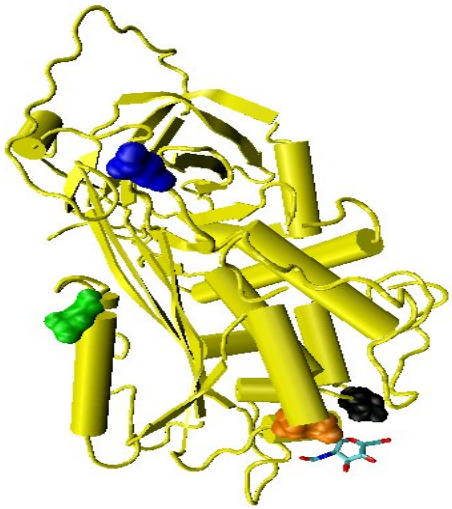


FIGURE 1. Structure of antithrombin.

on the dosage of the drugs, and the dosage, in turn, depends on up to what extent the vessels are blocked. Every patient has a different rate of blockage and calculating the right amount of dosage is difficult. A study conducted by Meijer et al. [7] showed that, even after the use of thrombolytic drugs, up to 30% of previously open arteries are re-occluded within just three months, which, clearly shows the inefficiency of this type of treatment to cure CVD [7].

Angioplasty

Angioplasty is one of the fundamental procedures used to clear a blocked artery. Angioplasty uses a process called catheterization, where a small tube is inserted into an artery in the leg, arm or the neck. This tube is then threaded until it reaches a coronary artery (an artery that goes to the heart). To the end of this tube, a small balloon is fixed. This whole procedure is performed by a doctor and is monitored on a special X-Ray screen.

Once a blockage is found, the small balloon is dilated so that it pushes the plaque and the arteries open up. This procedure eases blood flow and clears the block completely. To avoid the artery from collapsing again, a collapsed wire mesh (also called as a stent) is mounted on the balloon. Once the balloon is dilated it pushes the plaque away and locks the stent in its position.

This stent acts as a support and prevents the artery from collapsing again. However, in extreme cases, the artery is damaged so badly that a coronary artery bypass grafting should be performed [8].

Angioplasty is the most common procedure performed for treating cardiac arrests. From 1997 to 2014, there was an average of 200,000 angioplasties performed on a yearly basis. The average yearly cost of angioplasty was calculated to be $84,813. Apart from this, there are few other drawbacks to angioplasty. After the angioplasty is performed, it was found that, in 30% of the cases, the artery became narrow again resulting in a condition known as restenosis. In other cases, clotting of the blood was reported due the placement of the stent. The stent is a foreign particle and the body's defense mechanism kicks in to protect itself. Also, heavy bleeding could occur at the site where the catheter is initially inserted into the body, resulting in the requirement of additional medical care [9].

Coronary Artery Bypass Grafting (CABG)

Coronary artery bypass grafting is one of the most common open-heart surgeries performed by surgeons in America. This type of surgery is known as a cardiothoracic surgery and is performed by a cardiothoracic surgeon. In extreme cases where the above-mentioned treatments are not sufficient, considering the medical history of the patient, a CABG is performed. It is a highly complicated open heart surgery where depending on the location of the blockage, the particular artery or vein is bypassed and flow is restored with the help of a graft vein or artery from another part of the body [10], as shown in Figure 2.

Vein grafting. This type of grafting is done when a major blockage is found in the coronary vein. The procedure is performed by grafting a vein from the leg and is stitched between the aorta and the coronary artery.

Artery graft. This type of grafting is done when a major blockage is present in the coronary artery. An artery taken from the walls of the chest is stitched to the coronary artery bypassing the

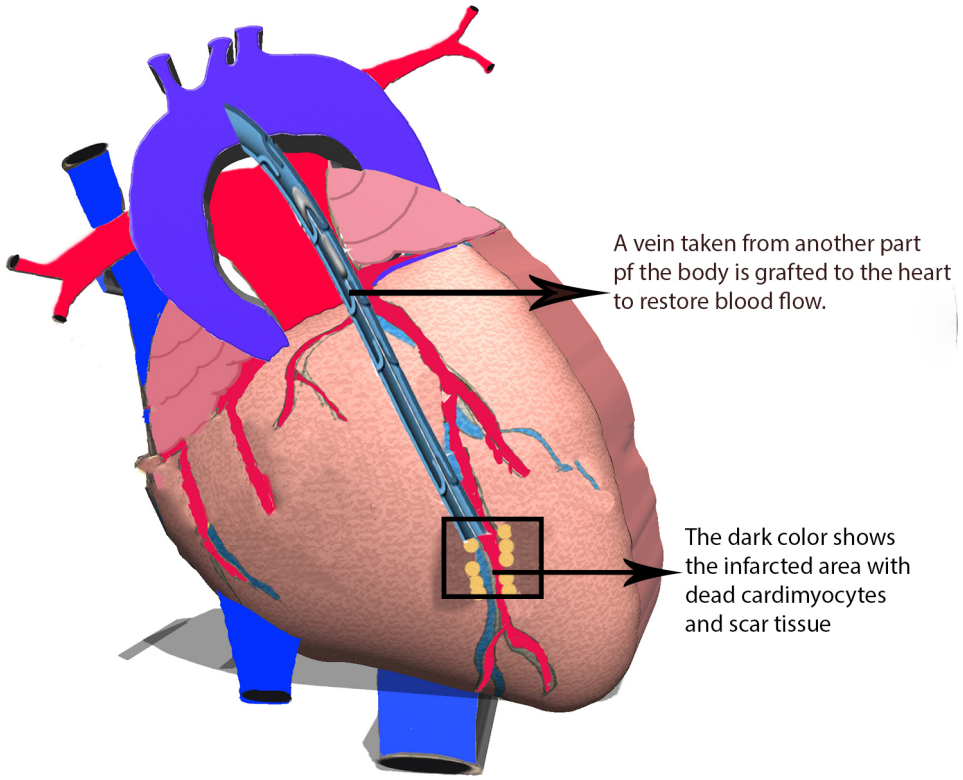


FIGURE 2. Vein grafting.

blockage.

In 2014, about 371,000 CABG procedures were performed. The average cost of a CABG surgery is around $75,000. Therefore, around $28 billion dollars were spent on CABG surgeries in the United States alone. Even after spending billions of dollars on this specific type of treatment, full recovery of the patient is not guaranteed [9]. Apart from this, there are post-surgery medical costs that the patient has to incur. Therefore, this calls for a new perspective to solve the problem permanently which is cost effective when compared to complicated surgeries.

Left Ventricle Assisting Device (LVAD)

A left ventricle assisting device (LVAD), sometimes simply known as a ventricular assisting

device is a mechanical pumping system that helps the heart to pump blood to the rest of the body. This type of treatment is used in extreme cases where the patient needs an immediate heart transplant but cannot receive one due to the inability to find a suitable donor or other medical conditions. The LVAD consists of a pump that collects blood from the left ventricle and when it senses that the chamber is full, it pumps blood to the main aorta which further sends it to the other parts of the human body [11].

This device is surgically placed in the abdomen below the heart during an open heart surgery. However, the control systems and the battery pack are outside the body near the abdomen. The main advantages of an LVAD are that it allows the patient to live a close to normal life even when the heart fails to function. It helps patients from not going out of breath. It also allows the heart to be at complete rest, improving the performance of the heart in the later stages.

However, the LVAD has a certain lifespan and it could get very complicated when it comes to implanting the device inside the patient [12]. A study was conducted by Hlatky et al. [13] where they compared the life of patients who had undergone angioplasty with patients who had undergone a bypass surgery. In this study, 465 patients were assigned to angioplasty and 469 were assigned to bypass and their life after the procedures were compared. The improvement in functional status was more in patients who underwent bypass than in patients who underwent angioplasty after one year (7.0 vs 4.4, P = 0.02), after two years (5.5 vs 3, P = 0.02) and three years (5.6 vs 3.2, P = 0.04) of follow up. There was no significant difference after the fourth year [13].

There was a significant decrease in the employment rate in both cases over the span of five years. The decrease was from 87% to 45%. The study also showed that the patients who had undergone angioplasty returned to work earlier than the other group [13]. The total cost after the five-year follow-up was found to be $56,225 in angioplasty and $58,889 in bypass surgery.

In conclusion, even after investing so much time and money and undergoing highly stressful procedures, there is no guarantee that the patient will survive because the disease is not com-

pletely cured and the occurrence of another infarction is still significantly high as the heart muscle is still weak. Hence, it is a strong belief that the main area of focus should be in trying to repair the heart muscle at the location of the infarction and the methods mentioned below directly target that.

Materials Application for CVD

Latest innovation and discoveries in the field of science have led to a new path for the treatment of CVD. One such innovation is the use of different type of materials, especially nanomaterials for the treatment of CVD. Materials application to CVD has to led to the emergence of a new field of engineering known as tissue engineering. Numerous novel materials and nanomaterials have been developed and studies have been conducted to show that they possess great potential in becoming the primary treatment for end-stage heart failure, i.e., CVD. An overview of materials application to CVD is illustrated in Figure 3. The following are the different approaches that are in the limelight of research for cardiovascular tissue engineering [14].

Scaffolds

One of the crucial structural supports for the cell is its extracellular matrix (ECM). The ECM consists of both structural and regulatory proteins in the form of a fibrous matrix that gives both mechanical and biochemical support to the cell to carry out their regular functions. A lot of research is being conducted in order to build scaffolds with structures similar to the ECM so that stem cells can be seeded onto those scaffolds.

Therefore, the scaffolds need to be strong enough to endure the cyclic stress caused by the continuous beating of the heart and also should be electrically conductive to maintain the electric signals between cardiomyocytes so that the new cells can adhere and proliferate and the heart is restored to its normal functioning. The scaffolds are usually constructed from both synthetic and natural materials. Commonly used materials for scaffolds are, biodegradable polymers, which are synthesized using electro-spinning and natural materials like gelatin, fibrin, carbon-fiber and gold

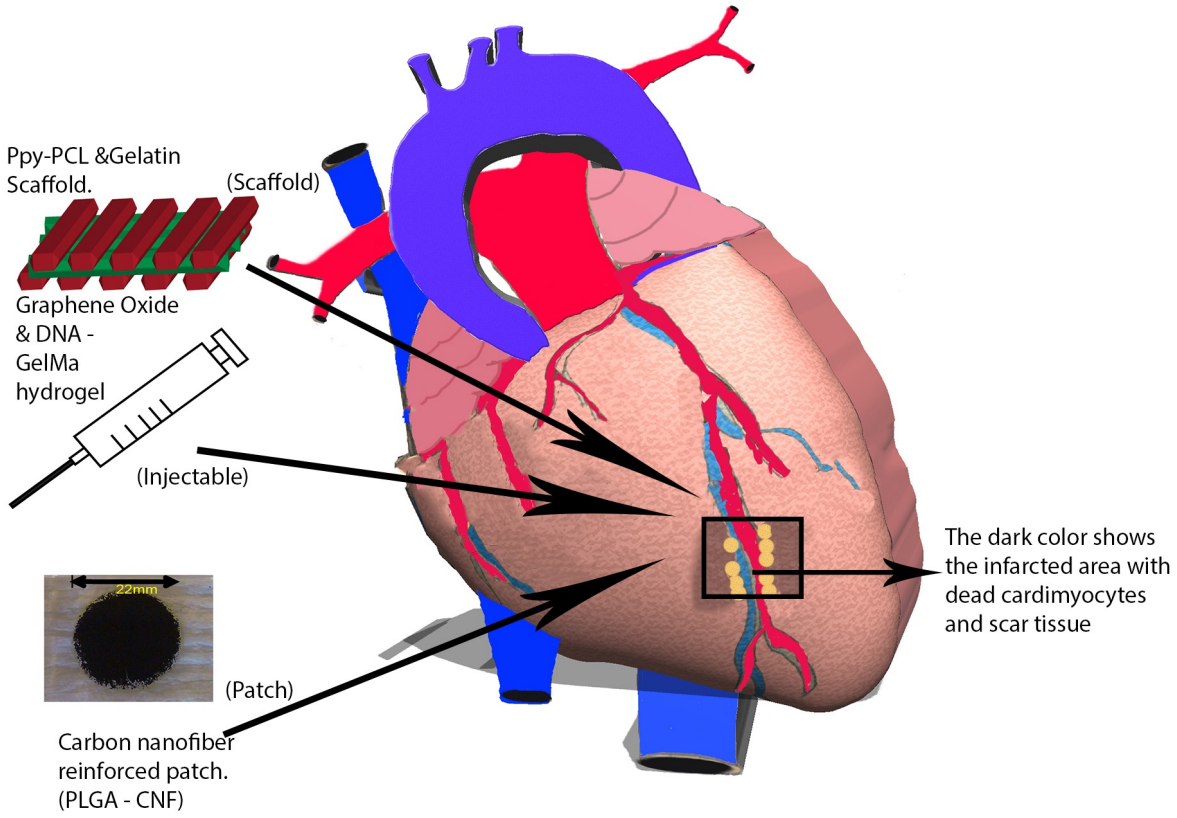


FIGURE 3. Materials application for CVD.

nanoparticles.

One such material application was studied by Kim et al. [15], where they used Poly(ethylene glycol) (PEG) to develop a nanopillar topography and observed increased cardiomyocyte adhesion, compared to conventional PEG substrates [15]. Another study was conducted by Aghdam et al. [16], where they developed a Polycaprolactone (PCA) Polyglycolic acid (PGA), nanofibrous scaffold to culture cardiac progenitor cells. They found optimal cell viability at 6 days of culture and a 65:35 PCL: PGA weight ratio. The main factor that enhanced the viability of this scaffold was attributed to its hydrophilic nature [16]. An illustration of the aforementioned studies has been shown in Figure 4.

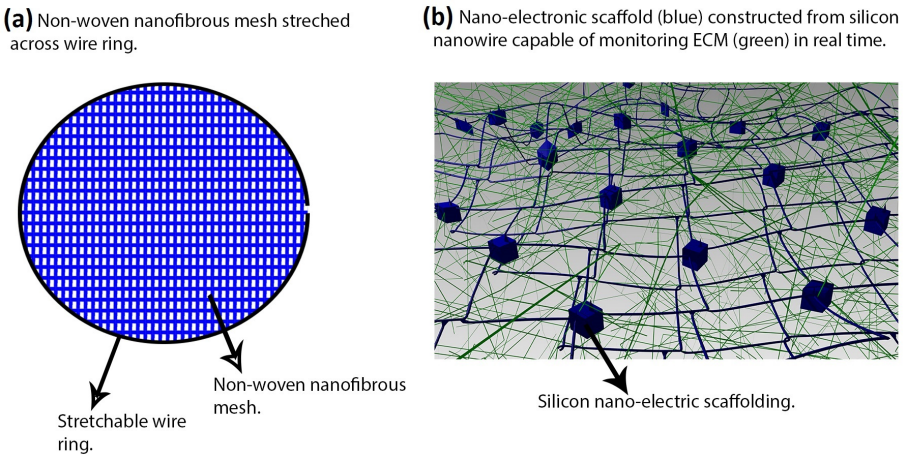


FIGURE 4. Two novel studies on scaffolds.

Patches

Another approach towards cardiovascular tissue engineering through material science is the development of patches. The reinforcement is usually in the micro or nanoscale. The patch is proposed to be placed on the infarcted area with stem cells seeded on it so that, it can structurally support the heart and the seeded stem cells will adhere to the cardiomyocytes and restore the functioning of the heart. The main constituents of a patch are a biodegradable matrix and a structural reinforcement that is in the nanoscale. A perfect patch has to have a conductive matrix and a mechanically strong reinforcement. Therefore the matrix is usually a conductive biodegradable polymer like poly(lactic-co-glycolic acid) PLGA or Polypyrrole (PPy), and the reinforcement is usually carbon nano-fiber [17], carbon nano-tubes or gold nanoparticles.

One such patch was developed by Kharaziha et al. [18], where carbon nanotubes were embedded in GelMA. The conductive network formed by the carbon nanotubes were responsible for enhancing the properties of the porous gelatin material. This resulted in increased cell adhesion, organization, and cell-cell coupling [18]. Figure 5 illustrates a cardiovascular patch embedded with Carbon Nanofibers (CNFs). The patch was developed by Stout et al., where they reinforced PLGA with CNF and observed increased cell viability and protein adsorption.

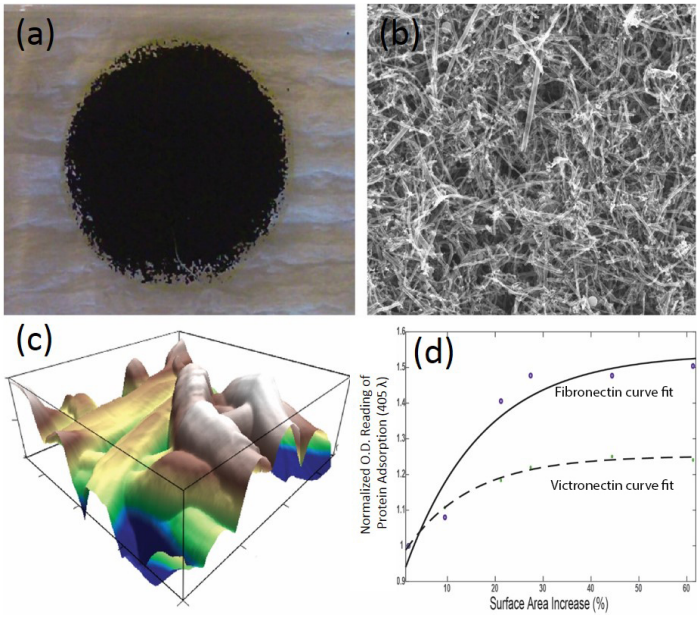


FIGURE 5. CNF cardiovascular patch.

In another study, a gold nanoparticle-based patch was developed by Dvir et al. [19], where they dispersed gold nanoparticles in alginate. They found this patch to be highly viable as it closely mimicked the heart's contractions, systolic and diastolic pressures. It was also found that gold nanoparticles improved electrical communication between adjacent cells, therefore enhancing cell adhesion and proliferation [19].

Injectables

All of the above-mentioned approaches to treating CVD require invasive surgery in one way or the other. A lot of research is being conducted on coming up with techniques and methods to approach CVD without invasive surgery. One such potential approach is using injectable materials. The material, usually in the form of a hydrogel, is directly injected into the myocardium where an infarction is present, as shown in Figure 6.

The injectable material is usually created by decellularizing the ECM. The injected material re-assembles to form a nanofibrous scaffold after being passed through the catheter. However, some of the main drawbacks of this approach is maintaining the shape of the scaffold and main-

taining its biodegradability rate. Nonetheless, a lot of research is being conducted to improve the synthesis of injectable materials as they have great potential to treat CVD.

One such injectable scaffold was developed by Wang et al. [20], where they used oliog[poly(ethylene glycol)fumarate] (OPF) as an injectable hydrogel embedded with mouse embryonic stem cells to treat myocardial infarction in a rat model [20].

In another study conducted by Paul et al. [21], they developed a graphene oxide nanosheet complexed with the human vascular endothelial growth factor to create a GelMA hydrogel capable of being injected into cardiac tissue to help retain the required mechanical strength for optimal function [21].

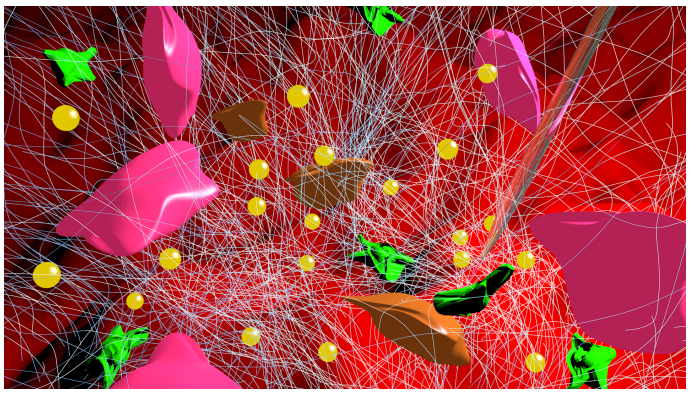


FIGURE 6. Injectable scaffold.

Conclusion

In conclusion, the aforementioned methods (see Table 1) show great promise in improving the condition of a patient after the diagnosis of a CVD, however, the occurrence of cardiomyopathy, the damage of the muscle tissue, is not addressed in any of the current methods used for treatment. Cardiomyopathy is one of the primary reasons why CVD has become a chronic disease as the recurrence of a heart attack is really high due to the presence of a weak muscular system of the heart where the cardiomyocytes bear the extra load and need more energy to beat in unison.

On the other hand, cardiovascular tissue engineering jumps right into the process of healing the scar tissue. The cardiovascular patch proposed in this research will be placed directly on the scar tissue and the patch will act as an extracellular matrix which will hold the cells and will give structural strength to the whole system. The cell will start to grow and the patch will slowly disintegrate as it is biodegradable.

Hence, it is strongly believed that cardiovascular tissue engineering has great potential in becoming the primary treatment for end-stage heart failure as it is much less complicated than the treatments currently available, and the biggest advantage is that it does not involve highly invasive surgery.

The advent of nanotechnology, the technology that involves the synthesis and applications of materials whose characteristics are in the nanometer (i.e. 10^{-9} meters) range, has opened a new domain for research for nano-biomaterials. So far, research has been conducted only on the characteristics of conventional materials in the nanoscale. Studies on biomaterials, which are the future of nanomedicine, are still in its infancy.

Nanomaterials, specifically nano-composites, are the future of material science research. The main reason being the enhanced material properties of nanomaterials compared to their bulk counterparts. Especially when it comes to electrical properties, nanomaterials possess a phenomenon known as ballistic conductance where the conductivity of the material is directly proportional to the applied voltage. Studies on gold nanowires have shown to drastically improve its conductivity as the thickness of the wire decreases to the nano range [22]. This enhanced electrical property is key to the biocompatibility as cells in the human body, especially cardiomyocytes, communicate with each other using electric signals.

Another interesting enhanced material property of nanomaterials is their mechanical properties. Studies have shown that this enhanced property is directly related to grain size and as we decrease the grain size of the material to the nano range, the newly formed material develops

TABLE 1. Modern Techniques Currently Used to Combat CVD

Type of Intervention	Advantage	Disadvantage
Thrombolytic Drugs	Swift response rate and ease of use.	High rate of recurrence of infarction. Damaged muscle is not targeted.
Antithrombin Drugs	Great for post attack recovery. Slightly inexpensive.	Any other external injury may lead to critical blood loss.
CABG	It is a surgical re-vascularization which is longer lasting. Effective even when aorta is severely damaged	Very expensive and complicated surgical procedure. Still does not target damaged muscle.
LVAD	Enables recovery of the patient even when the heart has almost completely failed.	Highly expensive device that the patient has to carry at all times. Limits movements to certain places.
Nanomaterials	Directly targets damaged infarct area. Doesn't require complicated surgery.	FDA approval for trials require a lot of time.

a new property known as superplasticity. This special property makes the material very strong and enhances its deformation properties compared to its bulk counterparts [22]. This property is leveraged and applied in designing composite materials where porous polymers are reinforced with nanoparticles to drastically enhance mechanical strength and electrical conductivity.

Research has found that the topography in the human body at the cellular level is in the nano range. Therefore it can be said that, if we use nanomaterials to repair damage to the heart muscles and cells, due to the nanotopography, the cells will adhere successfully and proliferation rates will be higher [23]. The added improved mechanical strength and electrical conductivity will further improve the healing rate. Hence, we can conclude by saying that using nanomaterials to develop tools to treat CVD is the right path to take.

Therefore the main purpose of this study is to analyze the material characteristics of the novel cardiovascular patch with aligned carbon nanotubes, compare its characteristics with other

cardiovascular patches, and see how the alignment of the carbon fiber influences the material characteristics and the biocompatibility of the patch. The main hypothesis of this study is that the alignment will strongly increase the mechanical strength of the patch and in turn increase its durability. To verify the hypothesis, the patch will be subjected to all types of mechanical loads and the strength of the patch will be found.

In Chapter 2, the concept of composite materials and their mechanical properties as well as different types of reinforcements will be discussed. Apart from this, a study on various composites with aligned versus random fiber dispersement will be conducted and their properties will be examined. Also, various techniques used to align these fibers will be elaborated.

In Chapter 3, the different experimental setups used in this project will be explained, beginning with the material synthesis procedure. The experimental setup for the electro-magnetic field generation and then the setup for an electric field generation will be thoroughly explained. The advantages and disadvantages of each method will also be discussed.

In Chapter 4, the results of the alignment experiments will be discussed. SEM images of the sample images will be displayed and discussed. Finally, the scope of future work and an overall summary of the thesis will be provided.

CHAPTER 2

COMPOSITE MATERIALS

Basic Definition

Any material that is a result of the combination of two or more physically and chemically distinct materials, where the combination of these materials leads to a significant increase in the properties of the resultant material, can be defined as a composite material. An illustration of different types of composite materials is shown in Figure 7. One of the main advantages of a composite is that, upon appropriate design, the best of the properties of the base materials can be exhibited and sometimes the composite can have the property that neither of its constituents possess. Below are some of the properties that can be improved by synthesizing a composite [24]:

Strength: One of the major properties that are enhanced by synthesizing a composite material is its specific strength. Specific strength is defined by the strength to weight ratio. In other words, composite materials have very high strength, but, weigh much less compared to conventional material. Therefore, due to this high strength of composites, it has become commonplace in the aerospace industry. This enhancement also protects the material from wear resistance, fatigue and improves the stiffness.

Thermal conductivity: One of the most commonly used composites are the fiber reinforced polymer composites. Polymers are not thermally conductive, however, reinforcing them with thermally conductive fibers helps the composite material to become conductive. This is mainly applied to sophisticated electronic devices that require complicated thermal management systems for Micro-Electro-Mechanical Systems (MEMS) and also heat sinks and heat removal applications.

Electrical conductivity: Fiber reinforced polymer composites have major applications in the aerospace industry. However, one major drawback of using polymers is that they are very poor conductors of electricity. Therefore, these polymers are reinforced with conductive fibers, mainly,

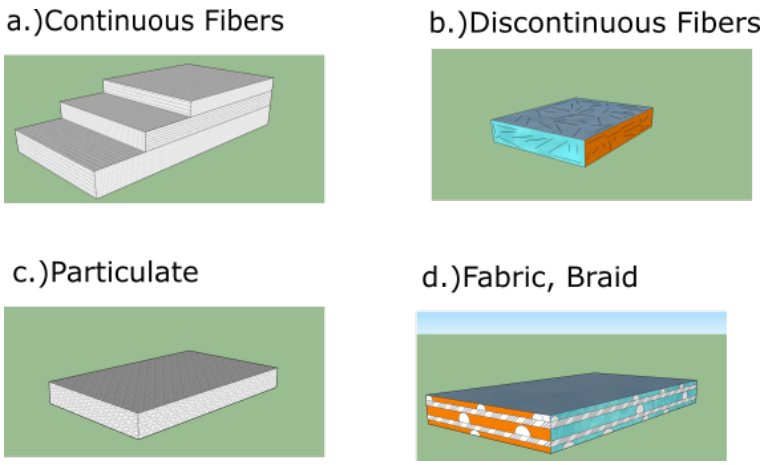


FIGURE 7. Different types of composite materials.

carbon fiber to increase the conductivity of the material. As this is used in the aerospace industry, this increased conductivity also protects the aircraft and spacecraft from lightning strikes and electromagnetic field (EMF) shielding.

Therefore, due to the above-mentioned enhancement in material properties, composite materials are in the limelight of material science research. Novel composite materials are being developed for biomedical purposes because of their unique behavior.

Classification and Characteristics of Composite Materials

Fibrous Composites

Fibers are physical entities that have a very high length to diameter ratio. A lot of research has shown that fibers highly enhance the mechanical strength of the composite. For example, ordinary plate glass fracture at around 20 Mpa, however, glass fibers have strengths of around 2800 Mpa to 4800 Mpa. The main reason for this is that the fiber has a more perfect structure, where the crystals are aligned along the fiber. But, in a bulk material, this is not the case and the random distribution of the crystals results in a large number of internal defects compared to individual fibers.

One of the most recent advances in the field of fibrous composites is the use of carbon com-

pounds i.e (carbon fiber and carbon tubes) as the fiber. Carbon fiber and carbon tubes have been known to have high aspect ratios and this empowers them to be one of the most strong fibers to be ever used in composites.

However, fibers are very weak by themselves when it comes to structural strength. A structural manifold needs to be added in between the fibers to bond them together, the element that is added to bond them together is called the *matrix*. The matrix can be metal, polymers, ceramics or carbon. Typically the matrix is very weak and has low stiffness and strength, but, when bonded with the fibers, there will be a significant increase in the stiffness and the strength of the composite and will have a lesser density.

Laminated Composite Materials

This is a type of composite material where multiple laminates (layers) of two or more metals are bonded together as shown in Figure 8 to obtain a significantly stronger material. The properties that can be improved by lamination are stiffness, strength, weight, and corrosion resistance. Some of the applications of laminated composites are listed below,

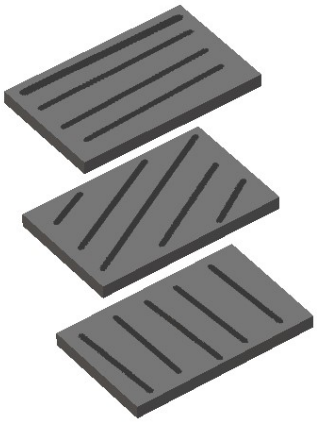


FIGURE 8. Composite laminates.

1. *Bi-metals*: As the name suggests, it consists of two strips of metals bonded together. This is

mainly used as thermostats.

2. *Clad metals*: A metal is clad or sheathed with another metal to enhance the properties of both the metals. For example, pure aluminum and some aluminum alloys are very corrosion resistant but very weak. So, high-strength aluminum alloys are claded with pure or corrosion-resistant aluminum to obtain a corrosion resistant with high strength.
3. *Laminated glass*: The above concept of clad metals is taken a level further to automotive safety glass. Two sheets of glass are taken and a high strength, a ductile polymer called, polyvinyl butyl is sandwiched between the two sheets of glass. This process makes the brittle glass more ductile and the glass protects the polymer from scratches hence creating a far more enhanced material.

Particulate Composites

This type of composite is designed by suspending particle of one type of material (metal/non-metal) in the matrix of another material. The four different types of particulate composites are,

1. *Nonmetallic particles in nonmetallic matrix*: The best example of this type of composite would be concrete. Gravel and sand particles are embedded in a cement and water mixture. The cement mixture acts as the matrix and the gravel and sand act as reinforcement, therefore, making it a composite material.
2. *Metallic particles in nonmetallic matrix*: The best example to illustrate this type of composite is solid-rocket propellants. Inorganic particles like aluminum are dispersed in organic binders like polyurethane. This composite form of propellant provides steady burning with uniform and controlled thrust, which is crucial for a rocket's trajectory.
3. *Metallic particles in metallic matrix*: These composites are different from alloys in a way where they do not dissolve in the matrix. One major application of this type of composite is to improve the machinability. Brittle material particles are added to a ductile material matrix to be able to improve the behavior of the ductile material at higher temperature. This is

process is called liquid sintering.

4. *Nonmetallic particles in metallic matrix*: In this type of matrix, ceramics are suspended in a metallic matrix. These materials are called cermets. They usually contain oxide or carbide based particles in a metal or a metal oxide matrix. These type of materials are used in nuclear reactors as control rods and fuel elements.

Fiber Alignment and Influence on Structural Properties

The structural properties of a composite depend on the respective properties of its constituents. The basic structural mechanism of a composite is that the load applied is transferred from the matrix to the fiber. Since fiber is the reinforcement, its main function is to bear the load and prevent the composite from failing, even if the matrix fails. Therefore, fiber orientation plays a crucial role in optimizing the overall strength of the composite.

In one such study conducted by Chand and Dwivedi [25], the influence of fiber orientation on high-stress wear in an epoxy composite was tested. The composite was reinforced with SASI fibers and three different fiber orientations were prepared and wear resistance was checked by sliding the composites on an abrasive surface. It was found that when the fibers were orientated normal to the sliding surface, the composite had maximum wear resistance and it was minimum when the fibers were parallel to the sliding direction [25].

Another study conducted by Jou et al. [26], focused on the influence of fiber orientation on the electromagnetic shielding of Liquid Crystal Polymers (LCP). The LCP and Nylon66 were first melted and carbon fiber was added to the melted mixture to avoid the breaking of the fibers. To obtain parallel alignment of the fibers, the carbon fibers and the LCP-nylon composite were manufactured by injection molding. The carbon fibers oriented themselves according to the flow direction during the injection molding process. Different orientation and fiber ratios were tested. It was found that, by aligning the fibers, fewer carbon fibers can be incorporated in the composite and the shielding effectiveness can be enhanced significantly by aligning the fibers parallel to the

field. This can also save the cost as the fiber content can be reduced [26].

Godara and Raabe [27] studied the influence of fiber orientation on the global mechanical behavior of advanced composites. In this study, an epoxy resin composite reinforced with 35 wt.% aligned borosilicate glass fibers. In this study, the material was tensile tested and the surface displacement was measured using digital image correlation. This study showed significant effects on material behavior due to fiber orientation [27].

Therefore, from the above studies, it is evident that fiber orientation thus has a huge impact on material properties on a large scale. Hence it can be hypothesized that aligning the carbon nanotubes in the PLGA matrix will greatly enhance the mechanical properties and the biocompatibility of the cardiovascular patch.

Different Methods Used to Align Fibers in a Matrix

Therefore, as discussed earlier, it is evident that aligning the fiber has great potential in enhancing the material characteristics. Below are a few methods researchers have used [28].

Ex Situ Alignment

In *Ex Situ* processes the fibers are aligned in advance, and then they are compounded into the polymer via *In Situ* Polymerization. Some methods used to align fiber *Ex Situ* are explained below.

1. *Filtration*: In this process, the fibers are drawn through a fine mesh along their lengths so that they can align parallel to each other and then these fibers are transferred onto the polymer surface. Another way they can be aligned is, while filtering through a membrane, the fibers are subjected to a strong electro-magnetic field [29].
2. *Plasma enhanced chemical vapor deposition*: Studies have been conducted where Carbon nanotubes were aligned by shooting a high power direct current generated plasma-enhanced chemical vapor deposition. Here microwave plasma was used to align the CNTs [30].

3. *Template*: This process can be compared to a die-casting process where a template is made using iron-nano particles and the CNT's are embedded in these templates to be aligned [31].

Some of the advantages of this type of alignment procedure are that fiber alignment can be controlled precisely, due to the fact that the fibers are aligned before they are embedded in the polymer. Depending on the design requirement, the fibers can be pre-aligned very accurately. However, one major drawback is that the procedure to perform ex-situ alignment is very complicated and requires sophisticated experimental setups.

Force Field Induced Alignment

This is a process where, as the name suggests, an external force is applied to the fibers so that the fibers are aligned in the desired orientation. For example, Vigolo et al. [32] dispersed single-walled carbon nanotubes (SWCNTs) by slowly injecting them into a Polyvinyl Alcohol (PVA) solution matrix with the help of a syringe needle [32]. Since the PVA solution is a highly viscous solution, the shear contribution during the flow maintains the alignment of the SWCNTs in the PVA solution. The above results can be obtained by even melt extruding processes [33]. Even though this seems pretty straightforward and easy, the fibers might get severely damaged due to the shear forces. This damage can further weaken the composite material.

Magnetic Field Induced Alignment

Research has shown that aligning the fibers with a magnetic field increases the electrical conductivity and the mechanical anisotropy of the composite significantly. The basic process is that the fibers are incorporated in the matrix and then the solution is introduced to a electromagnetic field. After it is exposed to the field for a certain amount of time, the solution is then polymerized so as to freeze the fibers in place to maintain the fiber orientation. Kimura et al. [34] dispersed multi-walled carbon nanotubes (MWCNTs) in a monomer solution and exposed it to a constant magnetic field of 10 Tesla to obtain aligned nanotubes [34]. Magnetic field induced

alignment is one of the most commonly used procedures due to its simplicity, however, due to the requirements of a high magnetic field it is optimal for use in only an industrial setup.

Electro-Spinning Induced Alignment

Electrospinning is a fiber production process where a high voltage direct-current is used to draw charged threads of polymer fibers by melting them. The process is illustrated in Figure 9. This process usually generates fibers in the magnitude of a hundred nanometers. In recent processes, a high direct current voltage is generated between a negatively charged polymer fluid and a metallic fiber collector. This produces induced alignment of CNTs [35]. The main advantage if this process is that it yields a highly accurate aligned fiber roll. However, this process is limited to the production of polymer fibers only. To produce any other type of fiber would require a very complicated setup.

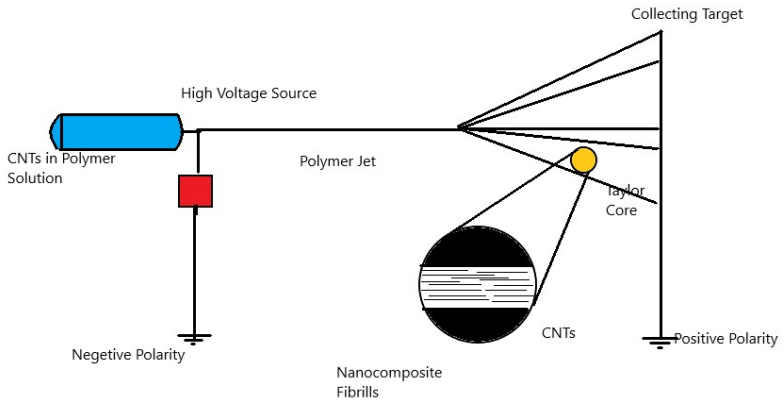


FIGURE 9. Electro-spinning.

Liquid Crystalline Phase Induced Alignment

This method is mainly used to align fibers in a liquid crystal polymer. Due to their unique molecular structure, they are easy to orient along the applied field such as force, electric and magnetic fields. In simple words, this is the exact opposite of the alignment of fibers as here the ma-

trix is aligned using an external force and the fiber are incorporated later into an already aligned matrix to in turn align the fibers [36]. One major drawback of this process is its lack of ability to produce composite materials with polymer matrices. Due to the inability of polymers to conduct electricity, it is impossible to align them using this method.

Methods Used in This Research

From the above-explained methods, a magnetic field induced alignment approach and an Electric field induced alignment approach was used for this project. These particular approaches were selected based on their ease of being conducted in a laboratory environment.

Electro-Magnetic Field Induced Alignment Approach

In this research, the magnetic field was generated by using a ferrofluid and an electric current. The ferrofluid was encased in a circular duct and an alternating voltage source was given as input to both the coils. The setup was similar to that of the study conducted by Krauss et al. [37], where they studied the pumping stresses on the ferrofluid due to the electric field.

However, this approach failed to align the carbon nanotubes in our composite as the voltage was very low and because of this, a strong magnetic field could not be generated. This was one of the main drawbacks of using a magnetic field to align the fibers. Studies have shown that a magnetic field of 10 Teslas or higher is required to align the fibers and it is almost impossible to generate such a high magnetic field in a simple laboratory set up.

Electric Field Induced Alignment

Due to the failure of the previous approach, electric field induced alignment method was finally selected to be used in this study. To generate the electric field, a set up which consisted of fifteen interdigited parallel electrodes were used. An alternating current with an input voltage of 15V was fed through the electrodes.

In conclusion, from the previously discussed studies, it is evident that fiber alignment does improve the electrical and mechanical properties of the composite material and the alignment

procedure is crucial to help develop our nano-composite with enhanced mechanical strength, electrical conductivity and biocompatibility.

CHAPTER 3

EXPERIMENTAL SETUP

Experimental Setup for Material Synthesis

A composite material has two main components, the matrix, and the reinforcement. Therefore, the composite synthesized in this research project consists of PLGA as the matrix and single-walled carbon nanotubes (SWCNTs) as the reinforcement. PLGA is chosen here due to its strong biocompatibility. One other key factor is that, even though PLGA is a polymer, it has conductive behavior, which is important as it is being used for cardiovascular applications. However, PLGA is structurally very weak, therefore, SWCNTs were chosen as reinforcement due to their high strength to weight ratio and their cytocompatibility. The added SWCNTs further enhance not only the structural strength but also help in increasing the conductivity of the composite.

The process used to synthesize this composite is known as drop casting. Drop casting is widely used for the synthesis of composite for biological applications. The materials required and their respective roles in the process are explained in Table 2.

TABLE 2. Materials List for Composite Material Synthesis

No.	Material	Purpose
1	PLGA	The polymer matrix of the composite.
2	SWCNTs	The reinforcement of the composite.
3	Chloroform	Solvent used for even dispersement of CNTs.
4	Tetrahydrofuran	Solvent used to disperse PLGA.
5	Weigh Boats	Used to weigh the components.
6	Stirring Rods	used for proper preparation of the solutions.
7	50ml Vials	To prepare solutions of the matrix and reinforcements.
8	Disposable glass pipettes	For appropriate mixing of the components.

Procedure

The following sequence of steps were followed:

1. In a vial, 30 ml of Chloroform was taken and 250mg of SWCNTs were weighed using weigh boats and were mixed to form a solution and were sonicated for 60mins.

2. In a separate vial, 20ml of THF was taken and 500mg of PLGA was weighed in a weigh boat and was added to form a solution and was sonicated for 60 mins.
3. After the sonication of both the solutions, another vial was taken and a 50% weight ratio was measured and both the solutions were mixed into the third vial accordingly. The composite material solutions were then again sonicated for 60 mins.
4. After sonication, a disposable pipette was used and the material was drop cast onto 22mm diameter coverslips as shown in Figure 10.
5. The coverslips were then placed in desired electric/magnetic field for alignment purposes for a certain amount of time.
6. And finally, the material sample was cured in an oven at 36° C for 15 mins and vacuum dried further for 48 hrs.

FIGURE 10. CNT-PLGA cardiovascular patch.

Experimental Setup for Magnetic Field Induced Alignment

This part can be divided into 3 subsections i.e., the design of the circular duct, synthesis of the fluid and the final experiment.

Design of the Circular Duct

A circular duct with a mean diameter of 100mm with a 5mm x 5mm square cross-section was modeled in SolidWorks. The assembly was then converted to a .stl file and was 3D printed using a ProJet 3600 3D printer. For the ease of 3D printing, a base and legs were also designed for the circular duct.

Synthesis of Ferro-Fluid

The suspension of magnetic nanoparticles in a colloidal solution is known as a ferrofluid. The fluid for our experiment were synthesized using $FeCl_2$ & $FeCl_3$ nanoparticles. Due to their quick response to magnetic fields, ferrofluids' movement can be controlled using a magnetic field. The mixture of Fe(II) and Fe(III) salts in a basic solution, yields to the formation of Fe_2O_3 nanoparticles. A surfactant is then added to the particles to stop them from agglomerating with each other in the liquid medium to which they are added to. The materials required to perform this experiment are listed in the Table 3.

Procedure

The following sequence of steps was followed,

1. 4ml of 1M $FeCl_3$ solution and 2M $FeCl_2$ solution were combined in a 100ml beaker and stirring was initiated using a magnetic stirrer.
2. As the solution was being stirred, 50 ml of 1M aqueous NH_3 was added over a period of 5 minutes, with the help of a dropper. A black precipitate (Magnetite) was formed at the end of this step.
3. Stirring was stopped and the magnetic stirrer was pulled out by using a magnet. The precipitate was allowed to settle for some time.
4. After the magnetite had settled, the remaining fluid was decanted and disposed of.
5. The magnetite was then transferred to a weigh boat with the help of a squirt bottle.
6. The ferrofluid was held in its place with the help of a magnet and was rinsed a couple of times before discarding the excess liquid.

TABLE 3. List of Equipment for Ferro-Fluid Synthesis

No.	Material	Purpose
1	2 M $FeCl_2$ in 2 M HCl	The Fe(II) slat required for the synthesis of the Nanoparticles.
2	1 M $FeCl_3$ in 2 M HCl	The Fe(III) slat required for the synthesis of the Nanoparticles.
3	1 M NH_3 in water.	Basic solution required to dissolve the ferrous salts.
4	25% tetramethyleammonium hydroxide in water	Surfactant used to stabilize the ferro-fluid.
5	Beaker, 100ml	Used for making the ferrous salt solution
6	Plastic weighing boats	Used for weighing the components.
7	Dropper	Used to slowly add the basic solution to the salts.
8	Glass stirring rod	For appropriate mixing of the components.
9	Magnetic stirrer	For appropriate mixing of the components.
10	Strong magnets	To separate ferrous nano particles from the salt solution.

7. After all the unnecessary liquid was discarded, 1-2ml of 25% tetramethylammonium hydroxide was added to the precipitate and stirred continuously until spikes appeared when a magnet was held underneath.
8. The ferrofluid thus synthesized, was then stored in a glass vial for further use.
9. Figures 11a through 11f illustrate the experimental procedure.

Final Experimental Setup

An illustration of the experimental setup is represented by a CAD model in Figure 12. The circular duct was filled with ferrofluid up to the brim.The primary copper wire was wound around the fluid channel which will provide the component in the azimuthal direction and another set of

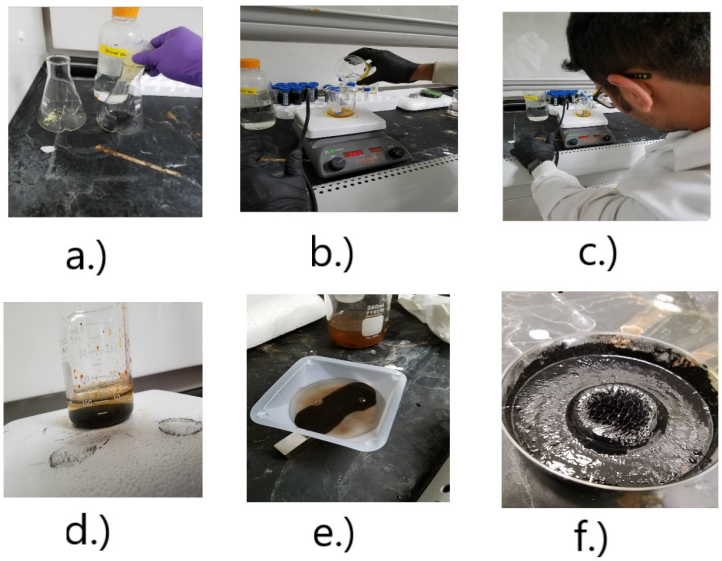


FIGURE 11. Ferro-Fluid synthesis procedure.

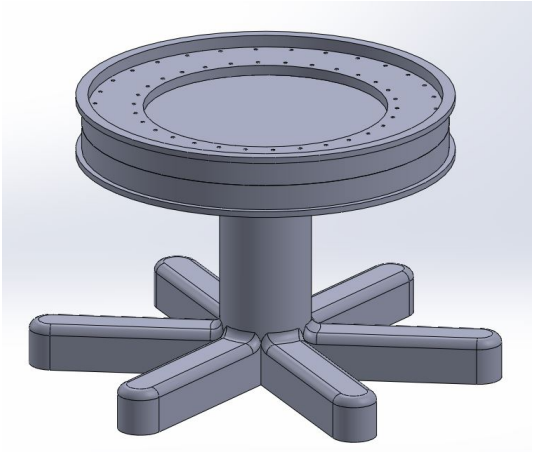


FIGURE 12. CAD model of the electro-magnetic setup.

copper wire, considered to be the secondary coil was lopped around the whole duct, providing the vertical component of the field. Figure 13 shows the actual setup that was constructed for the experiment.

Experimental Setup for Electric Field Alignment

The electric field alignment approach is quite simple and not as complicated as the magnetic field approach. It is very straightforward and less time-consuming. The setup is illustrated in Fig-

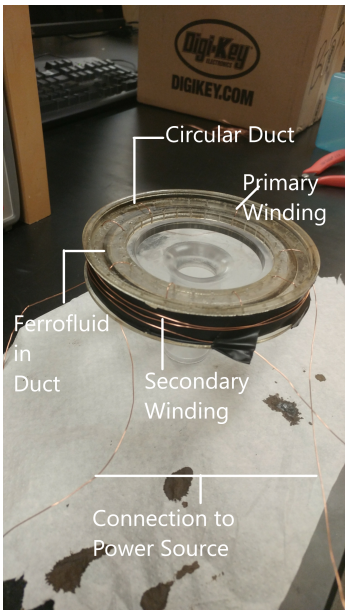


FIGURE 13. Setup for electro-magnetic field generation.

ure 14. The materials required for the experimental setup are displayed in Table 4.

TABLE 4. List of Equipment for Electric-Field Setup

No.	Material	Purpose
1	6x6x2 inch PMMA sheet	The base for placing all the components.
2	Copper wire	To run voltage across the circuit.
3	Gorilla glass plate	To protect the composite from touching the wires.
4	Breadboard	To make connections in the circuit.
5	Function generator	To supply the AC voltage.

Procedure

The following procedure was followed to setup the experiment:

1. Using the process of milling, 15 parallel grooves were milled on the face of the PMMA sheet to hold the copper wire electrodes.
2. Copper wires were placed in the grooves and were alternately connected to source and ground to create an array of interdigitated parallel electrodes.
3. The gorilla glass plate was placed over the wires so that the composite materials would not

touch the wires directly.

4. An input voltage of 15V was given across the electrodes.
5. The composite material samples were then placed on the glass plate so that it is in the electric field.
6. For successful alignment, the sample was placed on the sheet, in the field, for 24 hrs.

After the material was removed from the field, it was cured in an oven at 36° C, and then was imaged using SEM to confirm fiber alignment. The illustration is shown in Figure 14.

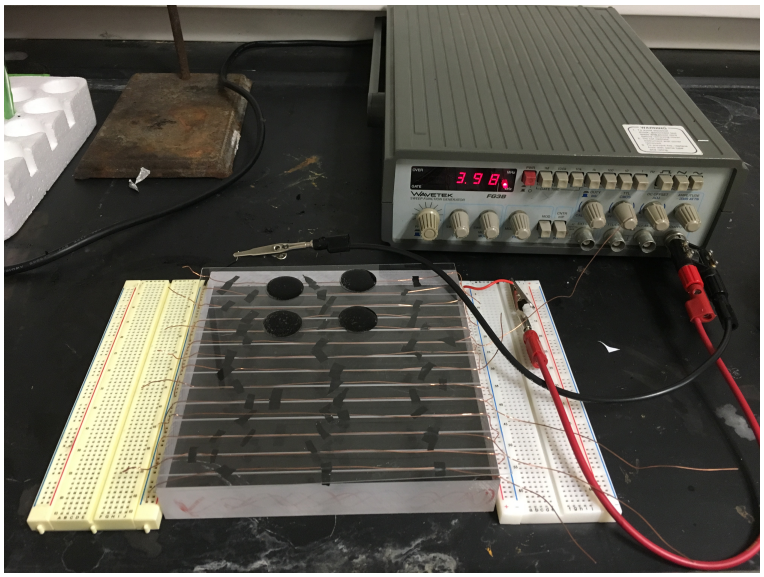


FIGURE 14. Parallel electrodes setup.

Experimental Setup for Electric Field Induced Alignment with Amplified Voltage

Due to the limitations of an academic function generator, the maximum output voltage that was supplied to the circuit was only 7 volts. Previous research has shown that at least voltage at a scale of around 20V was used to align the reinforcement. Hence there came a requirement for amplifying the voltage. The setup is shown in the Figure 15.

The same setup as the previous section was used, however, before the output voltage was sent into the circuit, it was sent into an operational amplifier (OP Amp), which was powered using a 25V DC power supply, which helped amplify the A.C voltage by using the gain from the

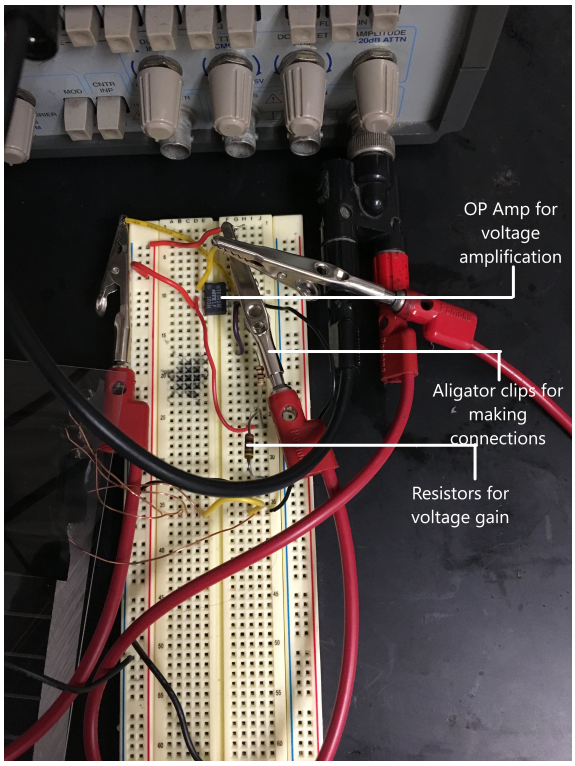


FIGURE 15. Amplified parallel electrode setup.

resistors that were connected to it.

Conclusion

The experimental setups for both the electromagnetic field and the electric field generation were successfully designed. All the required materials were brought in and some particular elements were fabricated. The circular duct for the ferrofluid setup was successfully 3D printed, circuit connections were made and the experiment was successfully conducted.

The required parallel electrode setup for the electric field generation was fabricated. All the required connections were made and the experiment was done and results were noted. For a higher voltage, a circuit with voltage gain was designed using an OP Amp and was successfully implemented. The results from all the setups are discussed in the next chapter.

CHAPTER 4

RESULTS AND ANALYSIS

In the previous chapter, all the experimental setups used for the project were discussed clearly. In this chapter we will present the results of the experiments in detail. Complete material characterization will be discussed. Young's modulus of the material will be calculated. Results from the conductivity tests will be shown and results from the Live/Dead cell culture assay will be discussed to show the biocompatibility of the patch. Future work and some of the lessons learned from the various experimental setups will be further discussed in this chapter. Finally, the properties of this proposed composite will be compared with previously developed patches and the significance of the patch synthesized in this project will be discussed.

Results from the Circular Duct Experiment

A CAD model of the Circular duct was created and then 3D printed using a Projet 6000 3D printer. The circuit was setup and a Voltage was supplied with the help of a function generator.

Unfortunately, there was no movement in the ferrofluid and we were unable to generate the required amount of electromagnetic field to provide sufficient torque to the ferrofluid. Therefore, a decision was made to move on from this setup to the parallel electrode setup, which looked far simpler and promising on paper.

A reason why this method did not work may be due to the fact that, we might have needed another function generator to provide a second phase of the A.C. voltage to help give more torque to the fluid and generate the sufficient amount of field.

Results from the Parallel Wire Setup

Four parallel copper wires were inserted into the slots in the Poly(methyle methacrylate) PMMA sheet. One end of the wires was grounded and a voltage was supplied to the other end with the help of a function generator. Since the function generator used was academic, the maximum peak-to-peak voltage achievable was around 7 volts. Due to the inability of the function

generator to reach a higher voltage, the OP amp circuit showed in figure 15 was used to amplify the voltage to 50 volts.

The setup was placed in the chemical hood and a gorilla glass plate was placed on the wires to act as a barrier to the material. The synthesized biomaterial was drop cast on the coverslips and then placed on the glass above the wire so that the material is in the electric field.

The material was left in the field for 24 hrs and then vacuum dried and cured in an oven at 36° C for 15 mins. The samples prepared were then viewed using a Scanning Electron Microscope (SEM) to check for alignment. The SEM image is shown in Figure 16.

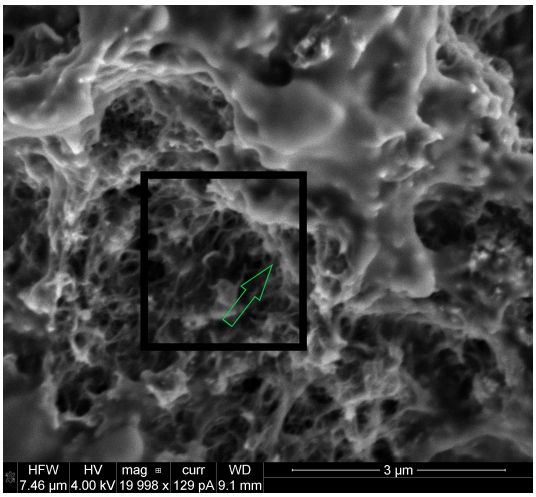


FIGURE 16. Biomaterial sample with aligned CNTs.

From Figure 16, it can be clearly seen in the highlighted box that there is a significant amount of alignment in the CNTs. The CNTs are also evenly dispersed in the PLGA matrix. This can be compared with Figure 17, which, is the SEM image of a sample that was not placed in the electric field. There is a uniformity in the dispersion of the CNT in the matrix, however, they are dispersed randomly. This shows that the electric field does influence the fiber alignment. This is mainly due to the conductivity of the CNTs and their high susceptibility. This susceptibility of CNTs is crucial when it comes to their reaction to electric and magnetic fields.

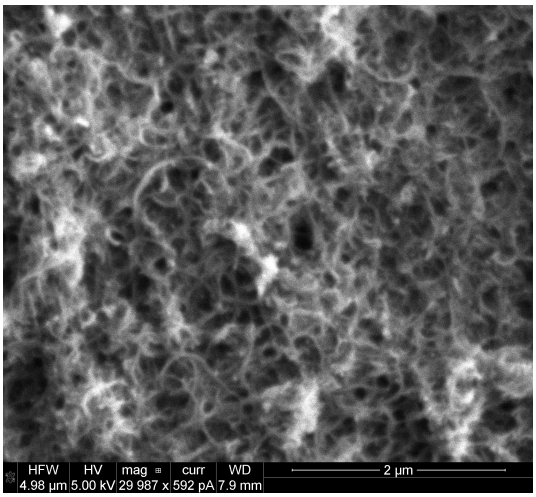


FIGURE 17. Biomaterial sample with randomly dispersed reinforcement.

Material Characterization

In this section, the material will be characterized with respect to its strength, material conductivity, and bio-compatibility tests. For strength, its Young's modulus will be calculated. A 4-probe conductivity test will be performed to deduce its conductivity and finally, a Live/Dead cell culture assay will be conducted and results will be displayed.

Young's Modulus Calculation

From the previous section, it is evident that the CNTs have been dispersed evenly in the composite polymer matrix and the following properties can be pointed out,

1. The fibers are uniformly distributed throughout the matrix.
2. There is perfect bonding between the fibers and the matrix.
3. Applied loads are either parallel or normal to the fibers.
4. There are no stress states in the material
5. Both PLGA and CNTs are linearly elastic materials.

Therefore, the rule of mixtures approach can be used to calculate the Young's Modulus of the material. Due to the complexity of the synthesis of the biomaterial, it is often difficult to pro-

duce an American Society of Testing and Materials (ASTM) standard test samples for tensile testing and the rule of mixtures approach has to be taken into consideration.

The Rule of Mixtures formula for a composite material is given by,

$$E_c = V_f E_f + (1 - V_f) E_m \tag{4.1}$$

where E_c is the Young's Modulus of the composite material, V_f is the fiber volume fraction and E_m is the Young's Modulus of the matrix. Therefore for our project the Young's Modulus of PLGA and CNTs are given by,

$$E_m = 1.3 \times 10^{-7} Mpa$$

$$E_f = 94 \times 10^{4} Mpa$$

$$V_f = 0.5$$

The V_f was taken as 0.5 as the ratio of filler to the matrix of our composite was chosen as 50:50.

Substituting the above values in equation 4.1 we get,

$$E_c = 4.7 \times 10^{5} Mpa$$

Therefore Young's modulus of our designed cardiovascular patch was calculated to be $4.7 \times 10^{5} MPa$.

Results from Conductivity Tests

The 4 probe sensing conductivity testing technique was used to measure the conductivity of the composite material synthesized in this project. Due to the unavailability of a 4 probe sensing source meter, a device was built in-house using a function generator and a hand-held multimeter.

The material sample was taken and 4 points were marked equidistant from each other. A constant current was supplied through the extreme endpoints using a function generator. A multimeter was taken and the voltage was measured through the inner two points. The circuit of the device is shown in Figure 18.

$$\rho = \frac{V \times 2 \times \pi \times S}{I} \tag{4.2}$$

The relationship showed in Equation 4.2 was then used to calculate the resistivity of the material sample, where, ρ is the resistivity, V is the measured voltage, I is the constant current and S is the distance between the probes/points. The required values were plugged into the equation and the resistivity of the material was found. The inverse of that resistivity gives us the conductivity of the material, which was calculated to be $8 \times 10^{-1} Sm^{-1}$. Research has shown that the conductivity of the native myocardium is around $1.6 \times 10^{-1} Sm^{-1}$ [23], which is less than the conductivity of the material developed in this project. Therefore as anticipated, the CNT alignment has significantly increased the conductivity.

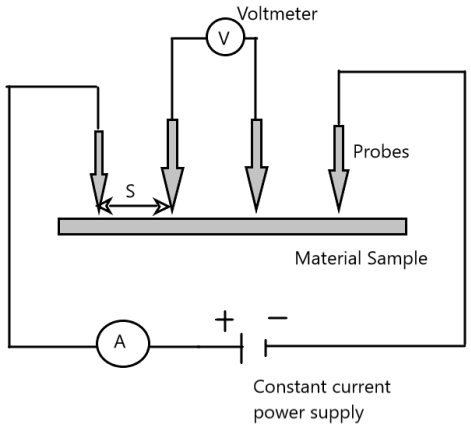
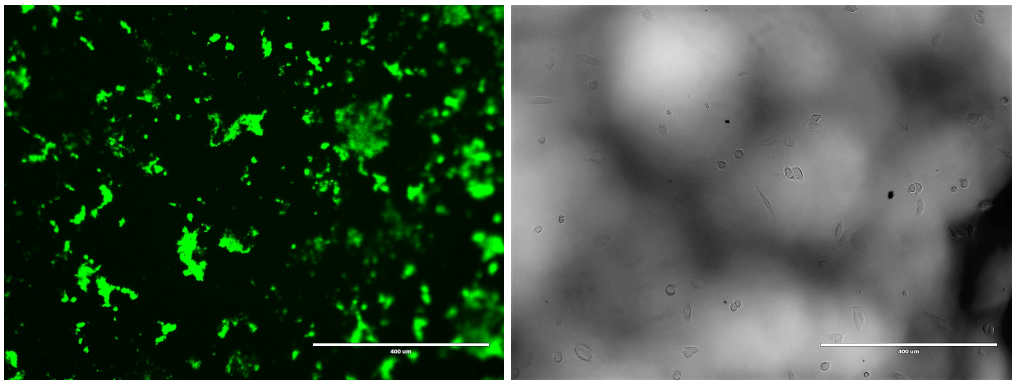


FIGURE 18. Four probe conductivity measurement circuit.

Results from Cell Culture Assays

A 24hr live/dead cell culture assay was performed on the samples to test their cell viabil-

ity and compatibility. A cell solution, with a concentration of 32,000 cells/ml was made and the composite was seeded with 1 ml of the cell solution with the addition of 5ml of cell media and was left in the incubator for 24 hrs. After 24 hrs, the cells were stained with calcien AM and ethidium homodimer-1 to evaluate live and dead cells. After coating with the aforementioned dyes, the cells were again incubated for 30 mins. The cells were then imaged using fluorescence microscopy as shown in Figure 19.



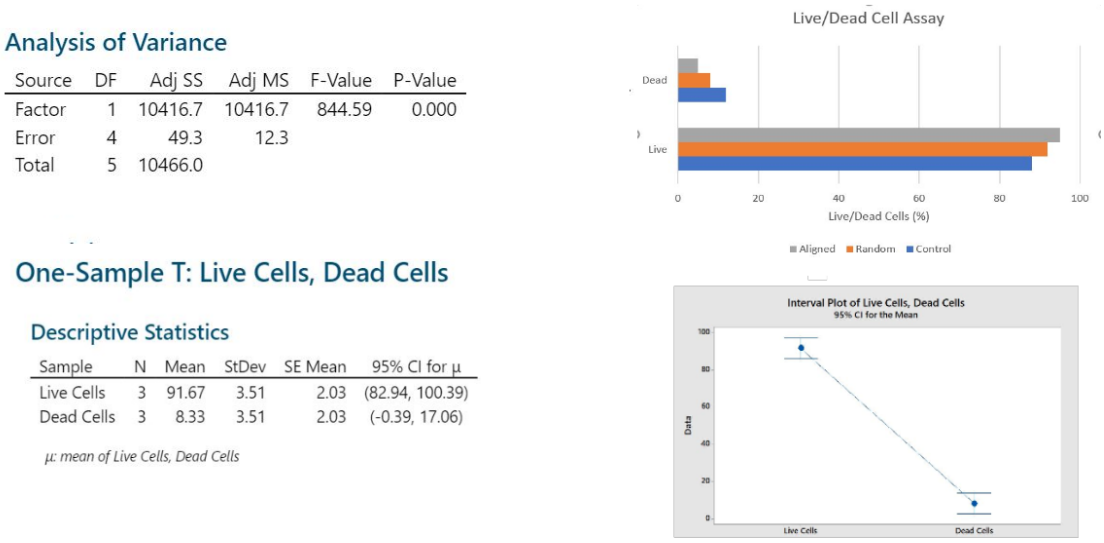
(a) Image of live cells. **(b) Transmission mode of microscope.**

FIGURE 19. Fluorescence microscopy images of cells.

From Figure 19a, it can be clearly seen from the presence of green color spots in the image, that the cells are alive. It also signifies that the cells managed to survive over a period of 24 hrs successfully. Also, the image clearly shows that the cells have successfully attached themselves to composite. Therefore, it can be said that the composite has a good topography for the cells to attach and survive. A quantitative analysis was performed and it was found that, from the 32,000 cells that were seeded on the composite material with aligned CNTs, the number of cells that were alive after 24hrs was found to be 30,400 cells, signifying a viability of 95%. Compared to this, the number of live cells on the sample with randomly distributed fibers was found to be 29,440, therefore signifying a viability of 92%. The control sample with plain PLGA had 28,160 live cells, therefore signifying a viability of 88%.

Figure 19b was taken through the transmission mode of the fluorescence microscope. This

is an image of the cells, seen through the material. The structure of the cells can be clearly seen and this solidifies the fact that the cells have successfully attached themselves to the material. Also, from the statistical data in Figure 20, it can be clearly seen that the composite material with aligned reinforcement has a significantly higher viability and lesser dead cells in comparison with the control and random fibers sample. The comparison is significant as both, the student t-test and the Analysis of Variances (ANOVA) tests were performed for a 95% confidence and interval and the p value was found to be significantly less than 0.05, therefore, validating the comparison.



Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	10416.7	10416.7	844.59	0.000
Error	4	49.3	12.3		
Total	5	10466.0			

One-Sample T: Live Cells, Dead Cells

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean	95% CI for μ
Live Cells	3	91.67	3.51	2.03	(82.94, 100.39)
Dead Cells	3	8.33	3.51	2.03	(-0.39, 17.06)

μ: mean of Live Cells, Dead Cells

FIGURE 20. Statistical analysis of the live/dead cell assay.

Overall Significance of the Project

The primary significant factor of the composite material thus designed is its property of anisotropy. Studies have shown that the muscles of the heart are highly anisotropic as they have different mechanical strength in different directions. Therefore, the composite material closely mimics the bio-environment, resulting in an increased bio-compatibility, which can be proved from the live/dead cell assays shown in Figure 19a. The material at a high viability of 95% was higher when compared to the patch with random fibers and the control sample with plain PLGA.

The second significant factor of this project is the design of the alignment procedure. The apparatus designed is simple and inexpensive due to the amplified circuit design. Unlike other setups, where they use expensive, high voltage power sources, this setup was successfully able to align the fibers by using a low voltage academic function generator, with the help of a simple op-amp setup for voltage amplification. Another aspect to point out is that, from Figure 14, it can be observed that more than one specimen can be synthesized at a given time, therefore, showcasing the ability of this setup to mass produce (manufacture) cardiovascular patches in the future.

Another significant factor of this project is the enhanced electrical conductivity of the composite material. The aligned CNTs will form an even network for electrons to communicate and the cardiomyocytes will be able to leverage this enhanced CNT network for better communication between adjacent cells, which will further aid in the rapid healing of the infarct area.

Scope for Future Work and Lessons Learned

Nanotechnology, specifically focusing on nano-composite and bio-materials has great scope in the future.

One major scope of improvement is to design the alignment fixture in such a way that it can output a higher voltage. Since CNTs have very high susceptibility, their alignment in an electric field is directly proportional to the voltage. The fixture in this project was able to output only 50V. This was sufficient since this was a nanocomposite. When it comes to bulk materials, a higher voltage has shown to do a better job.

Another aspect where there is scope for improvement for future research is to use multi-walled carbon nanotubes instead of SWCNTs. MWCNTs are proven to have higher electrical conductivity and mechanical strength compared to SWCNTs. Also, the addition of another dimension could give the cardiomyocytes an additional surface to adhere and grow in, therefore, greatly enhancing the biocompatibility of the biomaterial.

One major take-home lesson that this project has taught us is that aligning fibers in a poly-

mer matrix is difficult. It is even more difficult in this case due to the high filler ratio. Most research projects that developed aligned fiber composite materials have used filler ratios less than 1%. In our case, the filler ratio is 50%, which is significantly higher compared to previous studies.

This makes it worse in our case, as CNTs are highly unstable particles and tend to agglomerate in a solution. Therefore, making such a high concentration composite solution would result in the formation of Nanotube bundles, making them even harder to align to the field. Hence, a lower filler ratio and a higher voltage can be implemented in the future for better alignment.

Final Summary

Cardiovascular disease has always been a tough challenge that humanity has been trying to tackle for a long time. Scientists and doctors have put in tremendous efforts over the past two centuries in order to find the perfect cure for the disease. Various techniques have been developed that try to heal the damage through minimally invasive, though expensive, surgery. However, even with the availability of such sophisticated techniques, the recovery rate of patients is very low compared to the rate at which technologies are improving.

This research shows that the problem needs to be looked at from a whole new perspective and that being mainly of an engineer. An engineer would take the theoretical concept of that science and develop a practical tool that would help develop that science. Hence, the same approach can be used here to solve the problem of cardiovascular disease.

Therefore, in this project, the concept of composite materials was successfully implemented to develop a material that is not only structurally strong but also compatible with the cellular environment of the human body. One of the main challenges of any externally developed material is its compatibility. Even stem cells for that matter can be rejected by the body as it may detect it to be a foreign particle. Therefore, the challenge here was to mimic the bio-environment as close as possible to that of the human heart.

For the composite material developed in this project to be successful, it has to mimic the properties of the extracellular matrix (ECM), specifically, the ECM of a cardiomyocyte, as the material developed here is for cardiovascular applications.

One of the main characteristics of the ECM for the material to mimic is its porosity. The porosity is essential as it creates pathways in the scaffold to support the cells to grow and proliferate. This was achieved in the project by wisely choosing the polymer. PLGA, the polymer in the biomaterial, is highly porous. In addition to its porosity, PLGA is also FDA approved, making it an almost perfect polymer for tissue engineering applications.

The second most important factor for cells to successfully grow on the scaffold is its structural strength. Previously, scaffolds made from hydro-gels were extensively used in tissue engineering research due to its high biocompatibility, however, it lacked the structural strength needed to support the cells, as it would eventually break and the cells would fall off and die. This structural strength was provided to our scaffold by reinforcing PLGA with CNTs. CNTs are known to have extremely high Young's modulus due to its rod-like structure and nano-topography.

The third factor is the conductivity of the material. Since this scaffold is being used for cardiovascular applications, the conductivity of the material plays a huge role for the scaffold to be successful in its design as cardiomyocytes rely on a conductive path to beat in unison. This conductivity of the proposed scaffold comes from both the PLGA and CNTs. PLGA by itself is a slightly conductive polymer and this conductivity is further enhanced by the addition of CNT as a filler. CNTs are highly conductive as their nano-rod like structure helps in creating conductive paths allowing the successful flow of electron within its network.

The final factor which impacts cardiomyocyte survival on the scaffold designed in this project is its anisotropy. The myocardium is shown to be highly anisotropic in nature [23]. Therefore to successfully mimic the ECM, one of the main design characteristics which had to be considered is its anisotropy. This was achieved in our scaffold by aligning the CNT reinforcement in a par-

ticular direction with the help of an A.C voltage.

This research project was successfully able to design and synthesize a CNT reinforced polymer nano-composite cardiovascular tissue engineered with all the growth factors successfully implemented in the design.

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