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Study and characterization of conductive elastomers for biomedical applications

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STUDY AND CHARACTERIZATION OF CONDUCTIVE ELASTOMERS FOR BIOMEDICAL APPLICATIONS

By
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Dedication

I would like to dedicate this manuscript to my family.

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I would like to express my appreciation to Dr. Iman Noshadi for his guidance and help throughout this research. I would like to thank Vaishali Krishnados for her support in every minute of my journey. I would also like to thank my family for unwavering love and support through this endeavor.

Abstract

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Health issues have always been one of humankind's biggest challenges. Over the last century, there has been significant and monumental progress in health and biomedical science, with the end goal of alleviating and eliminating illnesses and ailments. For developing biomedical devices, polymers, and elastomers group among other types of biomaterials have been highlighted to be used due to high flexibility, stability, biocompatibility, and mechanical and rheological characteristics. In this work, the characterization of acrylated poly glycerol_sebacate (PGSA) polymer conjugated with bio ionic liquid (BIL) was investigated. Results showed high biocompatibility, high printability with tunable mechanical, adhesive, and conductivity properties. For example, without BIL, the conductivity is less than $0.05\times10-5\ S/m$, and it shows growth to more than $0.2\times10-5\ S/m$ for 60%PGSA/40% BIL. The swelling ratio is reported 70(%w/w) for 50% PGSA/50% BIL sample compared to 20% in the control sample. Biocompatibility of this composition in_vitro and in_vivo has been investigated by using C2C12 cell lines for in_vitro conditions and a rat animal model in_vivo. These results validate the biocompatibility of PGSA/BIL with 98% cell viability and its potential in cell adhesion, growth, and proliferation. The results show the potential of PGSA for various biomedical applications such as bioelectronics, sensors, and regenerative medicine.

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Chapter 1

Literature Review

1.1 Introduction

Health issues have always been the main priority for humans since an early age. Over the years, there have been various improvements in the field of medicine. Revolutionary methods and techniques have made allowed for quantum jumps in the field of medicine. Finding less invasive approaches to cure disease and aliments have led to people having more faith in modern medical methods. Various techniques and materials have been studied and developed in many laboratories all around the world. There are still many parameters to be considered to achieve ideal products. In this chapter, the role of bioelectronic devices, advanced biomaterials and biofabrication techniques in medical sciences are described.

Bioelectronic devices provide an interface of biology and electronics which has led to a new field over the past several generations. The developments in electrocardiography over the last 100 years represent big steps in the field of cardiology. Specifically, defibrillators that are used as implanted devices in the body to provide electrical pulses in failed hearts. The area of radiology is a prime example of the extensive presence of electronics in the medical field. Magnetic resonance imaging (MRI) captures images of the various soft tissues, while Computed tomography (CT) allows for three-dimensional visualization of areas of the body [1].

High health care costs have been sustained over the past ten years in the US. In 2008, the reported cost for domestic health care in the US alone was about $2.4 trillion and increased to $4.3 trillion in 2016. In that year, 1.4 million new cases of cancer were diagnosed. Additionally, the report shows 560,000 deaths from cancer alone. The National

Institutes of Health (NIH) reported the cost of these cases at nearly $90 billion for medical expenses and $130 billion in economic lost productivity. Reports on heart problems showed that almost 22 million Americans suffer from heart diseases and nearly 460,000 die from these problems annually, representing about 1 in 5 deaths. The direct cost of heart problems in the United States in 2008 was about $173 billion based upon NIH data. Furthermore, the American Diabetes Association reports that diabetes costs $174 billion each year, according to 2007 data [1]. These reports show large, diverse potential markets that can benefit from implantable bioelectronics to the well-being of society and individuals.

1.2 Bioelectronics

Electronics have had a significant impact on the study of biology and has led to vibrant activity in various research community recently [2]. Bioelectronics is a processing field at the conjunction of chemistry, biology, and physics. This relationship opened a huge area of research to develop materials and biomedical devices. The main feature of such bioelectronic devices is the monitoring of the interactions within biological networks and detecting the physiological signals by changing current, conductance and potential [1, 2]. The patch-clamp is an example of the necessity of bioelectronics, which is used for measuring the ionic current through single ion channels. As well as the electron microscope that made opportunities to image the minor parts of cells in detail [1, 2]. To produce high-quality bioelectronic devices with high performance, developing materials with desired properties is main challenge in this process.

1.2.1 Bioelectronics materials. A material platform for bioelectronics serves as the interface of biology and electronics and has been a sensational field for researchers in

numerous fields over the past decade. Bioelectronic devices have been developed to monitor physiological interactions and detect signals in the process of disease treatment. This platform brings biological species such as cells, antibodies, and proteins in interaction with electronic tools like transistors, resonators, and electrodes to realize the functionality of bioelectronic devices [3, 4, 5].

On the other hand, some mechanical properties of conventional electronics such as rigidity are in contrast with the soft nature of tissues and is a significant conflict and limitation in the field. To address this, materials like polymers and elastomers, applicable for both substrates and key components, have been highlighted to be used to develop high sensitivity and stability for a longer time in the related applications. Polymers are known as one of the best among existing materials with promising characteristics for use in bioelectronic devices. These characteristics make them a great choice as electrodes, substrate and optical transparency in the aspect of conductivity, and biodegradability in such devices [6].

Advantageous properties including flexibility, biocompatibility, stability, stretchability, and deformability are the main concerns of researchers to develop and satisfy various demands in this field of science [6, 7]. It has been opening vistas for sensor-based detection of diseases and help to treat them more beneficially than before.

1.3 Fabrication of Bioelectronics

Biofabrication is defined as the process of generating a composition of cells, biomaterials, and bioactive molecules, named bioink, as biological products via bioprinting [8]. Some biofabrication techniques including freeze-drying, particulate leaching and electrospinning conventionally were applied to generate 3D constructs but due to limited

versatility, reproducibility and the lack of control over the architecture during fabrication have been recently replaced with 3D bioprinting methods. 3D bioprinting proffers high reproducibility and significant control over the structure of the fabricated constructs through an automated process [8, 9]. Bioprinting has been developed by the possibility of pre-programming of the desired structure. Computer-aided design (CAD) patterns, based on 3D medical images such as computed tomography (CT), magnetic resonance imaging (MRI) and other techniques, can be created [3]. Using a mixture of targeted biomaterials and living cells as bioinks and synchronizing the mixtures with polymer crosslinking and motorized 3D printer movement, various methods of biofabrication and assembly can be developed and applied to a variety of tissue generation goals [8]. The versatility of this method helps to accelerate the application in biomedical sciences and tissue engineering, although in its primary stages [10]. The functionalization and development of bioink materials allow researchers to play with living cells and biological environments to provide complex and semi natural constructs [9].

Some basic features of an ideal bioink are printability, stability, insolubility, biodegradability, cell adhesion, non-toxicity, and non-immunogenicity. In addition, bioink materials should pose the favorable properties like chemical, rheological, mechanical, biological characteristics as well as easily processed and commercially available [7, 8]. Adjustments of these properties allow to create a proper environment for the generation of complex tissue constructs. Viability of cells during all steps of this process including cell culturing, proliferation, cell-cell interactions and cell-extracellular matrix (ECM) interactions can be considered as a remarkable challenge of this process [11].

Multiple bioprinting techniques have been developed recently to address the limitation of traditional methods. These techniques can be classified into some general categories, including extrusion-based, droplet-based, and laser-assisted bioprinting (LaBP).

Each of the modalities can support specific features. For instance, to avoid clogging in inkjet bioprinting, bioinks need to have a low viscosity. Alternatively, high viscosity bioinks can be supported in extrusion bioprinting [8, 11, 12]. Moreover, in multi-head deposition systems (MHDSs), there is the possibility of printing multiple materials. In all techniques, a computer-aided design system has been used to create a desired pattern for printing. The polymer in the bioinks as the main component is crosslinked during or after the printing to provide a final structure of tissue constructs [11, 12].

1.3.1 Bioinks materials and applications. Bioinks are known as cell-laden materials with the ability of accepting additional components as the main part of bioprinting process to generate tissue constructs. In bioprinting of three-dimensional tissue constructs and biomedical devices, two general classifications can be considered as natural-based and synthetic-based biomaterials. Also, bioinks can be multi-component, which is using more than one single type of biomaterial. It can be provided as a composition of variety of natural and synthetic polymers, biomaterials, biomolecules and cell lines. As single component bioinks have shown some drawbacks including poor mechanical and rheological properties, in order to ease this problem, different multi-component bioinks have been developed recently. For example, hydrogel-based biomaterials including collagen, gelatin, alginate, fibrin/fibrinogen, hyaluronic acid (HA), chitosan, decellularized extracellular matrix (dECM) can be used due to great biocompatibility and

biodegradability. Natural polymers consist of Arg-Gly-Asp (RGD) which is vital for cell attachment and through their natural structure and cell-binding sites are desirable for growth, and differentiation [13]. On the other hand, these biomaterials show low mechanical properties so that combining with other biomaterials with desired properties can be strongly resolvable. Synthetic bioinks such as pluronic and polyethylene glycol (PEG) can be good candidates for applications when strong mechanical properties are desirable. They also are tunable and facilitate the bioprinting process [11].

Rheological properties of bioinks generally can be considered as an important factor and should be controlled to facilitate the bioprinting process [12, 13]. Furthermore, to have a stable construct, bioinks can be crosslinked during or after printing through physical and chemical stimulus. For physical stimuli one example can be crosslinking of alginate by calcium ions and for chemical stimuli using a photo initiator to start crosslinking reaction in biomaterials under UV or visible light [8, 13]. Another important factor in selection of biomaterial is that hydrogel network should have an ability to respond to cell-mediated matrix remodeling. The rate of degradation can regulate remodeling and ECM production and can be highly considerable in bioinks preparation. Generally, bioinks can be degraded through enzyme, hydrolyte and ion exchange [12].

In another classification, two major types of bioink materials are defined, scaffold-based biomaterials and scaffold-free. In the former, hydrogels or exogenous biomaterials have been loaded with living cells. By these techniques, cell proliferation can happen in cell-laden hydrogel, and tissue will be formed. In the second type, cells are formed in neo-tissues first and then deposited in certain patterns, and they will be matured in order to

fabricate the larger functional tissues [11]. In this chapter, some of popular biomaterials are described.

Collagen is defined as one of the best natural-based hydrogels, widely used in tissue engineering and biomedical applications. Collagen is recognized as the main protein in the extracellular matrix of many tissues [14]. Collagen is synthesized by some cells such as fibroblast and osteoblasts [15]. Collagen consists of RGD (Arg-Gly-Asp) that is vital adhesion motif for interaction between the extracellular matrix and cells. Biodegradability of collagen in mammals is significant via collagenases and metalloproteinases and can be controlled by chemical cross-linking and enzyme [16]. Due to physiochemical properties of collagen and significant in vitro and in vivo biocompatibility has been widely used in different applications such as bone generation [17], mesenchymal stem cell-based therapies [18], such as cartilage repair [19] and skin repair [20]. WonJin Kim used collagen as the main character of a functionalized bioink. In his study, cell-laden collagen structure composing of collagen fibrillation and cell printing were fabricated by an extrusion-based 3D printing. In this work, Gly/KCL buffer solution was used to make alignment in collagen fibril to generate this biocompatible structure. The improved myogenic gene expression which resulted from significant aligned morphology and actin cytoskeleton compared to non-align collagen fiber was illustrated. This study reported that this effect can be due to both biochemical cues from collagen which has influence on cell attachment, differentiation, and physically topographical cues derived from aligned collagen fiber. At the end, this study claimed that this technique is a good alternative method in the process of generating different tissues [21].

Gelatin is a natural biomaterial used abundantly in various fields of medical sciences. Gelatin is synthesized by reverse hydrolysis of another commonly occurring protein, Collagen. It is derived from bone, skin and tendon of an animal [22]. It exists as a mixture of water-soluble protein fragments comprised of the same amino acid sequence as collagen [23].

Gelatin is a natural, nontoxic and, brittle polymer. Besides being a very low-cost product, gelatin has certain characteristics which makes it desirable to be used as a biomaterial. Gelatin is bioactive and biocompatible with no toxic products of metabolism. This biomaterial does not trigger any kind of immune reaction in the body and is harmless. Furthermore, gelatin is biodegradable, has the capability to break down after performing the required function. However, gelatin is not a very strong material, is brittle and may break very easily if very dry. Due to week mechanical strength, gelatin is usually mixed with other compatible materials to increase the overall strength [24].

In the biofabrication process, gelatin can be used as a bioink with different concentrations and blend with other polymers. Gelatin has the ability of being cross-linked chemically and adapted for three-dimensional bioprinting [8, 25]. For example, gelatin methacryloyl (GelMA) hydrogels have been widely used for various biomedical applications and tissue engineering. This hydrogel shows a very tunable physical properties and suitable biological properties. GelMA hydrogels provide three-dimentional support for native extracellular matrix and simulate natural environment. Gelatin due to having many arginine-glycine-aspartic acid (RGD) sequences can promote cell attachment effectively. Although collagen has the same characteristic, gelatin shows better solubility and less antigenicity [26].

A novel nanoparticle, which was used in situ drug delivery and targeting methods, has been studied by Naghamma et al. Gelatin-dopamine (Gel-Dopa) nano-gels (GDNGs) are loaded with Dox (doxorubicin) which is capable of efficiently penetrating the cell membrane. These nano particles can localize into the cells, release Dox molecules and kill the tumor cells. Gelatin due to properties including biocompatibility, cytotoxicity, low antigenicity and good solubility has been used in this composition [27].

In one study, a composition of gelatin and alginate was used as bioink with myoblast encapsulation to examine the mechanical strength of the tissue structures by Zhang et al [28]. The procedure included a physical crosslinking of the gelatin in a low temperature condition and ionic cross-linking of the alginate by calcium ions. Results showed the decrease in mechanical strength of the constructs whereas low porosity of the structure helped the durability [28].

Alginate or alginic acid is a polysaccharide, derived from different kinds of brown seaweed and obtained by two types of bacteria, Pseudomonas and Azotobacter [29]. Alginate is considered as a bioink in many bioprinting techniques either by itself or mixed with other biomaterials due to fast gelation property which results in good printability [8]. However, alginate has some limitations such as weak degradability and low cell adhesion properties [30, 31]. The former can be addressed by synthesizing a biodegradable alginate macromer using partial oxidation technique [32]. Cell adhesion can be improved by the addition of RGD or collaborating of alginate with gelatin [31, 33]. The viscosity of the composition depends on the concentration of alginate which also can specify the time of crosslinking and porosity. Preparing pre-crosslinked alginate by blending with a small amount of the crosslinker can promote the printability of the solution [34]. For improving

mechanical property of bioprinted constructs, alginate can be blended with other biomaterials.

Yu et al. studied a bioprinting techniques to print cell-laden construct with proper biological and mechanical properties. They encapsulated cartilage cells inside alginate and created a hollow tubular construct. They used extrusion-based bioprinting, running a coaxial nozzle with a CaCl2 solution in the core and an alginate solution through the sheath. Results showed that when they increased the alginate concentration from 2% to 6% (w/v) which results in surging shear stress, it can strongly reduce the cell viability [35]. More studies in developing various biomaterials for various applications are reviewed here.

Su Ryon Shi et al. have used conductive Carbon nanotube (CNT) ink in order of bioprinting 2D and 3D recently. This ink shows promising cell-binding sites with easy interfacing with living tissue. As they say in their publication, different biomedical devices, including supercapacitors, flexible actuators, transistors, and sensors have been supported by this bioink. This electrically developed Conductive CNT-based ink shows cytocompatibility property. They used surfactants such as DNA, and modified gelatin to improve CNT stability and solubility in water. Being stable in aqueous solutions and biological fluids are other features of this bioink [36].

In 2018, another work using CNT was studied by Se-Jun Lee and his team. They enhanced the interaction between biomaterials and the neural cells using carbon nanotubes (CNTs) to improve the scaffold surface properties. They highlighted that CNTs could provide nanoscale constructs similar to natural neural structures. They investigated the differentiation and proliferation potential of neural stem cells (NSCs) by seeding on a CNT scaffold. Their approach was that Amine functionalized multi-walled carbon nanotubes

(MWCNTs) in corporation with Poly (ethylene glycol) diacrylate (PEGDA) polymer to improve electrical properties and Nano- features on the surface of scaffold [37].

In 2013, Dogan Sinar and his team synthesized graphene-oxide (GO) from graphite powders, but non-conductive. The approach was that the electrical conductivity could be restored by the laser irradiation method for transforming the high resistance GO film to conductive oxygen reduced graphene oxide (rGO) [38].

To provide energy storage devices, high-speed electronics, and sensors, Adam E. Jakus and coworkers used a 3D graphene (3DG) composite including graphene and polylactide-co-glycolide as a new biocompatible, printable and conductive biomaterial. It was also used for drug delivery, imaging and osteo, stem cell differentiation, cardiac, and regeneration of neuro tissue engineering. In vitro, the viability of distinct cell types, including mesenchymal stem cells which developed neuron-like morphological characteristics, have been supported by 3DG. In vivo, it showed full biocompatibility, with no sign of any form of graphene deposited in some organs like the kidney [39].

Shweta Agarwala's new bio printing approach is deducted to create hydrogel-based bioelectronics. Stimulation electrically and monitoring temperature were their desired applications by incorporating GelMA with silver nanoparticles to print a soft and flexible platform [40]. Developing a GelMA based bioink conjugated with a gold nanorod (GNR) in order to fabricate cardiac tissue constructs was done by Kai Zhu and his team in 2017. The GNR-GelMA cardiac patch showed the progress in electrical signal speed [41]. Another study was done by Andrew R. Spencer and colleagues synthesizing a conductive and biocompatible biomaterial consisting of GelMA and poly (ethylenedioxythiophene): poly (styrenesulfonate) (PEDOT: PSS) to form 3D cell-laden constructs. Physical

properties relating to the specific bioink formulation described in Spencer's study were found to be tunable, such as conductivity and mechanical stiffness. Moreover, it showed the high biocompatibility both in vivo and in vitro. Lightening the interaction of PSS with the gelatin chain was achieved by ionic crosslinking and it led to cell attachment improvement [42].

Implantable electrochemical glutamate sensors were another study in developing bioelectronics using bioinks that has been recently done by Tran N.H. Nguyen and his team. Presented nanocomposite ink composed of PtNPs, MWCNT, PEDOT:PSS, and Ecoflex to print glutamate sensors in microscale. They demonstrated an economic and rapid way to fabricate a sensor which is sensitive to glutamate and has slow detection for those applications in in vivo conditions [43]. In 2016, Hyun Seok Song and his colleagues doped hydrogel with 2D graphene materials to overcome the limitation such as weak mechanical property and conductivity. Such composite materials support biocompatibility and tunability to cover requirements for applications like actuators, biosensors, and supercapacitors. Examples of these applications are stimuli-responsive graphene-hydrogel as actuators and hydrogel-graphene as supercapacitors [44].

Eduarda P. Oliveira specifically focused on such bioinks in bioprinting of application for neurological aims, specifically the central nervous system (CNS). The structural complexity of the CNS has limitations in using its full potential for bioprinting according to Oliveira [45]. He doubted on current stem cell-based regenerative methods for neurological problems. They believe that fabricating multi composites incorporation with stem cells within biomaterials can address some weaknesses like low cell viability and inaccurate engraftment [45].

In the case of heart tissue model, Sanskrita Das's team pointed out that the maturation of cardiomyocyte and interaction between cell and material rely on culture and matrix of the microenvironment. The regulation of signal transduction and extracellular matrices (ECMs) response were the main factors in tissue maturation according to Das [46]. They considered the biophysical properties of bioinks, which are dependent on interactions between cells and matrix, helpful to build cardiac tissue structures with improved functionality [46]. Yeong-Jin Choi et al. established a novel VML (volume muscle loss) treatment with dECM based bioink using 3D cell printing technology. dECM hydrogels and sponges, and muscle constructs were considered as the good candidates in transplantation. They pointed out that by fabricating pre vascularized muscle using coaxial nozzle printing with mdECM and vdECM bioinks, some functionality such as myotube formation, cell viability, and myofiber regeneration have been enhanced [47].

In 2019, Jacky F.C. Loo presented the development in POC (Point Of Care) sensor by printing microfluidic biosensors for the applications of diagnostics, healthcare, and even food safety. The capability of fast turnaround time and ease of customization of mentioned biosensors is another big advantage of it [48].

In 2017, Noshadi applied bio ionic liquids in a new class of electroconductive hydrogels (ECHs) used in cardiac applications as well as other bioelectronics. According to Noshadi [49], limitation that conventional methods show in terms of biodegradability, solubility and processability were addressed by this approach. He and his team functionalized the non-conductive polymers with a conductive choline-based bio-ionic liquid. The results showed descent mechanical properties, conductivity, porosity,

degradability and swellability as well as showed the ability of conjugated hydrogel with BIL in transducing physicochemical stimuli [49].

Ionic liquids (ILs) has been widely used in material science due to their properties such as high-water solubility, thermal stability and, high conductivity. Choline-based bio-ionic liquids (BILs), among other type of ILs have been considered as a promising alternative in biomaterials due to biocompatibility properties using choline. Choline is an essential nutrient, necessary for cell-membrane signaling (phospholipids), lipid transport (lipoproteins), neurotransmitter synthesis (acetylcholine) and methyl-group metabolism (homocysteine reduction) [50]. Furthermore, some studies showed the ability of choline in breaking smaller chain molecules physiologically and environmentally [51]. Choline-based ionic liquid due to nontoxic and biocompatibility properties has been considered as an alternative composition to apply in a variety of bio related applications [49].

The focus of this thesis is developing and conjugating PGSA with Bio Ionic Liquid (BIL) in order to have conductive and printable properties in fabrication of bioelectronic applications.

1.4 PGSA Properties

Poly (glycerol sebacate) (PGS) was developed in the lab of Professor Robert Langer in 2002 for the first time after the failure in mimicking the mechanical properties of natural tissue. This biodegradable elastomer has been used in a variety of implantable applications in the field of orthopedic, neurovascular, cardiovascular, and soft tissue [52].

In one study, PGS matrix was used for constructing cardiac patches in myocardial infraction issues. The adhesion and growth of both rat and human cardiac progenitor cells were studied within PGS biomimetic membranes in this work. It is also confirmed that

PGS characteristics such as biocompatibility, being bioresorbable with the capability to be tailored mechanically make it a good candidate for cardiac problems and tissue engineering applications [53].

This polyester is provided by the combination of polycondensation of glycerol and sebacic acid. PGS shows two highly needed properties in biomedical application, biocompatibility, and biodegradability. The mechanical properties of PGS also are adjustable to the requirement of the designed application as well as degradation kinetics of PGS by time and temperature modification, change in the concentration of reactants and acrylation used in PGSA [54]. For instance, Chen et al. illustrated the optimization of the physical characteristics of PGS in the application of myocardial tissue by changing the temperature. By showing an increase in Young's modulus by increase in cured temperature: 0.056 MPa at 110 °C, 0.22 MPa at 120 °C and 1.2 MPa at 130 °C [55]. Due to PGS properties, it has been used in cardiovascular, nervous, blood, soft, and hard tissue. Also new approaches were recently developed into some applications such as coatings for implantable devices [56]. In another study, Vitor Sencadas showed the promising mechanical performance of PGS under even dynamic conditions. Based on this study, the scaffold presented a Young modulus of 17.3 ± 3.4 kPa, a weight loss of $28 \pm 2\%$, excellent fatigue and resilience behavior [57].

The fabrication of PGS-based devices in the application with the aim of mimicking native microenvironments are also being pursued. Designs such as extracellular matrix (ECM) like constructs, cardiac patches in the shape of accordion-like honeycomb, and gecko-like surfaces for tissue adhesives have been considered so far; the new design will be developed to cover the demand for soft and hard tissue market [58]. It has been said that

PGS has become a material with great potential in the field of biomedical in less than a decade.

PGS acrylate (PGSA) was designed by introducing acrylate moieties into the PGS prepolymer, using UV radiation in the presence of the photo-initiator 2-dimethoxy-2-phenylacetophenone (DMPA) [55]. Both redox and photopolymerization of PGSA was studied by Ifkovits et al. Results showed the acceleration of the polymerization process from 48hr to a few minutes compared to the conventional method. The PGS synthesizing process has some limitation thermally that was also addressed by this approach. Thereby with the acrylate group incorporation in PGS, some application possibilities can be controlled as well as the mechanical properties of the PGSA [58, 59].

In order to mimic native physiological conditions, the encapsulating technique can construct a three-dimensional scaffold. Sharon Gerecht et al. used poly (glycerolco-sebacate)-acrylate (PGSA) as the three-dimensional scaffold for the growth of both neuroblastoma and human embryonic stem cells. This study showed PGSA properties such as mechanical and adherence can support cell differentiation and interaction [60].

In the process of providing a promising bioink in 3D bio printing, some biomaterials can be added to polymers to enhance desirable characteristics. Ionic Liquid (IL), due to desirable properties such as solubility and conductivity, has been widely used as a promising additive recently. Ionic liquid polymer shows favorable solubility and rheological properties [61].

In this work, the characterization of the biomaterial composed of PGSA conjugated with Bio-IL with possible application in wide range of bioelectronic applications are

investigated. The characterization is including verifying of mechanical properties, conductivity, adhesive properties as well as biocompatibility properties.

1.5 3D-Bioprinting Techniques

3D printing, also known additive manufacturing, has been improved recently in various fields such as engineering, manufacturing, medical and art [62]. This technology has been entered into the field of medical science since early 2000 [63]. 3D bio printing has been used for fabrication of various biocompatible materials, bioactive molecules, and cells in order to prepare tissue demands for different applications like transplantation [62], biomedical devices [64] and drug delivery [65]. Extra consideration should be taken for biofabrication in comparison with non-biological ones. Cell type, biomaterial, differentiation, degradability and technical issues can be the main challenges in this process due to the specific living condition of cells and tissue [62]. At first, a single biomaterial and a single cell type were used for 3D bioprinting but after development in technology to apply multiple biomaterials and 3D bioprinting techniques, regenerating of complex microarchitecture of tissue constructs have been possible [66]. In this work, three main types of 3D bioprinting techniques such as extrusion-based, droplet-based, and laser-based are briefly described.

Extrusion-based bioprinting technique, also named direct ink writing (DIW) has been known as the most common-used technique for 3D bioprinting due to affordability and versatility that this method has compare to other methods [67]. Extrusion-based bioprinter creates a continuous filament using extrusion force that can be classified as pneumatic, screw-driven and piston [67]. This method can be useful for a wide variety of biomaterials with different viscosities and cells with different concentrations and has been

widely used for tissue constructs which are desired with a good mechanical property [68]. Furthermore, extrusion-based bioprinting can perfectly support multi-material bioprinting by developing coaxial nozzle system [68].

Droplet-based technique, also named inkjet bioprinting is a technique using bioink droplets for building 2D or 3D printed constructs. Inkjet bioprinting technique classifies three main types including thermal ink jetting, electro-hydrodynamic and acoustic wave jetting. Some advantages have been recognized for these techniques such as high speed, high cell viability and low cost [69]. These methods also have some drawbacks such as head clogging, small range of material choice and the difficulty of keeping material in liquid phase [69].

Laser-based technique or Laser-assisted bioprinting (LAB) like two other techniques can be used for printing of biological materials including peptides and cells [70]. This technique proposes a non-contacting and nozzle free process [71]. In this technique, some parts of biomaterials are deposited on a substrate and a laser as a source of energy can print the selected pattern. The 3D printer is divided by three main parts: a pulsed laser beam, a ribbon coated with a layer of metal to absorb laser beam of energy and a receiving substrate. A laser-based bioprinter generates a laser pulse on the laser-absorbing layer and derive bioink to the collector substrate. One of the positive points of this technique is that clogging will not happen by this method as there is no nozzle involved and the possibility of cross infection is nothing. Furthermore, some range of viscosities (1-300 Pa/s) can be supported by this method. In this method, biomaterials can be printed at high speed and high accuracy. In addition, control over distribution of different cell types

can be achieved through laser-assisted bioprinting (LAP) which result in building complex constructs [70, 71].

Chapter 2

Characterization Methods

2.1 Introduction

To study the characterization of the biomaterial developed in this work, various instrumental methods were engaged. This chapter will provide a synopsis of each method and a brief overview of the theory behind each technique. The techniques include analysis of nuclear magnetic resonance (H-NMR), Fourier-transform infrared spectroscopy (FTIR) and adhesion properties of PGSA/BIL were examined by burst pressure test. Conductivity, mechanical characterization, and biocompatibility were other tests performed to make a complete set of tests for characterization of this biomaterial.

2.2 Analysis Nuclear Magnetic Resonance (H-NMR)

The electromagnetic field is formed due to the spinning of the nuclei around their axis. This phenomenon is true for some atoms and H and C are two atoms with the ability to respond to this technique. The direction of the spin is very random without the presence of an external magnetic field, but otherwise, they can be very aligned by setting in an external magnetic field. Two possible orientations are parallel or against the direction of the external field. At this level, radio wavelength energies, which are in the MHz range, are enough for bringing H nuclei into resonance. The basic explanation of an NMR spectrometer is that a sample, which is in a small glass tube, is placed between the poles of a strong magnetic field. A generator pulses the radio frequency to a sample and excites the nuclei. This causes the spin-flip that will be detected by the detector and send a signal to a computer [72]. H-NMR Varian Inova-500

NMR spectrometer was used to analyze the biomaterial structure. H-NMR spectra of choline bicarbonate and choline acrylate samples were tested.

2.3 Fourier-Transform Infrared Spectroscopy (FTIR)

Spectroscopy has been known as a strong technique in material analysis in the library for a long time. It represents a fingerprint for each sample by an absorption peak. This peak tells the frequencies of vibrations between the bonds of the atoms of a material. The concept is that each material has a unique combination of atoms. In fact, there are no two compounds that can produce the same infrared spectrum. Therefore, qualitative analysis can be performed by infrared spectroscopy. To overcome some limitations such as the speed of the scanning process, Fourier transform infrared (FTIR) spectrometry was developed. The process includes multiple parts. The beam from a glowing black body source passes through an aperture for controlling the amount of energy. There is an interferometer, which modulates all of the infrared frequencies "encoded" into it. It uses a reference laser for precise wavelength calibration, mirror position control, and data acquisition timing. The beam passes through the sample and reflects the surface of the sample off. The sample absorbs some of them and few are transmitted and made a molecular fingerprint of each sample. As each molecular structure has a specific infrared spectrum, this helps to study chemical bonding and functional groups. Ultimately, the beam reaches the detector and measures the interferogram signal. It is digitized by computer and be available for user interpretation [73].

For FTIR analysis, the infrared radiation is passed through the thin film of material, some of the energy are absorbed by the sample and a few are transmitted giving a molecular fingerprint of the observed sample. Each molecular structure has a different infrared

spectrum. The result shows as a graph of wavelength versus absorption. The proof of certain components can be specified by matching the IR spectrum with the known material [73, 74].

This technique (FTIR) was performed to demonstrate the acrylic moieties. PGSA, Bio ionic liquid (BIL) and the composition of these formers as the samples were tested using FTIR. All samples were 500 microliters. The analysis was done at room temperature. All the samples were run in a window from 800 to 4000 cm-1 through 512 scans to increase the signal to noise ratio.

2.4 Mechanical Test

Mechanical testing of biomaterials can be the main part of the evaluation and design of medical devices. Mechanical characterization of a biomaterial should be performed to evaluate the material's behavior in interface with tissue and the durability of the material in the natural space [75]. In this chapter, we will focus on the fundamental mechanical test, which is tensile test.

The tensile test is known as one of the most basic mechanical tests to characterize the material's strength and its mechanical behavior. In the test, an axial load is applied to the sample which can be a strip or cylinder shaped in the sample's long axis. The load is increased gradually, and displacement grows till rupture happens. Elastic Modulus is defined by stress and strain graph. It has been said this constant value is dependent on materials and called "elastic region". The relation of this constant, stress and strain are defined by the following equation:

$$\sigma = E\varepsilon$$

Where (σ) is the stress in Pascal, (ε) is the Strain and (E) is the Elastic Modulus or Young's Modulus [76]. In addition of tension properties of a material, the compressive characterization of the scaffold can be measured by this test.

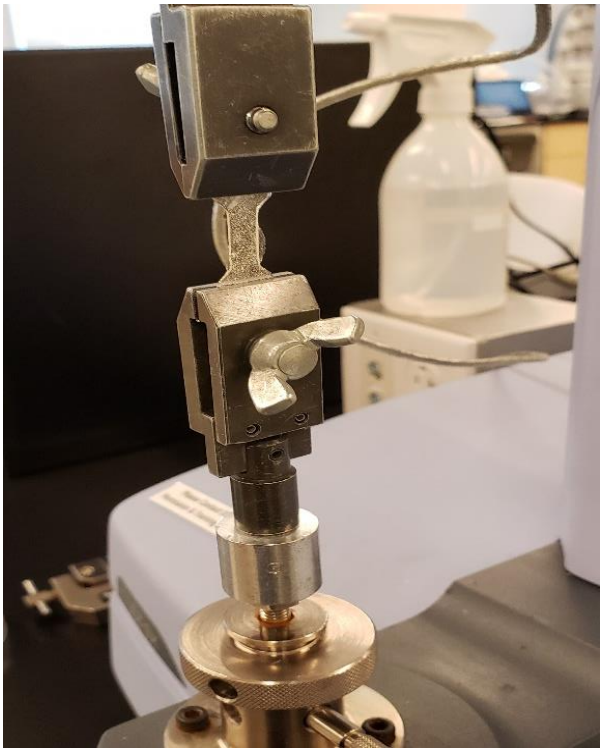


Figure 1. Tensile Test, example picture.

In this study, elastic moduli have been calculated by EZ-SX mechanical test instrument (Shimadzu). The shape of the samples was cylindrical, having a 5mm diameter and 5cm height using Polydimethylsiloxane (PDMS) molds. The samples were made in different compositions of PGSA and BIL in addition of 0.05% of a photo initiator, named LAP (lithium phenyl-2,4,6-trimethylbenzoylphosphinate) polymerized under visible light for 45 Sec. for each composition, which were 100% PGSA-0% BIL,90% PGSA-10% BIL,

80% PGSA-20% BIL, 70% PGSA-30% BIL, 60% PGSA-40% BIL and 50% PGSA-50% BIL, 3 samples were tested. The slope of the linear part of the graph was computed.

2.5 Degradation and Swelling Tests

The swelling is defined as an increase in the volume of a material by up taking a liquid or gas. The absorption of a solvent can results in change in mechanical properties of a material [7]. In polymers, the crosslinks create an elastic restoring force and prevent the dissolution of the polymer network by the solvent but increase in volume by absorbing the solvent [7]. The swelling process is one of main factors in scaffold's characterization.

Degradation ratio of biomaterials is an important factor as well as swelling ratio. More specifically, to perform a proper biological function such as providing regenerating tissue and risk of the presence of a biomaterial for a long time in the human body, the knowledge of degradation process of biodegradable materials is crucial to be investigated [7].

The cylinder-shaped samples which were the various composition of PGSA and BIL were prepared. They were weighed in both wet and dry conditions. The samples were placed in 24-well plate with 1ml of DPBS at 37 °C in an incubator environment for 2 weeks. At the times of 1, 7 and 14 days, the samples were taken out of the DPBS and freeze-dried overnight and then they were weighed again. The percentage of degradation was calculated of following equation:

$$D\% = \frac{w_i - w_t}{w_i}$$

Where w_i is the initial dry weight of the sample and w_t is the dry weight after time t. For swelling ratio, after 4, 8 and 24 hr, the weight of samples was measured. Afterwards,

the samples are immersed in DPBS at 37 °C in an incubator and rescale the weight. The fraction of mass of swelled samples divided by the mass of dried ones were defined as the swelling ratio [77].

2.6 Adhesion Test

Surface properties of biomaterials have a vital role in adhesion phenomena and a great impact on the inflammatory and wound healing responses to biomedical devices and biomaterials when they apply in the body (in vivo) [9]. For engineering, a proper biomaterial used in biomedical applications requires high mechanical strength and tissue adhesion. Therefore, to have a proper attachment to the tissue environment, the adhesion properties should be reliable [49]. Four techniques of shear strength, wound closure, peeling and burst pressure are performed to evaluate the adhesion properties of a biomaterial in vitro.

Shear strength of the PGSA/BIL composition was tested based on the modified ASTM F2255-05 standard. A rectangle-shaped piece of glass, 1cm by 2 cm, coated with a 1cm by 1cm layer of gelatin was used for testing shear strength of the PGSA/BIL compositions. The gelatin layer was kept overnight to be dried and considered as a base layer. 25 μL of the various combination of PGSA/BIL was placed between 2 pieces of gelatin-coated glasses and polymerized under visible light for 1 min. The mechanical tester machine applies a force to pull two pieces in the opposite direction. Shear strength was measured by drawing stress-strain curve for at least 3 samples for each composition [78].

Figure 2. Wound Closure Test, example picture.

For the Wound closure test, ASTM F2458- 05 standards have been referenced. Small strips of porcine skin were prepared and immersed into PBS to keep them fresh for the test. The tissue was cut in half and 100 μL of composition applied and photo cross-linked. The adhesive strength of the samples was calculated by using a mechanical tester machine. 3 samples for each composition have been tested [79].

Burst pressure calculation of the biomaterial was based on the ASTM F2392-04 standard. A square 5 cm by 5 cm skin was prepared to connect to a custom-built burst pressure apparatus. A hole that is 5 mm by 5 mm was made in the center of the skin. The apparatus is including a pressure meter and a syringe pressure setup. The syringe pump was used for making a flow of air with the speed of Air 0.5 ml/s. The skin was covered

with the composition of PGSA/BIL at the puncture area. Then the biomaterial was cross linked using visible light. The burst pressure was recorded when rupturing had occurred, and the airflow had to stop. Like the other tests, 3 samples for each composition ware performed [79].

2.7 Conductivity Test

Conductivity is known as the ability of passing an electric current in a material. This property can be defined by Ohm's law. Ohm's law is an empirical relation that describes the conductivity of the electrically conductive materials and represents the interrelation of voltage, current and resistance. It states the current through two points of conductive material is fraction of the voltage passing these points over resistance. The equation below shows the best of this definition:

$$I = \frac{V}{R}$$

Where I is the current (amperes), V is the voltage (volts) and R is the resistance (Ohm). To display the conductivity, definition of two other parameters may be critical to mention, cell constant and conductance.

Conductivity ($Cm^{-1}S$) = cell constant (Cm^{-1}) * conductance (S)

Where the conductance is a definition of the mutual of the resistance of a material between two electrodes, (1/R) and cell constant is the ratio of the distance between the electrodes to the area of the electrodes [80, 81].

To display the conductivity of this biomaterial, PGSA/BIL disc shaped samples with varying concentration were cross linked under visible light. The polymerized samples were placed in a two-probe electrical station connected to an electrochemical analyzer

instrument for measuring the conductivity of the samples. The voltage was applied in increments of 0.01 V from -25 to 25 V and the variation in the current was recorded [49]. Conductivity is defined by the product of cell constant and conductance. Conductance is defined where the imaginary impedance is zero. Cell constant is the fraction of distance between the electrodes over the area of the electrode which we consider 1mm as the distance between the electrodes and area of the electrodes is considered 0.0196 cm^2. Below shows the calculation for conductivity in this work:

$$C = \frac{l}{R*A} = \frac{1}{R*0.0196}$$

2.8 In-Vitro Biocompatibility Test

The ISO 10993-1:2018 standard gives a general definition for biocompatibility as the "ability of a medical device or material to perform with an appropriate host response in a specific application" [46]. To evaluate the harmful effects of device or material in human body, the performing biocompatibility tests is vital [82, 83].

Biocompatibility of PGSA/BIL composition were tested in 3 categories: Cell viability, metabolic activity and cell spreading. On the surface of adhesive, 5× 10^4 cells/well were seeded in 24-well plate with 500 µl of DMEM, supplemented with 10% FBS (fetal bovine serum), growth medium. The condition of keeping cultured cells was 37 °C in a 5% CO2 humidified incubator for 7 days. The DMEM as the growth medium were replenished 48 h. The cell viability of primary C2C12 cultured on the surface of PGSA/BIL was evaluated by an Invitrogen Live/Dead viability kit, following manufacturer's instructions. Cells were stained for 15 min at 37 °C with calcein AM (0.5 µl/ml) and ethidium homodimer-1 (EthD-1) in DPBS (2 µl/ml). Fluorescence images were collected

on days 1, 4, and 7 post-seeding using the Axio Observer Z1 inverted microscope (Zeiss). The number of live/viable shown as green and dead cells as red spots was determined by Image software. The ratio of the number of live cells to total number of cells was calculated as the cell viability. Cell metabolic activity was evaluated at 1, 4, and 7 days, using Presto Blue assay (Life Technologies). 2D cultured C2C12 were kept in an incubator for 2 h at 37 °C in 500 µL of 10% Presto-Blue growth medium. The fluorescence beams including excitation and emission were considered 560 nm and 590 nm respectively. To better understanding control wells were measured as well. For presenting surface spreading of the cells on the biomaterial, fluorescent staining of F-actin filaments and cell nuclei were applied. 2D cultures at days 1, 4, and 7 were incubated for 45 min with Alexa-fluor 488-labeled phalloidin (1:1000 in DPBS, Invitrogen). The cells were washed three times consecutively by DPBS. The samples were counterstained with 1 µl/ml DAPI (4′, 6-diamidino-2-phenylindole, Invitrogen) in DPBS for 5 min and the fluorescence images captured using an Axio Observer Z1 inverted microscope [79].

2.9 In-Vivo Biocompatibility Test

All animal experiments were reviewed and approved by the ICAUC (protocols 15-0521R and 15-1248R) at Northeastern University, and all experiments were performed in accordance with relevant guidelines and regulations. Male Wistar rats (200–250 grams) were obtained from Charles River (Boston, MA, USA) and housed in the local animal care facility under conditions of circadian day– night rhythm and feeding ad libitum. Anesthesia was achieved by 2.0 to 2.5% isoflurane inhalation, followed by 0.02 to 0.05 mg/kg SC buprenorphine administration. After inducing anesthesia, eight 1-cm incisions were made on the posterior medio-dorsal skin, and small lateral subcutaneous pockets were prepared

by blunt dissection around the incisions. PGSA/BIL composition (1 × 5 mm disks) were implanted into the pockets, followed by anatomical wound closure and recovery from anesthesia. Animals were euthanized by anesthesia/exsanguination at days 4, 14 and 28 post-implantations, after which the samples were retrieved with the associated tissue and placed in DPBS [79].

2.10 3D-Bioprinting Techniques

A DMD- based bioprinter was employed to do the printing process in this work and relied on photo crosslinking. Digital micromirror device (DMD) printing is also known as the Digital Micromirror Device-based Projection Printing (DMD-PP). This technique is including a digital mirror for reflecting laser light on a plane which can produce image. Firstly, desired patterns are designed in CAD software and converted to STL files. Black and white images named bitmap files are generated by slicing STL files. White parts are showing the material and black parts are known as void parts. After that, image is reflected on the resin which contains of the photo crosslinker and white parts starts to polymerize and cure the resin. This process is continuing till all layers are printed [84,85].

DMD-based is one type of DLP-based bioprinting devices that includes a digital micromirror device chip with the possibility of projecting layer after layer through a transparent plate. There is an elevator attached to a platform named as ‘build platform’ in order to move plate upward after layer exposure. The DMD chip can adjust the UV light intensity and polymerization within a layer [86].

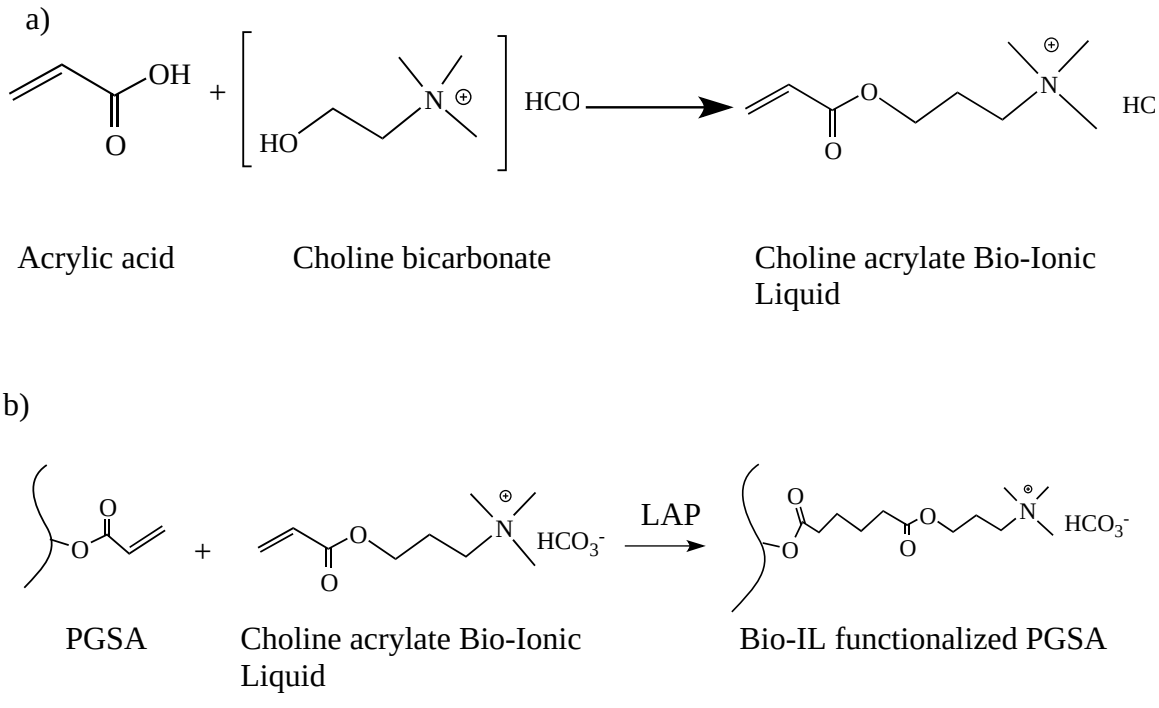
Figure 3. DMD-based 3D bioprinter, example picture.

Chapter 3

Results and Discussion

3.1 Synthesis and Characterization of the Bio-Ionic Liquid

The Scheme 1a shows the synthesis of BIL. The synthesis of BIL is done by the reaction of choline bicarbonate and acrylic acid. After synthesizing of BIL, the next step would be the conjugation with the polymer PGSA, Scheme 1b. Different concentration of these two compositions from 0% BIL to 50%BIL have been considered for all characterization tests.



Scheme 1. Synthesis of BIL and PGSA/BIL. The schematics show the structure of the proposed reactions for (a) Acrylation of choline bicarbonate to form Choline acrylate (BIL), and (b) reaction between PGSA and Bio Ionic Liquid to form PGSA/BIL.

The presence of acrylate group after reaction with acrylic acid has been confirmed by result from the proton nuclear magnetic resonance (H NMR) spectra of choline bicarbonate and choline acrylate (BIL), shown in Figure 4. The peak of hydrogen atoms of acrylate groups was shown between δ = 5.8–6.1 ppm. The sharp peak at δ = 3.1–3.2 ppm shows the three hydrogen atoms of choline (ammonium ion).

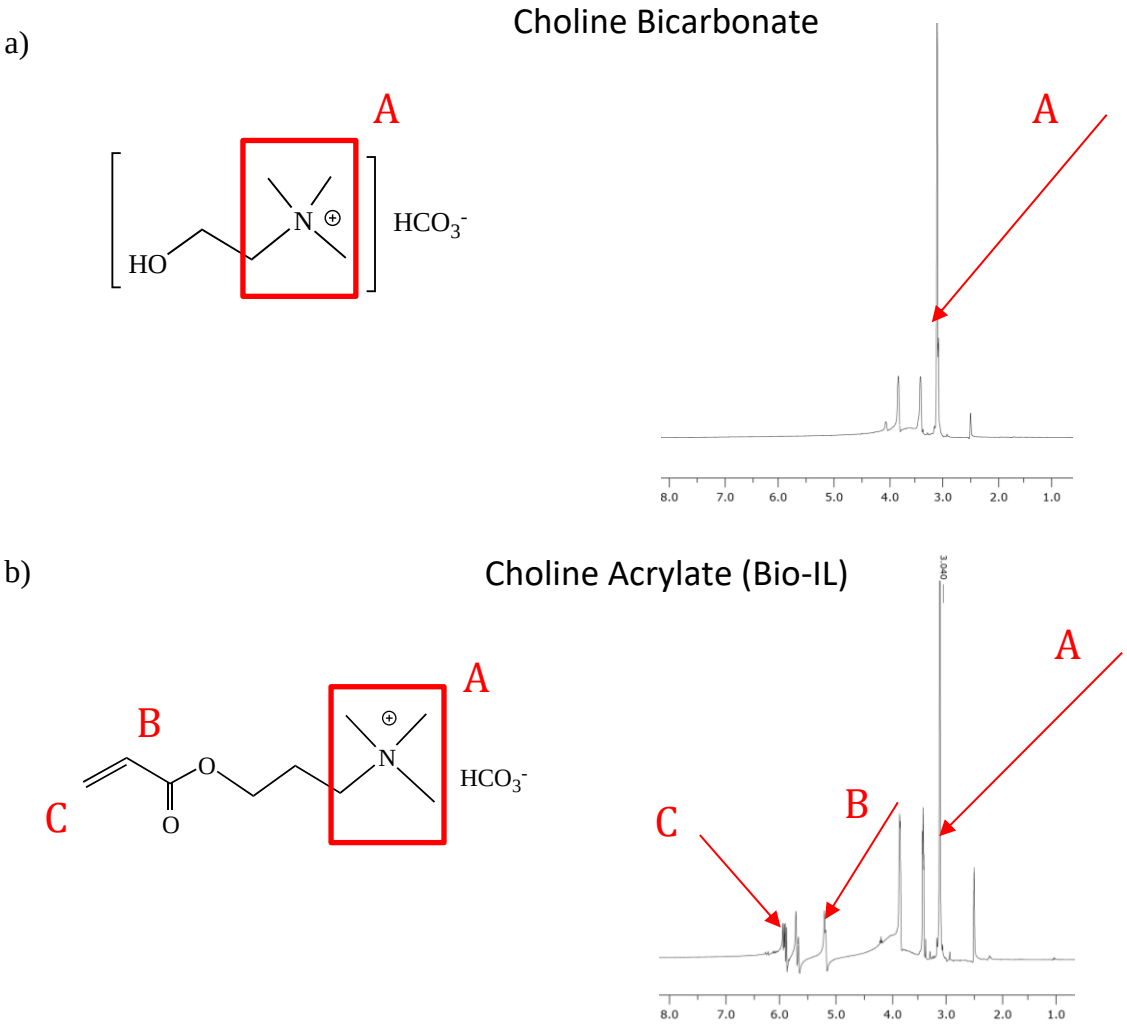


Figure 4. 1H-NMR analysis of (a) Choline bicarbonate (b) Choline acrylate (BIL).

Similarly, to confirm the presence of acrylate group in this composition, FTIR was measured for PGSA, BIL and PGSA conjugated with BIL (Figure 5). In the FTIR result, the appearance of a peak at 1580 cm-1 indicates the formation of acrylate group for all samples including BIL, PGSA and PGSA/BIL.

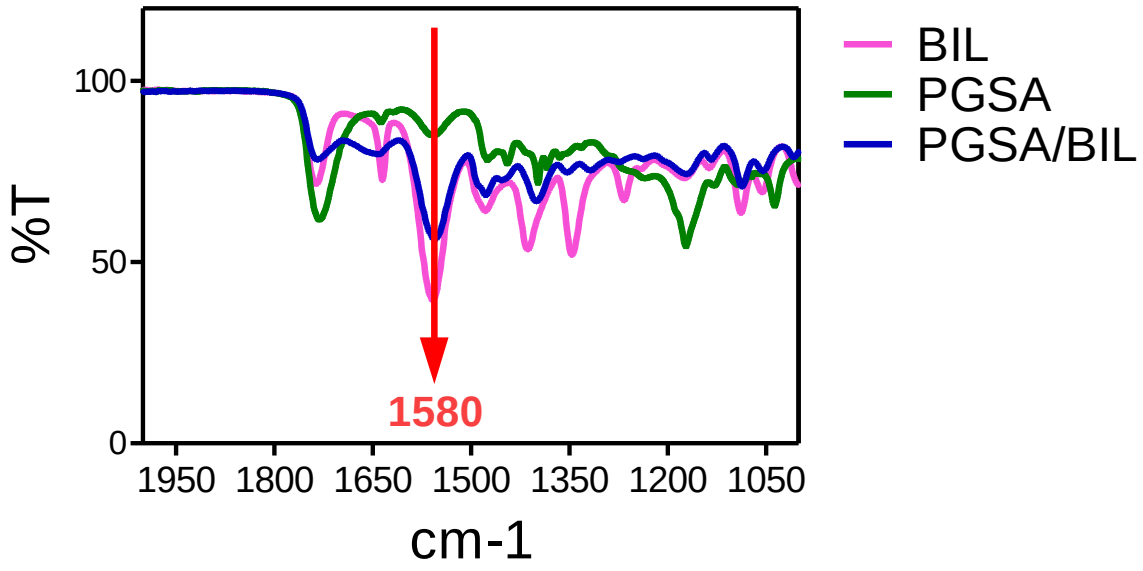


Figure 5. FTIR spectra of BIL, PGSA and PGSA/BIL. Representing acrylate peak at 1580 cm-1 for three samples including BIL, PGSA and 50% PGSA /50% BIL.

3.2 Mechanical Properties of PGSA/BIL and Results

3.2.1 Swelling and degradation results. To have an ideal biomaterial, mechanical properties and flexibility of a biomaterial should investigate to be matched with the dynamic environments such as tissues. For this aim, biodegradability and swelling properties were tested. In general, biomaterials can be metabolized by either enzyme system or hydrolysis at various pH conditions. Moreover, for preventing of rejection as a

foreign invasive object, biomaterials must eventually be hydrated via biological fluids. On the other hand, excessive water uptake could result in weakening in adhesive and mechanical properties. This preview leads to do the optimization in the aim of making more applicable and more suitable applications in in vivo conditions.

The biodegradation results show that the biomaterial with the concentration of 50% PGSA and 50% BIL were degraded by 25 % after 7 days. Whilst, the 20 % degradation were shown for control which is 100% PGSA in same period. The swelling and mechanical strength are presented in Figure 6. The swelling of PGSA/BIL versus the polymer/BIL concentration is shown in Figure 6 (a). The steady growth can be seen in the water uptake and swelling with time. Besides, there is a significant increase in swelling ratio versus PGSA/BIL concentration from 100% PGSA to 50 % PGSA, starting with 20% swelling ratio for former and almost 70% of that for later. The ideal requisites of a biopolymer would entail its flexibility to match the dynamic environment and movement of native tissues. This requires that the biodegradability be controlled, and the metabolites of biodegradation be noncytotoxic. Hydrogels can be metabolized through various pathways - enzymatic degradation or simply via hydrolysis at either acidic or basic pH conditions. For the hydrogel to prevent rejection as a foreign invasive object and be eventually metabolized, it must be adequately hydrated via body fluids. However, excessive water uptake and degradation could lead to impaired mechanical and adhesive properties. There is a steady increase in the water uptake and swelling with time. With 0% BIL incorporation, the polymers swelled much faster. With increasing BIL incorporation, the swelling rate decreased due to initial intrinsic physical cross-linking induced by electrostatic interactions mediated by the BIL functional groups. As the polymer absorbs water, in a semi dilute

solution, polymer chain conformations form overlapping correlation blobs with hydration spheres around each blob dictated by functional groups with water affinity. With BIL functionalization, there is an increased strong interaction between monomers inside a single correlation blob, as well as between the monomers in the periphery of the correlation blob, which act as additional physical cross-links conferring an initial resistance to polymer expansion with hydration.

3.2.2 Tensile test results. The mechanical properties of the biomaterial were tested through tensile test. Figure 6 (b) shows the elastic moduli versus the PGSA/BIL concentration. The trend shows the general decrease in mechanical strength by adding BIL to PGSA. With 0% BIL, the elastic modulus is 0.17 kPa. This value decreases to 0.04 kPa for 50% BIL. Tensile tests on the hydrogels show that the elastic moduli and compression moduli of the polymers could be modulated by varying the percentage of BIL. Hydrogels exhibit a tradeoff between stiffness and flexibility to resist shear, tension, or compression forces while maintaining structural integrity.

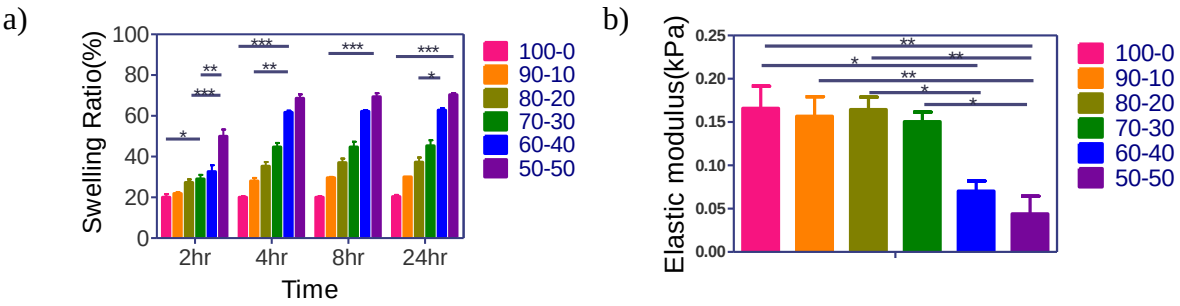


Figure 6. Mechanical Characterization of the PGSA/BIL (a) Swelling (b) Elastic Modulus. 100-0 represents 100% PGSA and 0% BIL, 90-10: 90% PGSA/ 10% BIL, 80-20: 80% PGSA/ 20% BIL, 70-30: 70% PGSA/ 30% BIL, 60-40: 60% PGSA/ 40% BIL, 50-50: 50% PGSA/ 50% BIL. Data are means ± SD. P values were determined by one-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001).

3.3 Conductive Properties of PGSA/BIL and Results

Conventional polymer-based biomaterials such as PEGDA and PGSA are non-conductive. This property is a limitation to be used in biological applications. To ease this issue, conjugating this polymer with a choline-based Bio-IL can add an electroconductive property to that. For this aim, impedance tests were run to show the conductivity of PGSA/BIL different compositions. Results demonstrates that as the ratio of BIL increases the conductivity of the PGSA/BIL increases, Figure 7. For control, we can see the conductivity less than $0.05\times10^{-5}\ S/m$ and a growth to more than $0.2\times10^{-5}\ S/m$ for 60%PGSA/40% BIL.

The lower portion of the Nyquist curves represents higher frequency inputs. The sample behaves as a parallel plate capacitor, with a double layer capacitance electrode/electorate, and a representative circuit with a film resistance and film capacitance in parallel, in series with a double layer capacitance. The total impedance is related to oscillation, and local motion of charged BIL groups, represented by the distorted circular portion. The straight-line portion of the curves relates to the diffusion and electric relaxation from flow of solvent in the polymer matrix. Low frequencies show BIL group alignment, migration, polymer chain distortion, solvent convection, and molecular vibration, leading to energy dissipation. Here, the material behaves like a resistor in the low-frequency parts. As the frequency increases, it becomes a resistance-capacitance transmission line circuit. Higher BIL concentration causes polarization of chains and mobility of chain segments. Each BIL pendant group is accompanied by a free counter ion resulting in higher conductivity. A greater solvent polarization also contributes to the increase in conductivity.

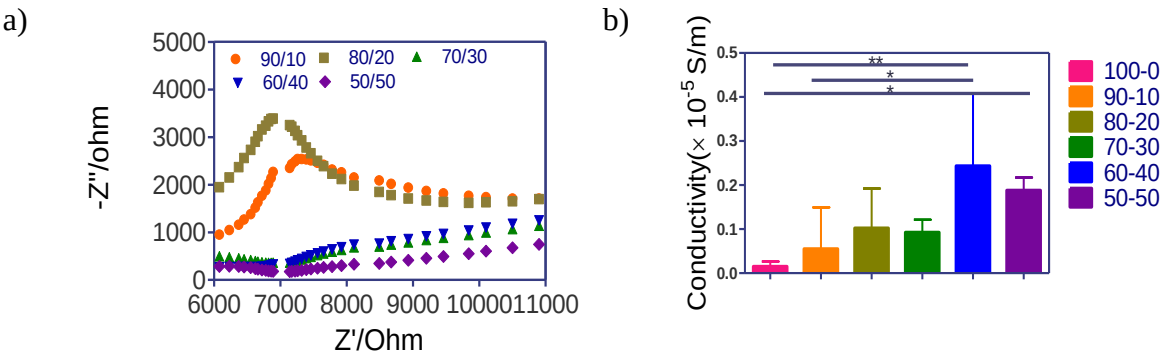


Figure 7. Conductivity of the PGSA/BIL (a) Nyquist Plot (b) Quantitative evaluation of the Nyquist plot to represent conductivity value in S/m. 100-0 represents 100% PGSA and 0% BIL, 90-10: 90% PGSA/ 10% BIL, 80-20: 80% PGSA/ 20% BIL, 70-30: 70% PGSA/ 30% BIL, 60-40: 60% PGSA/ 40% BIL, 50-50: 50% PGSA/ 50% BIL. Data are means ± SD. P values were determined by one-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001).

3.4 Adhesive Properties of PGSA/BIL and Results

To test adhesive properties of the PGSA composed of BIL, burst pressure test has been performed. This graph is presented the pressure tolerated by this material as the sealing part in the area of the ruptured skin versus concentration of both components, PGSA and BIL. Results show moderate adhesive properties of this composition at 80% of PGSA and 20% of BIL with 22KPa, Figure (8). The adhesive property is directly related to electrostatic interactions. It is also related to better film-forming properties, which increase with increasing overall molecular weight. The molecular weight, in turn, is dependent on the average molecular mass of repeat units in the polymer, which increases with increasing functionalization by bulky choline pendant groups.

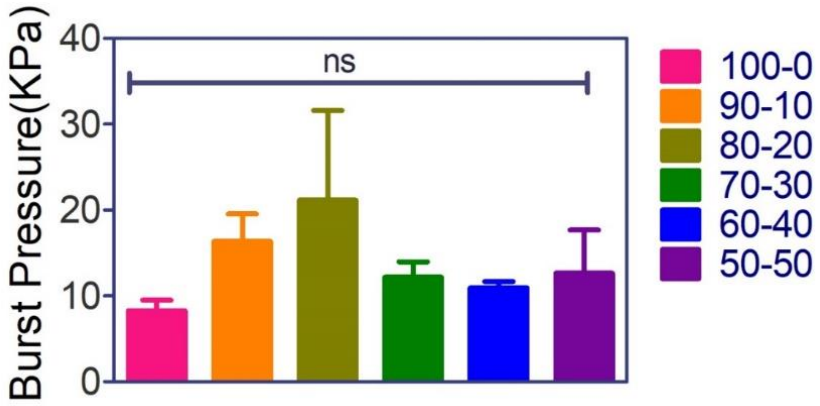


Figure 8. In-Vitro sealing properties of the PGSA/BIL, standard burst pressure test to determine the adhesive strength. 100-0 represents 100% PGSA and 0% BIL, 90-10: 90% PGSA/ 10% BIL, 80-20: 80% PGSA/ 20% BIL, 70-30: 70% PGSA/ 30% BIL, 60-40: 60% PGSA/ 40% BIL, 50-50: 50% PGSA/ 50% BIL. Data are means ± SD. P values were determined by one-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001).

3.5 In-Vitro Biocompatibility Test Results

The biocompatibility of PGSA/BIL was investigated in vitro through Cell viability, proliferation and metabolic activity done by some tests. The viability of C2C12 cells on the PGSA/BIL surface with the concentration of 60% PGSA/ 40% BIL was tested over 7 days by performing live/dead assay. To show the cell attachment and spreading on PGSA/BIL, F-actin/DAPI (Figure 9 a, d) immunofluorescent staining was performed. The observation of live/dead assay indicates (Figure 9 b, c) that the viability of cultured cells on day 7 was 98.5%±0.5%. PrestoBlue assay was performed to show metabolic activity of cultured cells. It shows (Figure 9 e) the significant growth from 5000 RFU in day one to 12000 RFU in day seven. These results validate the biocompatibility of PGSA/BIL and its potential in cell adhesion, growth, and proliferation.

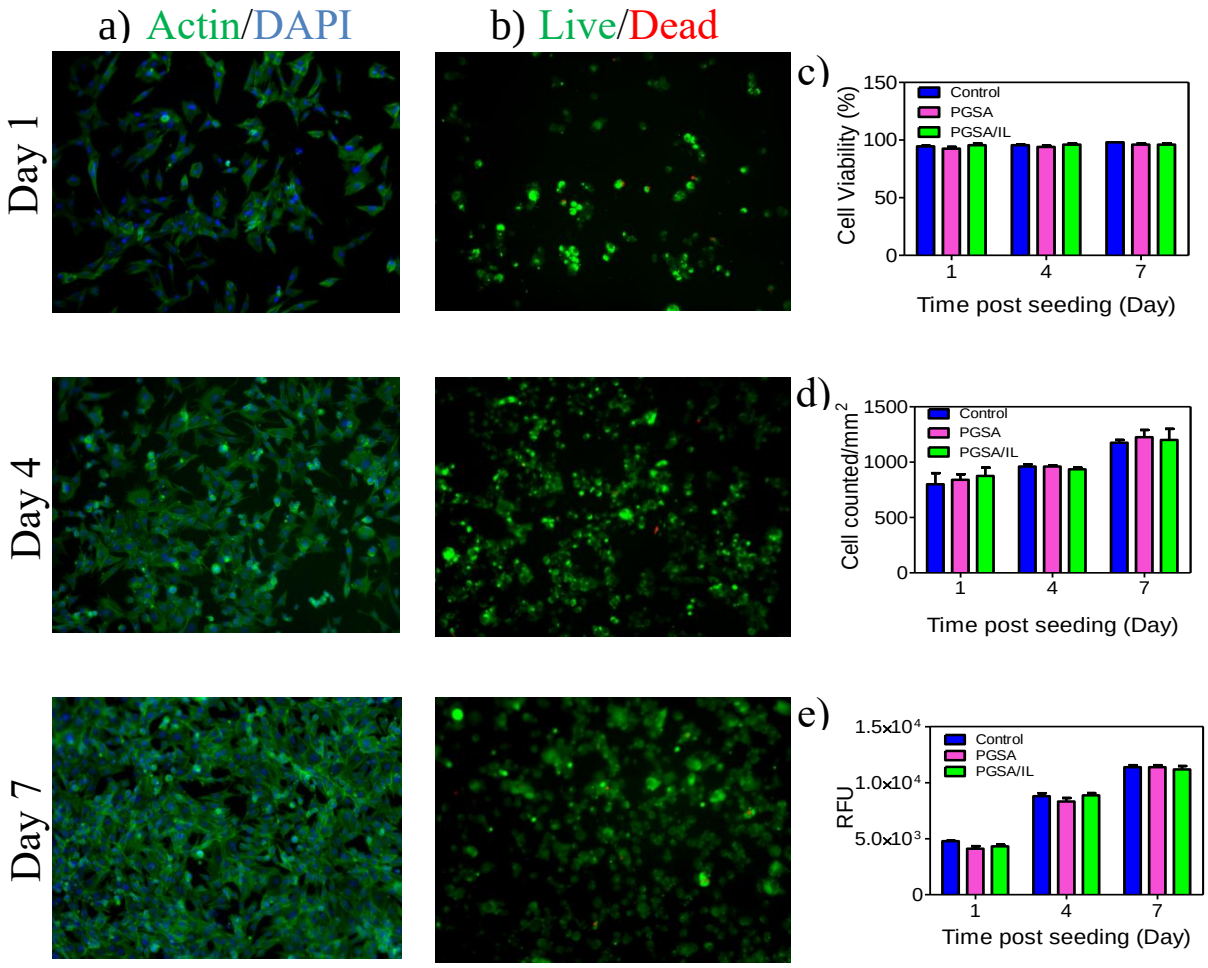


Figure 9. In-Vitro biocompatibility of PGSA/BIL. Representative a) F-Actin/DAPI fluorescent images and b) Live/Dead images at days 1, 4 and 7 post seeding of C2C12. c) Quantification of cell proliferation based on DAPI-stained cell nuclei d) Quantification of cell viability of live/dead images e) Quantification of metabolic activity, Relative fluorescence units (RFU), using PrestoBlue assay. Data are means ± SD. P values were determined by one-way ANOVA (***P < 0.001).

3.6 In-Vivo Biocompatibility Test Results

In order to perform the evaluation of in vivo degradation of PGSA/BIL, the samples with the concentration of 60% PGSA and 40% BIL have been prepared. The subcutaneous implantation applied into rats (Figure 10 b). Explanted samples on days 4, 14 and 28 have been studied for testing of the biocompatibility and degradation. Hematoxylin and eosin (H&E) staining have been done to characterize the morphological changes and in vivo degradation. The staining shows that the biomaterial is presented till day 4 (Figure 10 b (i)) and no significant macrophage infiltration has been shown by the tissue architecture. In other words, no severe adverse inflammatory has been caused by biomaterial. For characterization of the local immune response, Fluorescent immunehistological staining for macrophages (CD68) was used. CD68 macrophage invasion and infiltration was reported at day 4 but not at day 28 (Figure10 a). This result shows that the CD68 can cause the infiltration of the implanted material and degradation control. It shows the minimal inflammatory response.

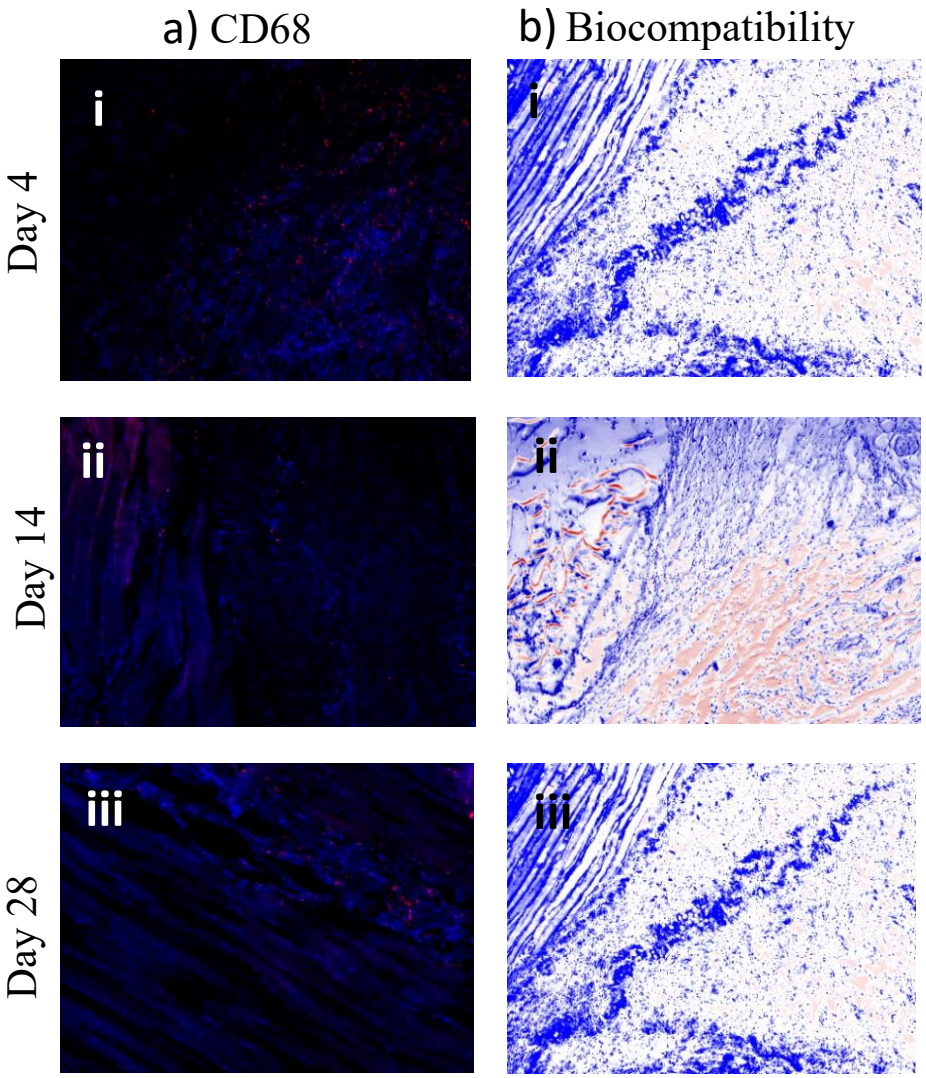


Figure 10. In-Vivo biocompatibility of PGSA/BIL. Representative a) Macrophage (CD68) b) H&E staining Fluorescent Immunohistochemical analysis, sample explanted with surrounding tissue after (i) 4, (ii) 14, and (iii) 28 days of implantation, counterstained with nuclei (DAPI).

3.7 3D-Bioprinting Results

Bioprinting is a powerful platform and has been used for wide variety of applications such as tissue regeneration, medicine, biomedical devices and drug delivery. There are various 3D printing devices that have been developed recently due to strong capability of this method for applying in different applications. In this work, digital micrometer device (DMD) has been used for 3D printing of this composite. Different patterns have been developed to show the ability of this biomaterial for 3D printing. Eight patterns were designed as the STL files and were combined on one bitmap file to be printed on one chip. Light intensity of 350 W/m2 was considered for this process. The time of polymerization was considered 3 seconds. The printed constructs were washed by Ethanol. Images were taken using the microscope (Figure 11).

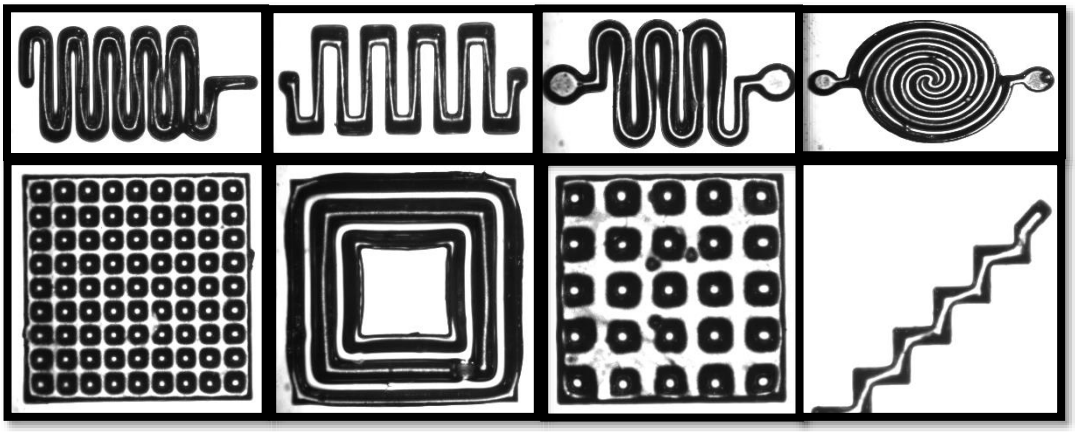


Figure 11. 3D-Bioprinting by a DMD-based bioprinter, microscopic images. Top view of 8 different patterns including Circuit, Square circuit, Squiggle, Coil design (first row, from left to right), Fine mesh grid, Square, Mesh grid and Triangle circuit (second row, from left to right)

Chapter 4

Conclusion and Future Work

The goal of this work to generate a bioink that can rectify the existing obstacles faced by the current bioinks used for 3D printable bioelectronics applications. To have a bioink for bioelectronics, it is vital to show the proper mechanical and desirable conductive properties. Moreover, the biocompatibility of the composition in in-vitro and in-vivo conditions is extremely important. For this purpose, a complete set of characterization tests were performed, and the capabilities of this biomaterial were shown. In this thesis, the characterization of PGSA conjugated with BIL has been investigated.

Mechanical properties have been investigated through tensile, swelling and degradation tests. The trend shows the general drop in mechanical strength by adding BIL to PGSA, showing the elastic modulus of 0.17 kPa for 0% BIL and 0.04 kPa for 50% BIL. There is a steady increase in water uptake and swelling with time. With increasing BIL incorporation, the swelling rate increased from 20% for 0% BIL to 70% for 50% BIL.

Adhesive properties of the composition have been shown by burst pressure. Results show moderate adhesive properties of this composition at 80% of PGSA and 20% of BIL with 22 kPa. Being conductive has been confirmed by conductivity test through electrochemical methods by showing the growth from less than $0.05\times10^{-5}\ S/m$ for 0% BIL incorporation to more than $0.2\times10^{-5}\ S/m$ for 60%PGSA/40% BIL.

Biocompatibility of the biomaterial in-vitro and in-vivo have been investigated by using C2C12 cell lines as in-vitro conditions and an in-vivo rat model. The results show the promising properties of this biomaterial by reporting 98% cell viability and its potential

for in cell adhesion, growth, and proliferation. Moreover, results show great printability property of this composition using a DMD-based bioprinter.

Biodegradable elastomers such as PGS have shown good potential due to their characteristics in various areas of the biomedical field mostly in tissue engineering, drug delivery, and biomedical devices. They show the ability to be matched with the desired applications by altering curing temperature and time, concentration, and the degree of acrylation in acrylated PGS. Moreover, acrylate polymers have been known as capable materials in additive manufacturing lately due to photocurable property. The result validates the potential of PGSA in the field of biomedical and considers it as a good candidate in various applications.

For future work, the PGSA/ BIL composition can be blended with other promising biomaterials in order to enhance mechanical and adhesive properties. Moreover, working on 3D printing capabilities shall be developed using different patterns to validate the proper printability for desired applications. Since biocompatibility is a vital characteristic in this class of material, it is necessary to develop biocompatibility tests in-vitro using human cells as well as other animal cells.

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