DEVELOPMENT AND CHARACTERISATION OF BIODEGRADABLE ELECTROSPUN POLYHYDROXYBUTYRATE (PHB)/ RICE HUSK DERIVED BIOACTIVE GLASS CERAMIC (RHBGC) COMPOSITE SCAFFOLDS

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ABSTRACT

Bioglass derived from rice husk ash (RHBGC) is considered to be natural bioactive glass. It is known for its remarkable properties that exhibits biocompatibility, biodegradable, bioactivity and excellent mechanical properties. Nanofibrous structure is one of the key factors that is considered in scaffold fabrication because it can resemble natural extra cellular matrix (ECM). Electrospinning technique enables the production of nanofibrous scaffold at low cost. RHBGC can be obtained by multistep preparation which involves silica extraction from rice husk and sol gel synthesis of bioglass. The RHBGC of various amount (0.2, 0.4 and 0.6g) is blended with Polyhydroxybutyrate (PHB) to strengthen the mechanical property of the scaffold. The scaffold is fabricated with electrospinning technique. The scaffolds were characterized using Field Emission Scanning Electron Microscope (FESEM), Fourier Transform Infrared Spectroscopy (FTIR) and contact angle. The FESEM results demonstrate that RHBGC in PHB produced nanofibrous electrospun scaffold with high porosity and interconnected fibers. However, increase in concentration of RHBGC can cause agglomeration which can lead to bead formation and reduce porosity. The formation of chemical bond between the bioactive glass and polymer were studied through FTIR. Water contact angle results shows variations in the angle due to different concentrations of RHBGC in the polymer. The results demonstrates PHB/RHBGC composite scaffold to be promising material for Bone Tissue Engineering (BTE).

ABSTRAK

Bioglas yang berasal dari abu sekam padi (RHBGC) dianggap sebagai kaca bioaktif semulajadi. Ia terkenal dengan ciri-ciri fenomena yang memperlihatkan biokompatibiliti, biodegradable, bioaktiviti dan sifat mekanik yang sangat baik. Struktur nanofibrous adalah salah satu faktor utama yang dianggap sebagai fabrikasi perancah kerana ia boleh menyerupai matriks selular tambahan semulajadi (ECM). Teknik elektrospinning membolehkan pengeluaran perancah nanofibrous dengan kos yang rendah. RHBGC boleh didapati dengan penyediaan multistep yang melibatkan pengambilan silika dari sekam padi dan sintesis sol gel bioglas. Amaun RHBGC yang berbeza (0.2, 0.4 dan 0.6 g) dicampur dengan Polyhydroxybutyrate (PHB) untuk mengukuhkan sifat mekanik perancah. Perancah dibuat dengan teknik elektrospinning. Perancah dicirikan menggunakan Mikroskop Elektron Pengimbasan Pelepasan Medan (FESEM), Spectroskopi Sinavan Infra-Merah Fourier (FTIR) dan sudut kenalan. Hasil FESEM menunjukkan bahawa RHBGC di PHB menghasilkan perancah electrospun nanofibrous dengan porositi tinggi dan gentian yang saling terhubung. Walau bagaimanapun, peningkatan kepekatan RHBGC boleh menyebabkan aglomerasi yang setevusnya membawa kepada pembentukan manik dan mengurangkan keliangan. Pembentukan ikatan kimia antara bioglas dan polimer bioaktif dikaji melalui FTIR. Keputusan sudut hubungan air menunjukkan variasi di sudut kerana kepekatan RHBGC yang berbeza dalam polimer. Kepritusan uikaji menunjukkan perancah komposit PHB/RHBGC menjanjikan potensi untuk bahan Kejuruteraan Tisu Tulang. Hasilnya menunjukkan perancah komposit PHB/RHBGC menjanjikan untuk kejuruteraan tisu tulang (BTE).

ACKNOWLEDGEMENT

All the praises be to the Almighty Allah, and appreciation for his prophet Muhammad Sallallahu Alaihe Wasallam, as I start writing my thankful note at the completion of this research paper. I would love to thank my project Supervisor Puan Norita, for all her patience and guidance throughout the project. And I must express my gratitude to Dr. Pedram Azari, for his support and assistance during the whole course of the research work. It would be so much harder for me to complete this research work, if it was not for them with their heartiest cooperation. My prayers for their blissful life and successful career. As the opportunity presents itself, I would love to state that, I am blessed with wonderful parents who gave me the courage and independence, and provided me the fortune to study on a subject that I have always dreamt of and aimed for. On this journey, I want to thank my friend Shahed Khan, for his supportive care and motivation throughout my study. And sincere appreciation to my friends Thiri Dong, Shazia Anis and Shaan Jallu, for all their support and contribution for completion of this project.

I have put my best and sincere effort on this research work, and I surely hope this will be helpful as future reference for researchers and/or professionals who shall study or work on this field of interest.

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LIST OF SYMBOLS AND ABBREVIATIONS

BTE	:	Bone Tissue Engineering
CA	:	Contact Angle
ECM	:	Extracellular Matrix
FESEM	:	Field Emission Scanning Electron Microscopy
FTIR	:	Fourier Transform Infrared
PHB	:	Polyhydroxy-butyrate
RHA	:	Rice Husk Ash
RHBGC	:	Rice Husk derived Bioactive Glass Ceramic

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background

Human bone is a mineralized rigid organ that supports, protects and provides structure to various organs of the body. Damages to bone tissue leads to conditions like fractures, bone tumors, osteomyelitis, scoliosis, congenital defects and degenerative diseases (Durgalakshmi, Subhathirai, & Balakumar, 2014; Roseti et al., 2017). These diseases significantly affect the quality of patient's life. Human bone has remarkable regeneration properties when it undergoes injury. However, if the injury is complicated and very little chance of self-healing then it requires additional treatment. Moreover, due to aging world population and longer life expectancy, there is a rapid increase in bone related disease such as osteoarthritis, scoliosis and fractures. Therefore, these issues have alarmed the researchers to look for implantable devices using tissue engineering strategies (Boyan, Cohen, & Schwartz, 2017). Over the years, tissue engineering has emerged as a promising technique for bone reconstruction. Due to increase in population and high demand for organ transplantation, there is shortage of donors. Hence, development of tissue engineered structures can be a potential solution to solve these shortcomings. The tissue engineered structures are composed of cells, scaffolds and growth factors.

In Bone Tissue Engineering (BTE), biomaterial is used as a temporary matrix or scaffold that provides suitable environment and architecture for the growth and development of bone as well as support structure for repairing and formation of tissues. An ideal scaffold should be biocompatible and should be able to possess features like cell adhesion, proliferation, vascularization and cell differentiation. If the scaffold has biodegradable features, then degradation products must be non-toxic and must be excreted out of the body

without any interference with other organs (Bowlin et al., 2002; Chan & Leong, 2008; Henkel et al., 2013).

One of the key feature of scaffold is bioactivity, which enables the scaffold to interact with the surrounding tissues. Bioactivity feature of a scaffold enables to induce growth factors and respond to the surrounding tissue for the formation of bone cells (Gerhardt & Boccaccini, 2010). The architecture of the scaffold plays a significant role in cell adhesion. An ideal scaffold must by highly permeable and possess interconnected porous structure for efficient growth of tissue and vascularization. In recent studies, nano-sized materials have the potential to form biomimetic candidates and enhances mechanical stability and provides greater surface area (Roseti et al., 2017). Scaffolds should be easily fabricated into complex or irregular shapes to match the specific requirements of the patient. Finally, the scaffold must be suitable for sterilization, cost effective and mass production for commercialization (Thomson et al., 2000; Velema, Kaplan, Lee, & Kaplan, 2006).

For BTE applications, majority of scaffolds are made of natural and synthetic polymers, bioactive glass and composites. Naturally derived polymers such as collagen and fibrin possess excellent biocompatibility and low immunogenicity. However, degradation rate is difficult to control which affects the mechanical stability. Synthetic polymers like poly(lactic-co-glycolic) acid (PLGA), poly (Ɛ-caprolactone) (PCL), polyvinyl alcohol (PVA), polyether ether ketone (PEEK) and polylactic acid (PLA) are commonly used for BTE applications. These polymers can be produced at lower costs, large quantities and have long shelf life. However, the critical drawback of these polymers are lower chances of interaction with cells due to their hydrophobic nature and low mechanical stability.

Bioglass / bioceramics such as hydroxyapatite (HA), bioactive glasses and composite materials combining bioglass and biodegradable polymers are known to be one of the promising biomaterials in BTE. These materials are capable to react with the physiological fluids that forms bone like structural layers which leads to effective interaction with cells and repairing the damaged bone tissue. Moreover, these materials can induce ions that can enhance cellular responses to promote rapid bone formation. Bioglass was discovered in 1969 by Hench. Hench and his team made silicate based glass compositions and discovered that rat bone can bond these glass compositions. Over the years, researchers examined various types of Bioglass / bioceramics composites that can be used for tissue engineering and clinical applications (Krishnan & Lakshmi, 2013; Wilson, 1985). The most applied bioglass for regeneration of bone are calcium phosphate, hydroxyapatite and bioactive glasses. These materials demonstrated superior properties including improvement in material strength, stiffness, biodegradability, osteoconductive, and bioactivity.

In addition, polymer / bioactive ceramic composite scaffolds have structures that resemble bone, where the inorganic component of these scaffolds mimics the hydroxycarbonate-apatite (HCA) motifs while the polymer component mimics the collagen-rich extracellular matrix. Although both HCA and bioactive glasses have been used to form composites with superior bioactivity, bioactive glasses have been preferred due to their superior osteoconductive properties. Furthermore, not only do bioactive glasses increase the hydrophilicity but they also increase the hydrolysis of the polymers, thereby increasing the composites degradation rate, albeit at a control rate sufficient for creeping substitution to occur. Along with other added features such as improved porosity with interconnectivity of the pores in this composite, such a product will be of great value for use in clinical applications.

Nanofibers have the potential to mimic the architecture of human tissue at nano-sized scale. They provide features like high surface to volume ratio and microporous structure. These features favor cell adhesion, migration, proliferation and differentiation. In 2003, Yoshimoto fabricated PCL scaffolds by electrospinning for BTE. Mesenchymal stem cells (MSCs) were derived from the bone marrow of the neonatal rats and seeded on the nanofibrous PCL scaffold. It was discovered that MSCs migration took place inside the scaffold and abundant extracellular matrix (ECM) was produced. These results demonstrated that nanofibrous scaffolds have the potential to be candidates for BTE (Vasita & Katti, 2006; Yoshimoto, Shin, Terai, & Vacanti, 2003).

1.2 Problem Statement

One of the critical concerns in tissue engineering is the biocompatibility and degradability of the scaffold. Due to the biodegradable characteristic of Polyhydroxybutyrate (PHB) and osteoconductive nature of Bioglass, it motivates us to produce composite scaffolds of these two materials and evaluate the resultant properties of the composites for potential applications in tissue engineering field. Furthermore, rice husk silica based bioglass (RHBGC) can reduce the cost of the composite scaffolds due to abundant availability of rice husk.

1.3 Report Organization

The report comprises of five chapters; introduction, literature review, methodology, results and discussion, and conclusion. Introduction gives the overview about bone tissue engineering, problem statement and objective of the project. Literature review gives narrative analysis about the past and current established solutions related to the problem solutions.

Methodology comprises of materials and procedures that are used to implement the project. Result and discussion gives comprehensive analysis based on the methodological approach. Conclusion gives the overall analysis about the proposed solution and also propose future studies that can be performed.

1.4 Objectives

The objectives of the study are:

1. To fabricate the polyhydroxy-butyrate/ rice husk silica-bioglass (PHB/RHBGC) scaffolds using electrospinning technique.
2. To characterize the various ratios of electrospun PHB/RHBGC composite scaffolds using Field Emission Scanning Electron Microscopy (FESEM) for the morphology, water contact angle for the hydrophilicity and Fourier Transform Infrared (FTIR) spectroscopy for the chemical complexation.

CHAPTER 2: LITERATURE REVIEW

2.1 Rice Husk Ash (RHA)

Rice husk is a hard-protective covering of rice gain which is an agricultural waste product generated during the rice milling process. In most cases, rice husks are either burned or dumped in open spaces which contributes to environmental pollution. However, due to raise in concerns related to environmental pollution and sustainable energy, rice husks have gained attention of many researchers to look for better waste management and alternative use of rice husks. Researchers have come up with wide range of applications for most common agricultural waste. At present, the common applications of rice husk include biofuel, soil remediation silicon battery material and many more (Shen, 2017). Similarly, rice husks have also shown promising benefits for biomedical applications.

Rice husk comprises about 87% to 97% silica and other impurities. Silica as a raw material is widely used in electronics and as a polymer in industries. Silica is obtained from rice husk by acid leaching of rice husk in boiling temperature and burning the rice husk at high temperature until it turns into white powder. Silica has quite many application such as drug encapsulation and manufacturing scaffold in tissue engineering. Presence of silica in the scaffold have enhanced the bioactivity nature. A biomaterial is manufactured out of silica which is called Bioglass that can come in many forms such as pellets, powder or mesh. In the earlier times, Bioglass was used in the form of solid for small bone replacement. However, at the present state it has a wide range of clinical applications mostly in the field of BTE due to its biocompatibility, biodegradability and osteoconductive characteristics. In vitro studies showed that Bioglass had non-toxic by products, no inflammation to the cells of both soft and hard tissues (Hench, 2006; Nayak, Kumar, & Bera, 2010).

2.1.1 Possible applications of Bioglass

The current clinical applications of bioglass are as listed below:

1. As a bone graft: because of the interconnected porous structure of the bioglass, it supports tissue growth and improved the stability of the implant.
2. As a carrier: bioglass is known for its biocompatible and good mechanical strength features which also makes it suitable to be a carrier for delivering drugs and growth factors in to the body to induce bone tissue growth or eradicate bone diseases (Chen, Chou, Chan, & Lin, 2017).
3. As a coating material: the biocompatibility feature of bioglass makes it suitable to be coated on a metal implant of bone. It can protect the metal against corrosion from the body fluids and improve the durability of the metal implant.
4. As a scaffold in bone tissue engineering: bioglass scaffolds are made out of nanofibers which exhibit high porosity and high surface area (Jones, 2013; Nayak et al., 2010). These features facilitate good cell adhesion, differentiation and proliferation.

Initially, bioglass is fabricated by sol gel technique or traditional melting process. However, there are various fabrication techniques available to produce bioglass nowadays as shown in Table 2.1.

Table 2.1: Advantages and disadvantages of various fabrication techniques for bioglass (Boccaccini et al., 2010)

Fabrication Technique	Advantages	Disadvantages
Sol-gel technique	• Versatile • Can produce variety of nanoscale bioactive glasses	• Time consuming process. • Batch-to-batch variations may occur.
Microemulsion technique	• Can obtain inorganic particle in nano size with minimal agglomeration.	• Low production yield
Laser spinning technique	• Very fast process. Nanofibers can be produced in microseconds.	• Expensive

2.1.2 Limitations

The applications of bioglass is certainly limited to non-load bearing parts of the body, due to their inherent brittleness. This can be enhanced by making composites of bioglass and other naturally derived materials like corals, which may give better mechanical strength and enhance biocompatibility. Since bioglass is degradable, more studies need to be carried out such as material degradation rate and waste material toxicity level for future establishment of bioglass as a potential biomaterial. However, the limitations of bioglass are minimal compared to its versatile strength (Krishnan & Lakshmi, 2013; Naghizadeh et al., 2014).

2.2 Polyhydroxybutyrate (PHB)

PHB comes from the group of polyhydroxyalkanoates (PHAs). It was first discovered as a component from bacterium Bacillus megaterium in 1926. It has excellent biocompatible and biodegradable properties (Yu, Lan, Wang, Fang, & Sun, 2010) and can be produced by both naturally from bacteria and synthetically. PHB has high melting point and crystalline structure. However, it has intrinsic brittleness which limits its applications. The properties of biodegradability of PHB can be controlled and its degradation product (hydroxybutyric acid) is a common metabolite in human body. The degradation of PHB happen by hydrolysis in environments where extracellular enzymes from microorganisms are present; transforming the polymer in oligomers. These are finally transformed in carbon dioxide and water by intracellular enzymes. By reinforcing the PHB with bioceramics, an increase of Young Modulus and micro-hardness were obtained with controllable degradation rate. Introduction of RHBGC will improve the bioactivity of the composites tremendously by using low cost and easily available source of rice husk.

2.3 Electrospinning

Electrospinning is an efficient technique for production of nanofibers out of polymeric biomaterials. It can generate 2D or 3D constructs and produces highly porous non-woven ultrafine fiber mats that can propose varieties of application in tissue engineering. The fibers can mimic the topologies of ECM. The parameters of electrospinning can be manipulated to achieve the desired properties of the scaffold such as fiber diameter and thickness. This method is cost effective and simple which can produce fibers in the range from 1 nm to 1000 nm.

Electrospinning is a process that can produce nano fibers out of polymer solutions by utilizing electrical forces. It was first observed in 1897 by Rayleigh and was patented by Formhals in 1934. This technique can produce fibers in the nano to submicron range and has gained attention in the last decade not only due to the production of polymeric fibers but also producing nano sized fibers which is difficult to attain by standard mechanical fiber-spinning techniques (Bhardwaj & Kundu, 2010). Electrospinning technology has been applied in various fields such as tissue engineering, pharmaceutical, clothing, healthcare etc. For biomedical application, electrospinning is mainly used for fabrication of scaffolds. Various types of scaffolds can be manufactured with differing pore structure, morphology and porosity. The design of the electrospinning device is unsophisticated which makes it cost effective (Doshi & Reneker, 1995). The electrospun fibers have high surface area, high permeability and high interconnectivity which makes it able to mimic the biological functions at the cellular level. In the field of tissue engineering, it can mimic the properties of ECM providing cell proliferation and differentiation (Bambole & Yakhmi, 2016). Electrospinning technique has been used to produce non-woven nano fibrous mats for tissue engineering using various natural polymers such as silk fibrin and collagen (Agarwal, Wendorff, & Greiner, 2008). Moreover, this technique is also been used for drug delivery system by encapsulating drugs with biodegradable fibers. (Chew, Wen, Dzenis, & Leong, 2006).

2.3.1 Electrospinning device

The electrospinning device is composed of three fundamental components which are a high voltage supply, a needle of small diameter and a stationary metallic collector. Currently, there are two types of experimental setup that is vertical and horizontal. The electrospinning setup is placed under a fume hood where unpleasant vapors are pumped and shields from air turbulence (Frenot & Chronakis, 2003). From Figure 2.1 represents the experimental setup pf electrospinning device. The high electric field which is positive charge electrode is applied across the needle containing the polymer solution and negative charge is applied on the metallic collector, this induces a charge in polymeric solution which then accelerates towards the collector. Initially, a cone shaped jet is ejected out of the needle, this is known as Taylor's cone. When critical value of electric charge is achieved, the Taylor's cone transforms into unstable whipping jet. As a result, the solvent evaporates and the polymer lays and solidifies on the grounded metallic collector (Bhardwaj & Kundu, 2010).

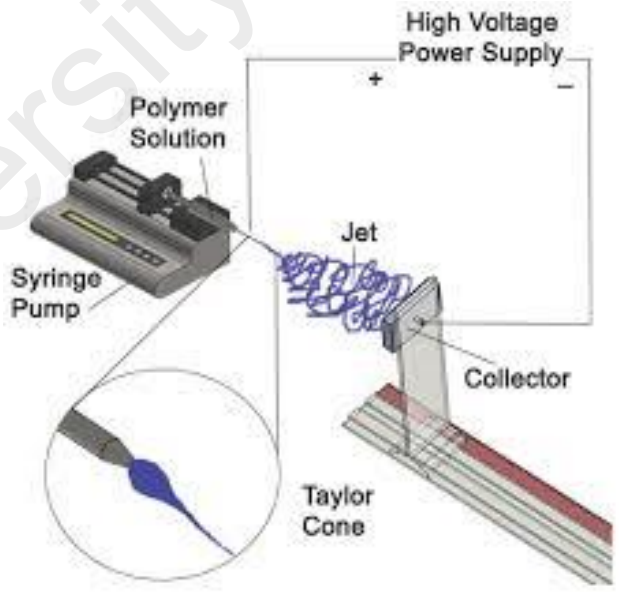


Figure 2.1: Electrospinning device (Bhardwaj & Kundu, 2010)

Electrospinning process is governed by many parameters which is classified into intrinsic parameters, processing parameters and ambient parameters. Intrinsic parameters depend on the nature of the material such as conductivity, molecular weight and viscosity. Processing parameters are electric potential, distance between collector and needle and flow rate. Ambient parameters include the environmental factors surrounding the electrospinning device such as temperature, air velocity and humidity. The parameters have notable effect on the fiber morphology and diameter of electrospun nanofiber (Bhattacharjee & Rutledge, 2017; Chew et al., 2006).

2.3.2 Limitations

Apart from beneficial properties of electrospun fibers, there is still a requirement for essential studies since there are many challenges remaining. For example, reproducibility of nano fibers with specific position and orientations. A lot of raising issues related to toxicity and interaction of scaffolds with biological tissues need to be thoroughly investigated before this technique can be used for real practical medical applications (Agarwal et al., 2008).

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 Extraction of rice husk silica (RHS)

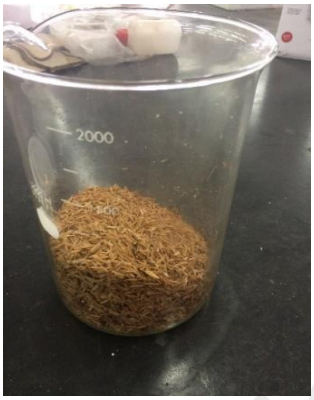
Rice husk was purchased from BERNAS rice mill, Tg. Karang, Selangor, Malaysia. The first step was removal of dirt and water-soluble impurities by washing some adequate amount of rice husk with sodium dodecyl sulfate solution (Figure 3.1 a). Furthermore, the rice was rinsed with distilled water and dried in air oven at 110 ºC for 24 hours. After the drying process, the washed rice husk goes under acid leaching process (Figure 3.1 b). At this stage, the un-leached rice husk was treated with warm hydrochloric (HCL) acid at a concentration of 0.5 M for 30 min with constant stirring. After the acid treatment, the acidic solution was drained off, the acid leached rice husk was rinsed with distilled water for several times and air-dried. The acid-leached rice husk was placed in the oven at 600 ºC for 2-3 hours to obtain the rice husk ash. After burning the rice husk, white ashes were obtained which are known to be silica (RHS) (Figure 3.1 c).

3.2 Fabrication of Rice husk silica based Bioglass (RHBGC)

For fabrication of RHB, 4 g of RHS was added to 0.1 M sodium hydroxide (NaOH) and dissolved in 100 ml of distilled water. The solution was constantly stirred and heated up to 60 ºC until clear solution is obtained. Acidic solution was prepared separately, 10 g of Calcium Nitrate ($Ca(NO_3)$) was added to 50 ml nitric acid (HNO_3). The sodium silicate solution was added drop by drop in to the acidic solution until gel was formed. The gel was left to age and dry which later turned into whitish material. This material was placed in the furnace for sintering at 600 ºC for 2 hours. The final product was obtained in the form of white solid which was crushed into powder form. To obtain homogeneous nano sized particles, the sintered RHB powder was freeze dried for 24 hours and sieved to extract the

fine powder (Naghizadeh et al., 2014). The schematic diagram for sol gel synthesis is shown in Figure 3.2.

a)



b)

c)

Figure 3.1: Unwashed rice husk (a), Acid leached rice husk (b) and White ashes of silica (c)

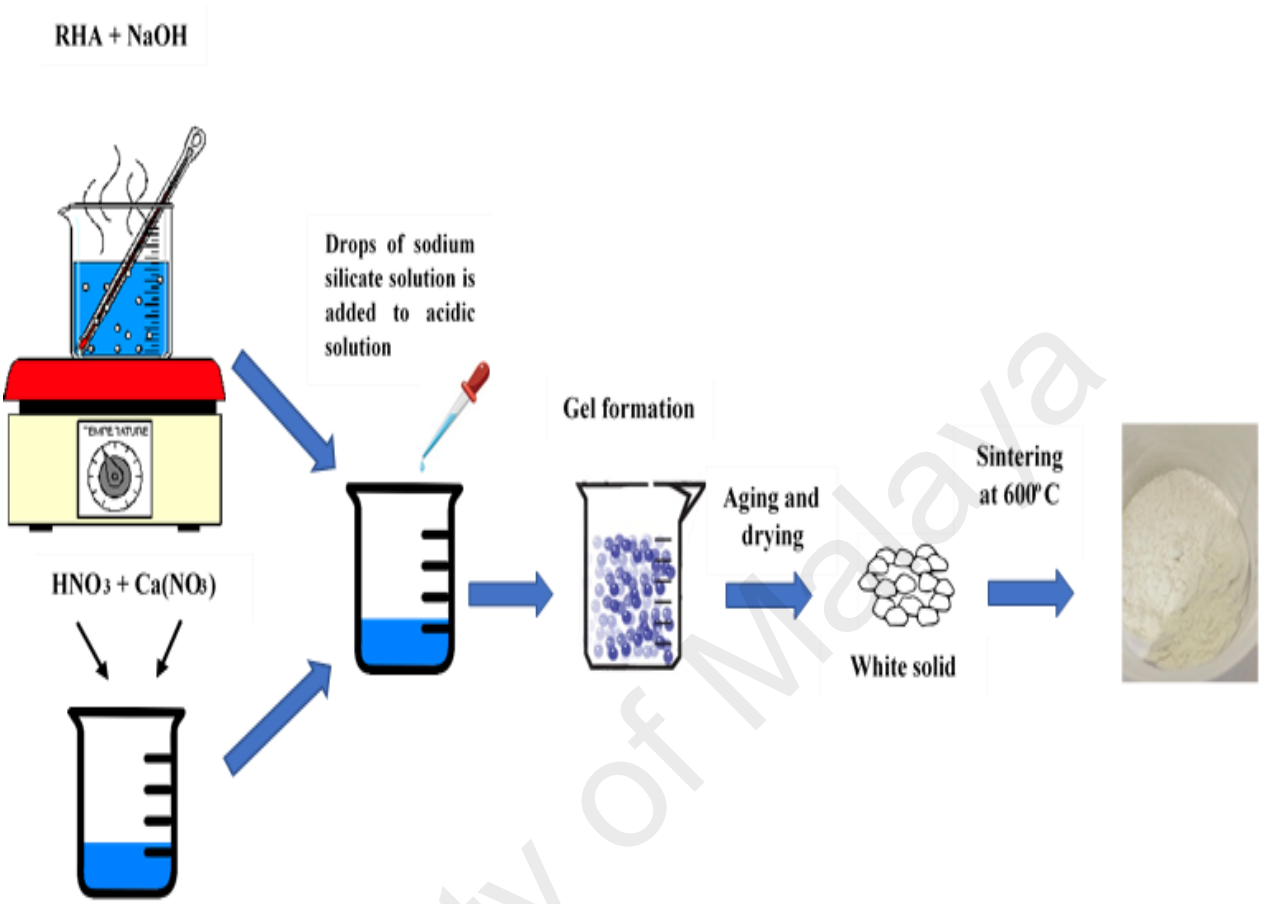


Figure 3.2: Schematic diagram of Sol-gel synthesis of bioglass

3.3 Electrospinning solution

PHB and RHB solution are prepared in three different ratios i.e. 90:10, 80:20 and 70:30. PHB solution was prepared by adding 2 grams of PHB powder (Sigma Aldrich) and were dissolved in 18 ml chloroform (anhydrous, contains 1% ethanol as stabilizer, Friendemann Schmidt). 2 ml of N, N-Dimethylformamide (DMF, Merck) was added to the solution to improve conductivity. According to the ratios, three separate PHB solutions were prepared and 0.2g, 0.4g and 0.6g of RHB were added to the PHB solutions respectively. The solutions were gently stirred using a hotplate with magnetic stirrer for 2 hours in a water bath of 60 ºC (Figure 3.3 a). The solutions were loaded in to a 10ml syringe.

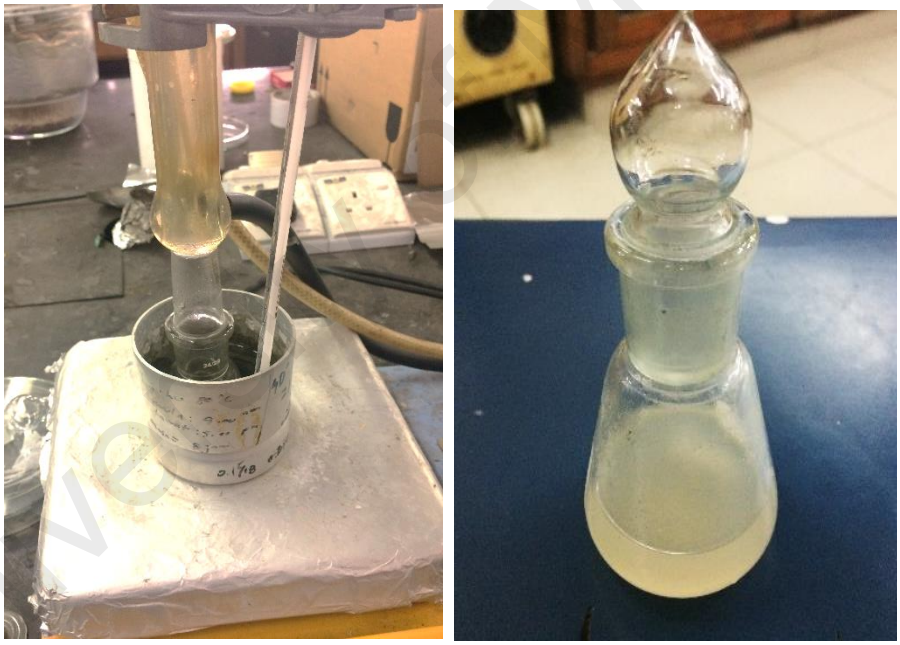


Figure 3.3: a) Electrospinning solution in water bath b) Electrospinning solution

3.4 Electrospinning device

The electrospinning devices comprises of step up transformer, syringe, syringe pump and collector. The step-up transformer supplied high voltage up to 12 V. In this setup, the needle of the syringe served as positive electrode and the collector which was an aluminum foil acted as negative terminal. The syringe filled with electrospinning solution and solution was spun at flow rate of 2.5 ml/hr. The distance between the needle and the aluminum collector was 18 cm. These parameters are considered as an optimal to achieve well aligned fibers. Before supplying high voltage, the syringe pump was operated to check if the needle is clogged. After the solution is dripping form the syringe, high voltage was supplied, a stable jet coming out of needle was observed which resulted in fibers (Naghizadeh et al., 2015).

Figure 3.4: Electrospinning device

The following Figure 3.5 exhibits the final product of three types of composites of PHB/RHBGC:

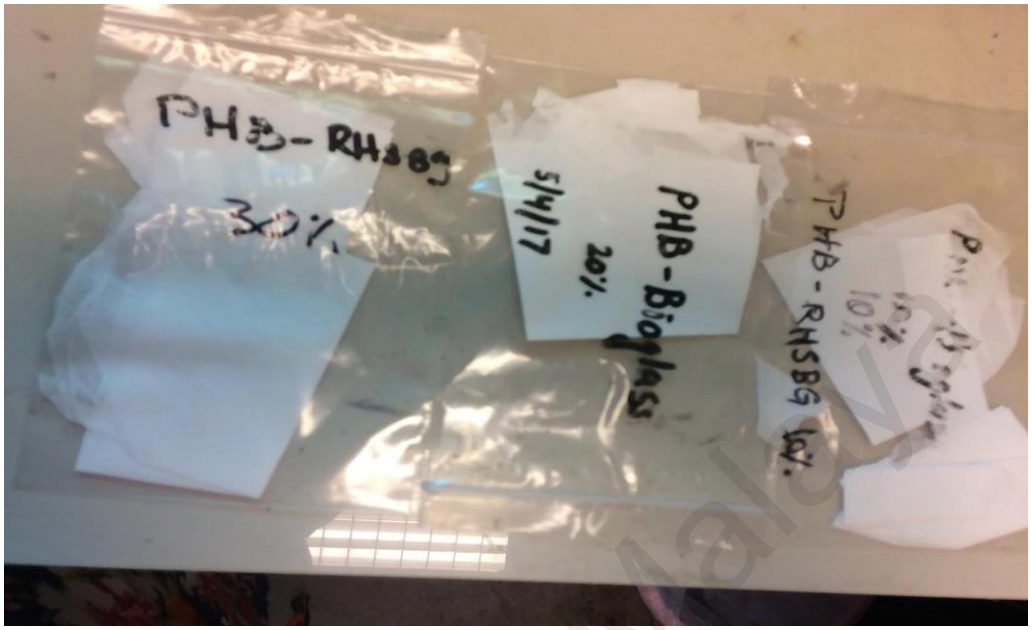


Figure 3.5 Electrospun PHB bioglass fibers

3.5 Characterization methods

3.5.1 Field Emission Scanning Electron Microscopy (FESEM)

The morphology and fiber diameter of the composite electrospun scaffolds were examined using FESEM. Samples were cut in desired shape and were kept in freeze dryer for 24 hours. Samples were sputter coated with a thin layer of gold before the scanning process.

Figure 3.6: a) Coating the samples b) placing the sample for FESEM examination

3.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR (ATR-FTIR, Perkin Elmer Spectrum 400) was used to examine the chemical compositions of the sample in the range of 4000-400 cm^{-1}.

Figure 3.7: FTIR spectroscopy

3.5.3 Water Contact Angle Goniometer

To test the hydrophilicity of the electrospun composites, a water contact goniometer is used. It is comprised of B. Braun 1 ml syringe, light source and computer system. Sample was placed underneath the needle, and 2µl of water is dispensed on the sample at a dosing rate of 1µl/s. SCA20 software was used to measure the water contact angle on the sample.

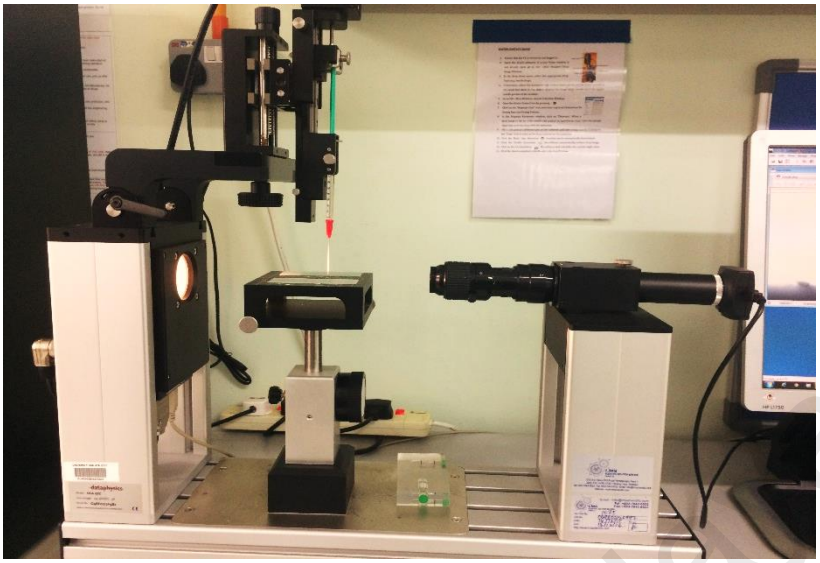


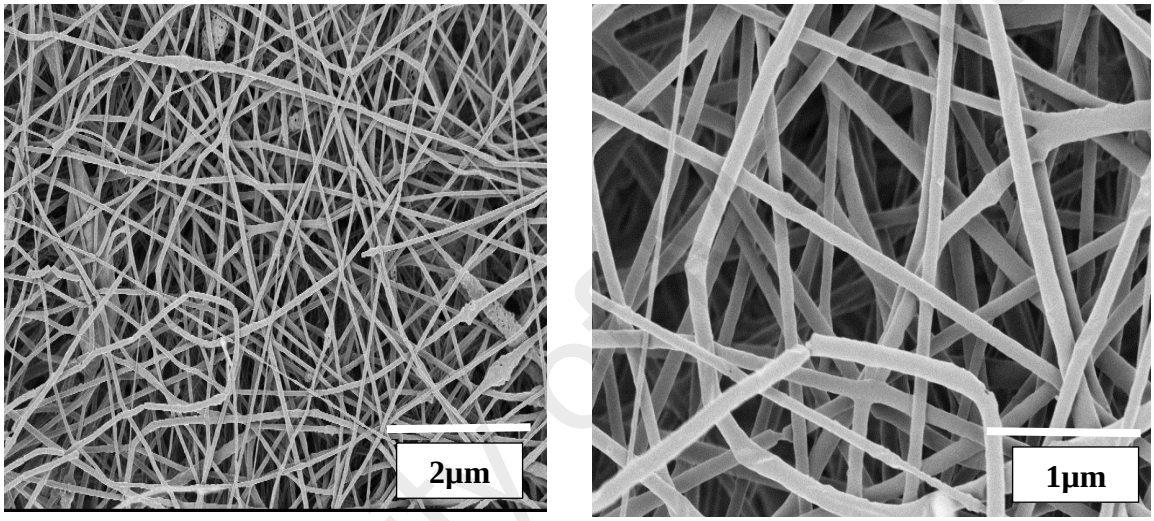
Figure 3.8: Water Contact Angle Goniometer

Figure 3.9: Drop of water on the sample to examine the hydrophilicity of the sample

CHAPTER 4: RESULTS AND DISCUSSION

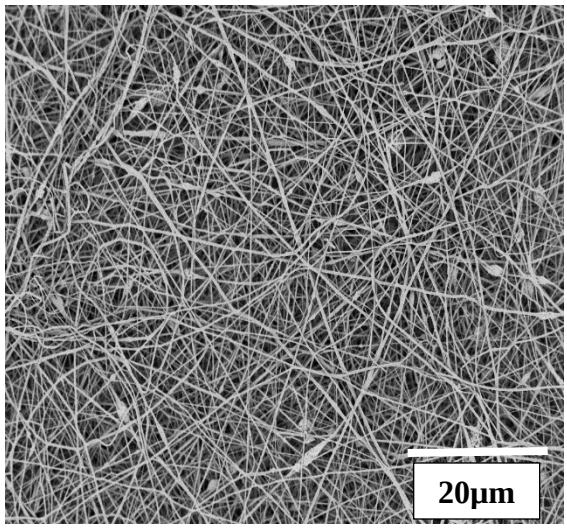
4.1 Morphology

Figure 4.1 shows the quite uniform distribution of PHB fibers with minimal beads and granules. The PHB fibers serve as reference for other composite fibers in terms of fiber diameter and formation of beads.

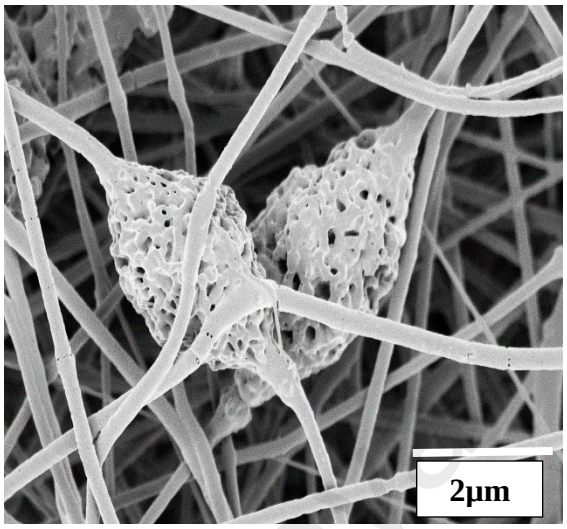


a) b)

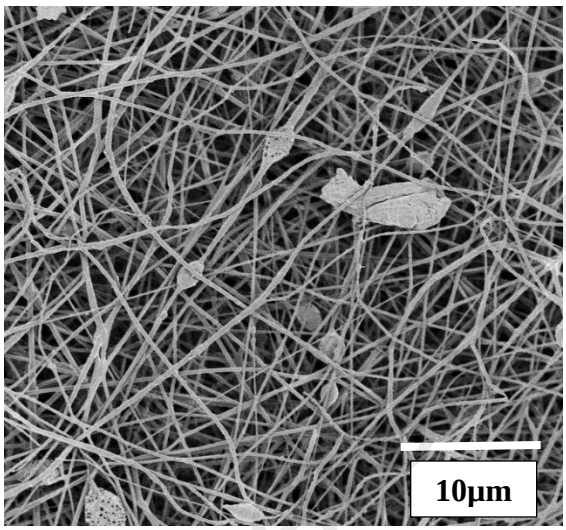
Figure 4.1: FESEM images of PHB scaffold (a) 500X magnification (b) 5000X magnification



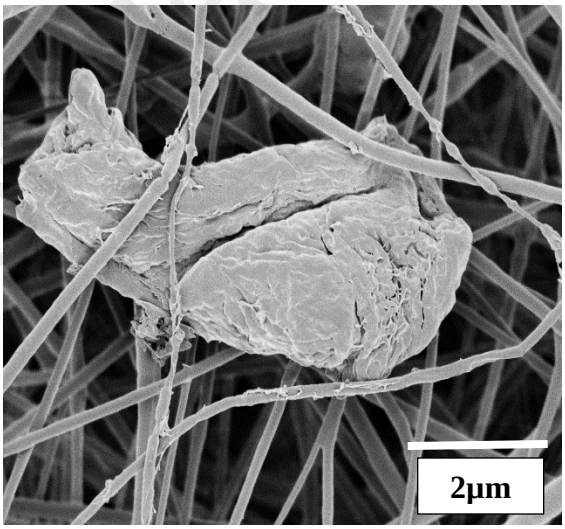
a)



b)

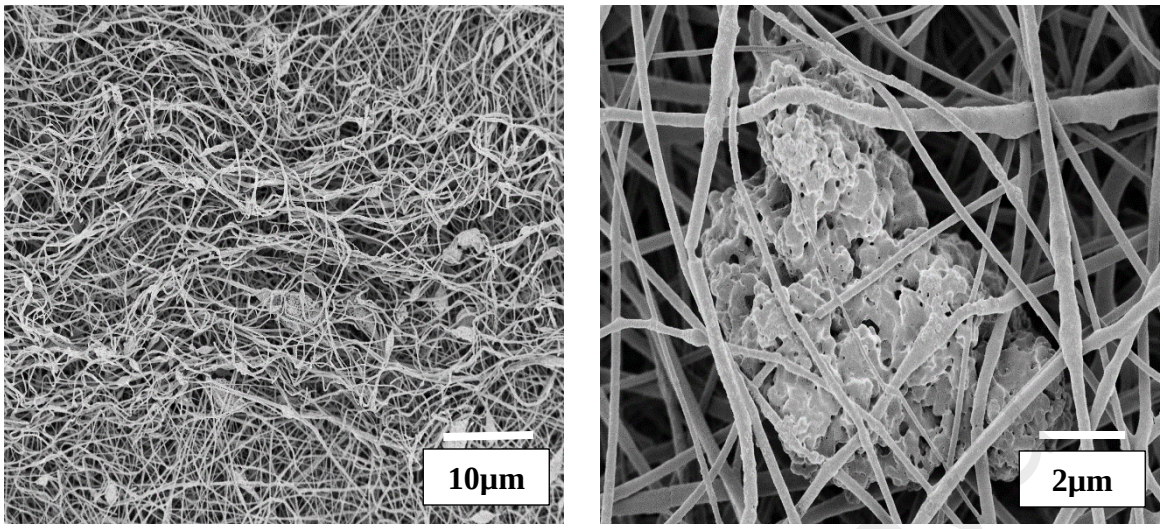


c)



d)

Figure 4.2: Fiber morphology (a) and (b) PHB/RHBGC (10%), (c) and (d) PHB/RHBGC(20%)



a) b)

Figure 4.3: FESEM of PHB/RHBGC (30%) composite (a) 500X magnification (b) 3500X magnification

Figure 4.2 (b), shows the presence of interconnected fiber and confirms porosity which is essential for bone cell regeneration. Presence of interconnected fibers promotes vascularization and enhances diffusion rate. From the images, it can be observed composite fibers are over lapping on each other, resulting in web like structures. This proves that the bioglass can generate nanofibers at small range of diameter which makes it eligible for cell attachment. Since RHBGC does not completely dissolve in the solvent, it is suspended in the polymeric solution and hence it can lead to formation beads. In Figure 4.2 (a) and (c), porous structures can be observed. In Figure 4.3, the composite fiber has more intense interconnection of fibers and porous than the other composite, the fibers are slightly twisted which makes it look little coiled. Figure 4.3(a), shows, the bioglass particle engulfed in PHB nanofibers. The intense nanofibrous network and inter connected pores enhance the mechanical stability of the scaffold (Bhattacharjee & Rutledge, 2017). It is observed that bioglass has agglomeration in their structure resulting in thicker fibers and limited the movement of polymer chains (Foroughi, Karbasi, Khoroushi, & Khademi, 2017).

Fiber diameter

Image J software was used to calculate the diameter of the electrospun fibers. Figure 4.4-4.7 show the diameter of 20 randomly picked fibers of pure PHB and PHB/RHBGC composites scaffolds.

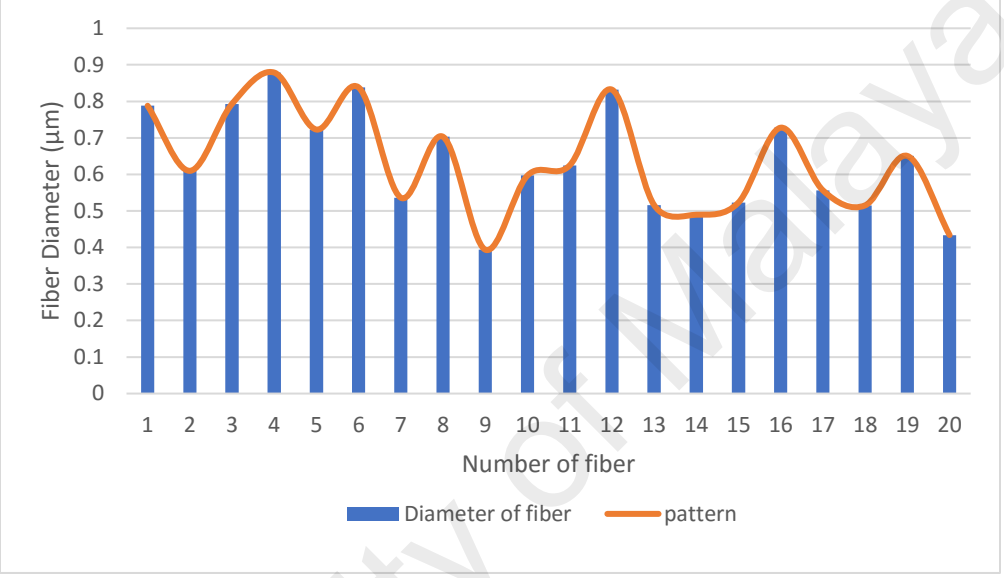


Figure 4.4: Fiber diameter of PHB electrospun fiber

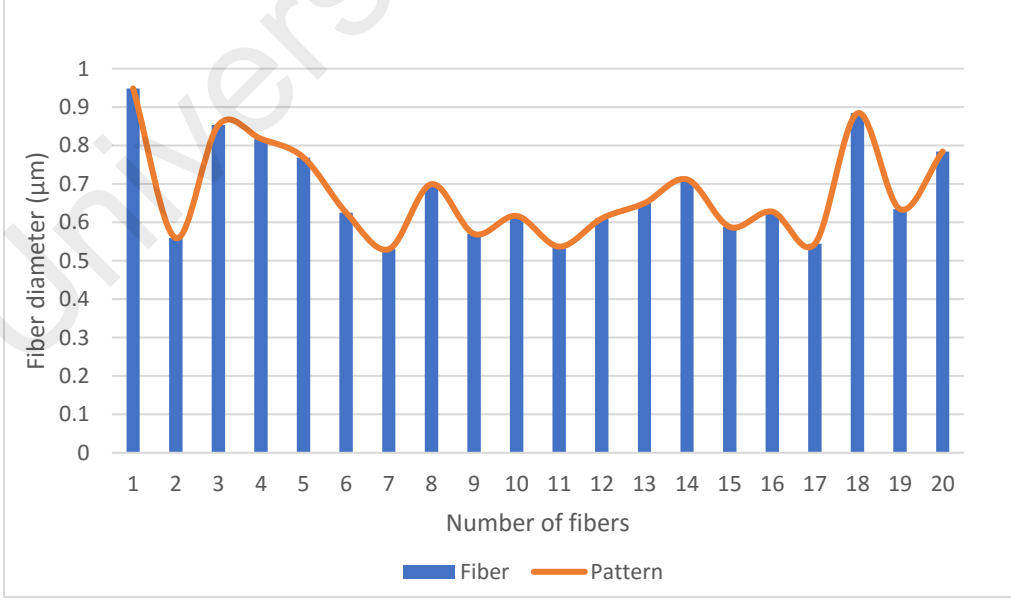


Figure 4.5: Fiber diameter of PHB/RHBGC (10%)

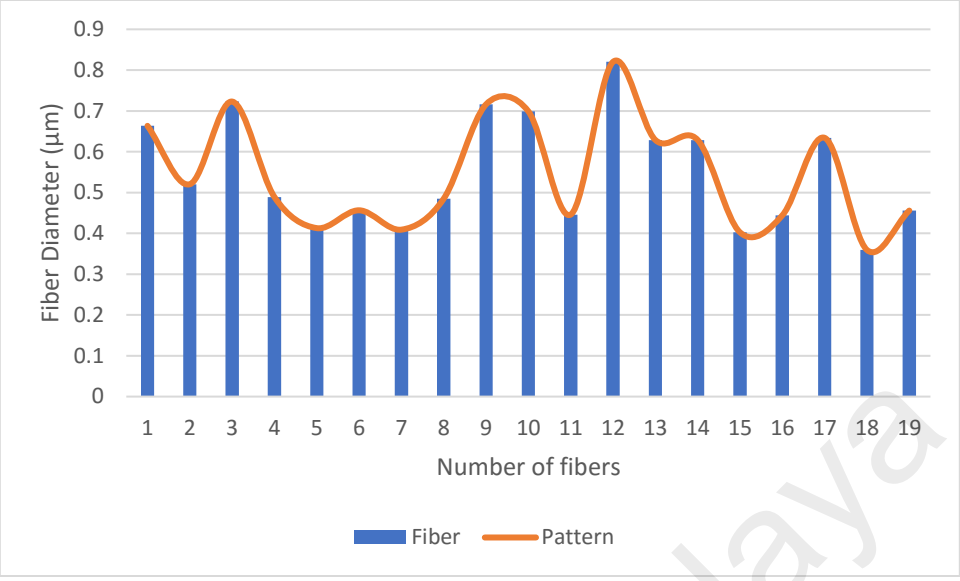


Figure 4.6: Fiber diameter of PHB/RHBGC (20%)

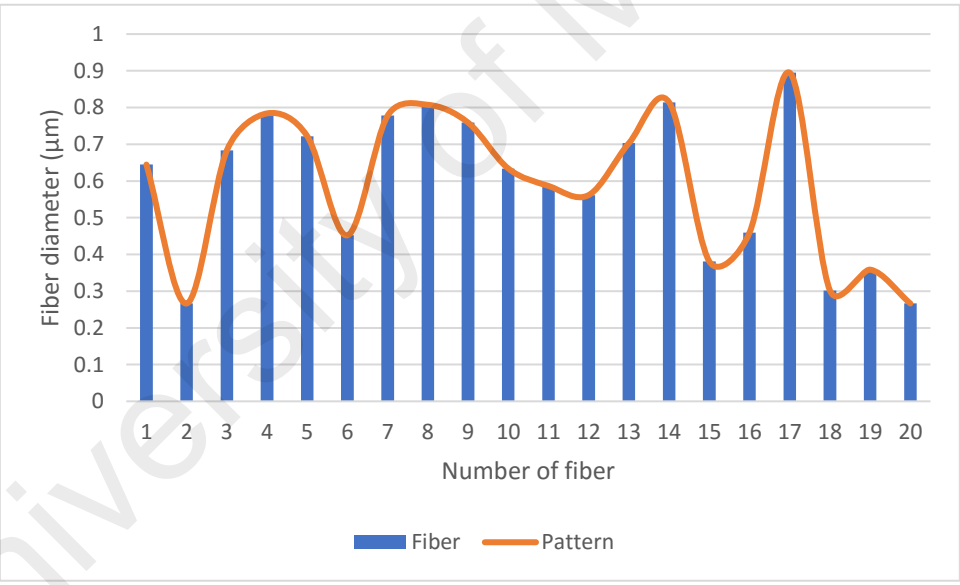


Figure 4.7: Fiber diameter of PHB/RHBGC (30%)

From Figure 4.4 to 4.7 it is shown that the pattern is not consistent. However, in Figure 4.7 it is observed that the fibers have more fluctuations than the rest. Table 4.1 displays the average diameter of fibers.

Table 4.1: Average Diameter of Fibers

Electrospun fiber	PHB	PHB/RHBGC (10%)	PHB/RHBGC (20%)	PHB/RHBGC (30%)
Average diameter (μm)	0.620±	0.465±	0.698±	0.612±

It is observed that addition of bioglass in the PHB polymer affects the diameter of the fibers. Thicker electrospun fibers can be achieved by increasing the concentration of polymer. However, it is reported that combination of microfiber and nanofibers in a scaffold enhances multiple cell interfaces and promotes different cellular functions.

The effect of pore interconnectivity, pore size and large surface area make the electrospun fiber highly conductive to adhesion and growth of cell (Rnjak-Kovacina & Weiss, 2011). The pore size and interconnectivity also affects the degradation rate of the scaffold. From Figure 4.8, all the composites have highest pore size distribution in the range of 0.41-0.8 μm. In Table 4.2 shows the average pore size, no. of intersection and total pore distribution of each electrospun fiber. The average pore size and total pore distribution have been calculated by using Image J software. PHB/RHBGC (30%) has the lowest average pore size which is 0.681± μm and PHB fiber has the highest average size of the pore which is 0.957± μm. Total pore distribution and highest number of interconnection is seen in PHB/RHBGC (30%) fiber compare to the rest of the fibers, therefore it is concluded that PHB/RHBGC (30%) has the highest porosity.

Table 4.2: Average pore size and total pore distribution of each fiber

Electrospun fiber	PHB	PHB 10% BG	PHB 20%BG	PHB 30% BG
Total pore distribution	686	836	850	1337
No of Intersections	1816	2047	2366	3330
Average pore size (µm)	0.957±	0.803±	0.786±	0.681±

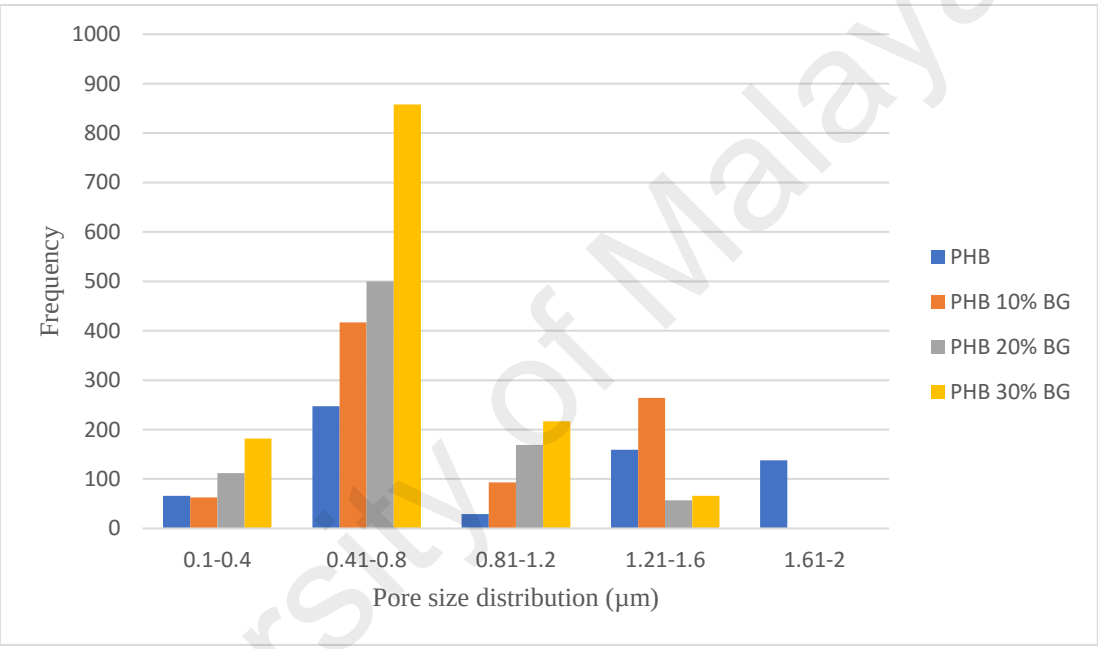


Figure 4.8: Histogram of pore size distribution

The morphology of electrospun fibers is affected by many parameters such as applied voltage, flowrate, concentration of solution etc. Researchers reported increase in solution concentration increase the fiber diameter which may also enhance the porosity (Essien, Adams, Shaibu, & Oki, 2013). Moreover, electrospun scaffolds inherent too small pores that may not be adequate for tissue growth, studies suggested that implementation of wet spinning instead of conventional electrospinning may lead to increase in pore size.

4.2 Fiber mat wettability

The results of water contact angle test are shown in Table 4.3. PHB is hydrophobic it is expected that addition of bioglass can affect the hydrophobic property of PHB. From the table, it shows addition of RHBGC has decreased the contact angle from 130.4 to 115.7. PHB/RHBGC (20%) has the lowest contact of 115.7. It is reported that there is a close relationship between hydrophilic surfaces and cell attachment on the surface. Cells generally favor to attach on hydrophilic surface. Contact angle less 90 indicates high hydrophilicity of material. Unfortunately, due to low concentrations of bioglass, significant differences cannot be noticed in the contact angle.

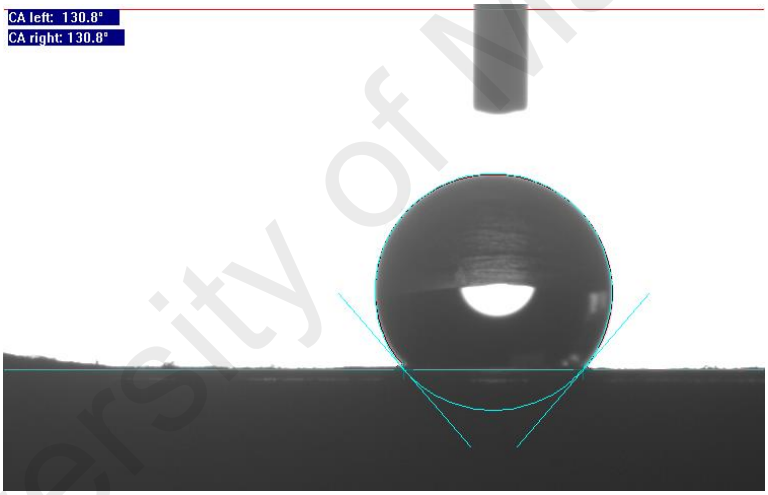


Figure 4.9: Contact Angle for pure PHB

Table 4.3: Contact Angle for Electrospun scaffold

Sl. No.	Electrospun Fiber	Contact Angle
1	PHB	130.4
2	PHB-10%BGC	125.8
3	PHB-20%BGC	115.7
4	PHB-30%BGC	121.6

4.3 FTIR analysis

For the assessment of functional groups FTIR tests were performed on pure PHB fiber, RHBGC and composites of PHB/RHBGC of three different ratios. The transmission spectra of PHB, PHB/RHBGC composites and RHBGC in the range between 400cm^{-1} and 4000cm^{-1}. Figure 4.10 The most prominent band for the recognition of PHB in pure PHB and PHB/RHBGC composite is the ester carbonyl group at 1722 cm^{-1}. The peaks at 979 cm^{-1} and 1279 cm^{-1} are sensitive to crystallinity and representation of C-O-C characteristic peak. The peak at 1132 cm^{-1} indicates to C-O-C asymmetric stretching vibrations. The peaks at 2930-2976 cm^{-1} have appeared due to carboxyl group. The bands which appear between 1380cm^{-1} and 1454 cm^{-1} represent the deformations of methyl (CH_3) and methylene (CH_2). Figure 4.10 shows the spectrum of PHB/RHBGC composite. Since the amount of RHBGC is very low, major differences in the spectrum cannot be noticed. However, small changes such as sharper and smaller peaks can be noticed due to interaction between PHB polymer and bioglass. This indicates the formation of O-Si bond between bioglass and carbonyl groups of PHB. The peak at 1056 cm^{-1} corresponds to C-O stretching (Foroughi et al., 2017).

Figure 4.10 shows the FTIR spectrum for RHBGC powder. The center band at 1349 cm^{-1} indicate the vibrations of non-bridging PO_2 in phosphate groups or carbonate groups, which emerged from air during sol-gel fabrication. The peak at 1013 cm^{-1} correlates to asymmetric Si-O-Si stretching vibrations of silanol groups. The peak located at 615 cm^{-1} corresponds to asymmetric vibration of PO_4.

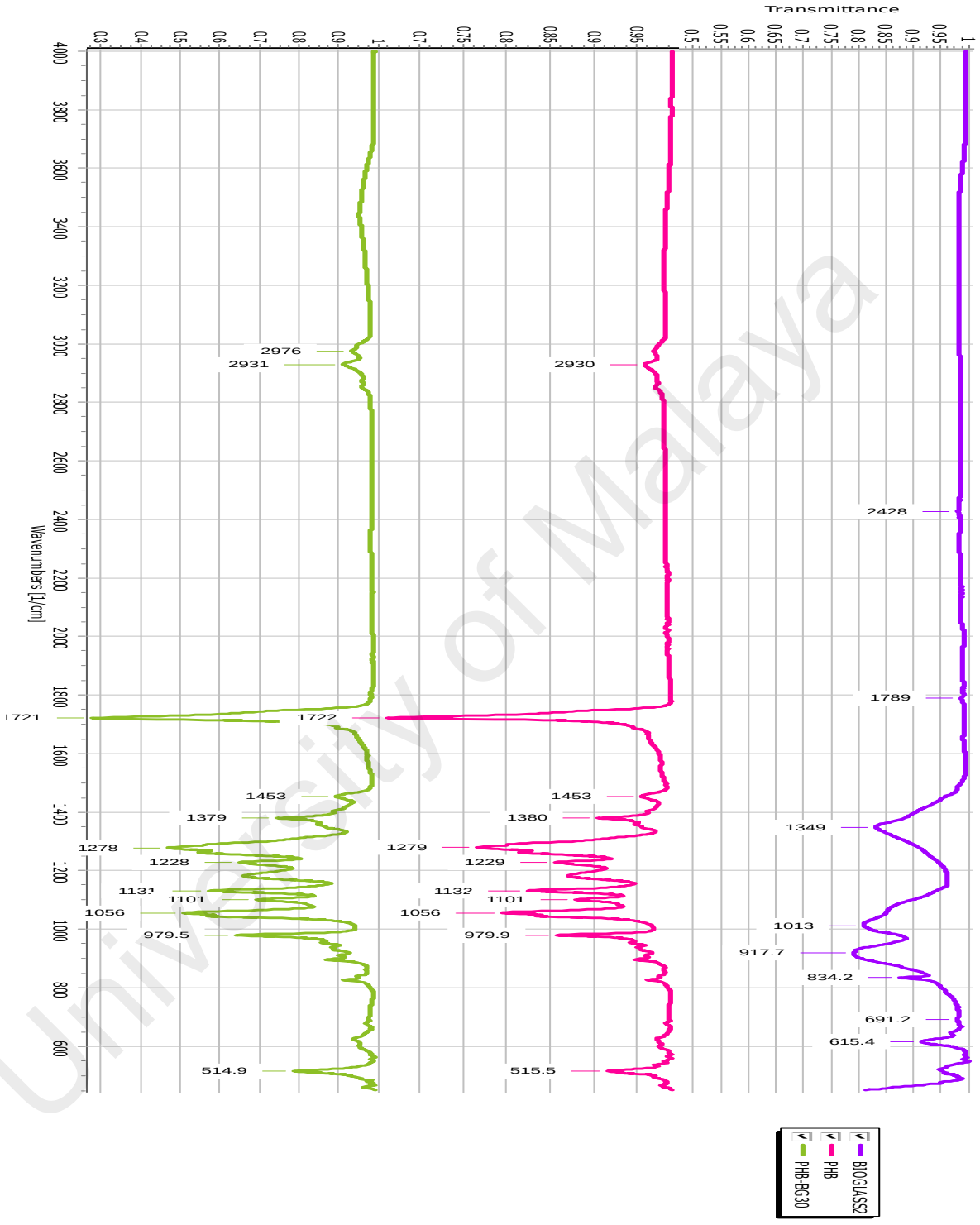


Figure 4.10: FTIR spectra for PHB/RHBGC, PHB and RHBGC powder

CHAPTER 5: CONCLUSION

The present study has shown that rice husk ash is rich in silica. Si plays effective role in bone tissue regeneration. The morphological studies reveal the presence of nano particle and microparticle in the scaffold composition. Porosity of the scaffold increases with increase in concentration of RHBGC which leads to enhancement of cell adhesion and migration on the scaffold. Average diameter of PHB/RHBGC fiber is in the range of 400-600 nm, hence this indicates that high surface to volume is achieved which leads to enhancement of cell bioactivity such as adhesion, migration, proliferation and differentiation. Addition of PHB/RHBGC into the polymer leads to intense interconnected fibers and increases porosity. However, due to low concentration of RHBGC in the composite, significant changes in the peak cannot be noticed between the FTIR spectra of pure PHB and PHB/RHBGC composite. Since bioglass doesn't completely dissolve in chloroform solvent, other solvent can be proposed to analyze the mechanical and chemical properties of bioglass.

Further test could be implemented to determine the degradation rate of PHB/RHBGC scaffold. The degradation rate is one the essential parts in tissue engineering. The PHB/RHBGC can be immersed in phosphate buffer saline for some days. To determine the mechanical strength of the PHB/RHBGC scaffold by using the universal testing machine to analyze whether PHB/RHBGC is suitable as biomaterial. To study the cell interaction and behavior in the PHB/RHBGC based scaffold, cytotoxicity test can be performed.

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