

Functionalized Polyvinyl alcohol – Click reaction compositebased Polymerized High Internal Phase Emulsion scaffold for Biomedical applications

Submitted in partial fulfillment of the requirements

for the award of

Bachelor of Technology degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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May – 2023



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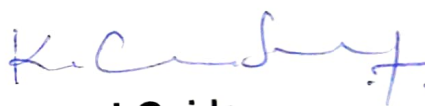
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Jesse Miracline Bincy D (39230019)** who carried out the project entitled "**Functionalized Polyvinyl alcohol-Click reaction composite based Polymerized High Internal Phase Emulsion scaffold for Biomedical applications.**" under our supervision from **January-2023 to May-2023.**


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DECLARATION

I **Jesse Miracline Bincy D (Reg. No - 39230019)**, hereby declare that the Project Report entitled "**Functionalized Polyviny alcohol – Click reaction composite based Polymerized High Internal Phase Emulsion scaffold for Biomedical applications**" done by me under the guidance of **Dr.P.Prakash Ph.D**(internal guide) **Dr.K.Gobi Saravanan Ph.D**(external guide) at **International Research Centre, Centre for Nanoscience and Nanotechnology**, Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology in **Biotechnology**

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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr.V.Ramesh Kumar Ph.D Head of the Department, Department of Biotechnology** for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr.M.Bavanilatha Ph.D** (Internal guide) and **Dr.K.Gobi Saravanan Ph.D** (External guide) for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of **Biotechnology** who were helpful in many ways for the completion of the project.

ABSTRACT

In the creation of scaffolds for the highly internal phase emulsion 5emplate polymers (poly HIPEs) provides a variety of potential benefits. A free- standing nanocomposites with 3D interconnected microporosity selectively decorating the scaffolds inner surface were produced by the free radical polymerization of HIPEs. Porous polymers that have been fabricated using high internal phase emulsion (HIPEs) have become one of the most popular techniques for creating three dimensional (3D) scaffolds because they make it simple to create interconnected porosity using a number of synthetic processes. In order to create polyHIPE, water- in-oil HIPEs are employed. On the basis of water soluble polyvinyl Alcohol (PVA), direct water-in-oil emulsions are produced. A thiol and an alkene are used to form polyHIPE. One of the key benefits of polyHIPEs is the ability to control the pore size and interconnectivity. Using conventional or additive manufacturing techniques, thiol-ene chemistry is the best way to create biodegradable photocurable poly HIPEs for the production of scaffolds. Using a suitable surfactant, commercially available multifunctional thiols are formed into water-in-oil high internal phase emulsions(HIPEs) and the HIPEs are photocured. According to its biocompatible material composition, PVA scaffold is non-cytotoxic and has been shown to be able to stimulate cell development. This research reports the work to fabricate thiol and Polyvinyl Alcohol based poly HIPE materials through a photoinitiated thiol-ene click reaction. The material is more helpful for biomedical applications due to its porous profile and biodegradability. Micromorphological characteristics, Hemocompatability, Cytotoxicity analysis have been investigated.

TABLES OF CONTENTS:

CHAPTER NO:	TITLE		PAGE NO:
	BONAFIDE CERTIFICATE		i
	DECLARATION		ii
	ACKNOWLEDGEMENT		iii
	ABSTRACT		iv
	LIST OF FIGURES		vi
	LIST OF ABBREVIATIONS		vii
01	INTRODUCTION		1
	1.1	Types of Scaffolds	2
	1.1.1	Natural polymers	2
	1.1.2	Synthetic polymers	2
	1.2	PolyHIPE scaffold	3
	1.3	Need for PolyHIPE scaffolds	3
	1.4	Click reaction	3
02	LITERATURE SURVEY		4
03	3.1	AIM	8
	3.2	SCOPE	8
04	METHODOLOGY		9
	4.1	SYNTHESIS	10
	4.2	Polymerization	11

	4.3	Characterization	13
	4.3.1	FTIR Analysis	13
	4.3.2	Raman Spectroscopy Analysis	13
	4.4	Invitro Studies	14
	4.4.1	Hemolysis	14
	4.4.2	Cell Cytotoxicity	14
05	RESULTS AND DISCUSSION		
	5.1	Characterization Results	16
	5.1.1	FTIR Analysis	16
	5.1.2	Raman Spectroscopy Analysis	21
	5.2	Invitro Studies	
	5.2.1	Hemolysis	22
	5.2.2		23
06	SUMMARY AND CONCLUSION		25
	REFERENCES		26
	PUBLICATION AND CERTIFICATE		37

LISTOF FIGURES:

FIGURE NO	NAME OF THE FIGURE	PAGE NO
4.1	Synthesis of PVA	10
4.2	PVA Poly (HIPE) Scaffold	12
5.1	FTIR analysis of scaffold	16
5.2	FTIR analysis of Thiol	18
5.3	FTIR analysis of Ene	20
5.4	Raman Spectroscopy analysis for scaffold	21
5.5	% of Hemolysis for scaffold	22
5.6	Fibroblast cells untreated	23
5.7	Fibroblast cells with scaffold	23
5.8	% of live cells	24

LIST OF ABBREVIATIONS:

ABBREVIATION	FULL FORM
PVA	Polyvinyl alcohol
HIPE	High Internal Phase Emulsion
BSA	Bovine Serum Albumin
DMEM	Dulbecco's Modified Eagle Medium
FTIR	Fourier Transform Infra Red
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

CHAPTER 1

INTRODUCTION

Globally, organ transplantation plays a significant role in medicine and has emerged as a crucial therapeutic approach for preserving and extending life in a wide range of clinical diseases. Regenerative medicine has a similar history to organ transplantation. One of the biggest accomplishments in the history of medicine maybe considered organ transplantation. In this specific discipline, moral principles relating to medicine, religion, and social customs are all considered. Organ trafficking, payment for organs, the delicate balance between the benefit to the receiver and the potential harm to the donor and others, as well as other important ethical dilemmas that call for strong action, are just a few of the pressing moral problems that need to be addressed. Definitions of death, particularly those related to brain death, are a significant problem in organ transplantation. Regenerative medicine refers to a variety of techniques for repairing or replacing damaged tissue or organs, including (i) in vivo tissue repair using host cells that grow into acellular natural or synthetic biomaterials, (ii) in vitro tissue engineering using seeded cultured cells to create biomaterial scaffolds, and (iii) therapeutic cloning and stem cell-based tissue regeneration. Currently, tissue restoration is typically done by either using the patient's own body or tissue from a healthy donor (allograft). (autograft). The shortcomings of these treatments, however, include the scarcity of donor tissues, the danger for infection, the poor allograft survival rates, to name a few. Tissue engineering is a developing science that potentially get beyond these restrictions. To repair injured tissues, tissue engineering uses natural, synthetic, or semi-synthetic tissues. Typically, tissue engineering uses a combination of scaffolds, cells, and promote cell ingrowth.

1.1 TYPES OF SCAFFOLDS:

There are two main types of scaffolds:

- Natural polymers
- Synthetic polymers

1.1.1 Natural polymers:

Natural polymers are proteins and polysaccharides. They show biocompatibility and biodegradability but they show poor mechanical strength. Eg. Collagen, Alg, HA derivatives etc.

1.1.2 Synthetic polymers:

Synthetic polymers are chemically derived polymers. Among them poly(lactic acid) PLA, poly(glycolic acid) PGA, poly(ϵ -caprolactone) PCL, poly(vinyl alcohol) PVA are the most common types. They show mechanical strength, surface morphology, and physiochemical properties than natural polymers.

Scaffolds for tissue engineering can be categorized into:

- Fibrous
- Porous
- Hydrogel

In our study, we use poly(vinyl alcohol) PVA as a polymer for creating porous scaffold-poly HIPE scaffold. Successful cell growth into functional tissues has been achieved using porous scaffolds. For cell attachment, porous materials offer a lot of surface area. For host cells as well as promoting cell development and proliferation, scaffold porosity and the connection of the pores within scaffolds are essential.

1.2 POLY (HIPE) SCAFFOLD:

The goal of tissue engineering (TE) is to come up with ways to fix damaged or ill sections of the host tissue that won't heal on their own. For the purpose of filling the faulty site, TE uses porous matrices known as scaffolds. To allow for cell penetration, nutrition flow, and integration of the material within the host tissue, scaffolds must have a porous design and high interconnectivity. Emulsion templating has attracted special interest as a method for fabricating scaffolds. It provides (i) high porosity (up to 99%) (ii) High interconnectivity. While high porosity and interconnectivity promote vascularization, cell migration, and the formation of new tissues. The method consists of two fundamental steps: creating an emulsion from at least two immiscible liquids in which one phase (the internal, dispersed phase) is mixed with the other phase (the continuous, exterior phase), and solidifying the emulsion's continuous phase. Droplets of the dispersed phase act as templates in this process, and they are removed after solidification to create porous matrices.

1.3 NEED FOR POLY(HIPE) SCAFFOLDS:

Porous polymers generated through emulsion templating are one promising kind of scaffold. These scaffolds meet the essential architectural standards for scaffolds used in tissue engineering. The primary benefit of HIPE polymerization is their capacity to create a three-dimensional, highly interconnected scaffold that is very porous and encourages cell ingrowth.

1.4 CLICK REACTION:

The thiol-ene reaction, also known as alkene hydrothiolation, is a chemical reaction in which a thiol (RSH) reacts with an alkene ($\text{R}_2\text{C}=\text{CR}_2$) to produce a thioether (RSR'). Free-radical additions are initiated by light, heat or radical initiators, which form a thiyl radical species. The radical then propagates with an ene functional group to form a carbon-centered radical. Photopolymerization is a useful radical-based reaction for applications within the nanotechnology.

CHAPTER 2

LITERATURE SURVEY

Rajeswari, *et al.* explained about Functionally Graded Materials (FGMs) that are being extensively investigated for tissue engineering applications, as tissues in the body exist as a gradient structure to meet versatile functional requirements. Also she explained that are particularly true for hard tissue substitutes, as they have a hierarchical graded structure with anisotropic properties. She concluded, a scaffold designed with gradients in chemical composition, mechanical properties, and spatial distribution would be ideal for use in bone repair.

Tianyu Yao, *et al.* explained that angiogenesis plays a pivotal role in tissue engineering and regenerative medicine for the successful survival of critical sized biological constructs. During tissue regeneration, blood vessels can supply oxygen and nutrients to sustain cell viability. He concluded that the cells located away from blood vessels suffer from hypoxia and apoptosis.

Petra Utrosa, *et al.* gives detailed explanation of optimal cell growth, differentiation, and function is a suitable scaffold stiffness that meets tissue-specific requirements. Heterogeneous or interfacial tissues such as osteochondral tissue, skin layers, and blood vessel walls exhibit a gradual change in stiffness. She had a conclusion of Osteochondral tissue is composed of soft articular cartilage that protects the underlying stiff subchondral bone plate by absorbing load when being subjected to high strains while minimizing transmission to the bone.

Atefeh Safaei-Yaraziz,et al. tells that the emerging engineering field of biomimetic extracellular matrices (ECM) is crucial in regenerative medicine and tissue engineering. The hierarchical network architecture of native ECM provides mechanical support and a biological interactive microenvironment for cellular and organ fusion. The interpenetrated network (IPN) structure of native ECM consists of crosslinked proteins interlocked with biomacromolecules.

Areli Munive-Olarte,et al. explained that in bone tissue engineering, the design of scaffolds is based on the understanding that bone is a mineralized collagen nanocomposite with a hierarchical structure from the microscale to the nanoscale. He points out the Current challenges include mimicking the chemical composition of the bone surface, simulate the structural properties of the bone extracellular matrix, provide sufficient mechanical strength and stiffness to support the proliferation and differentiation of osteoblast, and construct a 3D porous interconnected network for vascular tissue ingrowth and diffusion of nutrients and metabolites.

Viola Hobiger,et al. did research on porous polymers that has expanded into different directions, such as gas and energy storage, separation methods, heterogeneous catalysis and the biomedical sector. He said crosslinked polymers with micrometer-sized pores and an interconnected open porous structure are especially interesting for biomedical applications. They can be employed for tissue engineering, tissue regeneration, cell culturing, wound dressings, or controlled release.

Tucker J. McKenzie,et al explained that porous elastomers that possess structural or chemical properties in a spatially controlled manner are highly desirable for many applications including biomaterial interfaces. Also he said additive manufacturing, purification membranes, and acoustic manipulation as metamaterial wave guides. For example, polymeric foams possessing a porosity gradient have been prepared for controlled wave front shaping in gradient-index acoustic materials.

Betül Aldemir Dikici, et al. explained about the process for creation poly (HIPE). Solidifying of a monomeric continuous phase and subsequent removal of the internal droplet phase leaves behind a porous structure that is a 3D replica of the initial emulsion. He concluded that emulsions with an internal volume higher than 74% are defined as high internal phase emulsions (HIPEs), and substrates obtained by their polymerization are called polymerized HIPEs (PolyHIPEs).

Ilya Krizhanovskiy, et al. said that Organosilicon polymers are widely used in nearly all fields of human activity. Such a broad application range is due, in particular to the diversity of the chemical composition and architectural forms of these compounds. Also he said the hydrosilylation reaction is among the most versatile tools for creating Si-C bonds. This reaction occurs with participation of metal complex catalysts, most often those of the platinum group, on substrates containing double or triple bonds and organosilicon compounds with a functional Si-H group, is simple to implement, and features high commercial availability and versatility.

Ozgun Can Onder, et al. states that the ultimate goal of scaffolding for tissue engineering is to create functional and implantable scaffolds capable of providing suitable micro-environments for optimal cell growth, differentiation and function. He concluded that in this respect, the scaffolds should possess an interconnected macroporous structure for cell penetration and transfer of metabolites and nutrients.

Jennifer L. Robinson, et al. explained about the formation of injectable poly(HIPE) scaffold for bone tissue engineering. She developed the injectable and biodegradable PFDMA poly(HIPE). She tested her sample with human mesenchymal stem cell hMSC and also characterized the sample. She had a result of 90% viability for her sample and relatively slow cure with injury.

Maja Susec, et al. explained about the open porous monoliths for cell growth. He created the High Internal Phase Emulsion scaffold using low toxicity monomers D-Valine and pentaerythritol TT using thiol-ene photo polymerization. His porous scaffold resulted maximum porosity while analysed using SEM. Also he cultured osteoblast cells with his sample and the cell growth was observed.

Chiaoyi Chen, et al. created porous polymer scaffold by emulsion templating method using thiol-ene and thiol-yne photopolymerisation. He characterized the sample by Raman spectroscopy and analysed the porosity using SEM. He concluded that the acrylate monomers have significant levels of residual functionality and through the studies of mechanical properties, more highly functional monomers giving higher levels of crosslinking and therefore a more rigid material.

Su - Kyoung Chae, et al. explained the fabrication methods for creating porous PVA scaffold for Lung epithelial system. He characterized the material using FTIR to determine chemical structure and porosity was observed using SEM. Also he did cell culture using A549 cells with his sample and cell viability was determined.

Wei Luo, et al. explained about Poly(vinyl alcohol) hydrogels created by surfactant-free Co₂ in water emulsion using emulsion templating method. He created highly porous PVA scaffold without adding any organic solvents and surfactants. He studied cellular growth and proliferation with H9c2 cardiac muscle cells. Also he analyzed the porosity of the sample using SEM.

CHAPTER 3

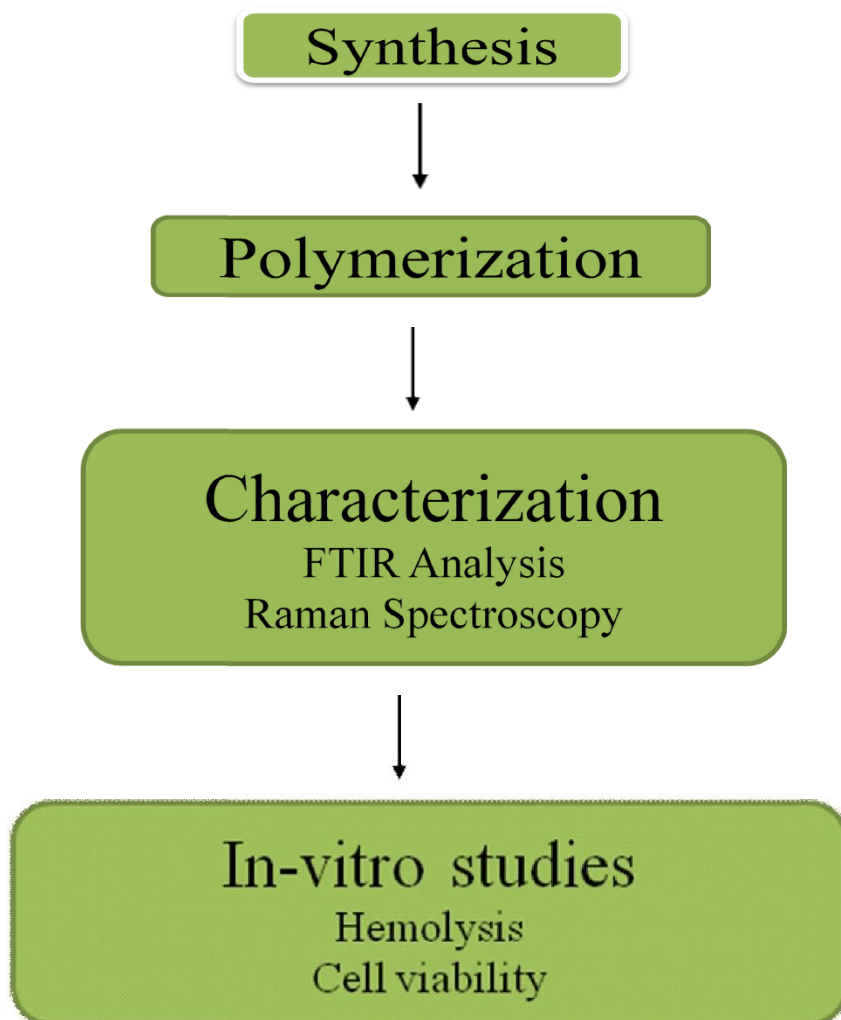
3.1 AIM

To develop biological substitutes to restore, replace or regenerate defective tissues. Scaffolds, typically made of polymeric biomaterials, provide the structural support for cell attachment and subsequent tissue development. Scaffolds for tissue engineering are support structures designed to facilitate cellular growth and proliferation upon implantation into the patient. Tissue engineering (TE) aims to regenerate critical size defects, which cannot heal naturally, by using highly porous matrices called TE scaffolds made of biocompatible and biodegradable materials.

3.2 SCOPE

- Polymerized high internal phase emulsions (polyHIPEs) have been utilized in the creation of injectable scaffolds that cure *in situ* to fill irregular bone defects and potentially improve tissue healing.
- PolyHIPEs have been investigated as tissue-engineered scaffolds because of their tunable mechanical properties and pore architectures that are appropriate for tissue regeneration.
- Scaffolds are of great importance in clinical medicine. It is an upcoming field, and usually associated with conditions involving organ disease or failure. It is used to rebuild organs and return normal function.

CHAPTER 4
METHODOLOGY:



4.1 SYNTHESIS:

PVA was dissolved in distilled water (100 ml) with ethyl acetate (1.5 g) under the flow of nitrogen and cooled it to 0° C in an ice bath. To this, PETMP (Thiol) (5 ml) was dissolved in 55ml of toluene and cooled to 0° C, was added dropwise with vigorous stirring. The solution was colourless and it was kept in room temperature for 18 hours. Then solution was observed to contain solid, which was removed by filtering over filter paper. The filtrate was then concentrated by rotary evaporation. The solution was extracted with KOH (3% w) and then with HCl (1%w). During the extraction process a thick, cream like emulsion formed which was slow to separate. To aid separation the emulsion was centrifuged (8000 rpm for 60 to 180 mins). The extraction organic phase was then dried over magnesium sulphate, filtered, and the DCM removed by rotary evaporation. This yielded a waxy yellow solid which was then further dried on a high vacuum line. The final product was obtained in 84% yield and stored at -4° C.

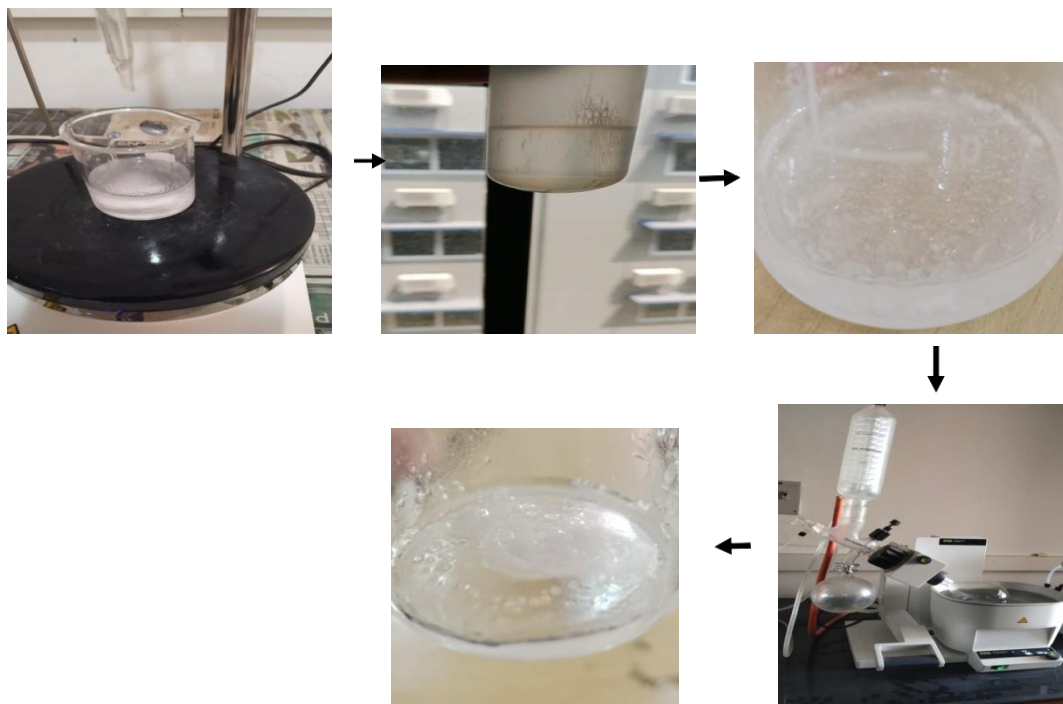


Fig 4.1 Synthesis of PVA

4.2 POLYMERIZATION:

Sample	PVA(g)	Thiol(g)	Ene (ml)	Surfactant (g)	Initiator (g)	Volume (ml)	Internal Phase(ml)
Synthesized PVA	1.7	0.55	4.0	0.22	0.32	6.79	90

Trithiol, PVA , Alkene, surfactant and the photoinitiator were combined to form an oil phase and the beaker wrapped in aluminium foil to exclude light. once the organic phase was mixed until the solution was free flowing, the volume of the organic phase was then measured with a measuring cylinder. The oil phase was stirred continuously. An aqueous phase of deionised water was added drop wise to form a HIPE. Once the aqueous phase was added the HIPE was allowed to stir for a further minute. The HIPE was then transferred and passed under a UV irradiator six times on each side of the emulsion. The resulting monolithic sheet was then carefully removed and immersed in an ethanol bath for 48 h.

Formation of (HIPE) High Internal Phase Emulsion:



Fig 4.2 PVA Poly (HIPE) Scaffold.

4.3 CHARACTERIZATION:

4.3.1 FTIR Analysis:

FTIR Analysis, commonly known as Fourier Transform Infrared Spectroscopy or FTIR Spectroscopy, is an analytical method used to distinguish between organic, polymeric, and, occasionally, inorganic materials. The FTIR analysis method scans test materials and examines chemical characteristics using infrared light. FTIR provides data based on the chemical constitution and physical state of the entire sample.

In our study , we did FTIR Analysis for our sample to confirm the chemicals i.e Thiol and Ene .

4.3.2 Raman Spectroscopy Analysis:

Raman spectroscopy is a method of chemical analysis that gives precise details regarding crystallinity, molecular interactions, phase and polymorphy of the substance being analysed, and chemical structure. Raman is a method of light scattering in which a molecule disperses incident light from a powerful laser light source. Rayleigh scatter is the term for when most of the scattered light has the same wavelength (or colour) as the laser source and offers no relevant information. Raman Scatter, on the other hand, is the little quantity of light (usually 0.0000001%) that is scattered at various wavelengths (or colours), depending on the chemical makeup of the analyte.

A Raman spectrum has several peaks that display the strength and location of the Raman scattered light's wavelength. Each peak, including specific bonds like C-C, C=C, N-O, and C-H, is associated with a particular chemical bond vibration.

In our study , we did Raman Spectroscopy analysis for our sample to identify the chemical structures.

4.4 INVITRO STUDIES:

4.4.1 Hemolysis:

The most popular technique for assessing a biomaterial's hemocompatibility is hemolysis testing. Hemolysis is the breakdown of red blood cells with the sample. our blood's red blood cells are a vital component. They transfer oxygen from the lungs to the tissues all over your body. This oxygen is used by our body to provide the energy required to carry out necessary tasks. In order to breathe it out, carbon dioxide is also transported back to our lungs by red blood cells from our tissues. Red blood cell apoptosis normally makes space for brand-new, healthy red blood cells. Red blood cells typically have a lifespan of 120 days.

In our study , we did hemolysis testing for our sample to determine the percentage of destruction of RBC with our sample.

Initially, test samples were put in a 25 ml beaker. Then, 10 ml of saline was placed into a beaker holding test samples, followed by a 30-minute incubation at 37°C. The supernatant (saline solution) was then collected and centrifuged at 1500 rpm for 10 minutes. 8 ml of anticoagulant-containing whole blood was diluted with 10 ml of normal saline solution, from which 0.2 ml was removed and placed into materials extracting saline solution. As a negative and positive control, normal saline and distilled water were employed.

A UV-spectrophotometer was used to measure the solution at 545 nm. The following equation was used to calculate the rate of hemolysis (HR).

$$\% \text{ Hemolytic ratio} = [\text{OD test} - \text{OD negative} / \text{OD Positive} - \text{OD Negative}] \times 100$$

4.4.2 Cell Cytotoxicity:

Cell cytotoxicity is the term used to describe a chemical's capacity to kill living things. Healthy live cells can be made to experience either necrosis (accidental cell death) or apoptosis (planned cell death) by employing a cytotoxic substance. Cell viability may be assessed in a variety of ways, but one method is to employ formazan dyes, which

are vital dyes. The dehydrogenases like lactate dehydrogenase (LDH) and reductases that are produced during cell death reduce tetrazolium salts to produce the chromogenic formazan colours. Eg MTT.

The MTT(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) test is based on the transformation of MTT into formazan crystals by live cells. The MTT test measures the metabolic activity of cells using colorimetry. Under specific circumstances, NAD(P)H-dependent cellular oxidoreductase enzymes may serve as an indicator of the quantity of alive cells. The tetrazolium dye MTT, or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, can be converted by these enzymes to its insoluble, purple formazan.

We used fibroblast cells for our study to identify the cytocompatibility of our sample.

CHAPTER 5

RESULTS AND DISCUSSION:

5.1 CHARACTERIZATION RESULTS

5.1.1 FTIR (*Fourier Transform Infra Red*) Analysis:

An important method that makes it simple to identify certain functional groups in an organic molecule is Fourier Transform Infrared Spectroscopy (FTIR).

Functional groupings have unique vibration frequencies. These vibrational frequencies belong to the infrared (IR) frequency band. Therefore, when an IR signal is sent through an organic complex, the functional groups vibrate at particular frequencies. In other words, an infrared signal that travels through an organic compound will be absorbed at these distinctive frequencies, resulting in a distinct spectrum.

In our study we did FTIR for our sample to identify the Thiol and Ene compounds in the scaffold.

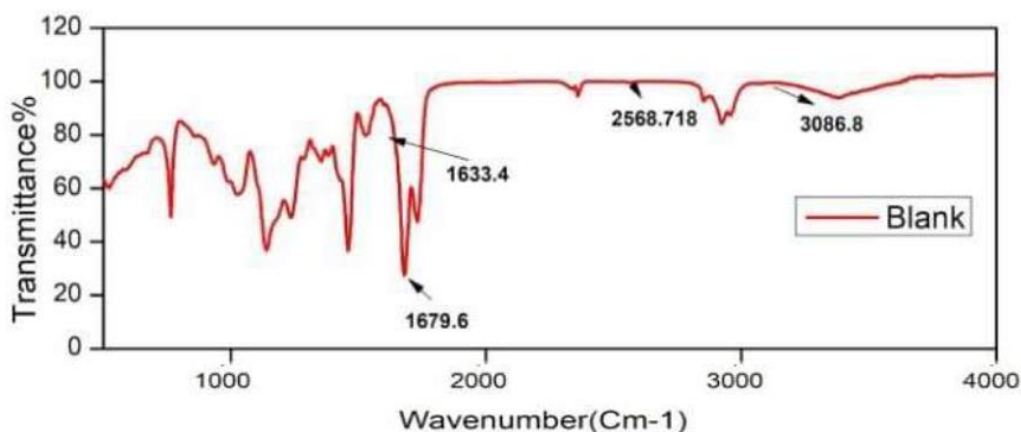
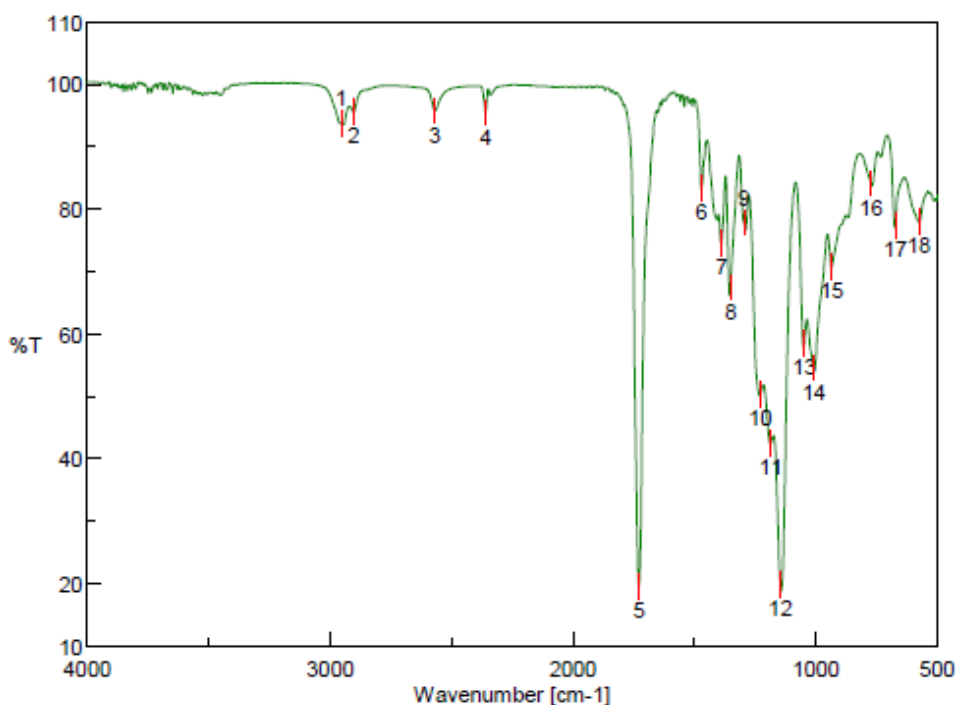


Fig 5.1 FTIR analysis of scaffold.

Peak Find - Memory-12



[Comments]

Sample name

Comment

User

Division

Company

Sathyabama Institute of Science and Technology

[Detailed Information]

Creation date 28-01-2023 15:03

Data array type Linear data array

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Vertical axis %T

Start 349.053 cm-1

End 7800.65 cm-1

Data interval 0.964233 cm-1

Data points 7729

[Measurement Information]

Model Name FT/IR-6600typeA

Serial Number B049361790

Accessory ATR PRO ONE

Accessory S/N C215161809

Incident angle 45 deg

Measurement Date 28-01-2023 15:03

Light Source Standard

Detector TGS

Accumulation 32

Resolution 4 cm-1

Zero Filling On

Apodization Cosine

Gain Auto (4)

Aperture Auto (7.1 mm)

Scanning Speed Auto (2 mm/sec)

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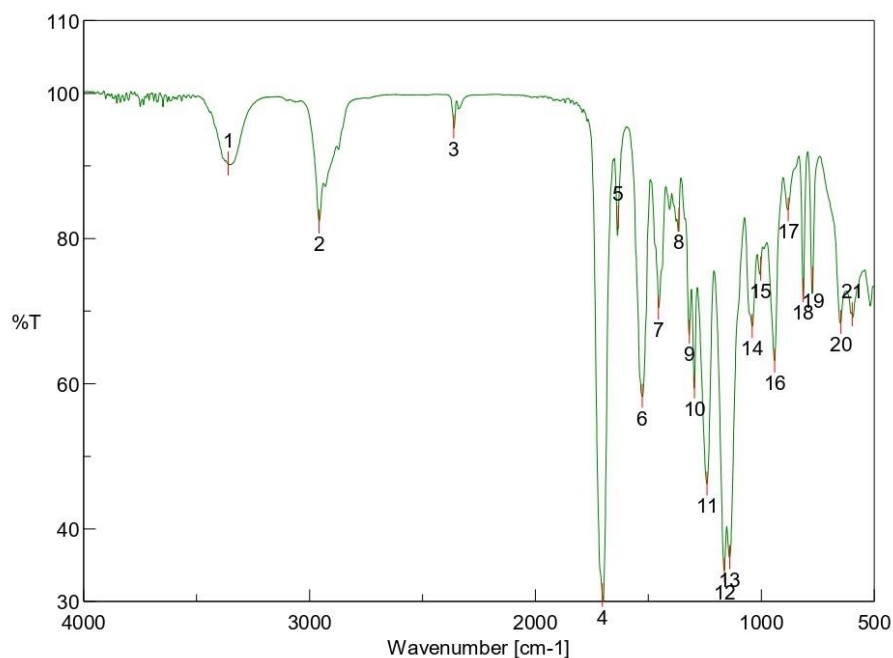
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5	1728.87	19.6118	6	1472.38	83.2796
7	1388.5	74.4396	8	1350.89	67.5048
9	1295.93	77.8403	10	1227.47	50.3436
11	1186.01	42.3909	12	1146.47	19.7418
13	1047.16	58.4843	14	1007.62	54.5416
15	934.342	70.7384	16	773.315	84.1549
17	672.071	77.4303	18	574.683	77.902

Fig 5.2 FTIR analysis for Thiol

From the above graph, vibration at 1728.87cm^{-1} and 1146.47cm^{-1} shows the highest absorbance of Thiol. The other peaks are at average of $1388.5, 1350.89, 1227.47, 1186.01, 1047.16, 1007.62\text{ cm}^{-1}$ shows the absorbance of Thiol.

Peak Find - Memory-13



[Comments]

Sample name

Comment

User

Division

Company Sathyabama Institute of Science and Technology

[Detailed Information]

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Data array type Linear data array
Horizontal axis Wavenumber [cm-1]
Vertical axis %T
Start 349.053 cm-1
End 7800.65 cm-1
Data interval 0.964233 cm-1
Data points 7729

[Measurement Information]

Model Name FT/IR-6600typeA
Serial Number B049361790
Accessory ATR PRO ONE
Accessory S/N C215161809
Incident angle 45 deg
Measurement Date 28-01-2023 15:07
Light Source Standard
Detector TGS
Accumulation 32
Resolution 4 cm-1
Zero Filling On
Apodization Cosine
Gain Auto (4)
Aperture Auto (7.1 mm)
Scanning Speed Auto (2 mm/sec)
Filter Auto (10000 Hz)

[Result of Peak Picking]					
No.	Position	Intensity	No.	Position	Intensity
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3	2361.41	95.4109	4	1703.8	30.8831
5	1633.41	82.9095	6	1527.35	58.321
7	1455.03	70.4389	8	1364.39	82.5572
9	1318.11	67.1453	10	1295.93	59.6061
11	1240.97	46.2317	12	1163.83	34.322
13	1139.72	36.0979	14	1040.41	67.9294
15	1002.8	75.8435	16	941.092	63.1527
17	881.309	83.9994	18	815.742	72.8405
19	771.387	74.4789	20	647.965	68.5021
21	596.861	69.5359			

Fig 5.3 FTIR analysis for Ene.

From the above graph, vibration at 1703.8, 1163.83, 1139.72 cm⁻¹ shows the highest absorbance of Alkene with the intensity of 30-36%. The other peaks are at average of 1527.35, 1455.03, 1318.11, 1295.93, 1040.41, 941.092 cm⁻¹ shows the absorbance of Alkene.

From these graph , we identify the compounds Thiol, Alkene and PVA in the scaffold.

5.1.2 Raman Spectroscopy Analysis:

Raman spectroscopy is a spectroscopic technique that may be used to examine the chemical makeup of materials by detecting vibrational, rotational, and other states in a molecular system.

The principle behind Raman spectroscopy is that the monochromatic radiation is passed through the sample such that the radiation may get reflected, absorbed, or scattered. The scattered photons have a different frequency from the incident photon as the vibration and rotational property vary. Chemistry uses Raman spectroscopy to identify molecules, investigate chemical bonds, and examine intramolecular bonds. Because the chemical interactions of a molecule determine its vibrational frequency. The Raman shift refers to the variation between the incident and dispersed photons. In our study we did Raman Spectroscopy analysis for the sample.

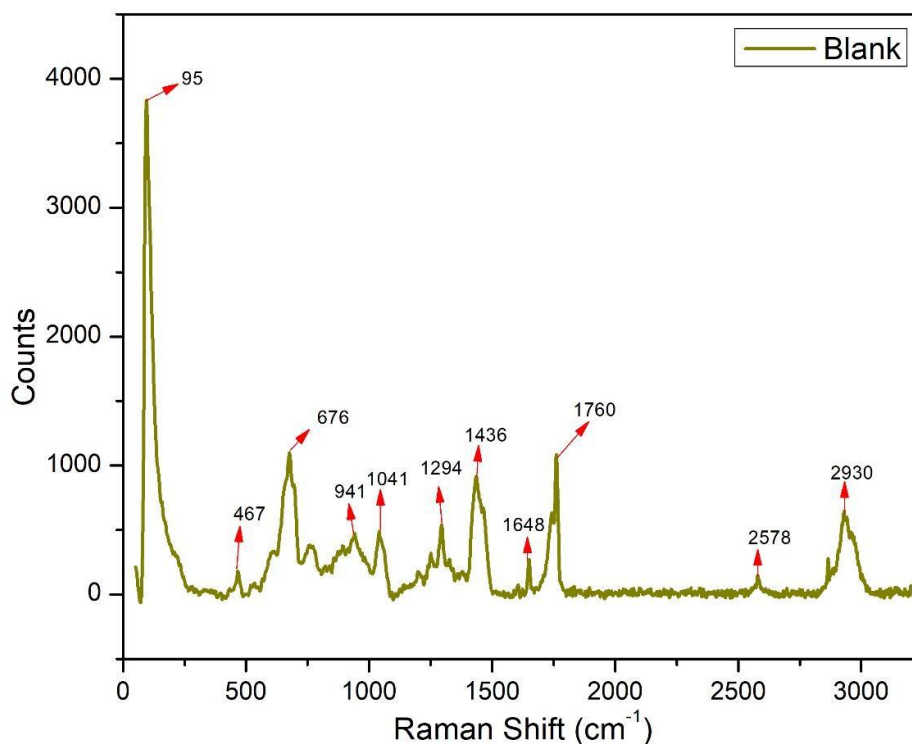


Fig 5.4 Raman Spectroscopy analysis for the scaffold

From the above graph, the highest peak of 95cm⁻¹ shows the absorbance of thiol and ene reaction(CH₂ – CH₂). Absorbance at 467,941,1041,1648,2578 cm⁻¹ represents the bonding of PVA (CH₂-CH-OH) . Absorbance at 676,1436,1760,2930 cm⁻¹ shows the bonding of PVA and Thiol-Ene.

Therefore these peaks represents the chemical makeup of the scaffold.

5.2 INVITRO STUDIES:

5.2.1 Hemocompatibility Test :

This test determine whether the scaffold can destroy the cells and whether it can digest the hemoglobin inside.

During rotation at 37°C , test material is incubated with an erythrocyte suspension for 24 hours. Samples are taken both before and after the incubation process, and the supernatant—which contains free hemoglobin—is obtained by centrifuging the mixture. A spectrophotometer is used to test the haemoglobin concentration.Both positive and negative OD values are measured. According to that % of hemolysis is calculated.

If a biomaterial is hemocompatible , it shows less than 5% haemolytic ratio i.e RBC break down less than 5% with the sample.Scaffold sample exhibits the haemolytic activity of <5% , indicates that the sample is hemocompatible.

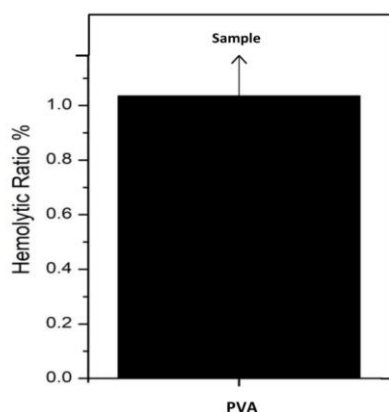


Fig 5.5 % of hemolysis for scaffold

5.2.2 Cytotoxicity Test:

Measurements of cell number or growth before and after exposure to a substance are used in cytotoxicity assays to determine if a substance causes cell death.

The majority of cytotoxicity tests operate under the assumption that dying cells have severely damaged cell membranes that permit the release of cytoplasmic substances or the entry of fluorescent dyes into the cell. Cytotoxicity is an essential consideration because killing off healthy, live cells in the area of the wound may delay the healing process.

Samples were washed 15 minutes with colourless test medium (Phenol Red-free DMEM). Then, polyHIPE discs were transferred to separate wells of 12-well cell culture plate in 1 mL of test medium and incubated overnight in cell culture incubator at normal conditions (5% CO₂ at 37°C). A day before the experiment, human fibroblast cell line S2S was seeded on a white 96-well microtiter plate at a density of 10 000 cells per well in 100 µL of the test medium with 10% BSA and incubated overnight in the cell culture incubator.

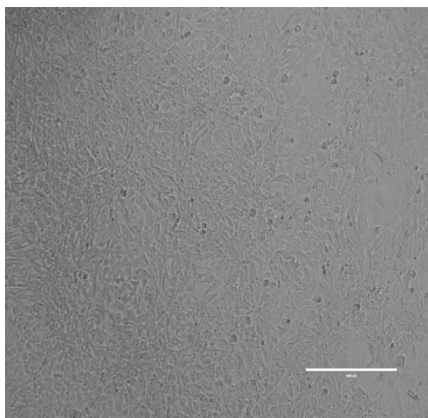


Fig 5.6 Fibroblast cells untreated

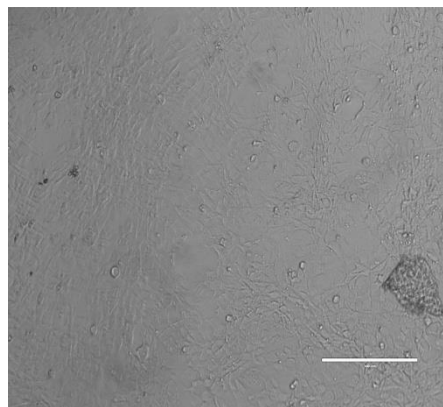


Fig 5.7 Fibroblast cells with scaffold

The next day, 100 μL of the media incubated in the presence and absence of the polyHIPE sample were transferred to the cells on a microtiter plate as the treatment and control respectively. Afterwards, 20 μL of a MTT reagent was added and incubated for an additional three hours to allow the living cells to form the fluorescent product. Read absorbances at 570nm.

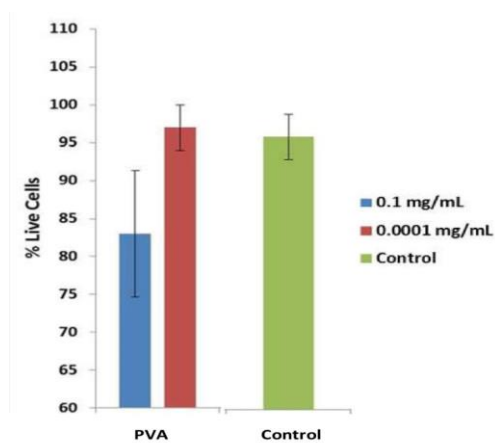


Fig 5.8 represents percentage of live cells.

83% of live cells obtained in 0.1mg/ml concentration and 97% live cells obtained in 0.0001mg/ml concentration.

CHAPTER 6

SUMMARY AND CONCLUSION

Tissue Engineering (TE) aims to devise solutions to restore or to improve the functions of injured / diseased parts of the host tissue which cannot heal naturally. TE utilize porous matrices that are called scaffolds to fill the defect site. Scaffolds serve as a guide for tissue regeneration as a three- dimensional substrate for cell attachment, proliferation, infiltration and they also provide temporary mechanical support.

As of the tests we have done so far,

- Our sample has a combination of thiol and ene & PVA ,is determined using FTIR analysis.
- We have detailed information of chemical structure of scaffold using Raman spectroscopy.
- Scaffold is hemocompatible as it shows < 5% hemolysis.
- Scaffold is cytocompatible by testing with MTT assay and 83 & 97% of cells were viable with scaffold.

Here we conclude that our sample is a poly(HIPE) scaffold . It is well polymerized and it is hemocompatible and cytocompatible. Further more studies on this scaffold will be useful for implantation. Further research focusing on the examination of PolyHIPE matrices' long-term behaviour in living organisms will also contribute to a better understanding of the potential of emulsion templated matrices for clinical application.

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PUBLICATION AND CERTIFICATE:



Presented Poster in SRM ICONN 2023 International Conference.

Abstract is published in ICONN 2023 Abstract Book:

Functionalized Poly Vinyl Alcohol click reaction composite based polymerized

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Abstract

In the creation of scaffolds for the highly internal porous emulsion templated polymers (poly HIPEs) provides a variety of potential benefits. A free- standing nanocomposites with 3D interconnected microporosity and non- functionalized NHA selectively decorating the scaffolds inner surface were produced by the free radical polymerization of HIPEs. Porous polymers that have been fabricated using high internal phase emulsion (HIPEs) have become one of the most popular techniques for creating three dimensional (3D) scaffolds because they make it simple to create interconnected porosity using a number of synthetic processes. Water-in-oil HIPEs are used in the preparation of polyHIPE. Direct oil-in-water emulsion based on water soluble poly Vinyl Alcohol(PVA) are created. The hydrophilic water-miscible thiol, ethoxylated trimethylolpropane tris(3-mercaptopropionate) (ETTTP) and Dichloromethane(DCM) are used to form polyHIPE. High interconnected porosity combined with additive manufacture are the properties that are shown by the emulsion templating polymers. One of the

key benefits of polyHIPEs is the ability to control the pore size and interconnectivity. Using conventional or additive manufacturing techniques, thiol-ene chemistry is the best way to create biodegradable photocurable poly HIPEs for the production of scaffolds. Using a suitable surfactant, commercially available multifunctional thiols are formed into water-in-oil high internal phase emulsions(HIPEs) and the HIPEs are photocured. According to its biocompatible material composition, PVA scaffold is non-cytotoxic and has been shown to be able to stimulate cell development. This research reports the work to fabricate thiol and Poly Vinyl Alcohol based poly HIPE materials through a photoinitiated thiol-ene click reaction. The material is more helpful for biomedical applications due to its porous profile and biodegradability. Micromorphological characteristics, Mechanical properties, Cytotoxicity analysis have been investigated.

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