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Preparation and characterization of poly (ε-caprolactone) PCL scaffolds for tissue engineering applications

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Thesis submitted in partial fulfillment of the requirements for the degree

Of

Master of Technology

In
BIOTECHNOLOGY AND MEDICAL ENGINEERING

By

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Rourkela-769008 (ORISSA)
May – 2009

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Under the guidance
Of
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May – 2009

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CERTIFICATE

This is to certify that the thesis entitled, ***"Preparation and characterization of polycaprolactone (PCL) scaffolds for tissue engineering applications"*** submitted by ***R.Sravanthi*** *(Roll No-207BM211)* in partial fulfillment of the award of Master of technology degree in Biotechnology and Medical engineering at the National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance. To the best of my knowledge the matter embodied in the thesis has not been submitted to any other university for the award of any degree or diploma.

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ACKNOWLEDGEMENT

I express my heartfelt gratitude to **Prof. Dr. Krishna Pramanik**, Prof of the Department of Biotechnology and Medical Engineering, NIT, Rourkela, for her constant encouragement, invaluable advice and guidance throughout the course of my research work. I must mention that without her timely help in writing and correction, this thesis could not have been submitted in time.

I am thankful to **Prof. G. R. Satpathy**, HOD, Department Of Biotechnology and Medical Engineering, NIT, Rourkela for all the facilities provided during the course of my tenure.

A special thanks to **Dr. Shubhankar Paul**, Asst.Professor, **Mr. Amit Biswas**, Lecturer, **Dr.B.P.Nayak**, Lecturer, **Dr.S.S.Ray,** Lecturer, **Dr. Kunal Pal**, Lecturer, for their valuable advices and constant support throughout my course work.

I am thankful to **Prof. Santanu Bhattacharya**, HOD, Department Of Ceramic Engineering and **Dr.S.K.Pratihar**, Asst.Professor, for permitting the usage of Mercury Porosimeter facility and **Dr.J.Bera**, Asst.Professor, for permitting to use the XRD facility.

I am also thankful to **Dr. B.C. Ray**, Professor, Department Of Metallurgical and Materials Engineering, for permitting the usage of DSC facility and **Rajesh Patnayak** for permitting the usage of SEM facility.

My heartfelt thanks to ***Tarangini, Archana, Jeevan, Ramakrishna, Kaleswar*,** and ***Chaitanya*** for their joyous company and for helping me in several ways.

I also thank my friends ***Gaurav Gupta, Navneet Kumar Dubey, Devendra Bramh Singh, Deepanwita Das, Sahitya, Ramya, Nadeem*** and ***Srinivas*** for their support all over my project work.

And it goes without saying, that I am indebted to my Beloved **Parents** and my little **Sister** R.Usha sree, whose patience, support and endurance made completion of my course a reality.

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NOMENCLATURE

M_n	Number average molecular weight
Tg	Glass transition temperature
Tm	Melting temperature
Tc	Crystallization temperature
ΔHm	Measured enthalpy of melting
ΔHm^0	Enthalpy of melting of 100% crystalline polymer
H_f	Heat of fusion
Ac	Crystallized area
Aa	Amorphous area
X_C	Degree of crystallinity
V	Volume of the scaffold
V_p	Volume of the polymer

ABBREVIATIONS

TE	Tissue engineering
ECM	Extracellular matrix
TIPS	Thermally induced phase separation technique
3D	Three -Dimensional
CAD	Computer aided design
CAM	Computer-aided manufacturing
SCPL	Solvent Casting & Particulate Leaching
ROP	Ring-opening polymerization
PCL	Poly (ε-caprolactone)
PVA	Polyvinyl alcohol
PHEMA	Polyhydroxyethymethacrylate
PNIPAAm	Poly (N-isopropylacrylamide)
PLGA	Poly (lactide-*co*-glycolide)
PLA	Polylactide

PGA	Polyglycolide
PDO	Polydioxanone
TCA	Tricarboxylic acid
SEM	Scanning electron microscopy
DSC	Differential scanning calorimeter
XRD	X-ray diffraction

ABSTRACT

The field of Tissue Engineering has developed in response to the shortcomings associated to the replacement of tissues lost to disease or trauma: donor tissue rejection, chronic inflammation, and donor tissue shortages. The driving force behind Tissue Engineering is to avoid these problems by creating biological substitutes capable of replacing the damaged tissue. This is done by combining scaffolds, cells and signals in order to create living, physiological, three-dimensional tissues. Scaffolds are porous biodegradable structures that are meant to be colonized by cells and degrade in time with tissue generation. Scaffold design and development is mainly an engineering challenge, and is the goal of this thesis.

The main aim of this thesis is to develop and characterize scaffolds for Tissue Engineering applications. Specifically, its objectives are:

- To study scaffold processing method: Phase Separation. This is done by experiment design analysis.
- To characterize the behavior of the scaffolds produced.

The scaffolds are prepared using a biodegradable polymer polycaprolactone by thermally induced phase separation technique using solid-liquid phase separation. The porosity, crystallinity and pore size was characterized using scanning electron microscopy (SEM), differential scanning calorimeter (DSC), Mercury porosimeter, and X-ray diffraction (XRD). The parameters that found to influence the architecture of the scaffolds were freezing temperature, freezing medium and polymer concentration. The freezing temperature was found to have a profound effect on the pore size and final morphology of the porous structures. The degree of crystallinity determined using XRD was comparable with that of the as received PCL. The porosity of the structures was found to be 90-97%. The porosity of the PCL structures can be controlled by the concentration of the polymer solution used. Micrographs of the samples from the SEM revealed that the pore size was smaller when the polymer solution was quenched to lower temperatures (-20^{0}C). Mercury porosimeter resulted in a pore size distribution from 50-100µm which makes them suitable for tissue engineering applications. PCL scaffolds therefore may have considerable potential as scaffold for tissue engineering.

CHAPTER-1
INTRODUCTION

INTRODUCTION

Accidents and diseases lead to devastating tissue losses and organ failures which represents a life threatening situation. Tissue repair by autologous cell/tissue transplantation is one of the most promising techniques for tissue regeneration. Autografting and allografting are the two main approaches currently used to repair or replace damaged or lost tissue and organs. However, autografts are associated with limitations such as donor site morbidity and limited availability. On the other hand allografts are not limited in supply; however, they have the potential to cause an immune response and also carry the risk of disease transfer. Tissue engineering has emerged as an excellent approach for the repair/regeneration of damaged tissue, with the potential to circumvent all the limitations of autologous and allogenic tissue repair

Tissue engineering is a new approach to resolve the missing tissue and organ problems. Therefore Tissue engineering represents an emerging multidisciplinary field which involves the "application of the principles and methods of engineering and life sciences towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes that restore, maintain or improve tissue function".

There are three strategies in tissue engineering;

(1) The use of isolated cells or cell substitutes to replace those cells that supply the needed function, including genetic or other manipulations before the cell infusion.

(2) The delivery of tissue-inducing substances, such as growth and differentiation factors, to targeted locations.

(3) Growing cells in three-dimensional (3-D) matrices (scaffolds) or devices, where cells can be either recruited from the host tissues *in vivo* or seeded (encapsulated) *in vitro.*

Biomaterials play a crucial role in tissue engineering by serving as 3D synthetic frameworks commonly referred to as scaffolds, matrices, or constructs for cellular attachment, proliferation, and in growth ultimately leading to new tissue formation. Both synthetic polymers and biologically derived (or natural) polymers have been extensively investigated as biodegradable polymeric biomaterials. In contrast, synthetic polymers have great design

flexibility because the composition and structure can be tailored to the specific needs. A number of novel approaches have been developed for the fabrication of biomaterial-based 3D scaffolds.

The scaffolds with high surface area to volume ratio favors cell adhesion, proliferation, migration, and differentiation, all of which are highly desired properties for tissue engineering applications. Therefore, current research in this area is driven towards the fabrication and characterization of scaffolds for tissue engineering applications.

CHAPTER-2
LITERATURE SURVEY

2.1 Scope and objective

In designing scaffolds for tissue engineering, the principal objective will remain the optimal recreation of ECM function in a temporally coordinated and spatially organized structure. The goal of this study is to fabricate scaffolds of the resorbable polymer poly (ε-caprolactone) and successfully characterization of these scaffolds. The idea of using this unique method of fabrication is that these scaffolds will provide a greater surface area for direct cell adhesion and provide even more guidance of cell growth. The resorbable nature of the polymer will allow for a minimum mechanical stability to support the initial cellular growth and provide an anchor for future implantation, but then allow the tissue to encourage regeneration at the site of implantation.

2.1.1 Tissue engineering: history, definitions and applications

The field of tissue engineering developed as a response to the problems associated with the replacement of tissues lost to disease or trauma. Currently, tissue replacements must overcome important challenges such as rejection, chronic inflammation and severe organ donor shortages [1]. In fact, thousands of patients die every year in waiting lists for organ transplantation [2]. The driving force behind tissue engineering is the desire to avoid these problems by creating biological substitutes capable of replacing the damaged tissue.

Nowadays, damaged tissue can be replaced by xenografts, allografts or autografts. A xenograft is a graft of tissue proceeding from another species. Xenografts offer the advantage of availability in a variety of shapes and sizes, but they also imply a nonnegligible risk of immunological reactions and infections. Allografts are grafts made of tissue from a human donor, usually post-mortem. This tissue must be thoroughly sterilised in order to avoid immunological reactions in the receiver and infections. Their limitations include donor shortages and risks of infections mentioned above. Autografts are grafts made of tissue obtained from the patient who receives the graft: a self-transplant of tissue in other words. Autografts are in some way a gold standard because they avoid most problems related to transfection and rejection. They do involve significant donor site morbidity and chronic donor shortages however. For example, in the case of bone replacement with tissue from the iliac crest, patients often complain of more pain in the hip area (iliac crest) than at the implantation site.

The idea behind tissue engineering is to create or engineer autografts, either by expanding autologous cells *in vitro* guided by a scaffold, or by implanting an acellular scaffold *in vivo* and allowing the patient's cells to repair the tissue guided by the scaffold. In both cases, the scaffold should degrade in time with tissue regeneration, so that once the tissue has matured the scaffold no longer exists as such and the newly created tissue can perform the function of the lost tissue [3]. This approach avoids some of the drawbacks of the grafting techniques discussed above. Namely, small numbers of cells are harvested from the patient, thus avoiding the problems of tissue shortage and donor-site morbidity. The cells are seeded into a scaffold which will eventually degrade completely, thus eliminating the presence of a foreign body at the implantation site and its consequent chronic inflammation. Finally, the use of autologous cells avoids problems of rejection and transfection **(Figure 1.1).**

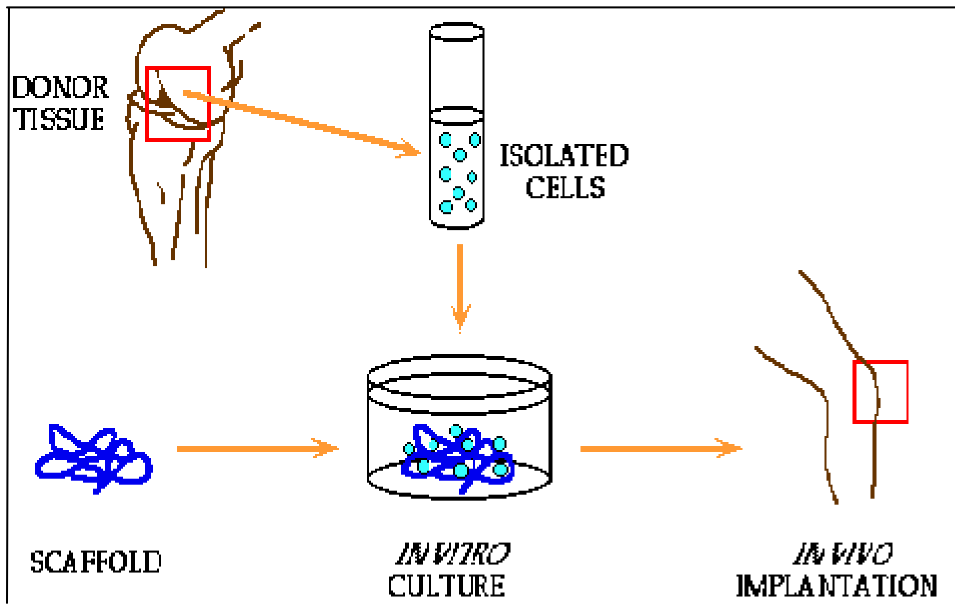


Figure 1.1: Schematic diagrams of the different phases in Tissue Engineering, from scaffold fabrication and cell isolation to *in vivo* implantation

The term **tissue** refers to: an aggregate of cells usually of a particular kind together with their intercellular substance that form one of the structural materials of a plant or an animal. This intercellular substance, or extracellular matrix, is a crucial part of a tissue and acts both as a structural framework and as a regulator of cell behavior. The word **engineering** is defined as: a) to contrive or plan out usually with more or less subtle skill or craft, and b) to guide the course

of. In effect, Tissue Engineering uses multidisciplinary tools to produce a surrogate extracellular matrix meant to guide cells into creating new tissue [4].

One of the classical definitions of Tissue Engineering was postulated by Langer and Vacanti in 1993 as:

• "…an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore maintain, or improve the tissue function.[5]"

Many other, more or less similar definitions of tissue engineering can be found in the literature. Being a relatively new field, tissue engineering, is not always clearly defined, and may span from decellularised extracellular matrices, to exclusively cellular implants or non-biodegradable biomaterial scaffolds.

Some examples of these definitions are:

• "... The process of creating living, physiological, three-dimensional tissues and organs utilizing specific combinations of cells, cell scaffolds, and cell signals, both chemical and mechanical. [6]"

• "...some combination of cells, scaffold material, and bioactive peptides used to guide the repair or formation of tissue. [7]"

• "…the three-dimensional assembly over time of vital tissues/organs by a process involving cells, signals and extracellular matrix. [4]"

• "The field of tissue engineering exploits living cells in a variety of ways to restore, maintain, or enhance tissues and organs [8]"

• "…products or processes that (1) combine living cells with biomaterials, (2) utilize living cells as therapeutic or diagnostic reagents, (3) generate tissues *in vitro* for therapeutic implantation, and (4) provide materials or technology to enable any of these approaches.[9]"

Already, one can infer two of the basic building blocks of tissue engineering: a) cells, and b) scaffolds. The third building block is signalling; biochemical and biomechanical signals which will coax the cells into creating tissue. Alternatively, these concepts can be interpreted as: a) biological and b) engineering challenges [8], bearing in mind that engineering challenges span both cell, scaffold, and signal treatment and vice versa. Thus the field of tissue engineering must

combine the knowledge and practices of life scientists and engineers in order to create viable tissues.

Cells are one of the basic components of tissue and are critical in all tissue engineering applications [4; 8; 10]. Whether cells are directly implanted into the body or are cultured in vitro before implantation, their source and type must be chosen carefully. Furthermore, the harvesting, expansion and differentiation of cells imply many challenges which have retarded the implementation of cellular grafts. Skin tissue engineering grafts such as Apligraf® and Dermagraft® are the exception, partly due to the relative simplicity of the structure of skin as an organ, and partly due to the ease with which skin cells can be cultured and expanded in vitro maintaining the appropriate phenotype [8].

If one considers the cellular approach, the first issue is the cell source: autologous, allogenic, or xenogenic, with the advantages and disadvantages discussed above. Due to the problems associated with the expansion and maintenance of the phenotypes of cells, cell type is also critical. Cells can be adult or embryonic stem cells (pluripotent, totipotent …) capable of self-renewal and differentiation into various cell lineages. They can also be adult cells at different stages of maturation and differentiation. Cells can also be generated by nuclear transplantation or manipulation ex-vivo [4]. Though stem cells hold enormous promise for this application, stem cell technology is still rather recent and must solve numerous engineering and ethical shortcomings. The chosen cell source and type should also guarantee sufficient supply and be free of pathogens and contamination.

Once cells are harvested they must be kept alive and expanded for a certain time *in vitro.* During this phase, cells must retain the desired phenotype be it undifferentiated or differentiated. Finally, the cells must be seeded onto a scaffold and should retain their function within the construct. Thus, the construct must also provide the mechanical and chemical cues the cells require.

The signals the cells receive from their environment (in this case the scaffold) will in fact determine whether the scaffold turns into integrated tissue. First of all the right cell types must adhere to the outer surface of the scaffold and be able to migrate into it. This is achieved if the

scaffold has cell-adhesion sites distributed with the appropriate density to promote cell migration [11].

Once the cells have colonised the scaffold, they should begin proliferation or differentiation in order to produce the tissue which is being replaced. Cells receive the cues for proliferation or differentiation via the integrins with which they anchor onto the extracellular matrix (ECM) or scaffold, and via growth factors and cytokines. The mechanical stimuli they receive also induce mechanotransduction which allows them to behave and thus remodel tissue in function of the mechanical environment. The integrin-mediated signalling pathway is indeed complex. Cells attach onto proteins of the ECM via integrins and apply traction forces on them, thus stretching the ECM which in turn extends proteins revealing hidden binding sites on the protein structure. The ECM is thus an active environment that interacts with the cells very differently than the relatively passive artificial scaffolds [12-16] (Figure 1.2). The addition of growth factors in scaffolds may solve the challenges of inducing cell proliferation and differentiation. The dosage and distribution of the growth factors within the scaffolds, however, is not straightforward. In any case, signalling and cellular mechanotransduction are critical issues in tissue engineering. They determine cell phenotype, proliferation and differentiation [3; 4].

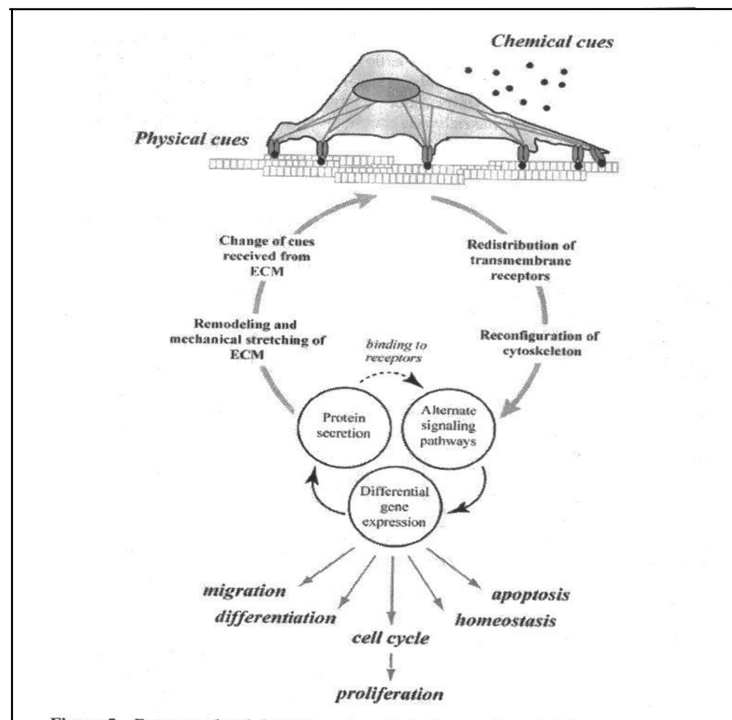


Figure 1.2: Schematic image of the dynamic reciprocity between cells and their extracellular matrix [11].

Scaffolds are the other major component of the tissue engineering approach; the choice of scaffold includes its constitutive material, its design and the surface or molecular treatment it may carry. First of all, the biomaterials the scaffolds are made of must be biocompatible. In addition to its biocompatibility, the material's chemical and physical configuration must be adequate for the application, this includes its degradability. The degradation of the biomaterial should be in phase with and of course should not harm tissue regeneration. Degradation by-products should not be toxic and should be easily and rapidly removed or diluted at the implantation site. The ability to eliminate the degradation by-products will largely depend on scaffold design. The design of the scaffold is another crucial issue. This design will determine its structure, porosity and interface with the cells and surrounding materials. The design must also be adapted to the application creating scaffolds with cubic or tubular pore shapes, for example.

Furthermore, the biomaterial must be processable, a major challenge in the case of brittle ceramic biomaterials for example. Ease of processability, such as easy conformation or injectability in the case of plastics can often determine the choice of a certain biomaterial. The combination of biomaterial and scaffold design will in turn determine the ease and cost of manufacture, manipulation and sterilisation of the construct.

2.2 Biomaterials for Tissue engineering applications

A biomaterial is a "material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body" [17]. As Hench and Polak describe in their key article [18] published in 2002, biomaterials have evolved during the past 50 years, and can now be considered "third-generation biomaterials". Initially, biomaterials were chosen because of their biological inertness, the goal was to minimize the body's immune response to the foreign material. Though this goal is still valid today, scientists have come to understand that complete biological inertness is synonym to non-recognition by the body. This lack of biological recognition is often accompanied by fibrous tissue encapsulation and chronic inflammation, which in turn compromise the mechanical performance and long-term biocompatibility of the prosthesis. Thus, second-generation biomaterials were developed seeking to tailor or enhance biological recognition in an attempt to improve the biomaterial-body interface. Second generation biomaterials used bioactive components that could elicit a

controlled action and reaction in the physiological environment. Two very typical examples of these components are synthetic hydroxyapatite and Bioglass®. Both were used as porous scaffolds, coatings or powders, and by the mid-80s these new bioactive materials had attained clinical use for various dental and orthopedic applications. The biomaterial-body interface problem was also addressed by exploiting resorbable materials, thus eliminating the interface all together. Resorbable polymers are the main example of these resorbable materials, namely polylactic and polyglycolic acid which decompose hydrolytically into H_2O and CO_2. They are used as sutures, screws in orthopedics and in controlled-release drug-delivery systems. Third-generation biomaterials are being designed at present, expanding the concept of biological recognition to specific biological recognition. Thus, third generation biomaterials aim to stimulate precise cellular responses: interaction with distinct integrins, stimulation of cell differentiation or the activation of certain genes. It is also important to emphasize that these biomaterials are being designed. That is, third generation biomaterials are no longer borrowed from existing materials and adapted to a medical application. Instead, they are being designed prior to their development. In this way, the properties of bioactivity and resorbability are being combined to create materials capable of helping the body repair itself better or faster than it could do on its own.

Typically, biomaterials can be divided into: polymers, metals, ceramics and natural materials. The material used in this thesis is a typical third-generation biomaterial. A resorbable polycaprolactone polymer has been used in this study. This material has then been shaped and processed into a scaffold for tissue engineering applications. A detailed description about this material has been given in the next section.

2.2.1 Polymer-based scaffold materials

The meaning and definition of the words biodegradable, bioerodable, bioresorbable and bioabsorbable (Table 1) are of importance to discuss the rationale, function as well as chemical and physical properties of polymer-based scaffolds [19]. In this thesis, the polymer properties are based on the definitions given by Vert [20].

The tissue engineering program in this research curriculum has been classified into two phases (Table 2). Each tissue engineering phase must be understood in an integrated manner across the

research program from the polymer material properties, to the scaffold micro- and macro architecture. Hence, the research objectives in each phase are cross-disciplinary and the sub-projects are linked horizontally as well as vertically.

Table 1

Biodegradable are solid polymeric materials and devices which break down due to macromolecular degradation with dispersion in vivo but no proof for the elimination from the body (this definition excludes environmental, fungi or bacterial degradation). Biodegradable polymeric systems or devices can be attacked by biological elements so that the integrity of the system and in some cases but not necessarily, of the macromolecules themselves, is affected and gives fragments or other degradation by-products. Such fragments can move away from their site of action but not necessarily from the body.

Bioresorbable are solid polymeric materials and devices which show bulk degradation and further resorb in vivo; i.e. polymers which are eliminated through natural pathways either because of simple filtration of degradation by-products or after their metabolization. **Bioresorption** is thus a concept which reflects total elimination of the initial foreign material and of bulk degradation by-products (low molecular weight compounds) with no residual side effects. The use of the word 'bioresorbable' assumes that elimination is shown conclusively. **Bioerodible** are solid polymeric materials or devices, which show surface degradation and further, resorb in vivo.

Bioerosion is thus a concept, too, which reflects total elimination of the initial foreign material and of surface degradation by-products (low molecular weight compounds) with no residual side effects.

Bioabsorbable are solid polymeric materials or devices, which can dissolve in body fluids without any polymer chain cleavage or molecular mass decrease. For example, it is the case of slow dissolution of water-soluble implants in body fluids. A bioabsorbable polymer can be bioresorbable if the dispersed macromolecules are excreted.

Table 2

The research program for tissue engineering is classified into two phases:

I-Fabrication of bioresorbable scaffold
II-Characterization of the scaffold

2.2.2.1 ***Natural polymers for scaffolds***

Many naturally occurring scaffolds can be used as biomaterials for tissue engineering purposes. One example is the extracellular matrix (ECM), a very complex biomaterial controlling cell function that designs natural and synthetic scaffolds to mimic specific functions. Natural polymers include alginate, proteins, collagens (gelatin), fibrins, albumin, gluten, elastin, fibroin, hyarulonic acid, cellulose, starch, chitosan (chitin), scleroglucan, elsinan, pectin (pectinic acid), galactan, curdlan, gellan, levan, emulsan, dextran, pullulan, heparin, silk, chondroitin 6-sulfate, polyhydroxyalkanoates, etc. Much of the interest in these natural polymers comes from their biocompatibility, relative abundance and commercial availability, and ease of processing [21]. Natural polymers possess several inherent advantages such as bioactivity, the ability to present receptor-binding ligands to cells, susceptibility to cell-triggered proteolytic degradation and natural remodeling. The inherent bioactivity of these natural polymers has its own downsides. These include a strong immunogenic response associated with most of the polymers, the complexities associated with their purification and the possibility of disease transmission [22].

2.2.2.2 ***Synthetic polymers for scaffolds***

Natural polymers are typically in short supply because they are expensive, suffer from batch-to-batch variation, and are susceptible to cross-contamination from unknown viruses or unwanted diseases. On the contrary, synthetic polymeric biomaterials have easily controlled physicochemical properties and quality, and have no immunogenicity. They can also be processed with various techniques and consistently supplied in large quantities. In order to adjust the physical and mechanical properties of tissue-engineered scaffolds at a desired place in the human body, the molecular structure and molecular weight are easily adjusted during the synthetic process. Synthetic polymers are largely divided into two categories: biodegradable and

non-biodegradable. Some non-biodegradable polymers include polyvinyl alcohol (PVA), polyhydroxyethymethacrylate (PHEMA), and poly(N-isopropylacrylamide) (PNIPAAm). Some synthetic biodegradable polymers are the family of poly(α-hydroxy esters) such as polyglycolide (PGA), polylactide (PLA) and its copolymer poly(lactide-*co*-glycolide) (PLGA), polyphosphazene, polyanhydride, poly(propylene fumarate), polycyanoacrylate, poly(ε-caprolactone) (PCL), polydioxanone (PDO), and biodegradeable polyurethanes. Of these two types of synthetic polymers, synthetic biodegradeable polymers are preferred for the application of tissue engineered scaffolds because they minimise the chronic foreign body reaction and lead to the formation of completely natural tissue. That is to say, they can form a temporary scaffold for mechanical and biochemical support [23]. Synthetic biomaterials on the other hand are generally biologically inert, they have more predictable properties and batch-to-batch uniformity and they have the unique advantage having tailored property profiles for specific applications, devoid of many of the disadvantages of natural polymers. Hydrolytically degradable polymers are generally preferred as implants due to their minimal site-to-site and patient-to-patient variations compared to enzymatically degradable polymers [24].

2.3 Polymers for Tissue engineering applications: Polycaprolactone

Polycaprolactone (PCL)

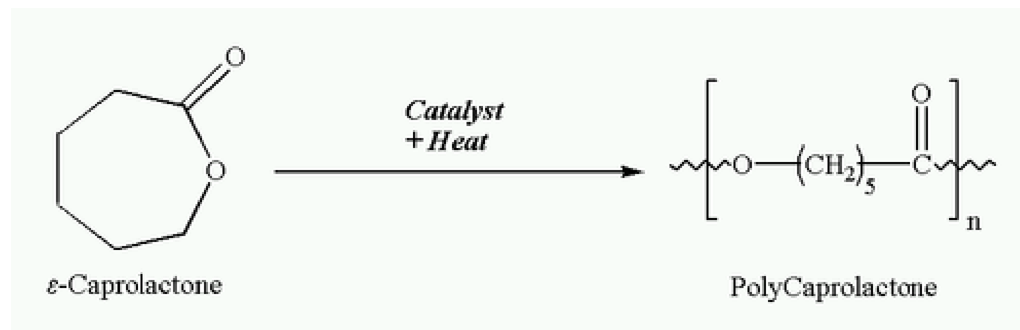


Figure 2: Synthesis of polycaprolactone by ring-opening polymerization (ROP) of ε-caprolactone.

PCL is a semi crystalline aliphatic polyester and is of great interest as it can be obtained by ROP of a relatively cheap monomeric unit "ε -caprolactone" and is known for its extremely low Tg (-60°C) and long-term degradation properties (>24 months to lose total mass). The low melting-point makes the material suited for composting as a means of disposal, due to the temperature

obtained during composting routinely exceeding $60^{O}C$ [25]. PCL is an attractive polymer to use based on its elastomeric properties and high elongation [26]. For biomedical applications, PCL has been approved for use by the FDA since the 1970s and can be found in many common sutures and suture components. In the recent past, PCL has been used more and more by tissue engineers. Specifically with PCL, some of its attractive qualities are its enhanced solubility in organic solvents, ability to be processed at low temperatures, and its non-toxic degradation byproducts. One of its most attractive qualities for use in biomedical applications is its slow rate of degradation. PCL degrades by hydrolysis and will lose about 50% of its strength in 8 weeks using an *in vitro* degradation test [27]. Because of its attractive mechanical properties and ability to blend easily, many researchers have turned to using co-polymerizations of PCL with various starches to reduce production costs and to also encourage cell growth with the presence of a natural biopolymer. When blended with starch, the non-isothermal crystallization rate of PCL is increased. This reinforced PCL and its damping properties, provide attractive qualities for biomedical applications that undergo extensive mechanical strain, such as orthopedic implants [27]. PCL has also been blended with higher amounts of starch to increase its degradation rate. With higher starch content, PCL is more susceptible to enzymatic degradation by proteinase K49 [29]. PCL has been blended with several other polymers, including PEO, PLA, and PGA in the past and has been used in many studies relating to biomedical applications.

Like other polyesters, PCL will undergo auto-catalyzed bulk hydrolysis degradation because of the susceptibility of its aliphatic ester linkage. However, the hydrophobic, semi-crystalline polymer retards degradation and resorption kinetics when compared to other aliphatic polyesters such as PLGA, which makes it more suitable for long term implantable devices [31-32]. Bulk hydrolysis breaks the ester linkage, which creates fragmentation and the release of oligomeric species. Low molecular-weight fragments are eventually engrossed by giant cells and macrophages. The byproduct e-hydroxycaproic acid is either metabolized via the tricarboxylic acid (TCA) cycle or removed by direct renal secretion [33, 34, 35]. It is also possible for PCL to enzymatically degrade (enzymatic surface erosion) by lipases and esterases, though this is rare [36, 37].

2.3.1 Use in Tissue Engineering

PCL has been used for several tissue engineering endeavors. The advantage in using these PCL scaffolds deals with its desirable biostability and mechanical qualities which ensures its long-term presence and elasticity. One study in particular has shown the use of PCL for skin grafts as a mix of collagen and PCL in a composite film [38]. The objective was to monitor the degradation of the collagen in a cultured environment and monitor the cell adhesion over several days. The study followed the behavior of several variations of the mixture of PCL and collagen. The resorption rate of about one year of the PCL showed promise for the use for developing skin grafts for burn victims. The PCL phase also provided the necessary stability for a graft of this nature. Unique for this study, was the ability to control the cell growth and adhesion by the amount of collagen in the initial mixture with PCL. Solutions with a higher PCL content showed ECM production with higher cell adhesion. This also suggested that there can be a way to manipulate the PCL/collagen solution within the scaffolds to develop a drug delivery system to continue the healing of the wound site. The resorption of the PCL over a year's time also aided in the healing process of the wound site and made the wound more apt to heal effectively.

2.4 Importance of Scaffold Matrices in Tissue engineering

Scaffolds play a critical role in tissue engineering. The function of scaffolds is to direct the growth of cells either seeded within the porous structure of the scaffold or migrating from surrounding tissue. The majority of mammalian cell types are anchorage-dependent, meaning they will die if an adhesion substrate is not provided. Scaffold matrices can be used to achieve cell delivery with high loading and efficiency to specific sites. Therefore, the scaffold must provide a suitable substrate for cell attachment, cell proliferation, differentiated function, and cell migration. The prerequisite physicochemical properties of scaffolds are many: to support and deliver cells; induce, differentiate, and channel tissue growth; target cell-adhesion substrates; stimulate cellular response; provide a wound-healing barrier; be biocompatible and biodegradable; possess relatively easy processability and malleability into desired shapes; be highly porous with a large surface/volume ratio; possess mechanical strength and dimensional stability; and have sterilisability, among others [39,40]. Generally, three-dimensional porous scaffolds can be fabricated from natural and synthetic polymers, ceramics, metals, composite biomaterials, and cytokine release materials.

Scaffold design and development is mainly an engineering challenge and is in fact the **goal of this thesis.**

Tissue engineering scaffolds are meant to be colonised by cells and should transmit the chemical and physical cues necessary to ensure adequate tissue growth. An ideal tissue engineering scaffold should fulfill a series of requirements [41]:

• It should have a reproducible microscopic and macroscopic structure with a high **surface/volume** ratio suitable for cell attachment.

• The material it is made of should be **biocompatible**. The scaffold should perform its function with an appropriate response of the host and it should not induce adverse responses [42].

• The scaffold should have an adequate **porosity**; this includes the magnitude of the porosity, the pore size distribution and its interconnectivity.

- ➢ This will allow cell in-growth and vascularisation, and
- ➢ Promote metabolite transport

• The scaffolds should have appropriate **mechanical properties and support** to resist physiological forces within the implantation site and similar flexibility to the surrounding tissue. Ideally it should support the mechanical load on the damaged tissue while it regenerates.

• The scaffold material should be **biodegradable.** Its degradation products should not be toxic and should be easily eliminated from the implantation site by the body.

• The scaffold's **degradation** rate should be adjusted to match the rate of tissue regeneration, so that it has disappeared completely once the tissue is repaired.

2.4.1 Essential Scaffold Properties

The porosity and pore size are key factors to the success of scaffold performance. The surface of the scaffold is the site of first contact for the cells, and therefore surface topography and surface energy are the next most important variables to be considered. When examining the porosity, the cellular in growth is a very important variable to consider. A highly porous scaffold will allow for cellular migration and good cell adhesion [43, 44]. The scaffold needs to allow for capillary ingrowth as well. With the fresh blood supply, comes the supply of fresh oxygen and nutrients necessary for cellular survival. Along with the vascularization, the ability to dispose to cellular waste also becomes available. Adequate porosity promotes vascularization and encourages angiogenesis [45]. However, too much porosity can cause the scaffold to lose all

mechanical integrity and be unable to support the cellular growth. Most previous researchers have demonstrated that highly porous structures are required with overall porosity values in the order of 90% as to have successful results [46]. The pore size is another important aspect to consider. When hoping to encourage the growth of a 3-D structure, there needs to be consideration for the size of the target cell. Surface coatings are another important aspect to include in the scaffold production. There has been significant success with the inclusion of natural polymers in conjunction with biodegradable synthetic polymers. The natural polymers and growth factors encourage the cell growth with no concern for cytotoxic effects. Through the fabrication process and ensuring the scaffold has 3-D shape and appropriate porosity and pore size distribution, as well as the necessary mechanical and tensile strength, the scaffold can also be made to include vital biological factors. These factors can be native ECM proteins or growth factors to stimulate tissue regeneration and healing. The most effective method to date has been the recent discovery of the advantages of coculture techniques. The field of tissue engineering tissue-specific extracellular matrices has come a long way since its beginning and still has a great potential to revolutionize the field of biotechnology.

2.5 Fabrication of Tissue engineering Scaffolds.

Different processing techniques have been developed for the design and fabrication of three-dimensional (3D) scaffolds suitable for TE implants. Conventional techniques for scaffold fabrication include gas foaming, fiber bonding, emulsification/freeze-drying, solvent casting/particulate leaching, thermally induced phase separation (TIPS), 3D-printing [47–50], Nanofiber Self-Assembly, Textile technologies [50], CAD/CAM technologies [51].

Detailed descriptions regarding each technique have been given below.

Each of these techniques presents its own advantages, but none is devoid of drawbacks.

- ***Nanofiber Self-Assembly***: Molecular self-assembly is one of the few methods to create biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix (ECM). Moreover, these hydrogel scaffolds have shown superior in vivo toxicology and biocompatibility compared with traditional macroscaffolds and animal-derived materials.

- ***Textile technologies***: these techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers. In particular non-woven polyglycolide structures have been tested for tissue engineering applications: such fibrous structures have been found useful to grow different types of cells. The principal drawbacks are related to the difficulties of obtaining high porosity and regular pore size.
- ***Solvent Casting & Particulate Leaching (SCPL)***: this approach allows the preparation of porous structures with regular porosity, but with a limited thickness. First the polymer is dissolved into a suitable organic solvent (e.g. polylactic acid could be dissolved into dichloromethane), then the solution is cast into a mold filled with porogen particles. Such porogen can be an inorganic salt like sodium chloride, crystals of saccharose, gelatin spheres or paraffin spheres. The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast the solvent is allowed to fully evaporate, then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen: water in case of sodium chloride, saccharose and gelatin or an aliphatic solvent like hexane for paraffin. Once the porogen has been fully dissolved a porous structure is obtained. Other than the small thickness range that can be obtained, another drawback of SCPL lies in its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold.
- ***Gas Foaming***: to overcome the necessity to use organic solvents and solid porogens a technique using gas as a porogen has been developed. First disc shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold. The discs are then placed in a chamber where are exposed to high pressure CO_2 for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge like structure. The main problems related to such a technique are caused by the excessive heat used during compression molding (which prohibits the incorporation of any temperature labile material into the polymer matrix) and by the fact that the pores do not form an interconnected structure.

- ***Emulsification/Freeze-drying***: this technique does not require the use of a solid porogen like SCPL. First a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane) then water is added to the polymeric solution and the two liquids are mixed in order to obtain an emulsion. Before the two phases can separate, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure. While emulsification and freeze-drying allows a faster preparation if compared to SCPL, since it does not require a time consuming leaching step, it still requires the use of solvents, moreover pore size is relatively small and porosity is often irregular. Freeze-drying by itself is also a commonly employed technique for the fabrication of scaffolds. In particular it is used to prepare collagen sponges: collagen is dissolved into acidic solutions of acetic acid or hydrochloric acid that are cast into a mold, frozen with liquid nitrogen then lyophilized.
- ***Thermally Induced Phase Separation (TIPS)***: similar to the previous technique, this phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example dioxane could be used to dissolve polylactic acid, then phase separation is induced through the addition of a small quantity of water: a polymer-rich and a polymer-poor phase are formed. Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent a porous scaffold is obtained. Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying.
- ***CAD/CAM Technologies***: since most of the above described approaches are limited when it comes to the control of porosity and pore size, computer assisted design and manufacturing techniques have been introduced to tissue engineering. First a three-dimensional structure is designed using CAD software, then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer melt.

The mixing of different polymers or polymer solutions is often accompanied by the phenomenon of phase separation. Thermally induced phase separation (TIPS) is currently

utilized to fabricate microporous membranes or microcellular foams [52-54]. This technique is based on the principle that a single homogeneous polymer solution made at elevated temperature is converted via the removal of thermal energy to two-phase separated domains composed of a polymer-rich phase and a polymer lean phase [55-57]. Subsequent freeze-drying of the liquid-liquid phase-separated polymer solution produces microcellular structures as a result of solvent removal. A schematic temperature-composition phase diagram for a binary polymer-solvent system is represented in **Fig. 3**. It shows the expected variations in polymer foam morphology depending on the final thermodynamic state of the polymer solution to be thermally quenched. According to whether the quenching end point is located in the metastable region between binodal and spinodal curves or in the unstable region below the spinodal curve, two distinctive morphologies can be obtained: (i) a poorly interconnected bead-like structure by a nucleation and growth mechanism or (ii) a well-interconnected open porous structure by a spinodal decomposition mechanism.

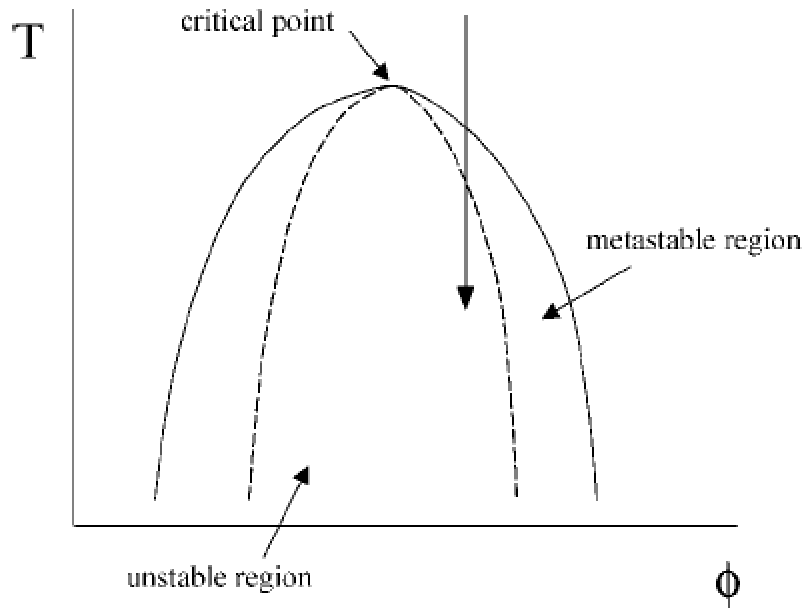


Figure 3: Schematic temperature-composition phase diagram for a two phase system with an upper critical solution temperature indicating a quench (arrowed) from the one-phase region into the unstable region where spinodal decomposition is the mechanism of phase separation. Nucleation and growth is the active mechanism or smaller quenches in to the metastable region **[58].**

A thermally induced phase separation (TIPS) process has been used in this work for tailoring a biodegradable polycaprolactone support. Phase separation takes place on cooling and removal of the solvent results in a porous morphology. When the phase-separated system is stabilized by polymer crystallization of the polymer-rich phase, no shrinkage of the porous structure occurs upon solvent removal. Otherwise, the phase-separated system has to be frozen at a low enough temperature and the solvent has to be removed by sublimation.

Polymer-solvent phase separation can be divided into

(a) liquid-liquid phase separation, which may occur prior to the solvent freezing, and

(b) solid-liquid phase separation,

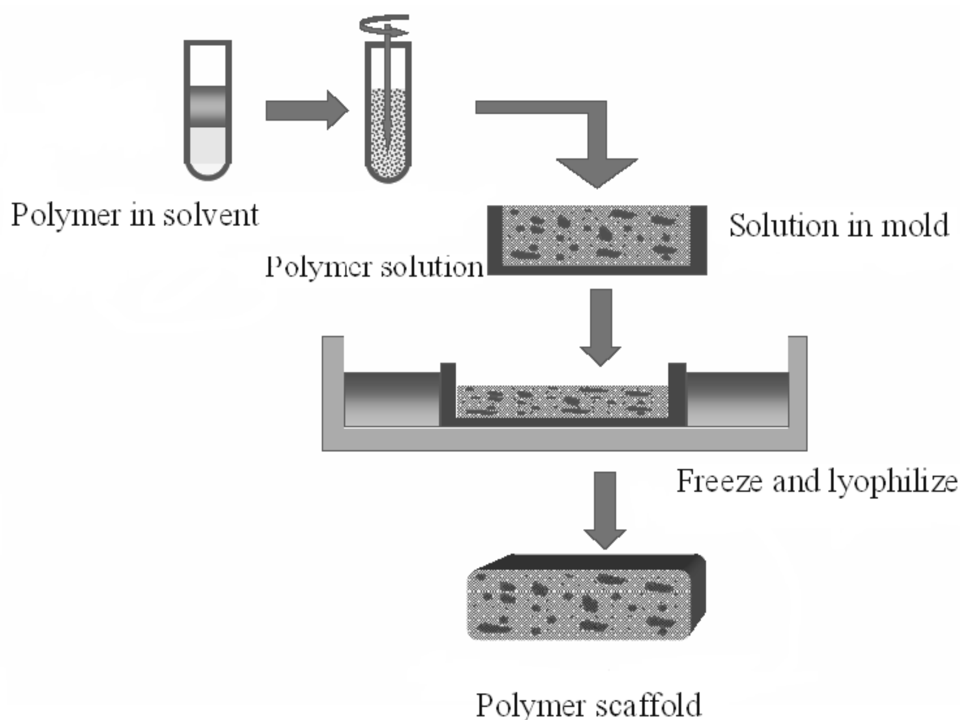


Figure 4: The schematic diagram of solid-liquid phase separation.

Solid-liquid phase separation, also called emulsion freeze drying, could be achieved by lowering the temperature to induce solvent crystallization from a polymer suspension (solid phase formation in a liquid phase). This process takes place by a nucleation and growth mechanism. After the removal of the solvent crystals (sublimation or solvent exchange), the space originally taken by the solvent crystals becomes pores which is observed when the solvent has been completely frozen. This removal must take place at a temperature below the solvent

solidification curve in order to avoid the solvent re-dissolving the polymer. The removal is typically performed by freeze-drying, also called lyophilisation. During this process, the phase-separated mixture is maintained at low temperatures and a high vacuum is applied in order to sublime the solvent. Alternatively, the solvent can be extracted by soaking the mixture in a substance which dissolves the solvent but not the polymer. This method, called freeze-extraction, was applied to polymeric scaffolds by Ho et al. [59] using ethanol.

1,4-dioxane is used as the solvent for creating PCL phase-separated scaffolds. Its physical properties are very convenient for this procedure and have a high melting point and a low boiling point, 11.8°C and 102°C respectively [60]. These temperatures allow for thermally induced phase separation at high temperatures, and facilitate sublimation.

The addition of a non-solvent to the polymer solution may induce liquid-liquid phase separation.

Phase separation relies on no special equipment and allows for three-dimensional scaffolds to be created within the sub-micron range. This process forms a continuous fiber network that can be tailored to any application. Pore structure can easily be controlled and batch-to batch consistency is high. Pore structure is easily varied by changing the solvent used in the process [61]. This change in solvent can change the phase separation process from liquid–liquid to solid–liquid depending on the solvent mixtures used thus changing the final pore morphology. Initial polymer concentration plays an important part in porosity of the final scaffold. High initial concentration of polymer leads to less porous network [62]. The ease with which these scaffolds can be created and the demonstrated ability of these highly porous networks to support cell growth makes them an attractive solution for creating the porous structures.

CHAPTER-3
MATERIALS AND METHODS

3. EXPERIMENTAL

3.1 Materials

Poly (ε-caprolactone) was purchased from Sigma-Aldrich with a molecular weight of M_n=80,000 in pellet form. The solvent used was 1, 4 dioxane ReagentPlus Grade, ≥99% purity, was purchased from Sigma Aldrich Pt Ltd.

3.2 Scaffold fabrication

3.2.1 Porous polymer material fabrication using freeze drying

Typically three processing steps were taken to prepare a porous polymer material from a polymer solution:

(A) Accurately weighed polymer was added in to a flask and calculated amount of solvent was added in to the flask to make a polymer solution of desired concentration. In an embodiment the polymer solution (polymer/solvent mixture) contains about 1%, 3% and 5% polymer. Typically the polymer was dissolved for an hour or longer to ensure a homogeneous solution when stirred with a magnetic stirrer at either room temperature or an elevated temperature. The petri dish containing the polymer solution was then rapidly transeferred into a cooling device and frozen at -20^{0}C using a freezer and at 6^{0}C using a refrigerator.

(B) The frozen polymer solution was then freeze dried at a predetermined vacuum and predetrmined temperature for the removal of solvent.

(C) The dried porous polymeric material was then placed in a dessicator for further charecterization.

3.3 Characterization Methods

3.3.1 *Morphology*

A qualitative study of the scaffold morphology was performed on a JEOL JSM-6480 LV Scanning Electron Microscopy (SEM). Frozen cross sections of the scaffold, were coated with platinum using a JEOL JFC-1600 Auto Fine Coater operated at 20 mA for 80 s prior to SEM analysis and examined.

3.3.2 *Differential Scanning Calorimeter*

The thermal transitions of the scaffold material were measured by a Mettler Toledo DSC822e Differential Scanning Calorimeter. The samples used weighed between 10 and 15 mg and were submitted to a heating cycle at 20°C/min under nitrogen atmosphere:

- First heating ramp: from -40°C to 100°C at 20°C/min. During this ramp, the material displays information on its actual physical and morphological state. This ramp is used to evaluate the heat of fusion, H_f, and melting temperature, T_m, of the material.

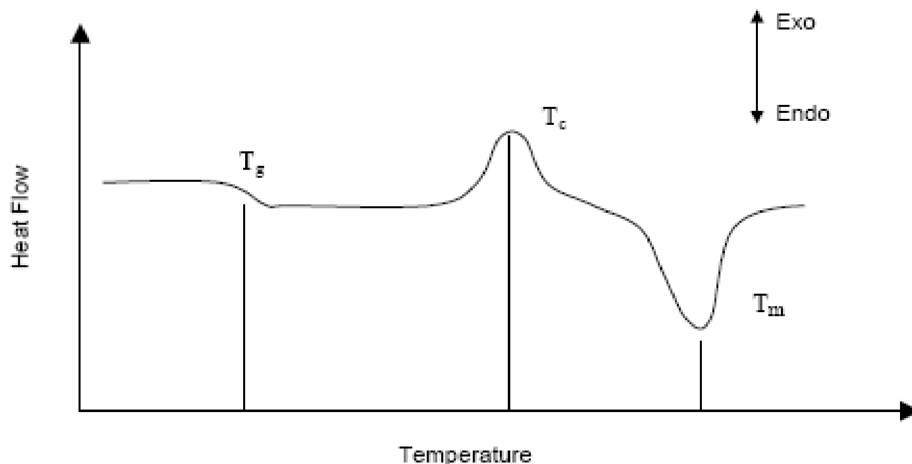


Figure 5: A schematic diagram of a heat flow curve of the polymer scaffolds.

The glass transition temperature, T_g, appears as an inflection in the heat flow curve, it is defined as the inflection point of the region. It is mainly concerned about the amorphous regions of the polymer.

Crystallisation is an exothermal transition, it occurs at temperatures slightly lower than the melting point of the polymer. The crystallisation of a polymer depends on the capacity of its chains to move and form a crystalline structure. There can be a crystallisation peak in both the first and the second heating ramp. The H_C is indicative of polymer chain length. The crystallisation temperature and enthalpy are defined as the maximum of the crystallisation peak and the area under the crystallisation curve respectively.

Melting is an endothermal transition, the melting point is defined as the minimum of the melting peak. The melting point of the PCL used for this study ranges between 60^0C. The heat of fusion is the area under the melting peak. Only the crystalline regions of the PCL have a melting temperature.

The heat of fusion is thus used to compute the percentage crystallinity, X_C calculated as:

$$X_C = \frac{\Delta Hm}{\Delta Hm^\circ}$$

Where, ΔHm was the measured enthalpy of melting and ΔHm° was the enthalpy of melting of 100% crystalline polymer. For PCL, ΔHm° = 139.5 J/g [63].

3.3.3 *Porosity and pore size*

The porosity of the scaffolds was calculated as follows:

$$\text{Porosity} = \frac{V - Vp}{Vp} \times 100\%$$

Where V= volume of the scaffold

V_p= volume of the polymer; obtained by dividing mass (M) by the density of the polymer. The density of the polymer was obtained from the supplier PCL (1.145g/cm^3).

3.3.3.1 Mercury intrusion Porosimeter

Mercury intrusion Porosimeter (PMI 30K-A-1, Porous Materials, Inc., Ithaca, NY) was used to determine pore size distribution, total pore volume of the foams. Pore size was calculated from the measurement of the intruded mercury volume by raising pressure.

3.3.4 X-ray diffraction (XRD) pattern

The crystal structure of the scaffolds was investigated by XRD analysis. The prepared scaffolds were cut in to slices and pressed into films which were then characterized using Philips X'pert MPD diffractometer .The sample were scanned from 5° to 60° at a scanning rate of 3.0°/min

The equation of the degree of crystallinity is calculated as follows:

$$X_C = \frac{Ac}{Ac + Aa}$$

Where: X_C refers to the degree of crystallinity;

Ac refers to the crystallized area on the X-ray diffractogram;

Aa refers to the amorphous area on the X-ray diffractogram

CHAPTER-4
RESULTS AND DISCUSSION

Results and Discussion

The phase separation processing parameters and the composition of the polymer solution strongly influence the various characteristics of the porous structures formed. Quenching at low temperatures reduces pore size due to the nucleation phenomena. Quenching at high temperatures, on the other hand, tend to create larger pores due to existence of less nuclei and the prevalence of the growth phenomena. The quenching rate and the temperature at which solvent removal takes place also affect the pore size. Polymer concentration influences the density and thus the overall porosity of the structure. Both quenching parameters and polymer concentration influence the occurrence of solid-liquid phase separation. Semi-crystalline polymers tend to create more porous structures than their amorphous counterparts due to the higher contraction of the semi-crystalline phase during solidification.

The influence of some of the key parameters such as quenching temperature, freezing medium, and polymer concentration on morphology and porosity of the scaffolds was studied.

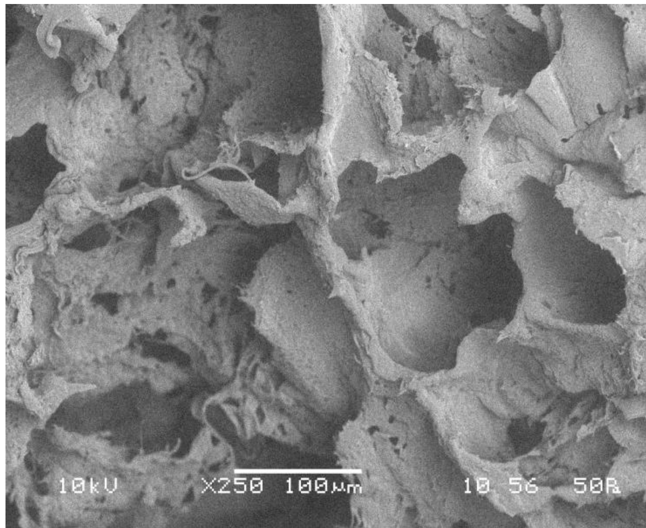
4.1 Effect of quenching temperature

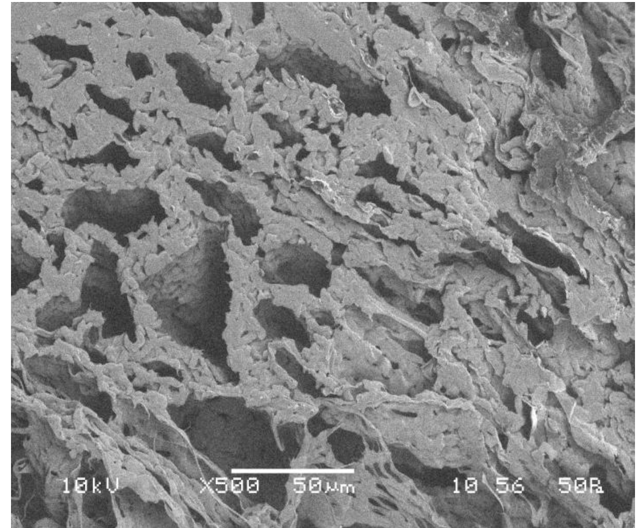
Quenching of polymer solution has a profound effect on the morphology of the scaffold. The effect of quenching temperature on scaffold morphology was studied by quenching the polymer solution at two different temperatures using a freezer (-20^{0}C) and refrigerator (6^{0}C). **Fig 8 s**hows the effect of quenching temperature on the resulting structures of scaffolds. Solid-liquid phase separation results in ladder or sheet like anisotropic morphologies which are strongly dependent upon the quenching temperature. Scaffolds quenched at lower temperature (-20^{0}C) were found to have well defined pores throughout the architecture of the scaffolds in comparison with those quenched at higher temperatures. This is evident from the SEM analysis of the scaffolds as shown in **Fig 6.** The is due to the fact that quenching at higher temperature is induced by lower nucleation rate and high crystal growth rate resulting in large pore size. The determination of pore size distribution of the sample using mercury porosimeter also confirms the higher pore size (22µm-60µm) obtained at lower freezing temperature as shown in **Fig 7.**

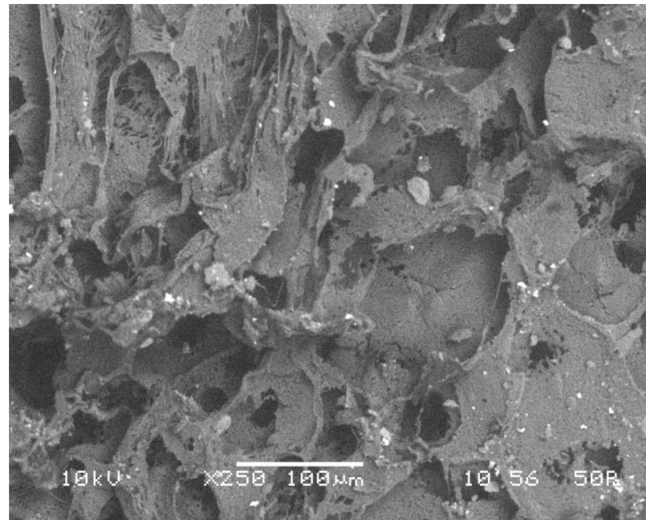
The quenching temperature did not affect the overall porosity of the samples. Low PCL w/v %(1 wt %) resulted in higher porosities (97%) than those with the high level of PCL w/v %(5 wt %) as shown in **Table 3.**

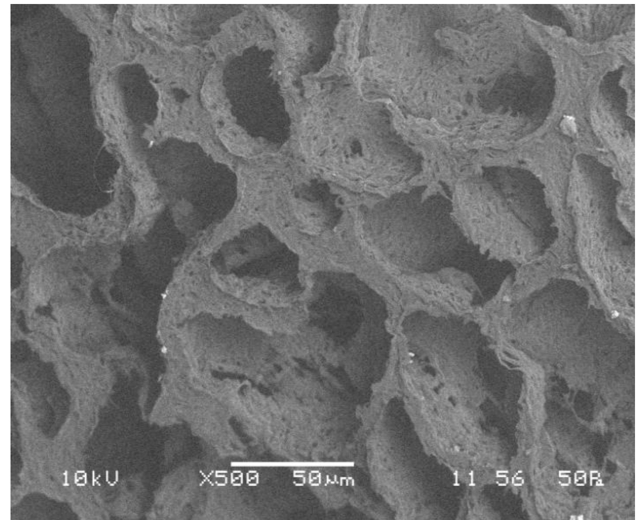
SEM

6^0C **-20^0C**









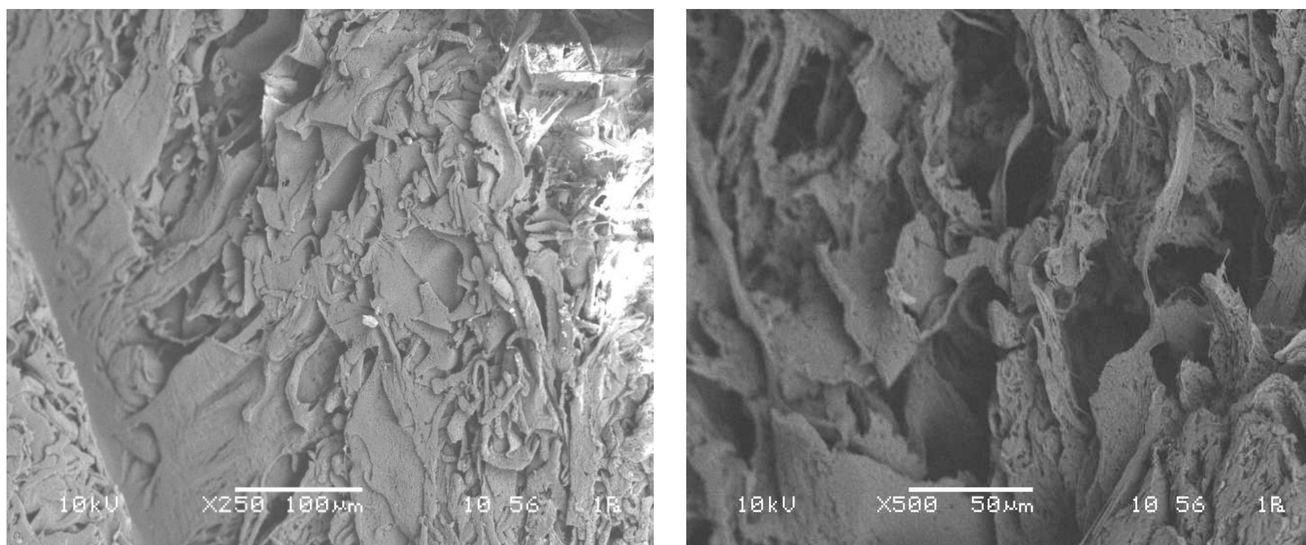


Figure 6: SEM micrographs of foams fabricated by freeze-drying of 5, 3, and 1 wt. % PCL/1, 4-Dioxane at -20^{0}C using a freezer and at 6^{0}C using refrigerator.

4.2 Effect of freezing medium

The freezing medium is another important factor found to influence the pore size and the architecture of the scaffolds. In the present study the effect of freezing medium on the prepared structures was investigated using freezer and refrigerator as the freezing medium. The experimental result is shown in **Fig 6.** The figure shows the effect of two freezing mediums observed at varying polymer concentrations (1wt%, 3wt%, 5wt %). All the structures resulted in a well defined porous architecture expect at lower concentration (1wt %). The intention in studying two freezing mediums is that with freezer the porous structure resulted in a higher interconnectivity than when observed using refrigerator. The effect of average pore size observed at two freezing mediums as shown in **Fig 7** is smaller in case of freezer rather than in refrigerator. A higher degree of supercooling resulted when the polymer solution was immersed in the freezer which gave rise to different crystallization kinetics, faster nucleation and slower growth of dioxane crystals in the polymer solution which resulted in different pore structures. The freezing mediums also had their effect on the porosities. A larger porosity of was obtained in case of freezer than the porosity obtained using refrigerator.

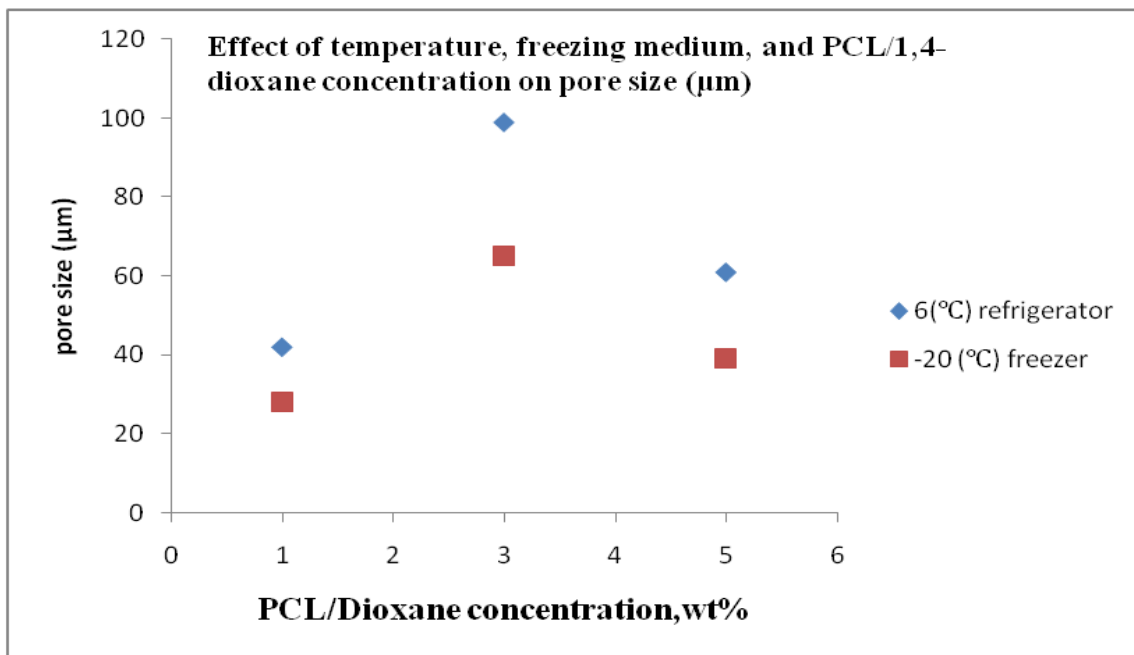


Figure 7: Effect of temperature, freezing medium and PCL/1, 4 dioxane concentration on pore size

4.3 Effect of polymer concentration

The polymer concentration is also an important parameter for the scaffold development. The different polymer concentration used in the study are 1, 3, 5 wt% to see their effect on the scaffold characteristics. The experimental results are tabulated in **Table 3**. The result shows that higher the concentration of PCL lower is the porosity. This is mainly attributed due to more matter remaining after solvent removal. Polymer concentration was found to affect the porous structures quenched at two different freezing temperatures (-20^{0}C, 6^{0}C). The scaffolds resulted were of highly porous with porosity values in the range of 90-97%. By varying the concentration of the polymer solution, as shown in **Table 3,** the porosity of the scaffolds could be controlled i.e. the porosity decreased with an increase in the polymer concentration and the density was found to increase (0.035-0.089 g/cm^3).

Table 3. Porosities of the prepared PCL scaffolds obtained by freeze-drying.

Polymer concentration wt%	Freezing temperature (°C)	Porosity (%)	Density (g/cm^3)
1	6	97	0.035
	-20	96.4	0.041
3	6	94	0.068
	-20	93.6	0.073
5	6	91.6	0.081
	-20	89.7	0.089

From **Fig 6** we can see that by increasing the polymer concentration the pore structure is being developed from 1 wt% to 5wt% as irregular to well defined structures.

4.4 Thermal properties of the scaffolds

Thermal Analysis is mainly done to detect the changes in the heat content (enthalpy) or the specific heat of a sample with temperature.The melting behavior of the prepared matrices was characterized using differential scanning calorimeter. All the scaffolds prepared from PCL have shown melting temperature, Tm, in the range of 60.5 to 63^{0}C. This melting temperature was similar to as-received PCL. The polymer crystal size in the scaffolds was similar to as the original pure polymer and was unaffected by the different processing parameters used during the fabrication process.

Table 4. Thermal properties (Tm) and porosity values of PCL scaffolds fabricated using freeze drying.

Concentration wt (%)	Freezing temperature(℃)	Freezing medium	Melting temperature	Porosity (%)
1	6	Refrigerator	61.36	97
	-20	freezer	63	96.4
3	6	Refrigerator	62.06	94
	-20	freezer	62.15	93.6
5	6	Refrigerator	61.75	91.6
	-20	freezer	60.50	89.7

The PCL scaffolds retained their semi-crystalline morphologies irrespective of the various freezing conditions used during the samples preparation.

4.5 XRD patterns

The crystalline phases present in the samples were identified by XRD. XRD patterns of the resulting PCL scaffolds are shown in **Fig 8.** There are no diffraction peaks of other substances and all diffraction peaks are corresponding to PCL. PCL is a semi-crystalline polymer with two diffraction peaks, around 21° and 23°. Moreover, the peaks were sharp and distinct, which indicated that the samples were highly crystalline materials. **Table 5** shows the degree of crystallinity obtained from XRD found to be in the range of 47-56%. Crystallinity range for the polymer polycaprolactone is in between 42-60%. The obtained results are in accordance with literature.

Table 5. Degree of crystallinity obtained from XRD

Polymer concentration wt%	Freezing temperature ^0C	Degree of crystallinity Xc (%)
1	6	56
	-20	54
3	6	62
	-20	52
5	6	51
	-20	47

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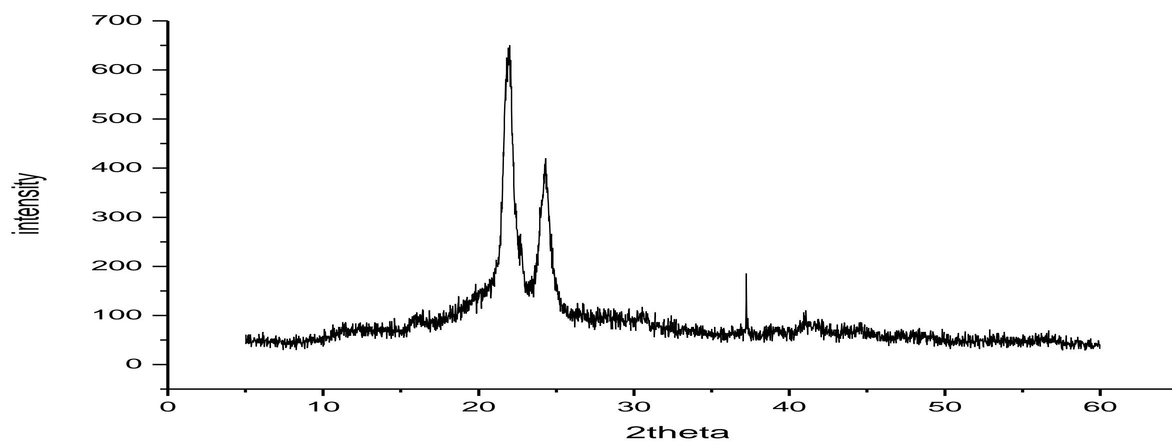

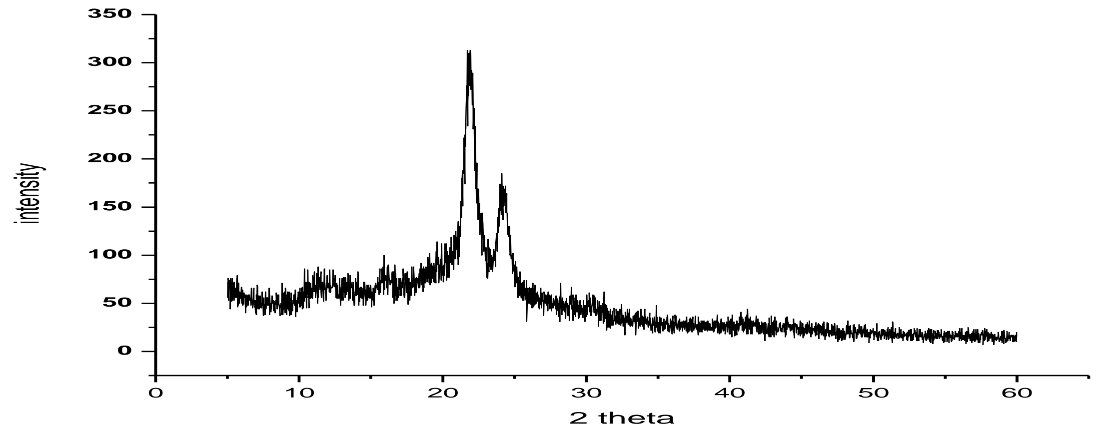
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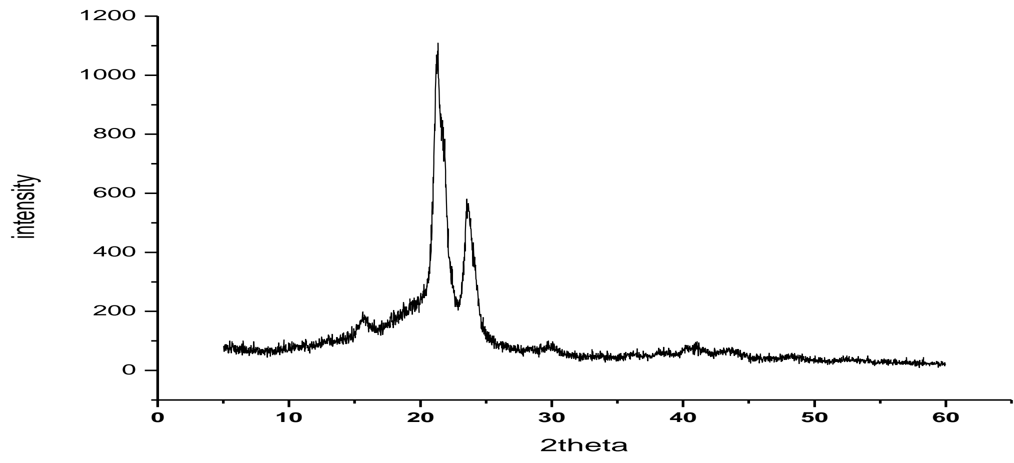
2 theta


1200
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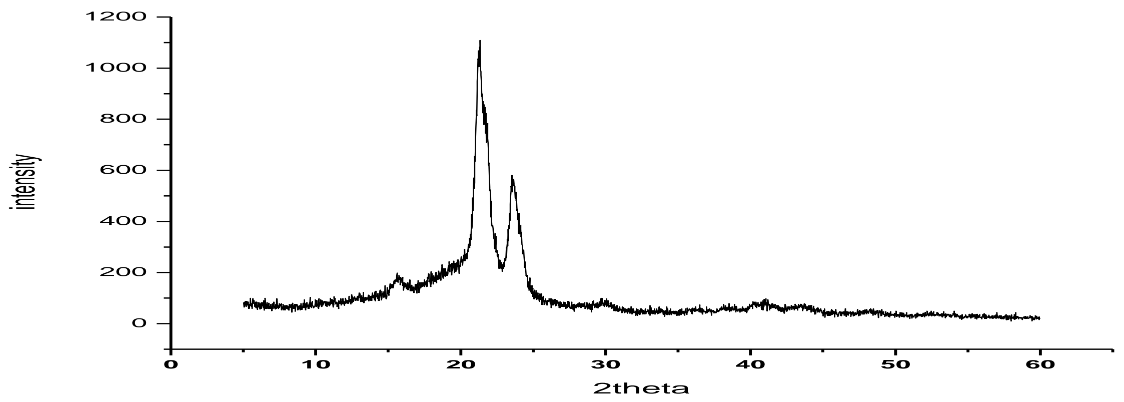
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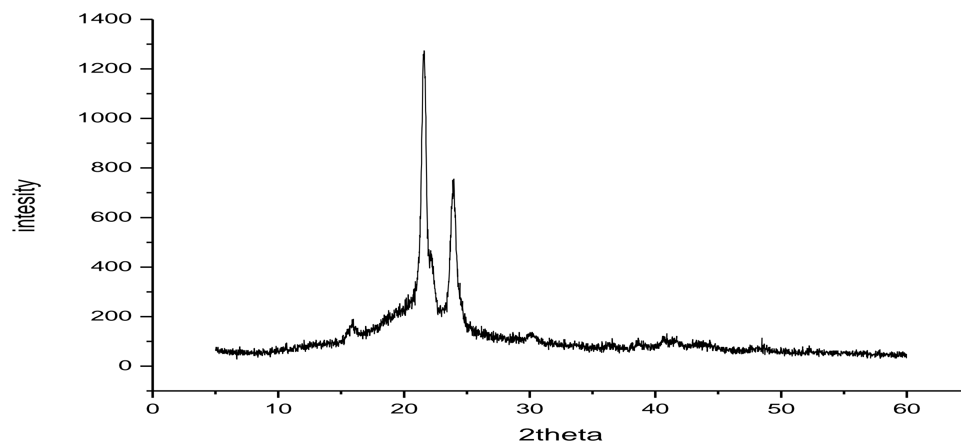

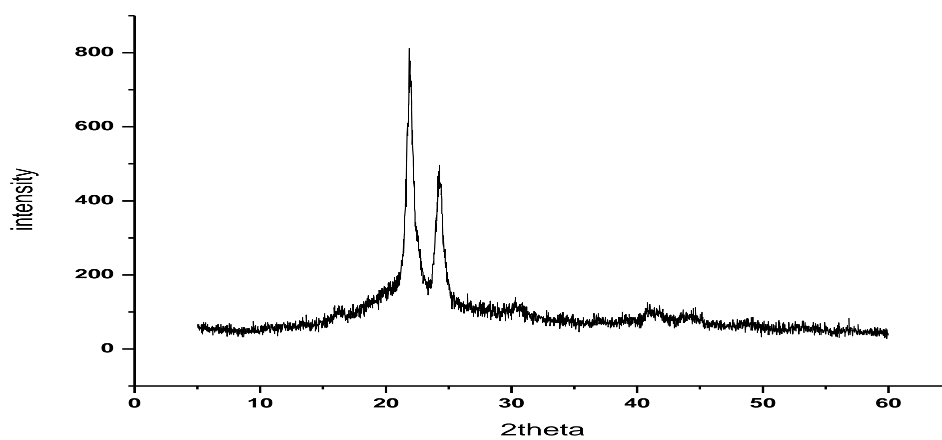

Figure 8: XRD patterns for PCL of 5, 3, and 1 wt. % PCL/1, 4-Dioxane

CHAPTER-6
CONCLUSION

Conclusion

The main aim of the present investigation was to prepare scaffolds from synthetic polymer, PCL, to be used as extracellular matrix in Tissue engineering. Micro porous scaffolds with high anisotropy were fabricated from PCL by thermally induced using solid-liquid phase separation method. The key parameters of the solid-liquid phase separation technique such as quenching temperature, freezing medium and polymer concentration were found to influence the scaffold morphology.

Interconnected porous structure of the scaffolds were obtained in the size range of 50 to 100 μm. Porosity and density of the scaffold structures are strongly dependent on the initial concentration of polycaprolactone. The pore interconnectivity was lower at lower polymer concentrations at 1 wt%. Freezing temperature had a major impact on the scaffold morphology and the porosity. At lower freezing temperatures the scaffolds structures were homogeneous. The porosity was higher in case of lower freezing temperature in the range of 96% to 97%. The average pore size and porosity of scaffold increased with decreasing polymer concentration. Ladder like structure was obtained at high polymer concentrations. This work suggests a useful technique to control the expected micropore formation of the scaffold. Therefore with high porosity and interconnectivity these scaffolds serve as potential candidates for various tissue engineering applications.

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APPENDIX

APPENDIX

Pore size of PCL scaffolds fabricated using freeze drying.

Polymer concentration wt%	Freezing temperature (°C)	Pore size (μm)
1	6	45±8
	-20	22±6
3	6	87±12
	-20	56±11
5	6	65±4
	-20	37±2

List of Instruments used during the whole experiment their make and function

Instruments	Manufacturer	Function
Magnetic stirrer	Spint	Mixing
Lyophilizer	lyolab	Freeze-drying
SEM	JEOL JSM-6480 LV	Morphology
DSC	Mettler Toledo DSC822e	Thermal properties
XRD	Philips X'pert MPD diffractometer	Crystallinity
Mercury intrusion porosimter	Quantachrome	Pore size analyzer