Poly (ε-caprolactone) PCL Scaffolds Preparation and characterization for tissue engineering

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Abstract— The main aim of the project is to develop and characterize scaffolds for tissue engineering applications are to study scaffold processing method and to characterized the behaviour of the scaffolds produced. The scaffolds are produced using a biodegradable polymer polycaprolactone by thermally induced phase separation technique using solid 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%.

Index Terms— Crystallinity, Pore size, Porosity, Scaffolds

I. INTRODUCTION

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 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 idea behind tissue engineering is to create or engineer auto grafts, either by expanding autologous cells in vitro guided by a scaffold, or by implanting an a cellular 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]. In effect, Tissue Engineering uses multidisciplinary tools to produce a surrogate extracellular matrix meant to guide cells into creating new tissue [4]. 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. [5]. some combination of cells, scaffold material, and bioactive peptides used to guide the repair or formation of tissue. [7] The field of tissue engineering exploits living cells in a variety of ways to restore, maintain, or enhance tissues and organs [8].

II. MATERIALS AND METHODS

A. 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.

B. Scaffold preparation

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 transferred into a cooling device and frozen at -20^0C using a freezer and at 6^0C using a refrigerator.

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

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

C. Characterization method

C.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.

C.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.

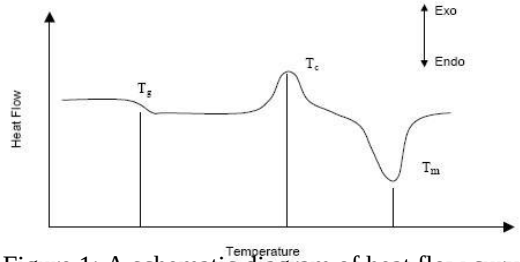

Figure 1: A schematic diagram of heat flow curve of the polymer sca

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.

C.3. Porosity and pore size

The porosity of the scaffold is calculated as follow:

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

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 ($1.145 g/cm^3$).

C.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.

C.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 werscanned from 5° to 60° at a scanning rate of 3.0° /min The equation of the degree of crystallinity is calculated as follows: $X_C = A_C/(A_C+Aa)$

Where:
$X_{C=}$ degree of crystallinity
A_C = crystallized area on X- Ray diffrectogram
A_a = amorphous area on X- Ray differectogram

III. 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.

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.

i Effect of quenching temperature

Quenching of polymer solution has a profound effect on the morphology of the scaffold. 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 2. 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 3.

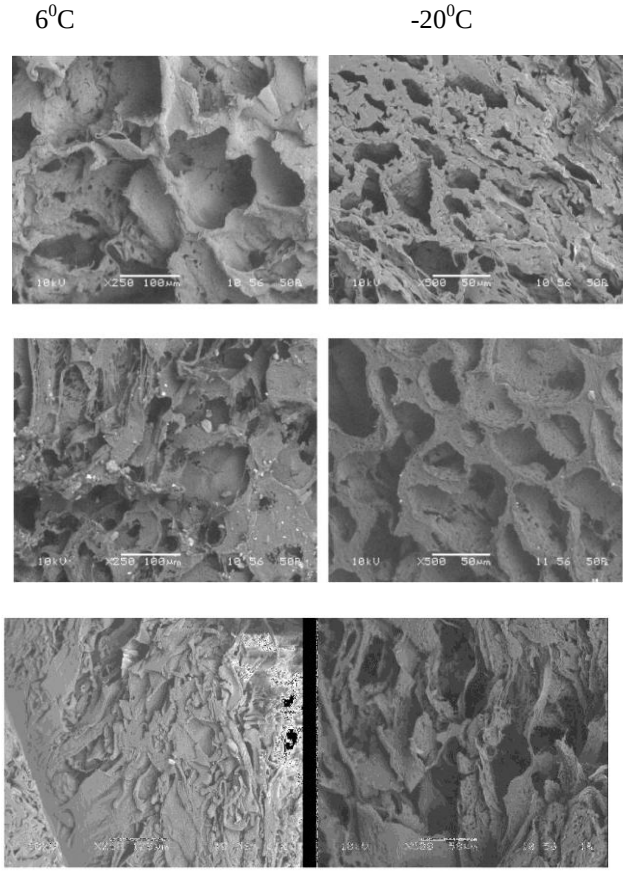

Figure 2: SEM micrographs of foams fabricated by freeze-drying of 5, 3, and 1 wt. % PCL/1, 4-Dioxane at -20^{0}C using a freezer and at 6^{0}C using refrigerator.

ii. 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 3 is smaller in case of freezer rather than in refrigerator

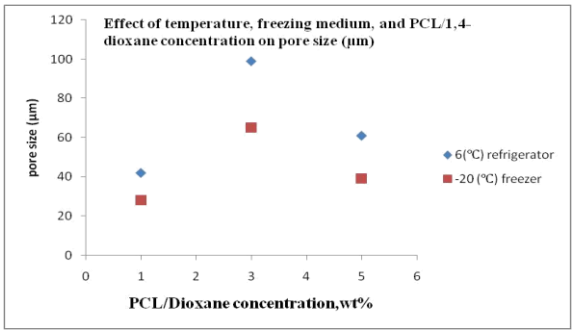

Figure 7: Effect of temperature, freezing medium and PCL/1, 4 dioxane concentration on pore size.

iii 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.

IV 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 application.

V. REFERENCES

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