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Fabrication of Nanocomposite Foam by Supercritical CO_2 Technique for Application in Tissue Engineering

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Abstract

Introduction: Microcellular foams are produced through polymer saturation by supercritical CO_2, then heating the sample at a temperature higher than the Tg of polymer/gas mixture after the pressure drop.

Objective: In this research, the polyethersulfone/graphene oxide nano-composite was initially made. Then the nanocomposite foamed with supercritical CO_2.

Material and Methods: The study on the effect of nucleation by carbonated nano-sheets is a relatively new topic. Polymeric foams particularly have proper dimensional stability. By changing the variables, the internal structure of the foam can be controlled. Due to the uniformization of energy distribution areas, the nucleating agent creates more uniform pores, higher porosity percentage, resulting in pores with a smaller diameter.

Result: In this study, the nanoparticles of GO were used as the heterogeneous nucleating agent in the foaming process. Also, the effect of foaming temperature, foaming pressure, foaming time and presence of nucleation agent were investigated. Adding 0.8% of GO reduces the average diameter of the PES foam cells from 6.99 to 3.7 µm. Besides, the increase of 40 °C foaming temperature (160 °C to 200 °C) also increases the diameter of the average cell from 3.14 to 7.2 µm.

Conclusion: The toxicity tests indicated that the product is non-toxic and can be used as a scaffold in the body of living creatures.

Keywords: Foam; Supercritical; Tissue Engineering; Graphene Oxide; Polyethersulfone

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1. Introduction

Polymeric foams are a new field of materials that have rapidly found a special place in human life in the last half century due to their unique characteristics. In addition, due to the lightness and low density [1], these materials have important properties such as mechanical strength and high stability [2, 3]; they are thermal insulation and show unique features in the electronic domain [4, 5]. The use of these materials greatly reduces the cost, energy, and consumption of raw materials [6, 7]. Thus, the great importance of this polymeric product is tangible and observable in different sectors such as the automobile manufacturing industry, aviation industry, and even at homes [8, 9]. This porous composite product consists of two solid and gas phases. The solid phase is usually

formed of the polymer and the gas phase is obtained by the foaming agent [6, 10, 11]. In recent years, due to the increasing global warming and environmental problems, it has been tried to use the new and safe sciences to make foams [12]. Supercritical fluids are one of these sciences. These fluids have a combination of gas and liquid properties together and can be reached to supercritical conditions with a low energy consumption [7, 13, 14]. Carbon dioxide can be known as one of the most important examples of supercritical fluids, which its effective role in the application of the above- mentioned cases is visible [15, 16]. The making of foam by using the supercritical fluids as a foaming agent is a very convenient method, since at low energy consumption and low cost as well as being harmless; the foam morphology can be controlled by changing the operating conditions[17, 18] .The mechanism for processing and production is a non-continuous process. In this process, the gas is dissolved in the polymer and subsequently by the formation of unstable thermodynamic conditions [19], a large number of voids appear inside the polymeric matrix. Eventually, the growth of voids from the previous stage will be controlled and sustained [20, 21]. One of the problems in the discontinuous method is the relatively long time required for polymeric matrix saturation. The reason for this limitation is the low penetration rate of gases into the polymer at ambient temperature.

In some cases, this amount increases with increasing temperature. The use of supercritical fluids can also improve the penetration rate into the matrix [22, 23]. One of the most important uses of polymer foam is the use as a scaffold in tissue engineering [24, 25]. Nowadays, there is a growing need to develop a method to replace lost tissues and organs. The tissue engineering has made fundamental efforts in this regard [26, 27]. Making microcellular foams by using supercritical fluids has advantages such as non-use of an organic solvent as well as the ability to control the structure. Hence, the microcellular foam can be a very suitable and effective option to be used as a scaffold [6, 28]. It is worth noting that these foams should be made from biocompatible and biodegradable materials [29]. In this study, the polyethersulfone/ graphene oxide nanocomposites were first made. The resulting nanocomposite was then turned into foam with the help of supercritical carbon dioxide. Finally, the morphological properties and biocompatibility of this material were examined.

2. Materials and Methods

2.1. Materials

The major raw materials used in this study included polyethersulfone, nanoparticles of graphene oxide and dimethylformamide as a solvent. The polymer used was Polyethersulfone, a product from the BASF Company, Germany, with an E6920P Ultrason grade. The modified Humer method [12] was used to synthesize the graphene oxide. Dimethylformamide is an appropriate solvent for dissolving the polyethersulfone. In this study, the dimethylformamide made by Merck Company, Germany, with a purity of 99.8% was used. Also in the use of the non-continuous process, solid carbon dioxide was used. The dry ice required was prepared from the CO_2 production unit of Pepsi Soft Drinking Plant in Shiraz.

2.2. Synthesis of polyethersulfone/graphene oxide nanocomposite

A solution method was used to prepare polyethersulfone/graphene oxide nanocomposites. A 15% w/w solution was prepared with heating and stirring. Three different weight percentages of nanoparticles (0, 0.4% and 0.8%) were synthesized with the help of ultrasonic apparatus for uniform distribution in the polymeric solution. The solution was immersed in a mold with a standard size as well as in dropping into distilled water for 24 hours, which dissolves the DMF, to remove the solvent from the composite.

2.3. Preparation of microcellular foams

The aim of applying Taguchi method in this research was to determine the percentage effect of the variables used on the size of the foam cells made and find the optimal operating conditions. The high-pressure reactor was used to make the microcellular foam. In the reactor mentioned, the solid CO_2 and sample were placed in the chamber inside the reactor so that after

receiving heat, the carbon dioxide would evaporate and the system would reach the desired pressure. The temperature and pressure must be selected in such a way that the CO_2 would be placed within the supercritical range. The Taguchi $L_9(34)$ experiment design was used. Hence, 4 variables with 3 modes for each of the variables were considered and 9 tests were performed. The percentages of nanoparticles, saturation pressure, foaming temperature, and foaming time are 4 variables, which values are given in Table 1.

2.4. Specifications

The scanning electron microscopy (SEM) was used for the morphological and structural study of the foam produced. The scanning electron microscope used in this research is located at the Faculty of Electrical Engineering, University of Tehran. The size of the foam cells produced was calculated using the MIP4student software. Using the qualitek-4 software, the optimal value of operating conditions and the percentage of the distribution of each of the variables were calculated. The software acts based on the Taguchi experiment design. The toxicity test was conducted in the toxicology department of the Faculty of Pharmacy of Shiraz. Thus, four different doses were injected into the body of 24 living mice. After 24 hours, the ALT, AST, and LDH enzymes were examined. The change rate of each enzyme was compared in different doses; hence, the toxicity or non-toxicity is determined by their amount and comparison with the reference group.

Table 1. Operating variables for samples

Row	Sample	percentages of nanoparticles *n* [%]	foaming temperature *T* [°C]	foaming time *t* [s]	saturation pressure *P** [bar]
1	A	0	160	15	75
2	B	0	180	30	100
3	C	0	200	45	125
4	D	0.4	160	30	125
5	E	0.4	180	45	75
6	F	0.4	200	15	100
7	G	0.8	160	45	100
8	H	0.8	180	15	125
9	I	0.8	200	30	75

3. Results and Discussion

3.1. Morphology

Polymer foam is produced by a process called phase separation. This is usually due to thermodynamic instability like a sudden change in temperature or pressure of the homogeneous polymer/foaming agent system. The supercritical CO_2 absorption reduces the glass transition temperature of the composite. With proper control of the operational variables, morphology can be adjusted [19, 30].

The Taguchi design of experiment was used to study the effect of variables simultaneously and separately and find the optimal test conditions. The operating constants were considered as follows:

- Saturation temperature: 40 °C
- Saturation time: 24 h
- Pressure reduction time: 0.5 seconds
- The interval between the first and second stages: Minimum possible time as about 1 minute

The results obtained using SEM images and the use of the MIP4student software are presented in Table 2. The following results represent the average diameter of each group of samples.

All samples had a diameter between 2 μm and 9 μm. Pore size distribution is narrow and the difference between the smallest and the largest size is only 7 μm and other samples are located in this narrow range. This narrow distribution is because of the heterogeneous nucleating model which is achieved by the use of graphene oxide as a nucleation agent in the foaming process [3, 12]. The mean diameter of all samples was also equal to 5.02 μm. The smallest diameter was related to the sample G as 2.115 μm. This sample simultaneously had the highest nanoparticle content and the lowest foaming temperature. The highest diameter was also related to the sample C as 9.75μm, which had the highest foam temperature without adding nanoparticles to the polymeric matrix before foaming. The set of images in each row occur in a composite group. In other words, each row has an equal nano-value. It is clear from the images that by adding nanoparticles to the polymeric matrix as the nucleating agent before foaming, the spherical shape of the cells becomes more uniform. Also, the dispersion of cells in the solid phase of foam is more uniform and homogeneous. With the presence of graphene oxide as a heterogeneous nucleating agent, the level of energy reduced, as a result, the rate of nucleating increased and subsequently the cell size decreased [9, 11]. The open structure of some cells is due to the presence of very low solvent content remained from the composite and nanoparticle synthesis. Figure 1 is related to the surface inside the foams.

Table 2. average diameter of microcellular foams for each sample

sample	A	B	C	D	E	F	G	H	I
diameter [μm]	4.850	6.420	9.750	2.505	4.605	6.450	2.115	3.195	5.450

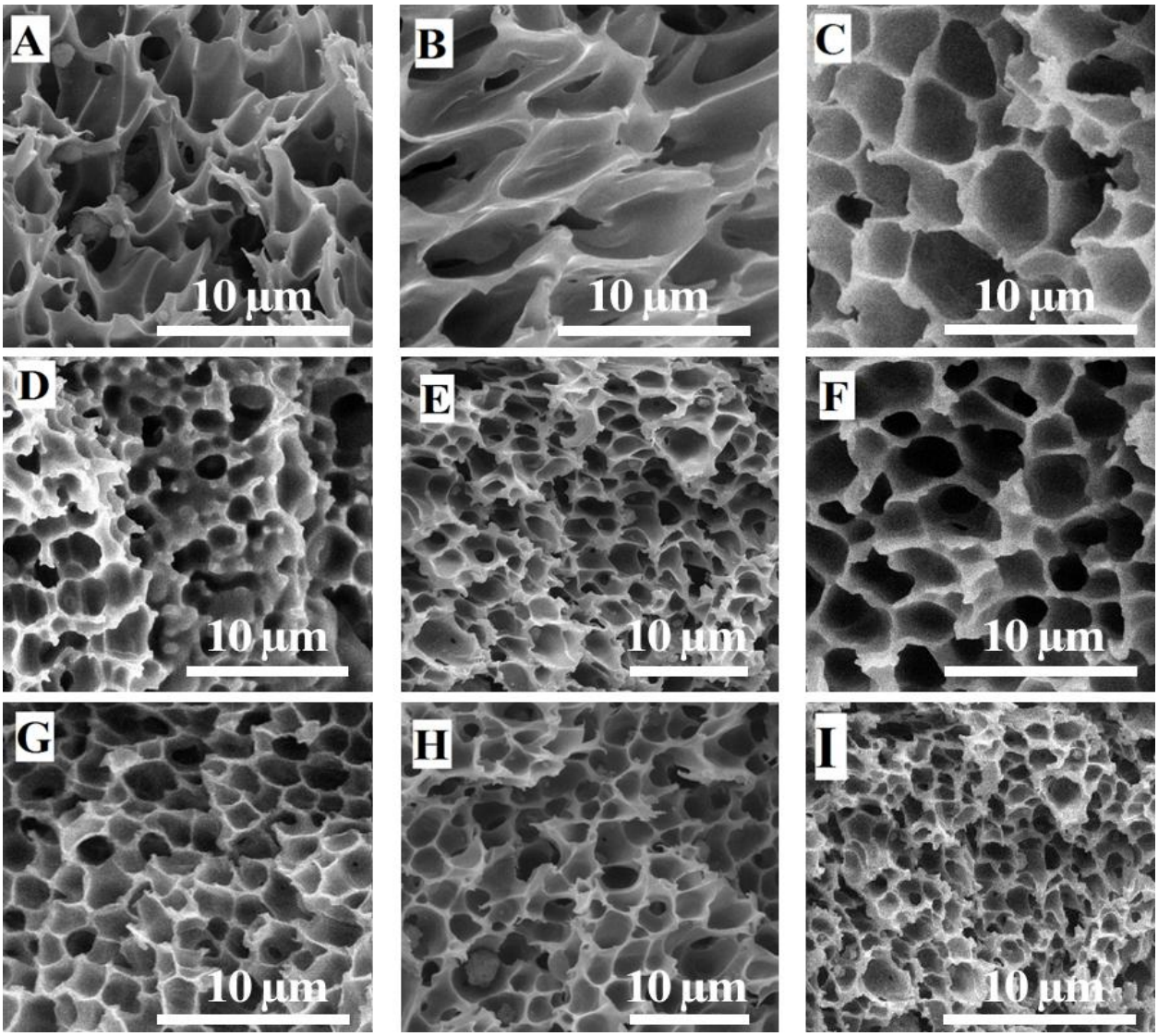

Figure 1. SEM micrographs for PES/GO microcellular foam produced according to Table 1 variables

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heterogeneous nucleating agent, the level of energy reduced, as a result, the rate of nucleating increased and subsequently the cell size decreased [9, 11]. The open structure of some cells is due to the presence of very low solvent content remained from the composite and nanoparticle synthesis.

3.2. Effect of foaming temperature, pressure, saturation time and the effect of graphene oxide on the morphology

The diagrams of the variation of diameter versus the pressure (fig2-A) / temperature (fig2-B)/ nano value (fig2-C) / time (fig2-D) are as follow:

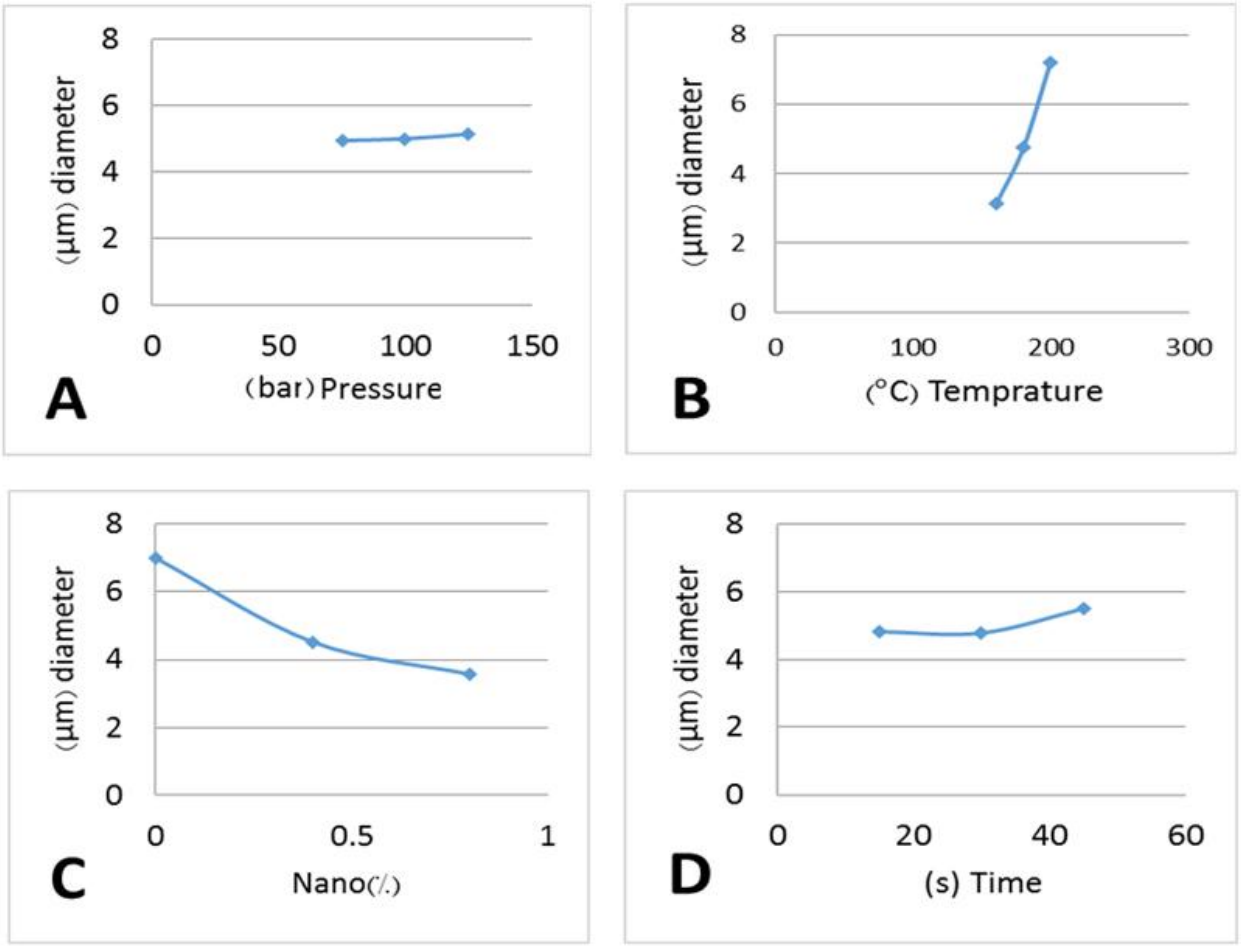

Figure 2. The diagram of the variation of diameter versus the pressure (fig2-A) / temperature (fig2-B)/ nano value (fig2-C) / time (fig2-D)

3.3. Toxicity

One of the methods for checking the toxicity of a substance in the body is the examination of blood enzymes that detect inflammation and damage to the liver. The liver damage markers in the plasma, including the activity rates of AST, ALT, and LDH enzymes in different groups were measured using the standard kits of the analyzer apparatus (Mindray BS-200), which is a device for obtaining the results of enzymatic kits. Each column is the average results of the measured enzyme value results of 6 mice (in a simple term, 4 groups with different doses, each group 6 mice) with the same sample dose compared with other groups that have received the same dose of suspension. The first column was considered as the base group that no injections applied. The enzyme level of the base group is the same level of the natural enzyme in the blood circulation. If the enzyme value measured for each group, after injection, does not have a significant difference in the enzyme value of the reference group in terms of toxicology, it means the occurrence of no toxicity in the living organism. Otherwise, this material will cause toxicity in the body, and it cannot be used in the living body in any way. Usual standard levels of AST, ALT, and LDH may slightly vary depending on the individual laboratory's reference values. Typically the range for normal AST is reported between 10 to 40 units per liter, ALT between 7 to 56 units per liter and LDH between 100 to 430 units per liter. [36] Until now nano-composite foam of Polyethersulfone/Graphene oxide has not been used in the body. All the enzymes measured are in the permissible range. This means that the made foam which injected into the body at different doses did not cause toxicity in the mouse body. The following three diagrams as shown in Fig3 are the plasma levels of LDH (Fig3-A), AST (Fig3-B), and ALT (Fig3-C) enzyme, respectively.

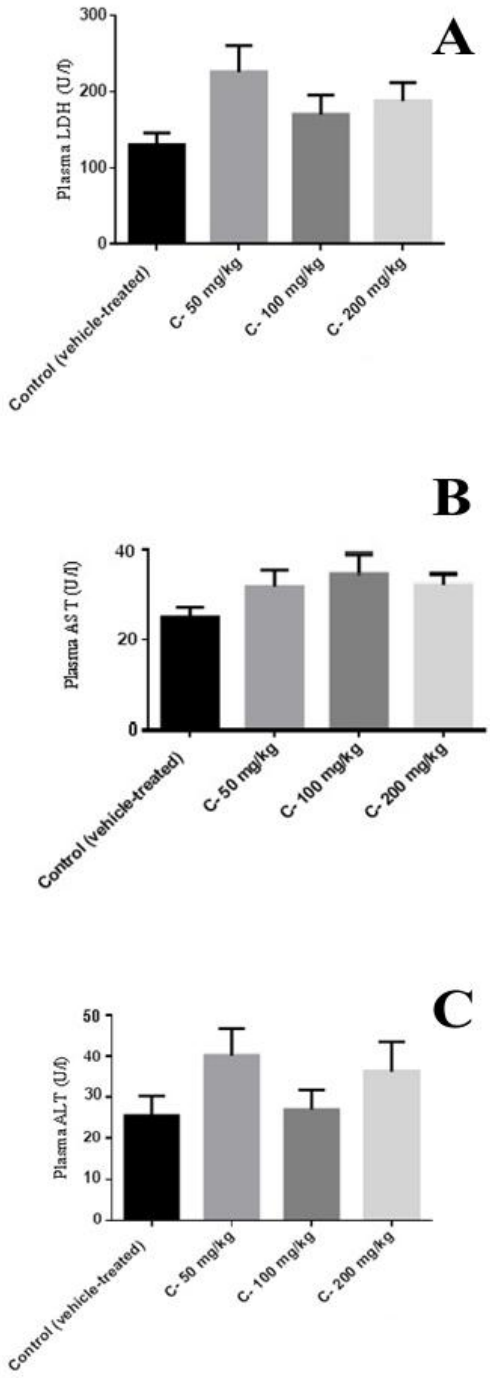

Figure 3. Plasma level of the enzyme LDH (A), AST (B) and ALT (C) In the control group and receiving different doses of the product.

4. Conclusion

According to studies findings and the results obtained from tests and observations, the following conclusions can be outlined:

- The major point in the synthesis of polyethersulfone/graphene oxide nanocomposite is the uniform distribution of nanoparticles in the polymeric matrix. The use of Sonicator provides an acceptable distribution.
- The use of anti-solvent separation method provides better results for nanocomposites synthesis for the reasons mentioned.
- The average particle size obtained in this research is about 5.02 μm. The maximum value in sample C was obtained as 9.75 μm, while the minimum value in the sample G was reported to be 2.115 μm.
- The size of the cells increases as the temperature rises.
- As the amount of nanoparticles increases, the size of the cells decreases. This trend represents the effective impact of nanoparticles on the nucleation of samples. In general, with higher nanoparticles concentration in the polymeric matrix, the size of the cells would be smaller, and consequently, the cellular density will increase.
- The average pore size in the pure polyethersulfone foam is equal to 7 μm; it is equal to 4.52 μm and 3.57 μm in the 0.4% and 0.8% nano-foams, respectively.
- Based on qualitek-4 software analysis, the effect of saturation temperature on the porosity of the foam is 54.8%, and nanoparticles are 40.54%. The effect of two other variables can be ignored due to being below 5%.
- Among the variables, the foaming temperature has the greatest effect on the porosity variations. Then, the percentage of nanoparticles contained in the composite has a significant effect on the diameter of the cavities. Foaming time and saturation pressure have a negligible effect on these changes, respectively.
- The density of the foams was significantly reduced compared to the crude polymer.
- The polyethersulfone/graphene oxide composite did not cause toxicity in the body.
- Due to the fact that the synthesized composite foam did not cause toxicity in the living body, it can be used as wounds covering (dressing) by changing its hydrophilicity value. Also, if the cells size is lowered (less than 5μm), it would have the usability as a hemodialysis membrane.
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Conflict of interest

The authors declare that they have no conflict of interests.

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