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Effects of Multi-Wall carbon Nano-Tubes (MWNTs) on structural and mechanical properties of electrospun poly(3-hydroxybutyrate) scaffold for tissue engineering applications

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Abstract. The aim of this study is to evaluate the effects of Multi-Walled Carbon Nano-Tubes (MWNTs) on the structural and mechanical properties of poly-3-hydroxybutyrate (P3HB) electrospun scaffolds. To achieve optimal properties of the electrospinning machine, P3HB polymer solutions were prepared at different concentrations and spinned in different electrospinning parameters. After optimization, MWNTs in different weight percentages (0.5%, 0.75%, 1%, and 1.25%) were added to the polymer solutions and electrospinned. The effects of MWNTs on the structure of fibers were investigated using Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR) techniques. The addition of MWNTs increased the average fiber diameter from 210 (neat P3HB) to 700 nm at 1.25% MWNTs. In addition, SEM photomicrographs and the MATLAB software program showed an increase in porosity from 81% to 84% in the presence of MWNTs. Tensile strength of P3HB/MWNTs composites revealed 158% improvement over pure P3HB scaffold. According to mechanical and structural properties, the best amount of MWNTs was 0.5 wt%. Therefore, MWNTs with low percentages can significantly improve the mechanical properties of pure P3HB scaffold, so that they can become favorable mechanically for tissue engineering applications.

1. Introduction

Tissue engineering developed with the idea of using biomaterials and cells to help tissues repair themselves [1-3]. As this branch of science developed further, its aim changed toward the development of rational strategies in order to optimize formation of new tissues to finally induce the formation of new tissues or organs [4]. In this context, three-dimensional porous scaffolds, which are components of different aspects of tissue engineering, can pave the way for adhesion, migration, and proliferation of the cells in question by creating an empty surface or space and function as a framework for the growth of the tissue [5-6]. Different natural and synthetic materials have been used for the manufacture

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of three-dimensional scaffolds [7-10]. Since the sizes of the majority of components of the extracellular matrix, such as porosities and the diameters of the fibers comprising them, are at nano-scales, use of nano-fibers in tissue engineering has drawn a lot of attention in recent years [11-13]. Different techniques are available for the synthesis of nano-scale fibers of which the electrospinning technique is more important than others due to its controllability and low cost. Electrospinning is a process to synthesize nano-fibers and micro-fibers from polymer or composite solutions. One of the most important applications of this technique is the synthesis of three-dimensional scaffolds for use in tissue engineering [14]. The chief components of this equipment are a power source with the capacity to supply a high voltage (in order to create an electric charge in the polymer or molten solution), a collecting plate, and a syringe pump. One of the electrodes of the power source is attached to the polymer solution, and the other electrode is connected to the collecting plate, creating an electric field [15]. The parameters of the equipment can be adjusted based on the conditions of the laboratory and the behavior of different materials; in this context, the conditions should be adjusted optimally in order to synthesize homogeneous and regular fibers.

Of all the bio-environmental polymers, P3HB, which has a long history of clinical uses, such as tissue engineering applications, has undergone extensive research [16-17]. This polymer which is synthesized by bacteria is polyester and is hydrolyzed in the human body to form butyric acid which is one of the metabolites of the body [18]. However, given the mechanical properties necessary for a scaffold, this polymer does not in itself has sufficient strength necessary for the synthesis of three-dimensional scaffolds. Iron et al. prepared a scaffold from P3HB scaffold and bioactive glass nano-particles using the electrospinning technique [19]. Evaluation of the properties of the scaffold showed that the bioactive glass nano-particles improved the mechanical properties of the scaffold by the weight percent of about 10.

On the other hand, studies have shown that carbon nano-tubes (MWNTs) exhibit unique and extraordinary properties and structure; therefore, they have widespread and different uses in the fabrication of different composites. In addition, these materials exhibit proper biocompatibility when they contact with cells, blood, and tissues [20,21]. Another important property of MWNTs is their strength and resistance, which is 100-fold of that of steel; but, their specific gravity is 1/6 times that of steel [22-24]. Therefore, MWNTs can give rise to a significant improvement in the properties of composites. By using MWNTs, optimum and high mechanical properties can be obtained with low percentages [25-29]. The incorporation of MWNTs into polymers has not been without complication as MWNTs are naturally chemically inert and dispersed poorly in numerous solvents [30]. Biofunctionalisation of MWNTs surface with bioactive molecules, such as carbohydrates or peptides, can help improve the biocompatibility and bioactivity of the scaffold. This should prevent MWNT aggregation and allow for their incorporation into polymer scaffolds. Once again, their large surface area makes MWNTs useful for tissue engineering purposes as large amounts of biomolecules can be placed onto the nanotubes [30-31]. Jang et al. manufactured nanocomposite scaffolds made of polylactic glycolic acid and MWNTs using the electrospinning technique [32]. The results showed a 54% increase in the strength of the scaffold in the presence of only 0.5% of multi-walled carbon nano-tubes. In another study, Yoo et al. added 1% of MWNTs to polyvinyl alcohol scaffolds and reported an improvement in the mechanical properties of this scaffold [33]. Mechanically, nanoparticles of bioceramics are stronger than polymers and play a critical role in providing mechanical stability. Researches have shown that in order to enhance mechanical properties of polymers by nanoparticles, high amount of nanoparticles has to be added to the basic polymer [34-38]. Shor et al. added 25% of hydroxyapatite to poly(caprolactone) scaffold to increase the mechanical properties of scaffold [36]. By addition of 20 wt% to three different ceramic nanoparticles, namely Calcium Titanate (CT), Strontium Titanate (ST), and Barium Titanate (BT), Bagchi et al. significantly increased the moduli and strength of poly(ε caprolactone) [38]. In this context, a large number of other studies have shown that MWNTs, with low percentages, can significantly improve the mechanical properties of different polymers [39-46]. Li et al. prepared the MWNT/ poly(3-hydroxybutyrate-co-3-hydroxyvalerate) scaffolds by Melt Molding method, and reported that MWNTs can significantly improve the mechanical properties of the polymer [47]. The difference between these studies is in construction method. As a result, the present study was undertaken to evaluate the effect of MWNTs on the structural and mechanical properties of electrospinned P3HB scaffolds for tissue engineering applications.

2. Materials and methods

2.1. Materials

Polyhydroxy butyrate (Sigma-Aldrich, USA), multi-walled carbon nano-tubes measuring 525 nm in diameter and 0.5-2 μm in length (Nanosunazma, USA), dimethylformamide (DMF), chloroform (Merck, Germany), sulfuric acid (Merck, Germany), and nitric acid (Merck, Germany) were used for the purpose of this study.

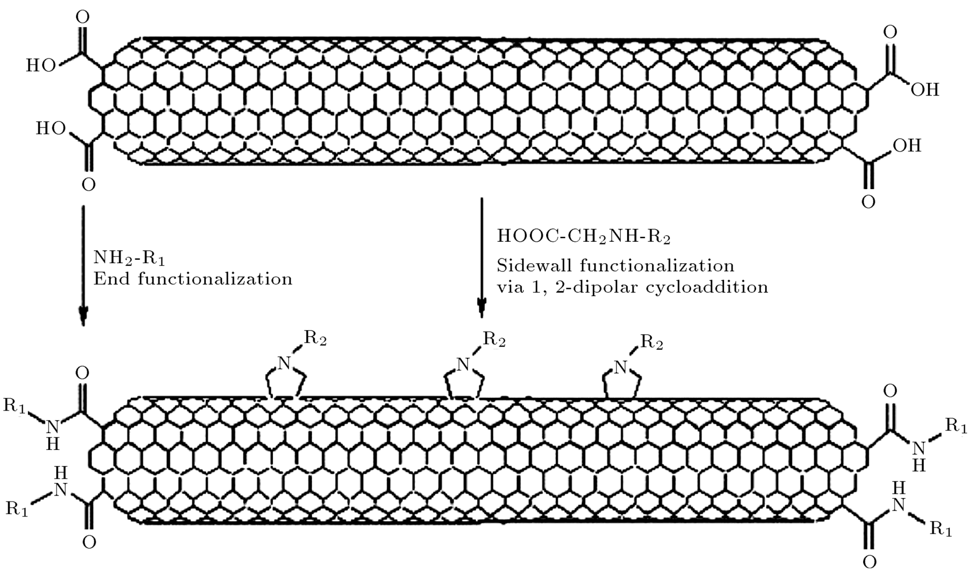

Figure 1. Heterogeneous functionalisation of MWNTs. This shows both tip and side-wall adaption, shown to reduce MWNT toxicity and increase MWNT biocompatibility [29].

2.2. Electrospinning of P3HB/MWNTs nano-composite scaffold

The electrospinning equipment used in the present study consisted of a 1 mL syringe, a needle with an internal diameter of 0.27 mm, an injection pump, an aluminum plate, and a power source. To dissolve the polymer, the chloroform solution and DMF were selected at a ratio of 7:3. Then, 6 wt% optimal concentration of P3HB was added to the solution and mixed for 30 minutes at 60°C. Next, MWNTs were added to the solution at different concentrations of 0.5, 0.75, 1, and 1.25 wt% and mixed for 30 minutes. MWNT functionalisation is thus required and involves the addition of functional groups such as carboxyl or alcohol groups to the walls and ends of the nanotubes. This should prevent MWNT aggregation and allow for their incorporation into polymer scaffolds [30] (Figure 1). Then, the P3HB/MWNTs solution was loaded in the syringe, and the syringe was placed in its special container. The distance between the tip of the needle and the collecting plate was adjusted at 25 cm, and the injection rate was set at 0.01 mL/min. These parameters had already been optimized. Then, a 12.5-kV current was applied to bring about a continuous current at the tip of the needle, and the fibers were electrospinned on the collecting plate (Figure 2).

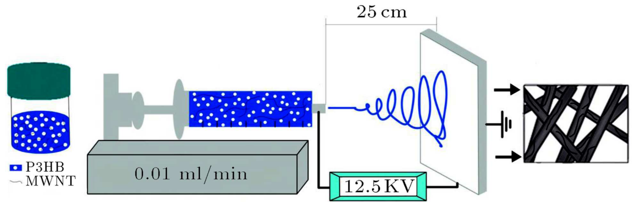

Figure 2. The optimum electrospinning parameters for P3HB/MWNTs nanocomposite scaffolds.

2.3. Evaluation of the structure and morphology of scaffolds

The morphology of the surface of scaffolds was evaluated under a scanning electron microscope. The mean diameter of fibers was determined by measuring the diameters of 40 single fibers on the SEM photomicrograph. The porosity of the scaffolds was also investigated on the SEM photomicrograph using MATLAB software program. Transmission Electron Microscopy (TEM) technique was used to evaluate the position and distribution pattern of MWNTs within the fibers. FTIR technique was used to evaluate changes in the chemical structure of scaffolds.

2.4. Evaluation of mechanical properties

Tensile strength test was used to evaluate the mechanical properties of scaffolds based on ISO 1798 specifications. The tensile rate of the machine was set at 10 mm/min with a load cell of 50 N. The samples were prepared at a dimension of 10 $\times$ 60 mm and underwent a tensile force at specifications mentioned above until the sample failed at which the strength of each sample was recorded.

3. Results and discussion

3.1. Evaluation of the morphological structure of scaffolds

Figure 3 presents the photomicrographs of pure P3HB scaffold and P3HB/MWNTs nano-composite scaffolds with 0.5, 0.75, 1, and 1.25 weight percentages, with 20$\times$ 30-mm dimensions. Pure P3HB scaffold is completely white; however, the color of the fibers turned gray

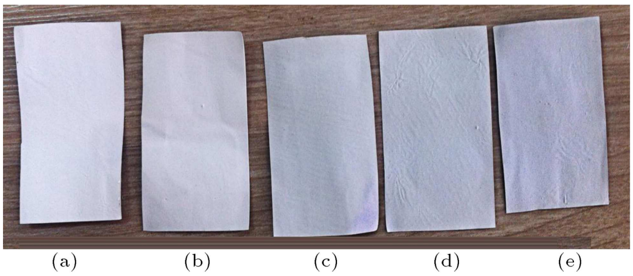

Figure 3. The photomicrographs of pure P3HB (a), P3HB/0.5% MWNTs (b), P3HB/0.75% MWNTs (c), P3HB/1% MWNTs (d), and P3HB/1.25% MWNTs (e) scaffolds.

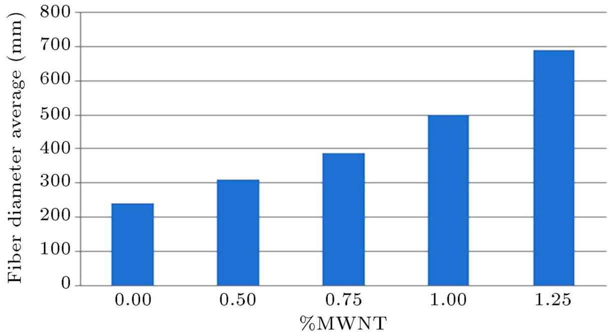

Figure 5. An increase in the mean diameter of scaffold fibers proportional to the concentration of MWNTs.

with an increase in the concentration of MWNTs in the composite.

Figure 4 presents the SEM photomicrographs of pure P3HB and P3HB/MWBTs nano-composite scaffolds. As shown in the figure, the synthesized fibers resemble extracellular matrix (ECM) which consists of fibers without any beads that are elongated and continuous and can serve as a basement membrane for cellular growth. However, based on the photomicrographs and the morphology of the fibers, it can be concluded that the fibers lose their integrity with an increase in their MWNTs content. Such a phenomenon is obviously seen in samples with 1.25% MWNTs. In addition, the viscosity of the solution increased with an increase in MWNTs concentration up to 1.25% in the polymer solution; such an increase during electrospinning decreased the width of fibers, resulting in pooling of MWNTs at one point of the fibers, while there was no change in the parameters of the equipment. On the other hand, an increase in viscosity sometimes caused the solution to be entangled in the needle, which made the formation of fibers difficult; therefore, the morphology of fibers lost its homogeneity. Such a phenomenon is a common occurrence, and the viscosity of the solution is an important parameter during the electrospinning process [45]. Therefore, the P3HB/0.5% MWNTs nano-composite scaffold was the most homogeneous of all the nano-composite scaffolds.

Figure 5 presents the diameters of scaffold fibers. The diameter of the majority of fibers in pure P3HB scaffolds was 200-280 nm, with a mean of 240 nm. Incorporation of 0.5 wt% of MWNTs to the scaffold resulted in an increase in the thickness of fibers; in this context, the mean diameter of fibers increased to 310 nm. By incorporating 0.75%, 1%, and 1.25% MWNTs, the mean diameters of fibers increased to 390 nm, 500 nm, and 690 nm, respectively. As seen in Figure 5, incorporation of only 1% MWNTs to P3HB resulted in a two-fold increase in the diameter of fibers.

3.2. Porosity of scaffolds

The only aspect of electrospinning that is not easy to directly control is the size of the scaffolds pores. This can be controlled indirectly by creating smaller diameter fibers, as smaller fibers result in smaller, more tightly packed pores [48-49]. Stephen et al. charac-

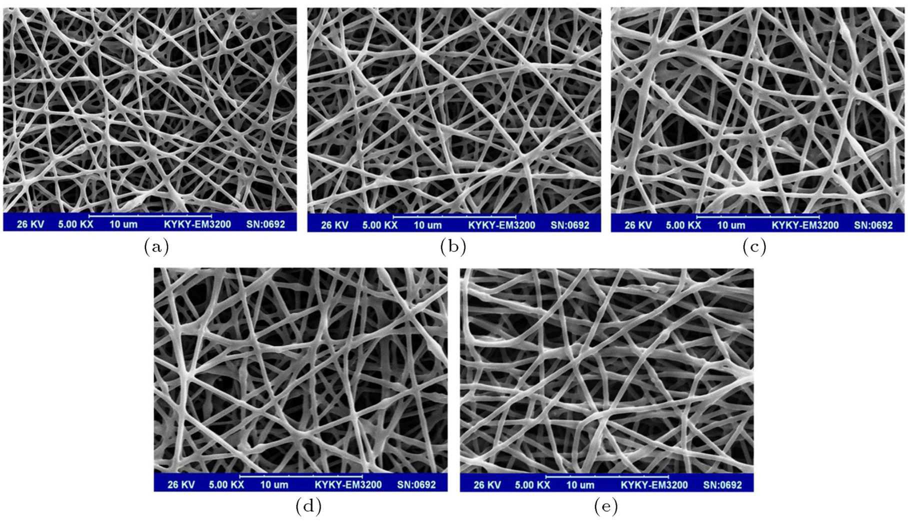

Figure 4. SEM photomicrographs of pure P3HB (a), P3HB/0.5% MWNTs (b), P3HB/0.75% MWNTs (c), P3HB/1% MWNTs (d), and P3HB/1.25% MWBTs (e) scaffolds.

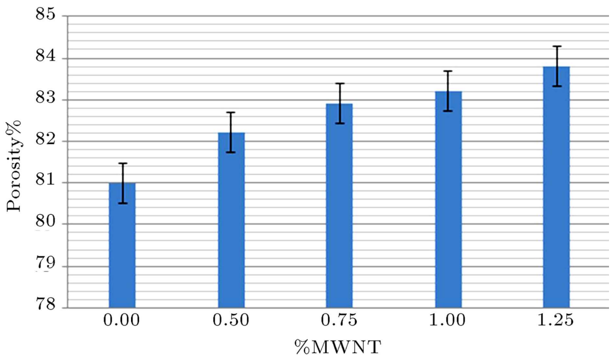

Figure 6. Porosity rate of scaffolds in different amounts of MWNT.

terized dominant role of fiber diameter in controlling the pore diameter of the networks, and reported that increasing fiber diameter results in an increase in mean pore radius [49]. However, it is not possible to alter pore size without changing any of the other electrospinning parameters [50]. The results of analyses carried out using MALAB software program in relation to the porosity of samples showed that the porosity rate in all the scaffolds was over 80%, which is favorable for tissue engineering purposes. Considering the porosity rates presented in Figure 6, it can be concluded that the porosity increased from 82% to 85% with an increase in the diameter of fibers from 240 nm to 700 nm. Therefore, the MWNTs have no negative effects on porosity of scaffolds.

3.3. Morphology of MWNTs in the structure of fibers

Transmission Electron Microscopy (TEM) technique was used to evaluate orientation and position of MWNTs in electrospinned fibers. Because of the homogeneity of fibers in the P3HB/0.5% MWNTs nanocomposite scaffold, this sample was selected for TEM photomicrography (Figure 7). Nano-tubes are fine tubes that preserve their shape and are lodged within the fibers. MWNTs are readily visible within the fibers. A proper equilibrium has been achieved with

Figure 7. TEM photomicrograph of P3HB/0.5% MWNT nano-composite scaffold.

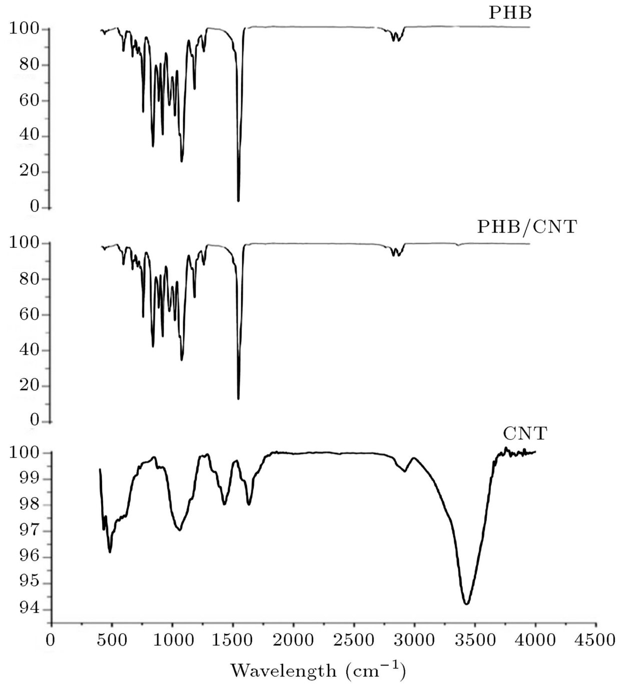

Figure 8. FTIR of P3HB, P3HB/MWNTs nanocomposites and MWNTs.

the MWNTs within the P3HB fibers during the electrospinning process, in which MWNTs are properly distributed and longitudinally oriented within the fibers. Strength of MWNTs is in its longitudinal direction. When the MWNTs were in the longitudinal direction of the fibers, they can take on their role. In this study, in the P3HB/0.5% MWNTs nano-composite scaffold, the MWNTs have been in the longitudinal direction of fibers.

3.4. Identification of the chemical structure with FTIR

Changes in the chemical structure of the pure MWNTs powder, pure P3HB scaffold, and P3HB/MWNTs scaffold were examined by the FTIR machine (Figure 8). In the spectrum obtained, vibrational modes were observed in 970 cm^{-1}-1725 cm^{-1} that indicate the structures and bonds of CH, CH_2, CH_3, C-O, and O-H of P3HB. In MWNTs-COOH functionalized, characteristic vibrational modes of MWNTs, C_C bond (1427 cm^{-1}-1630 cm^{-1}), O-H bond (2910 cm^{-1}-3430 cm^{-1}), and C_O bond (870 cm^{-1}-1150 cm^{-1}) are apparent in the spectrum shown in Figure 8. The electrospun P3HB/MWNTs nanocomposite scaffold spectrum consists of nanotubes surface peak (O-H bond) at around 3430 cm^{-1}.

3.5. The results of tensile strength of the scaffolds

Figure 9(a) presents the tensile strengths of the scaffolds with different percentages of MWNTs. In addition, Figure 9(b) presents the scaffold modulus for the

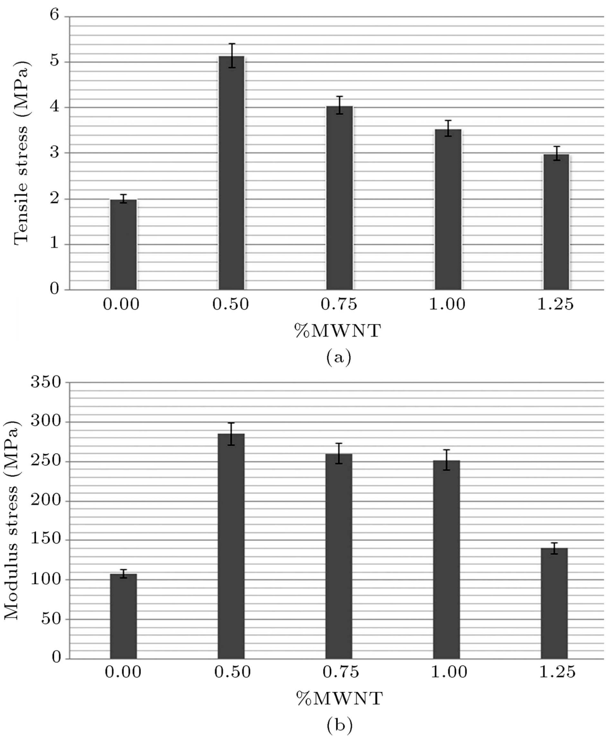

Figure 9. (a) Changes in the tensile strengths of scaffolds in terms of changes in MWNT concentrations. (b) The moduli of scaffolds in terms of changes in MWNT concentrations.

determination of the role of MWNTs in the scaffolds. The role of MWNTs in increasing the strength of nano-composite fibers is clearly evident. The highest strength was recorded in the P3HB/0.5% MWNTs, which increased 157% (from 2 MP to 5.15 MP) in comparison to pure P3HB scaffold. The Young's modulus increased 163% (from 108 MP to 285 MP). The results indicated that only 0.5% of MWNTs can significantly increase the tensile strength and the modulus of nano-composite scaffold. In addition, the results showed the highest improvement in the mechanical properties of P3HB/0.5% MWNTs nano-composite scaffolds compared to pure P3HB and other P3HB/MWNT nano-composite scaffolds. Jang et al. added MWNTs at different concentrations to polylactic glycolic acid and evaluated the mechanical properties, reporting the highest increase in strength in the presence of 0.5% MWNTs; an increase in concentration of MWNTs compromised the mechanical properties [32]. Such a phenomenon was attributed to the low diameter of nano-tubes and their distribution and orientation within the fibers. The nano-tubes exhibit very high strength in the longitudinal direction up to 100 times that of steel. Therefore, the best orientation of the nano-tubes within the fibers is in the longitudinal direction of the fibers. When the concentration of nano-tubes exceeds 0.5 wt%, their orientation within the fibers in the longitudinal aspect is disrupted. As mentioned, in order to enhance mechanical properties of polymers by nanoparticles, high amount of nanoparticles must be added to the basic polymer [34-38]. Shor et al. added 25% of hydroxyapatite to poly(caprolactone) scaffold to increase the mechanical properties of scaffold [36]. By addition of 20 wt% to three different ceramic nanoparticles, namely Calcium Titanate (CT), Strontium Titanate (ST) and Barium Titanate (BT), Bagchi et al. significantly increased the moduli and strength of poly(ε caprolactone) [38]. In this research, the low percentages of MWNTs (only 0.5%) significantly improved the mechanical properties of P3HB scaffold.

4. Conclusions

A number of P3HB/MWNTs nano-composite scaffolds were successfully synthesized at different weight percentages (0.5%, 0.75%, 1%, and 1.25%) using the electrospinning technique. The mean diameter of scaffold fibers increased with an increase in MWNTs concentrations. However, such an increase in MWNTs concentration distrusted the morphologic homogeneity of the fibers. The porosity of the scaffolds was calculated at 84%. Evaluations of FTIR, XRD, and TEM showed proper distribution of MWNTs within the nano-composite scaffolds. The results of tensile strength test of the scaffolds showed that the presence of MWNTs significantly increased the tensile strength of scaffolds, with the maximum increase of 0.5% in MWNTs. Therefore, based on the results, P3HB/MWNTs scaffolds can be considered the best scaffolds for tissue engineering purposes.

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