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Miscibility of Poly(Ethylene-co-Vinylalcohol)/Poly(δ-Valerolactone) Blend and Tissue Engineering Scaffold Fabrication Using Naphthalene as Porogen

Waseem Sharaf Saeed, Abdel-Basit Al-Odayni, Abdulaziz Ali Alghamdi, Ahmad Abdulaziz Al-Owais, Abdelhabib Semlali, and Taieb Aouak

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ABSTRACT
Herein, various poly(ethylene-co-vinylalcohol)/poly(δ-valerolactone) blends were prepared at different ratios by solvent casting for use in tissue engineering. The miscibility of these polymers was studied in detail using differential scanning calorimetry, Fourier-transform infra-red spectroscopy, and X-ray diffraction. The Avrami model has been applied for determining the isothermal crystallization kinetics of poly(ethylene-co-vinyl alcohol), poly(δ-valerolactone) and their blend with equal compositions, in which the Avrami parameters, the maximum crystallization time and the half-time were deduced. Cell adhesion and cell proliferation of the resultant materials were examined by a (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)MTT assay; the blend containing equal amounts of the two polymers showed the best performance. Micropores and their interconnections were formed by using a new porogen under vacuum at temperatures slightly less than the glass transition temperature. The produced micropores and their interconnections were studied using scanning electron microscopy.

KEYWORDS
Cell adhesion; miscibility; naphthalene as porogen; poly(ethylene-co-vinylalcohol)/poly(δ-valerolactone) blend; tissue engineering scaffold fabrication

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Introduction

Polymeric biodegradable materials are frequently employed in various biomedical domains like tissue engineering scaffolds, artificial implants, wound dressings, and carriers in drug delivery systems. [1–4] Many of these medical applications require modification of the surface properties of the polymer to improve its biocompatibility and cell adhesion. It is well known that a biocompatible and biodegradable polymeric material could be eliminated from the human body as small and non-toxic molecules after its implantation via a fast decomposition process. [5] Therefore, such a material requires surface modification to improve cell adhesion and proliferation [6]. To achieve this goal, different methods have been employed. For example, Jacobs et al [7] and De Geyter et al [8] used oxygen or air plasma to treat the surface of a poly(ε-caprolactone) (PCL) material, and the results showed moderate increase in the surface energy, surface roughness, and total oxygen functional groups on the PCL surface. Different researchers using the same method observed a better attachment and proliferation of osteoblast cells. [6, 9] Wulf et al [10] used ammonia plasma and produced a modified PCL surface without affecting the mouse fibroblast cell viability. The authors have also employed argon and helium plasma to enhance the PCL surface to improve the cell adhesion and proliferation. It was found that both the plasmas lead to an incorporation of oxygen functional groups in PCL. However, in case of helium plasma, nitrogen functional groups were also found on the surface of the treated PCL. Polymer blends can also be used as materials to improve cell adhesion and proliferation. Blending polymers has indeed proved to be a useful and economical method that is widely used to produce new polymeric materials with desirable properties compared to those obtained by the copolymerization of the corresponding monomers, which requires complex conditions to obtain the appropriate copolymer. Recently, the versatility of polymer blends has enabled the implementation of new ideas in different biomedical fields. These systems are continuing to expand and play an important role in improving the solutions in multidisciplinary domains, notably implantable materials for medical applications as well as investigations on bone [11, 12], cartilage [11, 13], prosthetic dentistry [11, 14], and drug release [11, 15].

However, most blend systems are immiscible and the resultant materials are of poor quality, considerably limiting their large-scale applications. Therefore, studying the miscibility and a thorough knowledge of the polymer blends before use is unavoidable. Consequently, the miscibility of polymer blends has been extensively investigated during the past 40 years. Most of these studies have focused on either two amorphous polymers or a pair of amorphous and crystalline polymers. However, the reported investigations on blends of two crystalline polymers are limited in the literature, [16–35] despite their determinant effect on the properties of materials. A crystalline-crystalline polymer blend is a complex system in which both the components are can crystallize; therefore, the morphology and crystallization behaviors of such pairs of polymers must be studied under various conditions.

However, it is also interesting to investigate a blend of two semi-crystalline components and observe the type of morphology produced on account of the sequential crystallization and determine the properties of the resultant material. For example, the blend of poly(butylene succinate) (PBSU) and poly(ethylene oxide) (PEO) is an example of a miscible crystalline/crystalline blend that was investigated by Qiu et al [35] and Shen et al [34] using differential scanning calorimetry (DSC), optical microscopy, and small-angle X-ray scattering. The studies revealed that the crystallization of PEO was affected by the presence of the PBSU crystals formed through different crystallization processes.

Poly(δ-valerolactone) (PDVL) has attracted less attention than PCL even though their chemical properties are similar. This polymer has a semi-crystalline aliphatic structure, is characterized by a low melting point and glass transition temperature, and exhibits a less elastomeric behavior than PCL. PDVL is an important aliphatic polymer because of its good biodegradability, biocompatibility, and permeability characteristics. Generally, this polymer is obtained via a ring opening polymerization of δ-valerolactone(DVL) using different catalytic systems. [36–38] To prevent the toxicity of the traces of the metal complexes employed as catalysts in this reaction, which are embedded in the polymer, an enzymatic ring opening polymerization has been recently established to produce clean PDVL that can be applied in biomedical applications. [39–43] Despite the advantages that characterize this polymer, practically no investigations have been reported on the use of PDVL individually in the biomedical field. However, this polymer is used in combination with other materials as a hydrophobic block in amphiphilic block copolymers, which are suitable for developing micellar delivery systems for hydrophobic antitumor drugs [44] and employed for the regeneration of soft tissues and production of various clinical implants [45–47]. Poly(ethylene-co-vinyl alcohol) (PEVAL) is a random copolymer and semi-crystalline over the entire range of composition despite the irregularity and non-stereo-specificity of the vinyl alcohol units distributed in the copolymer chain [48]. This copolymer is synthesized by copolymerization of ethylene with vinyl acetate to produce
the ethylene vinyl acetate copolymer, followed by hydrolysis. PEVAL is one of the best known flexible thermoplastic materials and used in a wide range of fields such as food packing, funnel tanks, and medical applications owing to its high gas-barrier, biocompatibility, and non-toxicity [49–53]. Such a copolymer that contains a few ethylene units is susceptible to degradation and can undergo complete biodegradation, notably in the presence of enzymes [54–57]. The presence of ethylene units in PEVAL considerably reduces the hydrophilic character of the original poly(vinyl alcohol); therefore, this material exhibits an excellent performance in the biomedical field. Despite its importance, only some works are cited in the literature using this copolymer in the biomedical field, such as that reported by Young et al [58] in the drug delivery domain.

In this present work, PDVL combined with PEVAL was selected as the crystalline–crystalline polymer blend to develop a better understanding of the nature and physico–chemical properties of this distinct type of polymer blends for their potential applications in biomedical application. For this purpose, various PEVAL/PDVL blends containing different PEVAL contents were prepared by the solvent casting method and characterized via DSC, Fourier-transform infra-red (FTIR) spectroscopy, X-ray diffraction (XRD), thermogravimetry (TGA), and scanning electronic microscopy (SEM). An isothermal crystallization kinetics was also investigated in which the Avrami model was used for determining the Avrami parameters, the maximum time and the half-time of the crystallization of pure components and their blend with equal compositions. The cell adhesion and cell proliferation on the newly prepared polymeric material were examined by an MTT assay. A tissue engineering scaffold was then fabricated from this miscible blend as a raw material and using naphthalene microparticles as a new porogen. The interconnections between the micropores inside the polymeric matrix were realized under vacuum at temperatures slightly less than the glass transition temperature. The resultant micropores and their interconnections and uniformity were studied using SEM.

Materials and methods

Chemicals

Both PEVAL, containing 32 mol% ethylene (purity 99.3%), with molecular weight $M_W = 20,000$ g.mol$^{-1}$ and DVL (purity 98%) were provided by Sigma Aldrich. N,N-dimethylformamide (DMF) (purity 97%), phosphate buffer saline (PBS), isopropanol (purity 99.5%), and naphthalene (purity 99%) were purchased from PANREAC and Mothball. LoVo cells, Dulbecco’s modified Eagle’s medium (DMEM), and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. All the chemicals were used without further purification.

Polymerization of δ-valerolactone

10 mL (0.11 mol) of DVL was polymerized in a bottom flask through a ring opening route in the presence of 0.5 mL of hydrochloric acid at 40°C in nitrogen gas according to the reaction in the Scheme 1. A highly viscous solution was obtained at the end of the reaction, indicating the formation of PDVL. The reaction was then quenched by pouring this solution into hexane, after which white beets of PDVL were obtained. The polymer so obtained was dissolved in tetrahydrofuran and then precipitated in hexane. The beets of PDVL were then kept in a vacuum oven maintained at 50 °C for drying until a constant mass was attained. The molecular weight of the synthesized PDVL was measured in THF at 30°C by size exclusion chromatography (SEC) on a Varian apparatus equipped with a JASCO type 880-PU HPLC pump, refractive index, UV detectors and TSK Gel columns calibrated with polystyrene standards and the results obtained was $1.7 \times 10^4$ g.mol$^{-1}$.

Preparation of PEVAL/PDVL blend

In a 100-mL flask, a known amount of PEVAL was completely dissolved in 50 mL of DMF at 80 °C.

under continuous stirring to prepare polymeric solution-A. In another 100 mL-flask, a known amount of PDVL was completely dissolved in 5 mL of DMF with stirring until complete dissolution, forming solution-B. The two resulting binary systems were mixed together to form a ternary solution composed of two polymers and a solvent. The mixture was cast on a Teflon plate and allowed to dry at 25°C for 24 h and then under vacuum at 40°C for 48 h. Various PEVAL/PDVL blend systems (PEVAV) containing 10, 25, 50, 75, and 90 wt% of PEVAL were prepared by the same method, and the preparation conditions are listed in Table 1.

**Preparation of PEVAV with interconnected pores**

A known amount of naphthalene microparticles (naph) sized between 80 and 120 μm and used as the porogen was cooled at −10°C and then immersed in a highly viscous solution containing 10 wt% of PEVAV in DMF. The resulting suspension was then homogenized at the same temperature by sonication for 20 min using a Degas heating ultrasonic bath and then immediately dried under vacuum at −10°C for 24 h. Pure PEVAL, pure PDVL, and PEVAV films were then prepared by smoothly casting the polymeric solutions over a perfect horizontal Teflon-plate surface obtained using a spirit level. Note that the low temperature used in this procedure was necessary to minimize the dissolution of the naphthalene particles in DMF. The extraction of the porogen from the polymeric material was realized after solvent evaporation by sublimation under a reduced pressure using a vacuum oven maintained at 25°C. The connection between the micropores produced inside the polymeric material were formed under a reduced pressure using a vacuum oven maintained at a temperature slightly lower than the glass transition temperature (Scheme 2).

**Characterization**

**FTIR analysis**

The FTIR spectra of the PEVAV blends and their components were obtained at 25°C using a Perkin Elmer 1000 spectrophotometer. In all the cases, at least 32 scans, with an accuracy of 2 cm⁻¹, were signal-averaged. The film specimens were sufficiently thin and transparent to obey the Beer–Lambert law. For qualitative and quantitative studies, the spectra were treated by the Grams 386 program.

**XRD analysis**

The crystalline structures of the pure polymers and their blends were examined by XRD analysis using an X-ray diffractometer (Rigaku Dmax 2000). The samples were directly used as films and analyzed using a Cu anode tube, tube voltage of 40KV/40 mA, and generator current of 100 mA. The specimens were then scanned in the 2θ range of 5–60° at a scanning rate of 1.0 °.min⁻¹.

**TGA analysis**

The TGA thermograms of the pure polymers and their blends were recorded under nitrogen gas on a TGA/DSC1 Mettler–Toledo thermogravimeter. Samples weighing between 10.0 and 13.0 mg were scanned between 25 and 600°C at a heating rate of 10°C.min⁻¹.

**SEM analysis**

The surfaces and the cross-section morphologies of the PEVAV blends and their components were examined by a JEOL JSM 6360 scanning electronic microscope using an acceleration voltage of 15 kV. The specimens were carefully coated with a thin layer of gold to reduce any buildup deposited on the film surfaces. The coating was realized using a JEOL JFC-1600 Auto Fine Coater operated at 20 mA for 80 s prior to the SEM analysis.

**DSC analysis**

The DSC thermograms of PEVAV blends and their components were obtained using Shimadzu DSC 60A, previously calibrated with indium. Specimens weighing between 8 and 10 mg were packed in aluminum DSC pans before placing in the DSC cell. The samples were scanned from −100 to +250°C under nitrogen gas atmosphere at a heating rate of 20°C.min⁻¹ and then

<table>
<thead>
<tr>
<th>Table 1. Preparation conditions of PEVAL/PDVL blends.</th>
</tr>
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<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>PEVAL</td>
</tr>
<tr>
<td>PEVAV90</td>
</tr>
<tr>
<td>PEVAV75</td>
</tr>
<tr>
<td>PEVAV50</td>
</tr>
<tr>
<td>PEVAV25</td>
</tr>
<tr>
<td>PEVAV10</td>
</tr>
<tr>
<td>PDVL</td>
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</tbody>
</table>
maintained at 200°C for approximately 5 min to destroy the nuclei that might act as the crystal seeds. The specimens were then cooled down to −30°C at the same constant rate. The obtained thermograms revealed that pure PEVAL, pure PDVL, and their blends did not undergo degradation. Glass transition temperature $T_g$ was derived accurately from the thermograms as the midpoint in the heat capacity variation with temperature. Melting point $T_m$ and crystallization temperature $T_c$ were taken exactly at the summits of their peaks. The isothermal crystallization of the samples was also characterized in the temperature range from −100 to +250°C under a nitrogen flow. The melting behavior of isothermally crystallized samples was recorded by heating these samples from their crystallization temperature up to 180°C at a rate of 20°C/min. The isothermal crystallization of PEVAV and their components were heated above the onset melting temperature and kept there for 5 min to erase the thermal history, then quickly cooled at a rate of 100°C/min to the isothermal target temperature then monitoring the resulting crystallization of the polymer as a function of time.

**Cell adhesion and cell growth tests**

The cell adhesion and cell proliferation tests of the PEVAL, PDVL, and PEVAL/PDVL specimens were performed using an MTT assay, as conducted previously by Semlali et al. [59, 60]. Briefly, the tests were performed on 12-well plates, of which one well was treated by collagen (5 mg.mL$^{-1}$) as a positive control of adhesion. Using a sterile technique, each material disk (15-mm diameter) was placed at the bottom of a well and exposed to $10 \times 10^3$ LoVo cells in 1.0 mL of 10% FBS-supplemented DMEM medium, and immediately incubated in a 5%-CO$_2$ humid atmosphere at 37°C for 24 h for the adhesion test and 24 h and 48 h for the cell growth test. After incubation, the material disks populated with LoVo cells were cultured in the presence of 1.0% (v/v) MTT solution (5 mg.mL$^{-1}$) for 24 h and 48 h before stopping the reaction. At the end of incubation, the test materials were washed twice with sterile

**Scheme 2. Fabrication of pores interconnected in PEVAL/PDVL material.**
PBS to remove the non-adhering cells, and then 500 µL of 0.04-N isopropanol solution was added to each well. The dissolved material was transferred from each well to a new 96-well flat-bottom plate and measured spectrophotometrically at 550 nm using an ELISA reader (Model 680, BioRad Laboratories, Mississauga, ON, Canada). The percentage of adhesion was calculated by Eq. (1)

\[ \text{Cell Adhesion (\%) = } \frac{\text{DO}_s - \text{DO}_{nc}}{\text{DO}_{pe} - \text{DO}_{nc}} \times 100 \]  

where \( \text{DO}_s, \text{DO}_{pe}, \text{and DO}_{nc} \) are the optical densities of the test sample, positive control, and negative control, respectively.

**Results and discussions**

**Miscibility**

**Preliminary test**

The preliminary tests of the miscibility of the solutions of PEVAL/PDVL blends in DMF indicated through the presence of single stable phases that blend was miscible at all the compositions. This observation was also confirmed by the transparency of the films obtained after solvent evaporation.

**FTIR analysis**

The FTIR analysis was performed in this investigation to prove the miscibility and highlight the type of interactions responsible to miscibilize this system. According to different authors [61–63], the specific interactions highlighted by this method were reflected by the broadening and/or shifting of the carbonyl band towards the lower wavelength numbers. Indeed, according to the literature [64–66], the absorption band observed in Figure 1 at 1730 cm\(^{-1}\) in the spectrum of pure PDVL was assigned to the free carbonyl stretching of the ester unit. However, the same absorption shifted towards lower frequencies from 1730 to 1721 cm\(^{-1}\) when the PEVAL incorporated in the blend varied from 100 to 10 wt% and, as shown in same figure, the half-width of this band decreased. On the other hand, the absorption band attributed to the hydroxyl group of PEVAL shifted from 3290 to 3297 cm\(^{-1}\) when the PDVL content in the blend varied from zero to 90 wt%. This fact confirmed that the miscibility of PEVAV system is principally due to the interaction forces created by the hydrogen bonds between the carbonyl of the ester group of PDVL and the alcohol group of PEVAL.

**XRD analysis**

The XRD spectra of the PEVAV blend with different compositions and the pure components are shown in Figure 2. The XRD pattern of pure PDVL reveals multiple absorption signals, in which the principals are centered at \( \theta = 22.2, 24.3, 30.5 \) and 41.3°, characterizing the semi-crystalline structure of this polymer, in perfect agreement with the literature [67]. However, the XRD pattern of pure PEVAL shows a single intense broad signal centered at \( \theta = 21.5° \) and another less intense at 23.7, indicating the semi-crystalline structure of this polymer [68, 69]. Practically the same absorption signals of both the polymers were observed in the spectra of the PEVAV blends, thereby, indicating the presence of two crystalline regions, one rich in PEVAL and the other in PDVL. Based on the DSC results and XRD analysis, it can be concluded that the components of this system crystallize separately in the blend and that the intensity of the diffraction signals of each polymer decrease with the increase in the other polymer component.

**TGA analysis**

In general, polymers are useful in a certain temperature range; they are brittle below the low temperature limit, whereas they soften, melt, degrade, and ultimately decompose above the high temperature limit. The TGA thermograms of the pure PEVAL polymer, pure PDVL polymer, and their blends in nitrogen gas atmosphere were scanned at a heating rate of 20°Cmin\(^{-1}\), and the thermograms thus obtained are collectively depicted in Figure 3. As can be seen from the thermogram of pure PEVAL, an onset of a minor weight loss is localized at 220°C, which is attributed to the elimination of the water embedded inside the polymer matrix. The first stage of the decomposition process occurring between 330 and 410°C involves a major degradation leading to the elimination of water and volatile products such as methanol, ethanol, ethylene, and propylene, resulting from the breakdown of the PEVAL backbone. [70] The final stage of the degradation process starting at 410°C is characterized by a relatively low weight loss owing to the char formation. The curve profile of the PDVL thermogram reveals practically an ultimate step in which the thermal degradation starting at 220°C involves a statistical rupture of the polyester chains, producing volatile molecules such as water, carbon dioxide, and 4-pentanoic acid [71]. In contrast, as can be observed from the curve profiles of the blend, the increase in the PEVAL content in the PEVAV blend leads to a drastic increase in the thermal stability of PDVL, except the blend containing 10 wt% PEVAL for which the degradation temperature of PDVL remains practically unchanged. As an example, the starting decomposition temperature of PDVL shifts from 220 to 265°C when 25 wt% PEVAL is incorporated in the blend. However, blending PEVAL with PDVL also
leads to a significant increase in its thermal stability. This is exhibited by the shift of the starting degradation temperature of PEVAL from 330°C to 370°C when the blend contains only 10 wt% PDVL.

DSC analysis

Glass transition temperature. The DSC thermograms obtained for pure PEVAL, pure PDVL, and their blends, presented in Figure 4, reveal only a single glass transition temperature, \( T_g \), between those characterizing the pure components, indicating the total miscibility of this pair of polymers in the amorphous portions for all the compositions. According to Qui et al. [35], the appearance of a single \( T_g \) for a blend indicates full miscibility in 20–40 nm scale.

The variation in the experimental \( T_g \) versus the composition plotted in Figure 5 reveals no particularly distinct interaction between the two polymers. Different approaches based on the experimental \( T_g \)s of the pure components have been suggested to predict the profile of the \( T_g \) change of a miscible blend versus the composition, among which those of Fox (Eq. (2)) [72] and Gordon and Taylor (Eq. (3)) [73] are selected in this work to study the glass transition behavior of this pair of polymers.

\[
\frac{1}{T_{g, blend}} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}} \tag{2}
\]

\[
T_{g, Blend} = \frac{w_1T_{g1} + k(1 - w_1)T_{g2}}{w_1 + k(1 - w_1)} \tag{3}
\]
where \( w_i \) and \( T_{gi} \) are the weight fraction and glass transition temperature of polymer \((i)\). \( k \) is an adjustable fitting parameter in the Gordon–Taylor equation, and \( \Delta \alpha_i \) is the change in the expansion coefficient at \( T_{gi} \). As can be seen from the data presented in Table 2 and plotted in Figure 5, the \( T_g \) values of the blends fit reasonably well with those calculated using both the Fox and Gordon–Taylor equations at \( k = 0.6 \) and slightly deviated from those calculated from the arithmetic mean, \( T_g^{\text{calc}} \). As such the behavior indicates the miscibility of the components in all the PEVAL/PDVL blends.

\[
k = \frac{\Delta \alpha_1}{\Delta \alpha_2}
\]  

\( \text{Equation 4} \)

**Melting temperature.** Figure 6 shows the DSC thermograms of the melting curves of pure PEVAL, pure PDVL, and their blends at different compositions. These curve profiles show two distinct exothermal peaks, indicating the presence of two melting points attributed to the polymer components. Such double melting temperatures are frequently observed in binary miscible blends involving a microbial polyester, such as poly(3-hydroxybutyrate) (PHB) prepared by the solvent casting method. [74, 75] The melting dynamics of a crystalline component in semicrystalline polymer blends is an inevitable tool to highlight the miscibility of the pair of polymers. In case of a miscible system, the thermodynamically favorable interactions result from the usual decrease in the melting
Figure 3. TGA thermograms of neat PEVAL, neat PDVL and their blend with different compositions.

Figure 4. DSC thermograms of neat PEVAL, neat PDVL and their blends with different compositions performed at a heating rate of 20°C.min⁻¹.
Figure 5. Dependence of $T_g$ on composition in binary PEVAL/PDVL blends.

Table 2. Variation of the experimental and Golden-Taylor glass transition temperature of blend versus the composition.

<table>
<thead>
<tr>
<th>System</th>
<th>PEVAL/PDVL Composition (wt%)</th>
<th>$T_g^{Exp}$ (°C)</th>
<th>$T_g^{Calc}$ (°C)</th>
<th>$T_g^{Fox}$ (°C)</th>
<th>$T_g^{G-T}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEVAL</td>
<td>100:0</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PEVAL/PDVL90</td>
<td>90:10</td>
<td>37</td>
<td>39.70</td>
<td>34.26</td>
<td>38</td>
</tr>
<tr>
<td>PEVAL/PDVL75</td>
<td>75:25</td>
<td>20</td>
<td>24.25</td>
<td>16.32</td>
<td>21</td>
</tr>
<tr>
<td>PEVAL/PDVL50</td>
<td>50:50</td>
<td>-8</td>
<td>-1.50</td>
<td>-11.40</td>
<td>-6</td>
</tr>
<tr>
<td>PEVAL/PDVL25</td>
<td>25:75</td>
<td>-25</td>
<td>-27.25</td>
<td>-34.00</td>
<td>-31</td>
</tr>
<tr>
<td>PEVAL/PDVL10</td>
<td>10:90</td>
<td>-42</td>
<td>-42.70</td>
<td>-45.77</td>
<td>-44</td>
</tr>
<tr>
<td>PDVL</td>
<td>0:100</td>
<td>-53</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 6. Melting behavior of PDVL and PEVAL components in the PDVL/PEVAL blends versus the mass fraction of PEVAL.
temperature of the crystalline polymer with respect to the pure component. According to the Flory–Huggins theory [76, 77], in case of a miscible blend, the extent of the melting temperature decrease allows to measure the interaction energy. However, the melting point of a polymer is typically affected by thermodynamic factors and morphological parameters, particularly the crystal thickness. To dissociate the thermodynamic effects acting on the melting temperature decrease from the morphological parameters, we should use the equilibrium melting point data. To achieve this condition, the equilibrium melting point, $T_m^\circ$, of a polymer was determined by the Hoffman–Weeks relation (Eq. (4)). [78] This method is based on the isothermal crystallization of the polymer at different crystallization temperatures, $T_c$, as presented in Figure 7 for the pure PEVAL, pure PDVL and their blend containing equal contents, and plot of the variation in the observed melting point, $T_m$, vs $T_c$.

$$T_m = \eta T_c + (1 - \eta) T_m^\circ \tag{5}$$

$T_m^\circ$ was deduced from the intersection of the straight line obtained with the $T_m = T_c$ equation and $\eta$ from the slope of the Hoffman–Weeks plot in Figure 8. $\eta$ takes a value ranged between 0 and 1 and can be considered as a parameter used to estimate the stability of the crystals undergoing melting. [78] $\eta = 0$ implies that the crystals are perfectly stable, so that $T_m = T_m^\circ$ at any $T_c$. In contrast, $\eta = 1$ reflects inherently unstable crystals. Table 3 summarizes the $\eta$ and $T_m^\circ$ for PEVAL and PEVAL blend with different compositions. The value of the equilibrium melting point of pure PEVAL obtained is 186°C which agrees with that reported in the literature (187°C). [68] For the PEVAL blend systems, the equilibrium melting point of the PEVAL phase dramatically decreased with PDVL content. The maximum extent of this melting point depression is 24°C in the PEVALV10, where the PEVAL has a $T_m^\circ$ of 162°C. In general, the small values of the Hoffman-Weeks slope of PEVAL in its blend is relatively small indicating the crystals stability of PEVAL in the blend. This characteristic decreased slowly with increasing the PDVL content in the blend. This suggests that the PEVAL crystals become relatively less stable, i.e., have smaller lamellar thicknesses, when blended with PDVL. This phenomenon is probably due to the morphological effect.
Comparable results were also reported by Lee et al [79] by blending poly(butylene succinate) with poly(vinylidene fluoride).

**Flory interaction parameter.** To determine the Flory interaction parameter, \( \chi_{1,2} \), and density of interaction energy, \( B \), characterizing the pair of polymers the Nishi–Wang equation [70] based on the Flory–Huggins theory was used. According to Qui et al [35] for crystalline polymer (1)/crystalline polymer (2) blends, the crystallization temperature used to obtain the equilibrium melting point for polymer (1) must be higher than the melting point of polymer (2), i.e., polymer (1) acts as a crystalline polymer, whereas polymer (2) acts as an amorphous polymer. For high-molecular weight polymers, the Nishi–Wang equation can be written as follows:

\[
\chi_{1,2} = \frac{\Delta H^o V_1}{R V_2} \left( \frac{1}{T_m^{o(\text{blend})}} - \frac{1}{T_m^{o(\text{pure})}} \right) \tag{6}
\]

or

\[
\frac{1}{T_m^{o(\text{blend})}} = \frac{R V_2 \chi_{1,2}}{\Delta H^o V_1 \varphi_2^1} + \frac{1}{T_m^{o(\text{pure})}} \tag{7}
\]

where \( T_m^{o(\text{pure})} \) is the equilibrium melting temperature of the pure crystallizable component having the higher melting point, \( T_m^{o(\text{blend})} \) is the equilibrium melting point of the blend, \( \Delta H^o \) is the enthalpy of fusion of the perfectly crystallizable polymer per mole of its repeat unit, \( V_1 \) is the molar volume of the repeating units of polymer (1) and \( \varphi_2^1 \) is the volume fraction of polymer (1) in the blend. Using the data collected from the literature, \( \Delta H_{u\text{PEVAL}} = 4.22 \text{ kJ.mol}^{-1}, \ V_{u\text{PEVAL}} = 37.80 \text{ cm}^3\text{.mol}^{-1} \) [79], and \( V_{u\text{PDVL}} = 85.98 \text{ cm}^3\text{.mol}^{-1} \) were calculated from the density of PDVL and molecular weight of a DVL unit. \( \chi_{1,2} \) and \( B \) values were deduced from the slope of the linear fit, indicating the variation in \( 1/T_m^{o(\text{blend})} \) versus \( \varphi_2^1 \) displayed in Figure 9. The \( \chi_{1,2} \) value deducted from the slope of the linear fit is estimated as \(-0.15\) determined with a \( R^2 = 0.994 \). and \( B \) value which is equal to \( \chi_{1,2} \) RTV \( 1^{-1} \) [80] was estimated at \(-15.14 \text{ J/cm}^3 \) of PDCL taken at 186°C. This value indicates the presence of strong interactions between PEVAL and PDVL components and confirms that the PEVAL/PDVL blend is thermodynamically miscible in its amorphous part and this pair of polymer can form a

**Figure 8.** Hoffman–Weeks plots for pure PEVAL and PEVAL/PDVL blend with different compositions.

**Table 3.** Hoffman Weeks data obtained for PEVAL/PDVL blend with different compositions.

<table>
<thead>
<tr>
<th>PEVAL/PDVL</th>
<th>( T_m^{o}(^\circ\text{C}) )</th>
<th>( \eta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>186</td>
<td>0.24</td>
</tr>
<tr>
<td>90:10</td>
<td>173</td>
<td>0.20</td>
</tr>
<tr>
<td>75:25</td>
<td>170</td>
<td>0.17</td>
</tr>
<tr>
<td>50:50</td>
<td>166</td>
<td>0.14</td>
</tr>
<tr>
<td>25:75</td>
<td>163</td>
<td>0.13</td>
</tr>
<tr>
<td>10:90</td>
<td>162</td>
<td>0.12</td>
</tr>
</tbody>
</table>
compatible blend system in the melt state, i.e., above the melting point of PEVAL, which has the higher $T_m$. 

**Crystallinity.** As is well-known in the polymer blend domain, the crystallization behavior of each component in a blend depends on its miscibility behavior, physico-chemical properties, and crystallization conditions. For example, according to Hu et al [81], the crystallization of one component in a blend affects the morphology, crystallization, and mechanical properties of the second component. Clearly, the DSC thermograms of pure PEVAL, pure PDVL, and their blends obtained during the non-isothermal crystallization process in their curve profiles presented in Figure 10 exhibit two endothermic peaks attributed to the crystallization of PEVAL and PDVL in the blends. A similar behavior was also observed in some miscible blends of biodegradable aliphatic polyesters, such as poly(butylene succinate), poly (hydroxybutylate), or poly(L-lactic acid) prepared by the casting method [82–84]. The variation in the normalized crystallinity of the blends with the PEVAL component reveals a shift toward low temperatures, accompanied by a drastic decrease in the crystallinity peak of PEVAL with increasing PDVL content. The presence of PEVAL with PDVL in the blend severely reduces their respective crystallinities.

**Isothermal crystallization kinetics.** The isothermal crystallization of pure PEVAL, pure PDVL and their PEAV blend containing equal compositions from the amorphous state was also investigated in their crystallization temperature ranges. The samples were first quenched to $-100^\circ$C to reach the amorphous state from the melt, and then heated to the crystallization temperature rapidly as described in Section 2.5.1. The exothermal crystallization patterns of the heat flow versus time are presented in Figure 7 where the maximum crystallization times, $t_{\text{max}}$ were deducted and summarized in Table 4.

The DSC analysis in its heating mode of pure PEVAL, pure PDVL and their PEVAV50 blend after isothermal crystallization runs are shown in Figure 11. As can be seen from these thermal patterns that the melting temperature for pure components slightly increased as the isothermal crystallization temperature increased. This tendency is practically observed for all polymer materials which is caused by the increase of the chain mobility when the crystallization temperature increases, allowing the formation of larger crystals and leading to higher melting temperatures. Different morphologies can be resulted by changing the crystallization conditions by varying the undercooling level. A higher number of nuclei could be obtained at lower crystallization temperatures, leading to the formation of a larger number of smaller crystals. Concerning the thermogram of the PEVAV50 blend, this tendency remains valid only in case of PEVAL, while, the PDVL in the blend preserve practically the same melting temperature at any isothermal crystallization.

*Figure 9.* Variation of the inverse of the melting temperature versus the square of the PEVAL volume fraction.
The estimation of isothermal crystallization parameters obtained from the crystallization exotherms is obtained by integration the enthalpy evolved during this process, a relation between relative crystallinity, \(\alpha(t)\), and time may be expressed by Eq. (8)

\[
\alpha(t) = \int_{t_0}^{t} \frac{dH_c}{\Delta H_c} \, dt
\]  

(8)

Here, \(t\) is the elapsed time and \(dH_c\) is the released enthalpy during crystallization for small time interval \(dt\). \(\Delta H_c\) is the total enthalpy of crystallization for a desired crystallization temperature which is given by Eq.(9)

\[
\Delta H_c = \int_{t_0}^{\infty} \frac{dH_c}{dt} \, dt
\]  

(9)

After substituting areas of DSC curves, Eq. (8) becomes

\[
\alpha(t) = \frac{A_t}{A_\infty}
\]  

(10)

where, \(A_t\) is the area under the DSC curves from \(t = t_0\) to \(t = t\) and \(A_\infty\) is the total area under the crystallization curve. Based on this equation, \(\alpha\) at a specific time is calculated. Integration of the exothermic peaks during the isothermal scans gives relative degree of crystallinity as a function of time for PEVAL, PDVL and PEVALV blends at different temperatures as

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**Figure 10.** DSC thermograms of neat PEVAL, neat PDVL and their blends recorded during the cooling process with a cooling rates of 20°C/min.

**Table 4.** Peak time of the crystallization exotherms (\(t_{max}\)) and the Avrami Parameters for Isothermal crystallization of pure PEVAL, pure PDVL, and in PEVALV50 blend.

<table>
<thead>
<tr>
<th>System</th>
<th>(T_c) (°C)</th>
<th>(t_{max}) (s)</th>
<th>(n)</th>
<th>(-\ln k (s^{-1}))</th>
<th>(t_{1/2}) (s)</th>
<th>(1/t(s)10^{-2})</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEVAL</td>
<td>150</td>
<td>18</td>
<td>2.00</td>
<td>6.43</td>
<td>21.12</td>
<td>4.76</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td>33</td>
<td>2.41</td>
<td>8.96</td>
<td>35.03</td>
<td>2.85</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>155</td>
<td>55</td>
<td>2.53</td>
<td>10.15</td>
<td>48.10</td>
<td>2.08</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>157</td>
<td>54</td>
<td>2.72</td>
<td>11.09</td>
<td>51.88</td>
<td>1.93</td>
<td>0.993</td>
</tr>
<tr>
<td>PDVL</td>
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<td>22</td>
<td>2.67</td>
<td>9.21</td>
<td>26.45</td>
<td>3.78</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>24</td>
<td>2.73</td>
<td>9.54</td>
<td>27.61</td>
<td>3.62</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>26</td>
<td>2.76</td>
<td>9.81</td>
<td>29.15</td>
<td>3.43</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>36</td>
<td>2.80</td>
<td>11.01</td>
<td>41.09</td>
<td>2.43</td>
<td>0.994</td>
</tr>
<tr>
<td>PEVAV50</td>
<td>125</td>
<td>123</td>
<td>3.00</td>
<td>11.84</td>
<td>46.56</td>
<td>2.15</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>127</td>
<td>120</td>
<td>3.20</td>
<td>12.17</td>
<td>38.67</td>
<td>2.59</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>150</td>
<td>2.75</td>
<td>9.80</td>
<td>37.84</td>
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<tr>
<td></td>
<td>135</td>
<td>120</td>
<td>2.63</td>
<td>10.00</td>
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<td>3.59</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>17</td>
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<td>2.75</td>
<td>12.41</td>
<td>84.37</td>
<td>1.19</td>
<td>0.995</td>
</tr>
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<td>67</td>
<td>2.50</td>
<td>11.16</td>
<td>76.68</td>
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<td>1.95</td>
<td>8.70</td>
<td>70.88</td>
<td>1.41</td>
<td>0.997</td>
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plotted in Figure 12. Because of the effect of retardation on crystallization, all curves have approximately sigmoid patterns and their slopes at each point correspond to the rate of crystallization. As can be seen from these sigmoid profiles the rate of crystallization keeps almost constant between 20 – 80% of the relative crystallinity. At the later stage, the curves tend to become flat due to the spherulite impingement. [85] The corresponding crystallization half times, $t_{1/2}$ were estimated directly from Figure 12.

The isothermal crystallization kinetics of PEVAV50 and their components were investigated using the linearized Avrami equation [86]

$$\ln[-\ln(1-\alpha(t))] = \ln(k) + n\ln(t)$$

(11)

where, $\alpha(t)$ is the mass fraction of polymer transformed crystallized at time $t$. The Avrami exponent, $n$, reflects the nucleation mechanism and growth dimension of the crystals. $k$ is an overall crystallization rate constant including the crystal growth and nucleation contributions. The Plots of $\ln[-\ln(1-\alpha(t))]$ versus $\ln(t)$ for pure PEVAL, pure PDVL and PEVAV50 system are presented in Figure 13. As can be seen, all specimens show straight lines thus confirming that the Avrami equation described perfectly the primary process of isothermal crystallization of these materials. $n$ and $k$ values determined from the

---

**Figure 11.** The DSC thermograms of pure PEVAL, pure PDVL and their PEVAV50 blend taken after isothermal crystallization runs at different temperatures.
slopes and the intercepts of these plots, respectively are gathered with $t_{1/2}$ and $t_{\text{max}}$ in Table 4.

According to typical polymer crystallization reports, the $n$ value close to 2 means that the crystallization process involves a two dimensional progression axial or lamellar with an instantaneous nucleation. [87] A $n$ value close to 3 indicates three dimensional spherulitic growth from instantaneous nuclei. Heterogeneous nucleation has been suggested for fractional $n$ values in polymer crystallization. [88] In this investigation the $n$ values of PEVAL at the same range of temperatures agree with those obtained by Alvarez et al [79] (1.93–2.36) indicating that the crystallization process involves a two dimensional crystal growth [89] and the crystal nucleating athermally [90]. The $n$ value of pure PEVAL increased rapidly and regularly from 2 to 2.72 when the temperature varied from 150 to 157°C, while, that of the pure PDVL slowly increased from 2.67 to 2.80 as the temperature varied from 20 to 27 °C indicating either thermal nucleation with two dimensional crystal growth or athermal nucleation with three dimensional spherulitic growth from instantaneous nuclei. On the other hand, a decrease in the $n$ values with temperature are observed for both PEVAL and PDVL in the PEVAV50 which practically involves a same crystallization behavior with a small modification for the PEVAL in the blend at 125 and 127°C, in which the crystallization process is purely three dimensional spherulitic growth from instantaneous nuclei. The decrease in the $n$ value of each component in the blend with the temperature reflects the effect of the miscibility of this pair of polymer. For all samples, the values of $k$ were very low and decreased continually with increasing crystallization temperature mirroring the slower cold crystallization process, except that of PEVAL in the blend in which this parameter randomly varied. The slowdown of the overall crystallization rate as the crystallization temperature increased, observed for the PEVAV50 blend, can be attributed to the fact that nucleation becomes more difficult at high temperature.

**Scaffold characterization**

**Assessment of cell adhesion and growth**

The results of the cell adhesion and growth screening tests performed with different PEVAL/PDVL ratios are

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**Figure 12.** Variation of the relative crystallinity of pure PEVAL, pure PDVL and PEVAV50 blend with time for various cold crystallization temperatures.
shown in Figure 14. These data revealed that the LoVo cell adherence after 24 h of culture was more visible on PEVAL50 that contains an equal amount of PEVAL and PDVL compared with pure PEVAL and pure PDVL (Figure 14(a)). Moreover, the specific growth during both the 24 and 48-h culture periods reflected the higher growth rate on PEVAL50 compared with the other tested specimens (Figure 14(b)). The increase in the adhesion and growth levels on PEVAL50 specimen may be a result of the porosity increase on the polymer blend surface and increase in its wettability owing to the increase in the hydrogen bonds between the water molecules and hydroxyl groups of the vinlyc alcohol units of PEVAL and between these hydroxyl groups and the cells. According to the investigation reported by Abdelwafa et al [91], the formation of functional groups such as hydroxyl or carboxyl groups on a scaffold promotes cell proliferation in a protein-mediated cell adhesion mechanism. This is a supplementary point showing that the PEVAV50 blend can be a potential candidate for use in scaffold fabrication for better adhesion and cell growth.

SEM analysis

The SEM micrographs in Figure 15 show the surface morphology of the naphthalene microparticles and the PEVAV50 specimens obtained during different steps of the scaffold fabrication. As can be observed from image-A, the naphthalene microparticles have hexagon forms that are mostly of uniform size between 90 and 120 μm. Image-B of the PEVAV50 specimen captured before the incorporation of the porogen microparticles showed a smooth or slightly rough, dense, and uniform surface morphology; thus, confirming the miscibility of this pair of polymers. Note that an immiscible blend was usually characterized by a specked surface morphology containing two or more distinct zones. However, the surface obtained after the second step of the scaffold fabrication (Image-C), when PEVAV50 was incorporated with 60% naphthalene microparticles, showed the naphthalene microparticles well covered by the polymer.

Micrograph-D, attributed to the surface morphology of the same specimen obtained after removing the porogen, exhibited the interconnections between the
Micocavities inside the material occurred under the action of reduced pressure. Clearly, the wall separating the different microcavities inside the polymer material disappeared under a high pressure difference between inside and outside the microcavities. These microcavities with diameters between 60 and 120 μm, produced under vacuum at −10°C (temperature slightly less than the $T_g$ of this blend), reflected more or less the dimensions of the porogen. At this temperature, the material becomes slightly soft and the walls separating the different microcavities easily disappeared without disturbing their geometry.

**Conclusion**

In conclusion, the intended objective of this investigation was attained, which was the preparation of a new material by blending two vital polymers, namely, PDVL and PEVAL to enhance some of their properties to use the blend in tissue engineering in the biomedical domain. The miscibility of this pair of polymers was preliminarily proved by the observation of a single phase in each blend and transparent films after solvent evaporation. This finding was also confirmed by the DSC analysis through the presence of a single $T_g$ for the blend compositions and by the Fox and Gordon–Taylor equations, in which the experimental $T_g$s slightly deviated from the ideal calculated $T_g$s. The miscibility of this pair of polymers was attributed to the interaction parameter that decreased by −0.27 from the Nishi–Wang equation. According the results obtained by the FTIR analysis, these interactions were owing to the hydrogen bonds developed between the hydroxyl groups of PEVAL and carbonyl groups of PDVL. For

**Figure 14.** Cell adhesion and growth screening tests on neat PEVAL, neat PDVL and their blend with different compositions. (A) Cell adhesion, (B) Cell growth. The Lovo cells adherence was examined after 24 h and 48 h of culture.
the isothermal crystallization, the rate constant and the crystallization rate for the virgin components decreased with increasing crystallization temperature, while, those of the both components incorporated in the PEVALV50 blend varied differently. Indeed, the rate constant of the PEVAL randomly varied and the crystallization rate increased. Concerning the PDVL in the blend, the rate constants have practically comparable values to those of the pure components but decreased as the crystallization temperature increased. On the other hand, at certain temperatures, the crystallization rate dramatically fall 2 to 4 times compared with those of pure components reflecting the effect of the miscibility on the crystallization behavior. In general, the Avrami exponent $n$ varied between 2 and 3 indicating a disc-like morphology formed by heterogeneous nucleation. The Avrami exponent ranged between 2 and 3 at these isothermal crystallization temperatures indicates a changing trend of the both components in the blend crystal growth from two- to three-dimensional with instantaneous nucleation and athermal. In general, the thermal stability of this new material analyzed by the TGA method revealed a drastic increase in the thermal stability of PDVL with increasing PEVAL content in the blend. In contrast, it was also observed that the addition of only 10 wt% of PDVL to the blend increased the decomposition temperature from 330 to 370°C. The cell adhesion and specific growth during the 24 and 48-h culture period reflected a higher growth rate on PEVAV50 compared with the other tested specimens. The use of naphthalene microparticles for the first time as the porogen to achieve tissue engineering was a success. Uniform and interconnected microcavities were easily obtained after removing the porogen from the prepared material by sublimation under vacuum at a temperature slightly less than the $T_g$. This was an important supplementary point supporting that the PEVAV50 blend could be a potential candidate to be used in scaffold fabrication for better adhesion and cell growth.

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References


[12] Li, Q.; Mai, Y.-W. Biomaterials for Implants and Scaffolds, American Chemical Society; Springer, 2017.


[26] Liu, L.-Z.; Chu, B.; Penning, J.; Manley, R. S. J. A Synchrotron SAXS Study of Miscible Blends of Semicrystalline Poly (Vinylidene Fluoride) and Semicrystalline Poly (1, 4-Butylene Adipate).


