Production of Poly(lactic acid)/Organoclay Nanocomposite Scaffolds by Microcompounding and Polymer/Particle Leaching

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Organoclay reinforced poly(lactic acid) (PLA)/poly(vinyl alcohol) (PVA) blend based porous nanocomposite scaffolds are prepared by microcompounding/injection molding and subsequent polymer (PVA)/particle (NaCl) leaching method. The objective of the study is to investigate the effect of clay content on the properties of scaffolds for tissue engineering. It is revealed from X-ray diffraction and TEM studies that incorporation of PVA to PLA matrix as a polymeric porogen enhance the state of dispersion of clay particles resulting in an exfoliated structure. The porosities of all the scaffolds are higher than 70% and the pores are well-interconnected as observed by SEM. The addition of clay to the scaffolds improves the compressive modulus. Differential scanning calorimeter analysis shows that \( T_g \) of neat-PLA is shifted to lower values when it is compounded with PVA. As a result, it is demonstrated that the method applied in this work can be a good candidate for production of polymeric scaffolds for tissue engineering applications. POLYM. COMPOS., 31:674–683, 2010. © 2009 Society of Plastics Engineers

INTRODUCTION

The scarcity and the limitations of autografts and allografts have given rise to the increased interest in developing tissue engineering approaches. Based on the Organ Procurement and Transplantation Network data as of July 2008, an excess of 99,000 patients are suffering in the waiting list to find an organ by transplantation or from a donor [1]. The economical figure behind this fact is estimated about $400 billion/year for the treatment of patients of end-stage organ failure or tissue loss [2]. In tissue engineering, new tissues can be produced by cultivation of cells in vivo or in vitro. According to the most commonly applied method of this emerging engineering field, cells are seeded onto the biomaterials and cultivated in a specific medium to have differentiation and proliferation. These biodegradable materials, also known as scaffolds, provide temporarily three-dimensional supports and a template for the tissue generation [3]. An ideal scaffold should be highly porous with an interconnected, open pore network to provide three-dimensional seeding and subsequent supply of nutrients to those seeded cells. It should have a hydrophilic surface, appropriate shape and size of pores that is critical in seeding and adhesion of cells and growing of new tissue and furthermore it should be strong enough to withstand external stresses before new tissue formation [4].

Numerous processing methods based on fiber technologies, solvent casting in combination with solid particle leaching, temperature induced phase separation, super critical fluid technologies, melt molding, fused deposition modeling and 3D printing have been introduced and applied to produce scaffolds from biodegradable polymeric materials [5]. However, most of them were just developed to demonstrate the feasibility of the tissue regeneration on biodegradable materials. There are still some issues related to scaffold fabrication needed to be solved. For example, in solution based methods, the inclusion of highly toxic organic solvents or nonsolvents are themselves a problem because of difficulty in removing those chemicals from the scaffolds by drying [6]. In addition to that, these processes are highly sensitive to process variables and therefore the precise control of pore size and pore shape becomes an issue. Another drawback of these methods is the difficulty in production of three-dimensional structures, rather than thin porous membranes.

To overcome these problems faced in solution based methods, conventional immiscible polymer blending technique was adapted to produce interconnected, porous,
three-dimensional scaffolds [7, 8]. In this method, a continuous polymer blend is processed in molten state. After processing, one of the phases is leached out by a selective solvent to form the porous structure. One of the advantages of this method is the final pore structure, which differs from the other techniques, consisting of interconnected cylinders that are strong enough to be used in bone and cartilage tissue engineering applications. In addition to that, the melt blending technique by utilizing extrusion and injection molding processes provides an economical and controllable method of producing scaffolds. On the other hand, about 50% of porosity is achievable in this method and this is not enough to be used in tissue engineering without further modification. Recently, Reignier and Huneault have introduced a method of scaffold production by combining melt blending technique and particle/polymer leaching to obtain highly porous interconnected networks [6]. In this method, poly(ε-caprolactone) was melt blended with a water soluble thermoplastic, polyethylene oxide, together with sodium chloride (NaCl) particles as the porogens in a conventional twin-screw extruder. The results of the study showed that porosity levels up to 88% can be achieved by a combination of polymer and NaCl particle leaching method.

Microcompounders enable researchers to work with a small amount of material (i.e. 3 to 15 g) with similar processing history as in a conventional twin-screw extruder. When the prices of the new developed biodegradable polymers are taken into consideration, microcompounders are much more economical in terms of material consumption in comparison with large scale conventional extruders. In combination with a microinjection molding device it is very easy to produce a three-dimensional scaffold with in a very short period of time. Moreover, it is cheaper to build a patient specific mold for a microinjection molding machine when it is compared with conventional injection molding systems. Therefore, microcompounding and microinjection molding devices may serve as a potential processing system in melt processed scaffold technology.

The strength is one of the important properties of the scaffolds. However most of the scaffolds made from biodegradable polymers by applying different techniques mentioned above are not strong enough for hard tissue regeneration [9–14]. During the biodegradation of the scaffolds, the growing tissue invades the resulting structure simultaneously; therefore the scaffold should withstand the in vivo and in vitro stresses during tissue regeneration in these applications [15]. To achieve high strength in polymers, recently nanocomposite technology has offered challenging opportunities to scientists. Incorporation of a small amount of high aspect ratio nanoparticles, i.e. clays or nanotubes, to a polymer matrix result in a high improvement in strength and stiffness of the system without significantly altering the density. Especially, organically modified layered silicate (OMLS) based nanocomposites (NC) have developed extensively since Toyota Researchers first polymerized caprolactam in the presence of clays [16]. However, NC have been produced by melt mixing using conventional devices [17–19]. In general, OMLS based NC are divided into three general types: conventional composites, in which the clay agglomerates dispersed in polymer matrix as in conventional particulate fillers; intercalated NC, in which a small amount of polymer enters into the clay layers; and exfoliated NC, in which the clay layers are fully dispersed in a continuous polymer matrix. The latter structure is important, because a great improvement in mechanical properties can be obtained [20]. The degree of exfoliation is highly dependent on the type of organic modifier to enhance compatibility between clay and polymer matrix to promote diffusion of polymer into clay platelets together with the processing conditions [21–24].

The purpose of this study is to evaluate the applicability of microcompounding/injection molding and subsequent polymer/particle leaching method in nanocomposite biodegradable porous scaffold production. Poly(lactic acid) (PLA), which can be obtained from fermentation of corn and corn based products, is a commercially available biodegradable polyester based thermoplastic. The biodegradable nature of PLA makes it a popular material in biodegradable scaffold production. In the current study, more specifically, PLA based nanocomposite scaffolds (NCS) are produced. NaCl is used as the particulate porogen and poly(vinyl alcohol) (PVA) is used as the immiscible polymeric porogen. OMLSs are used as the nanofillers to reinforce the scaffolds. The effects of clay addition and clay loading level on the morphology, porosity, wettability, mechanical and thermal properties of scaffolds are examined. An investigation of the state of dispersion of clay particles in the presence and absence of PVA is also conducted.

**EXPERIMENTAL**

**Materials**

PLA was provided by Cargill-Dow at a number average molecular weight of 186,000. Montmorillonite (MMT) (Cloisite® 30B, Southern Clay Products) was organically modified with methyl, tallow, bis-2-hydroxyethyl, quaternary ammonium by the supplier company. PVA (Sigma-Aldrich) and NaCl were used as the porogens. NaCl was sieved and the particles at a size range of 150–350 μm were used in scaffold preparation. All the materials were dried for 12 h at 65°C under vacuum before processing.

**Nanocomposite and Nanocomposite Scaffold Preparation**

PLA, PVA, NaCl and clay batches at prearranged compositions were dry-mixed at predefined amounts with a precision of 0.01 g. The PLA/PVA ratio of 50/50 was
kept constant for NC and NCS. The NaCl concentration was also kept constant at 70% by weight for NCS. The clay ratio was varied as 0, 1, and 3% with respect to polymer content in the NC and NCS. The samples were named for simplicity by giving corresponding amounts in the order of PLA/PVA/Clay. For example, 50/50/3 means that there exists 3% clay by weight in the nanocomposite, and the remaining matrix includes 50/50 PLA/PVA.

The mixtures were melt compounded in a co-rotating twin-screw microextruder (15 ml microcompounder, DSM Xplore) donated by a melt recirculation facility. The operating conditions of the microextruder were arranged as screw speed of 100 or 200 rpm and barrel temperature profile of 200–220–220°C (from top to bottom). At the end of mixing period of 3 min under N₂ atmosphere, the extrudate was taken. The melt was directly injection molded using the transfer cylinder of DSM Xplore 10 ml injection molding machine to obtain 80 × 10 × 4 mm³ bars. The injection and holding pressures were set to 10 bars. Melt temperature and mould temperatures were 220°C and 30°C, respectively. The rectangular specimens for further analysis were cut from the injected molded bars to obtain dimensions of 10 × 10 × 4 mm³.

To obtain porous scaffolds, the porogens NaCl and PVA were leached in water at 40°C for at least 72 h till constant weight was reached. The porous scaffolds were dried for 7 days at ambient temperature before characterization.

**Characterization of NC and NCS**

The morphology of NCSs was analyzed by using a low voltage SEM (JEOL JSM-6335F FEG). In order to prevent arcing, sample surfaces were coated with gold.

NCs and NCSs were examined by transmission electron microscope (TEM) (Philips CM200 TEM) at an acceleration voltage of 120 kV. Ultra thin sections of 70 nm in thickness were cryogenically cut with a diamond knife at a temperature of −100°C. All samples were trimmed parallel to the injection molding direction.

X-ray diffraction (XRD) analysis was conducted on scaffolds by using a RIGAKU D/MAX 2200/PC XRD using X-ray source of CuKα (λ = 1.54E) radiation generated at a voltage of 40 kV and current of 40 mA. The diffraction angle 2θ was scanned from 0.5 to 10° at a scanning rate of 0.5/°min. MMT was analyzed in powder form under same conditions.

The inorganic material content before and after leaching was examined by burning test of 1% and 3% Clay-PLA/PVA NC. The samples were burned at 600°C in an electrically heated furnace for 30 min to be sure that no organic residue remained. The materials were weighted before and after burning test in order to calculate the weight loss as a result of burned organic content.

To obtain the densities mercury pycnometry was used. The apparent density of the scaffold (ρ_app) and the solid nanocomposite (ρ_nano) were measured by means of the volume of mercury it displaced when submerged. The porosity of the scaffold is calculated as follows:

\[
\text{% Porosity} = 100 \times \left(1 - \frac{\rho_{\text{app}}}{\rho_{\text{nano}}}ight) \quad (1)
\]

Compression tests were performed on a Lloyd Universal Testing machine, with a 500N load cell at a constant cross-head speed of 1 mm/min according to ASTM D 695. The samples were rectangular prisms, whose average dimensions were 10 × 10 × 4 mm³ that were cut from injection molded bars. The compressive strength and moduli from the initial slope of the stress-strain curves were obtained. The results of the compression test were reported as the average of five replications.

Differential scanning calorimeter (DSC) analysis was performed using a Metler Toledo DSC 1 Star System between 0 and 200°C at a heating rate of 10°C/min under nitrogen atmosphere. The samples were dried at 65°C under vacuum before DSC analysis. Glass transition temperature, crystallization temperature, melting temperature and corresponding enthalpies of NCSs were obtained from DSC thermograms.

To observe the effect of clay addition on the wettabilities of the scaffolds, PLA and PLA based NCs as the matrices of NCSs are examined by water contact angle measurements. To measure the contact angle, the photograph of the droplet of distilled water put on the surface of the polymeric material was taken by a digital camera. The contact angles were measured on digital photographs using image analysis software (Image J).

**RESULTS AND DISCUSSION**

**Morphology of NC and NCS**

TEM micrographs NCs and NCSs are given in Fig. 1A–D. The intercalated clays are visible in the case of PLA-1% Clay and PLA-3% Clay NC in Fig. 1A and B. When PVA is introduced into the PLA NC, due to the possible interactions of PVA with –OH groups of Cloisite 30B, clays are exfoliated into single platelets in both PVA/PLA-1% Clay and PVA/PLA-3% Clay NCSs.

The XRD patterns of Cloisite 30B, and PLA, PLA/ PVA blends and PVA based NC are shown in Fig. 2. The neat-Cloisite 30B has an XRD peak at 2θ = 4.81, which corresponds to a basal spacing of 1.83 nm. PLA based NC including 1% and 3% Cloisite 30B exhibit intercalated structures which is revealed from the XRD peak appeared at 2θ = 2.38 corresponding a d-spacing of 3.71 nm (see Fig. 2B and C). A weak peak is also observed from these XRD patterns of PLA based NCs at around 4.5°. It means that most of the clays are intercalated in PLA, but there still exists some clay particles which are not intercalated.
When the XRD responses of the PLA/PVA blends based NC and PVA based NC are considered, the peaks seen in the case of PLA-NC are disappeared indicating an exfoliated structure (see Fig. 2D–F). One can expect that PVA molecules can extensively interact with Cloisite 30B surfaces through H-bonding, which can facilitate the diffusion of the PVA molecules into clay galleries resulting in exfoliation. One disadvantage of this may be the removal of clay particles during scaffold preparation from PLA/PVA/NaCl NC by dissolving PVA and NaCl in water.

The inorganic material content before and after leaching is examined by burning test for 1% and 3% Clay-PLA/PVA NC. One should note that the organic modifier on the surface of the clay is also burned out in this test by leaving a neat inorganic mineral (MMT). In the calculation of theoretical residual inorganic material (RIM) after leaching, it is assumed that there is no removal of clay particles during leaching. The organic modifier content of the Cloisite 30B is taken as 30% by weight [25]. The test results are shown in Table 1. Before leaching, the RIM is 0.73% and 2.15% for 50/50/1 and 50/50/3 NC, respectively. This indicates that the organic modifier removed in burning test is ~30%. After leaching, the experimental RIM becomes 0.92% and 2.92% for 50/50/1 and 50/50/3 NCSs, respectively. When the experimental RIM and theoretical RIM values are compared, it is revealed that ~35% of the clays are dispersed in PVA.

Figure 3 shows the morphology of the scaffolds investigated by SEM. Black holes are the pores left after removal of PVA and NaCl particles. Regardless of scaffold composition the morphology is cocontinuous. There exist some globular pores at an approximate size of 100 μm or smaller. These pores can be formed by broken and/or eroded NaCl particles during mixing in the microcompounder as a result of particle-metal friction and particle-particle collisions. Similar observation was also made by the other researchers [6]. The pore structures can

<table>
<thead>
<tr>
<th>Material (PLA/PVA/Clay)</th>
<th>Before PVA leaching</th>
<th>After PVA leaching</th>
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<tbody>
<tr>
<td></td>
<td>Theoretical&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Experimental&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50/50/1</td>
<td>0.73</td>
<td>1.41</td>
</tr>
<tr>
<td>50/50/3</td>
<td>2.15</td>
<td>4.33</td>
</tr>
</tbody>
</table>

<sup>a</sup> Theoretical residual inorganic material content after PVA leaching is calculated by assuming that there is no removal of clay particles during PVA leaching. The organic modifier content of the clay is taken as 30% [25].

<sup>b</sup> Experimental residual inorganic material content after PVA leaching is obtained by burning test.
be seen from the magnified SEM micrographs in the Fig. 4. Cocontinuity is valid and well-connected pores are apparent. When the pore morphology of the pristine PLA-scaffolds and NCS are compared, there is no significant difference observed in terms of pore wall-structure and overall morphology. The wall thickness which is important in withstanding stresses during tissue formation and implantation is $\sim 10\ \mu m$ for all scaffolds.

Porosity of Scaffolds

Porosity of the scaffolds has a crucial importance, since scaffolds should allow cells to be seeded on a three-dimensional geometry and high surface [3, 4]. Moreover, scaffolds should provide facile invasion of blood vessels for the supply of nutrients to the transplanted cells [5, 12]. Figure 5 shows the variation of porosity of scaffolds with respect to clay content and compounder screw speed. The porosity data for all of the scaffolds are between 75 and 80% by volume regardless of clay content and screw speed. The increasing clay content has a slight negative effect on the porosity of scaffolds at both screw speeds of 100 and 200 rpm Similarly, the increasing screw speed results in a slight decrease in the average porosity of scaffolds.

Mechanical Properties of Scaffolds

Mechanical properties are one of the important properties of the scaffolds. Since the strength of the scaffold walls decreases gradually during cell-growth as a result of degradation in-vivo or in-vitro, it is meaningful to consider modulus as the key parameter in designing the scaffolds structure to fulfill the structural requirement of scaffold applications [9]. In the current study, the mechanical properties of the scaffolds are traced by means of compressive modulus and compressive strength as well.

Figure 6 shows the dependence of clay content and screw speed on the compressive modulus and strength of neat-PLA and nanocomposite PLA scaffolds. Incorporation of clay results in an increase in modulus of scaffolds regardless of screw speed of compounder. It has been known that modulus of composites depend on filler...
volume fraction, filler aspect ratio, filler modulus, filler orientation, and interfacial adhesion. Fornes and Paul conducted theoretical studies, which were benchmarked against experimental data, to model the modulus of polyamide-6/clay NC by utilizing Halpin-Tsai Theory [26]. It is concluded in their study that modulus of a given nano-

FIG. 4. Magnified SEM micrograph of porous nanocomposite scaffolds prepared by selective polymer and NaCl particle leaching: (A) 0% clay, (B) 1% clay, (C) 3% clay (scale bar corresponds to 10 μ, screw speed of compounder was 100 rpm).

FIG. 5. Porosity of PLA/PVA nanocomposite scaffolds prepared by selective polymer (PVA) and NaCl particle leaching with respect to clay loading level and screw speed.

FIG. 6. Dependence of clay content and screw speed on the compressive modulus and compressive strength of neat-PLA and nanocomposite PLA scaffolds.

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composite system depends on the level of dispersion of the clay platelets and surface chemistry of clay.

For a given organoclay/polymer couple, the general approach to improve the extent of exfoliation is to create complex flow types by combining shear and elongational flow in presence of high shear rates during twin-screw extrusion compounding [27, 28]. This can be achieved by optimizing the processing conditions such as screw speed, barrel temperature, screw geometry and configuration [27, 28]. Based on our experiences on ABS/polyamide-6/clay NC processed by microcompounder used in the current study, increasing screw speed and residence time results in an increase in modulus of nanocomposite as a consequence of higher level of exfoliation [27, 28]. In the current study, the residence time is kept constant and screw speed is varied as 100 rpm and 200 rpm. The compression tests show that incorporation of clay particles results in an improvement in scaffold’s compressive modulus. Doubling the screw speed increased the compressive modulus of the scaffolds probably due to the improvement in the level of exfoliation due to higher shear rate at 200 rpm than that of at 100 rpm. The compression strength slightly increases with increasing clay content regardless of screw speed. The increase in screw speed does not affect the compressive strength significantly.

### Thermal Properties of Scaffolds

Figure 7 shows the DSC traces of neat-PLA and different scaffold systems based on PLA/PVA blends and their clay reinforced NC. It will be noted that all samples are characterized by a glass transition temperature ($T_g$), crystallization temperature ($T_c$), crystallization enthalpy ($\Delta H_c$), melting temperature ($T_m$), and melting enthalpy ($\Delta H_m$), which is typical of semicrystalline polymers. The thermal transitions and corresponding enthalpies are tabulated in Table 2. The estimates of degree of crystallinity ($\chi$) calculated using the value of 87 J/g for the heat of fusion of pure PLA crystal [29] are also shown in Table 2.

The $T_g$ value of neat-PLA (sample A) observed at 58.6°C is shifted to 48.5°C (sample B) when it is processed to produce scaffolds. The incorporation of 1% and 3% clays to the scaffolds resulted in an increase of $\sim 3^\circ$ in $T_g$ as observed in other nanocomposite systems [30]. The incorporation of silicate layers reduce the segmental mobility of polymer chains by suggesting that polymer chains developed interactions with organoclay at the interfacial zones [31]. As seen from DSC thermogram of PLA (see Fig. 7A), a small enthalpy relaxation peak that followed the $T_g$ as a result of nonequilibrium structure [32] is seen. This peak is disappeared in scaffolds. Cold crystallization peak seen at 128.5°C in DSC thermogram of neat PLA is shifted to 109.1°C when PLA is blended with PVA in the absence of clay particles to produce scaffolds. The incorporation of 1 and 3% clay particles slightly decreases ($\sim 1^\circ$) the $T_c$ of the scaffolds. This observation can be attributed to the hindered migration and diffusion of PLA chains to the surface of growing polymer crystal by the second phase (i.e. PVA) that negatively affects the crystallization and hence the $T_c$ of the blends. As can be seen from Fig. 7 and Table 2, neat-PLA has a single melting point at 152.1°C whereas the scaffolds have two melting peaks which are lower than that of PLA. This may imply that two types of less perfect crystal forms in comparison to neat PLA by the incorporation of both PVA and clays. Melting enthalpies and the degree of crystallinity values remain constant within the experimental errors.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$T_m1$ (°C)</th>
<th>$T_m2$ (°C)</th>
<th>$\Delta H_c$ (J/g)</th>
<th>$\Delta H_m$ (J/g)</th>
<th>$\chi$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A$^*$</td>
<td>58.6</td>
<td>128.5</td>
<td>-</td>
<td>152.1</td>
<td>19.1</td>
<td>22.3</td>
<td>25.6</td>
</tr>
<tr>
<td>B$^*$</td>
<td>48.5</td>
<td>109.1</td>
<td>139.5</td>
<td>146.3</td>
<td>19.2</td>
<td>21.9</td>
<td>24.8</td>
</tr>
<tr>
<td>C$^*$</td>
<td>51.7</td>
<td>107.9</td>
<td>139.2</td>
<td>145.9</td>
<td>20.68</td>
<td>21.0</td>
<td>24.1</td>
</tr>
<tr>
<td>D$^*$</td>
<td>51.8</td>
<td>106.7</td>
<td>138.6</td>
<td>145.8</td>
<td>19.7</td>
<td>21.4</td>
<td>24.6</td>
</tr>
</tbody>
</table>

$^*$ Neat PLA.

$^*$ Scaffold of 50/50 PLA/PVA blend.

$^*$ Scaffold of 1% clay reinforced 50/50 PLA/PVA blend.

$^*$ Scaffold of 3% clay reinforced 50/50 PLA/PVA blend.
Wettability by Water Contact Angles

The wettability of a polymer scaffold is considered one of the important issues for homogeneous and sufficient cell seeding in three dimensions. Since biodegradable synthetic polymers are relatively hydrophobic, it is difficult to distribute a cell suspension uniformly into the pores of the scaffolds [33, 34]. The wettabilities of the scaffolds produced in the current study are characterized by water contact angle measurements on PLA, PLA/1% Clay NC and PLA/3% Clay NC. Figure 8 shows the appearance of water drops on the samples indicating the water contact angles. Neat PLA has a hydrophobic surface with an average water contact angle of $\theta = 60.7^\circ$. Incorporation of 1% and 3% clay to the PLA reduced the water contact angles of NC to 42.8 and 31.4°, respectively. This implies that the presence of clay in the PLA reduces the interfacial tension between water/polymer interface and makes PLA surface more hydrophilic. Similarly, it is known from the literature that incorporation of the clays to the incompatible polymer blend systems reduces the interfacial tension that enhances the compatibilization of the components [35]. The modification of the scaffold surface properties by inclusion of clay particles to improve hydrophilicity may serve as a new method in scaffolding for tissue engineering.

Assessment of Applicability of the Scaffolds

Three dimensional scaffolds are prepared for the accommodation of cells, the guidance of the cell growth and the regeneration of three-dimensional tissues with proper structure and function [4]. The scaffolds are produced in proper chemistry, shape and structure that facilitate cell attachment (adhesion), migration, proliferation, differentiation (by the help of growth factors and biosignals) and neo-tissue growth in all three directions. The applicability of a scaffold in any field of tissue engineering (i.e. bone tissue engineering, cartilage tissue engineering, vascular tissue engineering, etc.) is characterized by its pore size, porosity, interconnectivity, surface chemistry and its mechanical properties as well. Besides, biodegradability is also important, because a scaffold is a temporary support of body’s structure and thereafter needs to be degraded over time as the wound is healed. The scaffolds prepared in the current study are assessed if they can be used in bone tissue engineering applications.

Pore size, porosity and interconnectivity has crucial importance in scaffold tissue engineering, since the scaffold architecture has to allow migration of cells in to the matrix after initial attachment, mass transfer of nutrients and metabolites, and provide enough space for three-dimensional vascularization of new tissue [5]. Large pores can be able to enhance the mass transport and vascularization more than small pores; whereas small pores can offer a large surface/volume ratio to seed high number of cell per scaffold. The recent studies show that for bone in growth the optimum range of pore size is 75–250 μm [36]. High porosity of materials is one of the essential factors for bone tissue engineering. It is seen in the literature that scaffolds for bone tissue engineering have been prepared at a porosity level of 75–95% [37]. The scaffolds produced in this study has an approximate pore size of 100 μm and the porosity range of 75–80% depending on processing condition and clay loading level. In terms of pore size and porosity, the scaffolds produced here fulfill the mentioned requirements to be used in bone tissue engineering.

Mechanical integrity of the scaffolds is another important property since a scaffold must act as a temporary support to withstand the external and internal stresses till the formation of the new tissue [37]. Therefore, it can be said that in vivo, a scaffold has to be a “structural member”. Moreover, in vitro, a scaffold has to be able to keep

![Figure 8. Water contact angles of (A) PLA, (B) PLA/1% clay reinforced nanocomposite (C) PLA/3% clay reinforced nanocomposite.](image-url)
its mechanical strength during static and dynamic culturing. When the compressive strength (CS) of the scaffolds produced here (varying between ~0.75 and 2 MPa) are compared with the CS of cancellous (CS\textsubscript{cancellous} ≈ 4–12 MPa, [38, 39]) and cortical (CS\textsubscript{cortical} ≈ 130–180 MPa, [37]) bones, the CS of the scaffolds is a long way off cortical bone and close but lower than cancellous bone. However, it is figured out in the review of Rezwan et al. that the CS of porous biodegradable polymer composite based scaffolds reported in the literature vary between 0.02 and 1.00 MPa [37]. This shows that CS of the scaffolds produced here is higher than most of the porous biodegradable polymer composite based scaffolds reported.

The surface hydrophilicity is one of the major factors that affects the cell adhesion and the efficiency of three-dimensional seeding of the cells. Since biodegradable synthetic polymers are relatively hydrophobic, it is difficult to deliver a cell suspension in a way that uniformly distributes transplanted cells throughout the porous scaffolds [33]. There is not any numerical value of surface energy or surface tension or water contact angle of an ideal scaffold reported in the literature up to our knowledge. However, it is mostly mentioned that the surface of a scaffolds must be hydrophilic. In the current study, the 3% clay incorporated PLA matrix yield water contact angle of 31.4° indicating a very hydrophilic surface.

**CONCLUSIONS**

Organoclay reinforced PLA/PVA/NaCl based NC are processed to produce porous scaffolds for tissue engineering applications. The morphologies, mechanical and thermal properties, porosities and wettabilities of the scaffolds are studied through appropriate experimental approaches. It is demonstrated that microcompounding/injection molding and subsequent polymer/particle leaching method is seen to be a feasible method for porous scaffold production for tissue engineering applications. Intercalated NC are achieved in the case of PLA based NCS. On the other hand, the addition of PVA as a polymeric porogen to this system yields an exfoliated nanocomposite system. It is found that the polymer/particle leaching process results in a 35% of clay loss. The porosity for all of the scaffolds is between 75 and 80% by volume regardless of clay content and screw speed. SEM images show that all the scaffolds have cocontinuous phase morphology. The existing globular pores are formed by broken and/or eroded NaCl particles during mixing in the microcompounder as a result of particle-metal friction and particle-particle collisions. Increasing clay content results in an improvement in compressive modulus of the scaffolds. Thermal properties of the scaffolds are significantly affected by the addition of the clay. The $T_g$ of the scaffolds is increased whereas $T_c$ is decreased. Besides, it is seen in the DSC thermograms that incorporation of PVA into the PLA results in formation of two melting peaks at lower temperatures in comparison to neat-PLA that is an indication of different crystal structures. Water contact angle measurements indicates that the wettability of the PLA based NCs are improved by the incorporation of the clay to the system.

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