Thermal Stability and Surface Wettability Studies of Polylactic Acid/Halloysite Nanotube Nanocomposite Scaffold for Tissue Engineering Studies

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Abstract. This paper reports the preliminary study about the incorporation of halloysite nanotubes (HNT) into polylactic acid (PLA) scaffold to improve the thermal resistance and surface wettability properties. The fabrication of the porous scaffold requires a simple yet effective technique with low-cost materials within freeze extraction method. The thermal stability of PLA/HNT scaffold compared to neat PLA scaffold achieved with increased content of HNT by 5 wt%. Moreover, the surface wettability of the scaffold also shows a positive impact with high content of HNT by 5 wt%. This new nanocomposite scaffold may have high potential as a suitable template for tissue regeneration.

1. Introduction

In medical technology, tissue engineering promising an alternative approach of malfunctioning or lost organs problems such as biodegradable porous scaffold, which patients cells itself can be used to treat them [1]. Tissue engineering emerged in the early 1990s aims to produce a successful scaffold that play a significant role by preserving tissue volume, providing temporary mechanical function, and delivering biofactors [2]. Since tissue and organ repair has been the ultimate goal of surgery from ancient time to the present, many attempts has been made by the tissue engineers to fabricate such ideal scaffold using various materials that can act as a template for the tissue regeneration. The success of scaffold for tissue regenerative mostly dependent on the properties of the materials which determine its morphology, biocompatibility, degradation rate, mechanical properties as well as its porosity.

Until recently, numerous synthetic polymers have found wider interest for tissue engineering as they have a range of degradation rates from very short (days) to long (several months) with good processing characteristics [3]. They also found to be more suitable for the replacement and repair of hard tissues by means of their inherent strength. Porous scaffold based on synthetic biodegradable polymer such as polylactic acid (PLA) appears to found a wide range of pharmaceutical applications
for tissue regeneration of skin [4], cartilage [5], blood vessels [6], and cardiac valve [7]. Basically, PLA which can be derived from the renewable resources [8] such as corn starch is one of the interesting material for scaffold fabrication due to its synthetically controllable degradation rate [9], good mechanical properties [10], and biocompatibility [11]. However, PLA has low crystallization rate, yet contribute causes to some limitations of high-performance applications. This parameter is particularly essential to control PLA degradation rate, thermal resistance, as well as optical, mechanical and barrier properties [12]. Due to these limitations, fabrication of successful PLA scaffold is almost impossible in order to meet the requirements for tissue regeneration.

Alternatively, in order to maximize the benefits and versatility of PLA, the physical, rheological, optical or other properties of PLA compounds can be modified by adding nano-fillers into the PLA matrix [13]. Halloysite nanotubes (HNTs), a type of nanofiller have found its significant potential as a new type of additive for enhancing the mechanical, thermal and fire-retardant performances of polymers [14]–[19]. HNTs can be used as an alternative for cost reduction, improvement of mechanical parameters, e.g. increase of modulus/stiffness at room temperature or elevated temperature, especially in those PLA grades which allow a high degree of crystallinity. HNTs are a type of natural aluminosilicate nanotubes with a similar structure to kaolinite. However, halloysite nanotube differs from kaolinite because of its multilayer tubular structure that results from the wrapping of the 1:1 layers structures. The unit layers in HNTs are separated by a monolayer of water molecules as shown in figure 1.

![Figure 1. Structure of halloysite nanotube (HNT).](image)
As reinforcing material, HNTs are considered as the ideal materials for preparing polymer composites. This is due to the fact that halloysites are rigid materials and that unique crystal structure of HNTs resembles that of carbon nanotubes (CNTs) in terms of aspect ratio. HNTs are more easily dispersed in polymer matrix by shearing due to their rod-like geometry and limited inter-tubular contact area, compared with other nanoparticles such as fumed silica, montmorillonite, and carbon nanotubes [20]. Moreover, due to several characteristic features such as nanoscale lumen, relatively high length-to-diameter ratio, and low hydroxyl group density on their surface, HNTs are considered as promising competitors and cheaper substitutes to both carbon nanotubes and organomodified layered silicates. Furthermore, incorporation of HNTs into various polymers including biopolymer can increase the strength and thermomechanical properties without loss if ductility [21].

Various fabrication techniques have been attempted to produce a 3D porous scaffold structure and acquire control over mechanical properties of the scaffold that may in turn contribute to define shape and function of the tissue [22]. This includes thermally induced phase separation [23], 3D printing [24], porogen leaching [25], freeze drying [26] and electrospinning [27]. Though freeze drying is the most popular applied technique, it is time and energy consuming. This technique also produced unwanted surface skin. However, there is another method to produce polymeric porous scaffold which is freeze extraction. Freeze extraction is a simple two-step process by which involves the freezing of polymer solvent allowing the phase separation process to occur, thus leading to formation of different phases of frozen solvent and concentrated polymer. In this method, the frozen solvent is removed which then resulted in formation of polymer scaffold [28].

Though there are few reports on inclusion of HNTs into PLA matrix, till today, there have been no study on the preparation of freeze extracted porous scaffolds of Therefore, this article aims in developing such PLA/HNTs nanocomposite scaffold by means of freeze extraction method. Inclusion of HNTs nanofiller in the PLA scaffold might open up opportunities in many biomedical applications such as tissue engineering. This preliminary study will investigate the thermal behavior and surface wettability of the nanocomposite scaffold.

2. Experimental

2.1. Scaffold fabrication
The porous PLA/HNTs scaffold was prepared by the solvent-freeze extraction method. PLA was dissolved in 1,4-dioxane for 24 hours to obtain a polymer solution. The required amount of HNTs powder (1, 3, 5, 7, 8 wt%) was added into the polymer solution and allowed to mixed by continuous stirring for 12 h. The solution was then poured into a glass round-shaped mold and cooled to -20°C until froze. The frozen PLA/HNTs polymer mixtures were then immersed in pre-cooled (-20°C) 80% ethanol for solvent extraction. During this process, the porous structure is obtained when the solvent was extracted out and replaced by the non-solvent (ethanol). The scaffolds were removed and rinsed with pre-cooled ethanol to remove excess solvent from the sample. Then, the samples were dried at room temperature. Following this procedure, neat PLA scaffolds and PLA/HNTs nanocomposite scaffolds with required HNTs filler loading were prepared. Figure 2 displays the freeze extraction method.

2.2. Thermogravimetric analysis
The aim thermogravimetric (TGA) analysis is to evaluate the effects of HNTs towards the thermal stability of PLA scaffold. Melting and crystallization behavior were studied using TGA analyser (TA Instruments Inc., New Castle, D.E. USA) under nitrogen gas flow over temperature range 30 to 600 °C at heating rate of 10 °C/min. The samples were prepared around 1 - 2 g each, and are heated. Differential peak temperature (DTp) was recorded as it represents the temperature derivative of the sample’s weight changes.
2.3. Surface wettability
The surface wettability was assessed using water contact angle applied sessile drop method. Contact angle measurement is basically used to measure wettability of solid surface when a drop of liquid is placed on it. In this procedure, the water contact angle was utilized with distilled water as the probe liquid. The contact angles were calculated from images captured by the camera of contact angle measuring system, VCA Optima, AST Products, Inc. Contact angle measurement is basically used to measure wettability of solid surface when a drop of liquid is placed on it. The result obtained may either spread out or remain in the droplet form. The contact angle depends on the hydrophilic moiety characteristic of the polymer either it is air interface or bulk of the solid oriented.

3. Results and discussion
3.1. Fabrication of scaffold
The PLA and PLA/HNTs scaffolds were successfully fabricated using freeze extraction method as can be captured in figure 3. The PLA/HNTs scaffold exhibit good porous structure based on the scanning electron microscope (SEM) as shown in figure 4. It is suggested that the successful inclusion of HNTs into the polymer scaffold during fabrication process may due to the formation of ionic and covalent bonds between both structures.

Figure 2. Freeze extraction method.

Figure 3. Fabricated of PLA/HNTs scaffold *via* freeze extraction method.
Figure 4. SEM of the cross-sectional of PLA/HNTs scaffold.

Figure 5. TGA curves of PLA/HNTs scaffolds.
3.2. Thermogravimetric analysis
The thermal stability of the PLA/HNTs scaffold is characterized by using the thermogravimetric (TGA) results as demonstrated in figure 5. Neat PLA degraded completely, while the PLA/HNTs composite degraded with forming residue. This residue can be assigned to the HNTs content of the sample, and it can be noticed that there was a gradual reduction in weight loss in PLA/HNTs composite between 350-450°C, which corresponds to the fact that HNTs starts degradation at approximately 400°C. The onset weight loss temperature for PLA was 304.5°C, which reduced to 295.07°C for PLA/5 HNTs (figure 5). As the onset weight loss temperature of the PLA/HNT composites decreased with the increase addition of HNTs, this indicated that the thermal stability of PLA/HNTs composites was reduced comparing with that of the neat PLA.

The reduction in thermal stability of PLA/5 HNTs compared to neat PLA may be due to presence of agglomerates in the scaffold. Compared to neat PLA, thermal stability of PLA/HNTs composites was achieved with high content of HNTs and uniformly dispersed HNTs in the polymer matrix. The uniformity dispersion is ascribed to the catalytic role of HNTs on the PLA pyrolysis with the existence of Si - OH and Al - OH acid sites on HNTs external surfaces [29], [30]. However, high content of HNTs in PLA/HNTs composite is not ideal for tissue engineering application. The reduction in thermal stability observed in current study may indicates that the space between PLA and HNTs allowed the volatile pyrolyzed products of PLA to accumulate, thereby accelerating degradation. Further analysis of other PLA/HNTs sample may need to be done to support this result.

![Figure 6. Water contact angle for each sample.](image)

3.3. Surface wettability
The water contact angle testing was conducted to analyze the capability of the nanocomposite scaffold to absorb water. The ability of scaffold to absorb water is crucial to maintain the scaffold shape and play major roles during cell culture studies as it may affects the cell growth [31]. As shown in figure 6, the water contact angle of the nanocomposites has a declining trend by raising the HNT concentration. The contact angle on neat PLA was found to be 110.10° which, basically, assigned to have hydrophobic surface. As the HNTs was introduced to each scaffold formulation, the water contact
angle of the scaffold surface reduces to 107.13°, 72.60° and 58.63°. Then, the contact angle increases back to 60.30° and 101.00° for PLA/1 HNT, PLA/3 HNT, PLA/5 HNT, PLA/7 HNT and PLA/8 HNT respectively. Therefore, based on the results, hydrophilicity properties of the scaffolds was improved. This is instigated by the capillary action of halloysite nanotubes and their good wettability properties [32]. This phenomenon can occur due to structure of HNTs that comprises hydrophilic groups (Al - OH and Si - OH) located on its surface beside alumina and silica groups at its crystal edges, thus ease the formation of hydrogen bonds between HNTs and water [33].

4. Conclusion
This study focused on the effects of HNTs on thermal behavior and surface wettability of fabricated PLA scaffold. PLA/HNTs nanocomposite scaffold was prepared by freeze extraction method. The introduction of HNTs into the PLA has increased the thermal stability of the scaffold which was achieved by the high inclusion of HNT and the well dispersion in the polymer matrix. Furthermore, the surface wettability of the PLA/HNTs scaffold also shows positive effect where the water contact angle on the surface increased with increasing of HNTs. Overall, this novel scaffold is easy to fabricate via freeze extraction method. This technique can be considered as the most cost-effective process since it requires only low-cost materials with minimum use of other utilities. In order to support the use of this nanocomposite scaffold in tissue engineering application, extension study such as the mechanical properties, porosity, and cytotoxicity test need to be conducted to ascertain its viability towards specific purpose.

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References
[1] Mironov V, Trusk T, Kasyanov V, Little S, Swaja R and Markwald R 2009 Biofabrication 1 22001
[2] Hollister S J 2005 Nat. Mater. 4 590
[3] Bonassar L J and Vacanti C A 1998 J. Cell. Biochem. Suppl. 30 297-303
[4] Mohiti-Asli M, Saha S, Murphy S V, Gracz H, Pourdeyhimi B, Atala A and Loboa E G 2017 J. Biomed. Mater. Res. Part B: Appl. Biomater. 105 327–339
[5] Hutmacher D W 2000 Biomaterials 21 2529–43
[6] Li Z, Zhao X, Ye L, Coates P, Caton-Rose F and Martyn M 2015 Chem. Eng. J. 279 767–776
[7] Iop L and Gerosa G 2015 BioMed Res. Int. 2015 1–13
[8] Yang S, Madbouly S A, Schrader J A, Srinivasan G, Grewell D, McCabe K G, Kessler M R and Graves W R 2015 Green Chem. 17 380–393
[9] Cui M, Liu L, Guo N, Su R and Ma F 2015 Molecules 20, 595–607
[10] Shi H, Gan Q, Liu X, Ma Y, Hu J, Yuan Y and Liu C 2015 RSC Adv. 5 79703–14
[11] Abdal-hay A, Hussein K H, Casettari L, Khalil K A and Hamdy A S 2016 Mater. Sci. Eng. C 60 143–150
[12] Murariu M, Dechief A L, Ramy-Ratiairison R, Paint Y, Raquez J M and Dubois P 2015 Nanocomposites 1 71–82
[13] Matusik J, Stodolak E and Bahranowski K 2011 Appl. Clay Sci. 51 102-109
[14] Deng S, Zhang J, Ye L and Wu J 2008 Polymer 49 5119–27
[15] Guo B, Lei Y, Chen F, Liu X, Du M and Jia D 2008 Appl. Surf. Sci 255 2715–22
[16] Ismail H, Pasbakhsh P, Fauzi M N A and Abu Bakar A 2008 Polym. Test. 27 841–850
[17] Liu M, Guo B, Zou Q, Du M and Jia D 2008 Nanotechnology 19 205709
[18] Marney D C O, L. Russell L J, Wu D Y, Nguyen T, Cram D G, Rigopoulos N, Wright N and Greaves M 2008 Polym. Degrad. Stab. 93 1971–78
[19] Ye Y, Chen H, Wu J and Ye L 2007 Polymer 48 6426–33
[20] Du M, Guo B and Jia D 2010 Polym. Int. 59, 574-582
[21] Schmitt H, Prashantha K, Soulestin J, Lacrampe M F and Krawczak P 2012 *Carbohydr. Polym.* **89**, 920-927
[22] Lee L, Zeng C, Cao X, Han X, Shen J and Xu G 2005 *Compos. Sci. Technol.* **65**, 2344–63
[23] Mannella G A, Conoscenti G, Carfi Pavia F, La Carrubba V and Brucato V 2015 *Mater. Lett.* **160** 31-33
[24] Rosenzweig D, Carelli E, Steffen T, Jarzem P and Haglund L 2015 *Int. J. Mol. Sci.* **16** 15118-135
[25] Choudhury M, Mohanty S, Nayak S 2015 *J. Biomater. Tissue Eng.* **5** 1-9
[26] Salerno A, Fernández-Gutiérrez M, San Román del Barrio J and Domingo C 2015 *J. Supercrit. Fluids* **97** 238-246
[27] Morelli S, Salerno S, Holopainen J, Ritala M and De Bartolo L 2015 *J. Biotechnol.* **204** 53-26
[28] Ho M H, Kuo P Y, Hsieh H J, Hsien T Y, Hou L T, Lai J Y and Wang D M 2004 *Biomaterials* **25** 129-138
[29] Kopinke F D, Remmler M, Mackenzie K, Möder M and Wachsen O 1996 *Polym. Degrad. Stab.* **53** 329–342
[30] Stoclet G, Sclavons M, Lecouvet B, Devaux J, Van Velthem P, Boborodea A, Bourbigot S and Salem-Idrissi N 2014 *RSC Adv.* **4** 57553–563
[31] Smetana K 1993 *Biomaterials* **14** 1046–50
[32] Abdullayev E, Price R, Shchukin D and Lvov Y 2009 *ACS Appl. Mater. Interfaces* **1** 1437–43
[33] Rawtani D and Agrawal Y K 2012 *Rev. Adv. Mater. Sci.* **30** 282–295