IOP Conf. Series: Materials Science and Engineering **369** (2018) 012013 doi:10.1088/1757-899X/369/1/012013

# Physical properties and morphology of electrospun composite fiber mats of polyhydroxyalkanoate containing nanoclay and tricalcium phosphate additives

**N Tanadchangsaeng[1,2,3] and S Boonyagul[1]**

[1]Faculty of Biomedical Engineering, Rangsit University, 52/347 Lak-Hok, Pathumthani 12000 Thailand
[2]Industrial Biomaterials Research Center, Rangsit University, 52/347 Lak-Hok, Pathumthani 12000 Thailand

E-mail: nuttapol.t@rsu.ac.th

**Abstract.** Recently, nanofiber research has gained substantial attention from scientists. In this study, the main component of the nanofiber sheet is polyhydroxyalkanoate (PHA) polymer, which is strong, ductile, flexible and adhesive to human skin. Two major additives of nanofiber sheet that we applied are nanoclay and tricalcium phosphate. The additives are generally synthetic substances that can be chemically synthesized and compatible with tissues body. Nanoclay has a low density, strong, durable to compressive strength and humidity. While, tricalcium phosphate is a calcium phosphate ceramic that is biocompatible to human tissue. From the reasons above, we proposed to choose both nanoclay and tricalcium phosphate for adding into PHA nanofibers for film formation. Thus, this study aims to investigate the morphological and mechanical properties of the fiber mat by using PHA added with various amount of nanoclay and tricalcium phosphate at 0.1%, 1% and 10% by weight, and fabricate nanofiber samples by electrospinning technique. The tested results of scanning electron microscope (SEM) morphology show that the fibers have a uniformed pattern. The PHA containing nanoclay of all additive contents exhibited micrometer diameter distributions, while PHA loaded with 1% tricalcium phosphate still had the nano-scale diameter range, and might be the optimum additive load for further nanometer medical applications. A tensile test was performed to determine the effect of nanoclay and tricalcium phosphate contents on the mechanical properties of the electrospun PHA films, and reflect the level of modularity. With nanoclay components being integrated into the polymer matrix, subsequent reduction in fiber crystallinity was occurred after addition of nanoclay with an increase of modulus value. The results confirmed that PHA fiber mat containing 1% nanoclay may have a potential for using as a rigid scaffold which bearing force loading in human organ system. Whereas, it can be indicated that PHA fiber mat containing 1% tricalcium phosphate might be employed as a flexible scaffold for biomedical materials application due to a high elongation at break value.

## 1. Introduction

Electrospinning, as shown in figure 1, is a simple and versatile method for the fabrication of polymer nanofibers. Nanofibers have been investigated as components in wound dressings, protective clothing, filtration, biomedical devices and as reinforcement fibers in composites [1]. Their high surface areas are one factor dictating their potential in tissue engineering applications, especially if the material used

is bioactive, biocompatible or nanoporous [2]. Electrospinning can also be used for processing non-woven nanofibers for advanced medical research, such as the fabrication of biocompatible nanofiber scaffolds with three-dimensional extracellular matrices for improving the function, or treatment of, damaged tissues or organs [3,4]. Scaffolds play a crucial role in commanding stem cells differentiation, therefore, electrospun scaffolds stands for a promising way for bone tissue engineering [5].

Until now, a variety of polyesters have been studied in biomedical applications due to their biocompatibility, bioresorbility, biodegradability and high mechanical propertiey [6-8]. Among them, polyhydroxyalkanoate (PHA), which is a family of biopolyesters produced by various microorganisms, can be processed similar to petroleum-based plastics, which their physical properties are of substantial attractiveness to polymer manufacturer [9,10]. The excellent biodegradability and biocompatibility of PHA bioplastics are desired properties for many short-life goods and applications in agriculture and health care [11,12].

Nanoclays have exhibited to improve physical properties of polymer morphology and structures [13,14]. Nanoclays are greatly employed to reinforce thermoplastics for hybrid composites. Incorporation of nanoclay into polymer matrices is still well-known to produce composite scaffolds. Tricalcium phosphate are bioactive and osteoconductive if fabricated with appropriate geometry [15]. The tricalcium phosphate can improve the mechanical properties of polymeric materials, and enhance cell adhesion for inducing the differentiation of cells. Due to a difference of mechanical properties of PHA scaffolds and natural bone, polymer matrix scaffolds made of inorganic materials are too brittle. The addition of these two inorganic additives to polymeric matrix can control the physical properties of the nanocomposite matrix [16,17]. Here, we proposed to choose nanoclay and tricalcium phosphate to add into nanofibers for film formations and assessed the morphological and physical properties of nanoclay/tricalcium phosphate-enriched electrospun PHA scaffolds. This work aims to investigate the strength of the nanofiber sheet after adding nanoclay and tricalcium phosphate by electrospinning nanofiber fabrication.

## 2. Experiments

### *2.1. Materials*

Polyhydroxyalkanoate (PHA) copolymer was produced from glycerol and levulinic acid by *Ralstonia eutropha* as described in a previous work [18]. Synthetic nanoclay (clay Cloisite 20A) was obtained from Southern Clay Products, Inc. Tricalcium phosphate was purchased from Sigma Aldrich (USA). Dichloromethane (DCM, analytical grade) was also purchased from Sigma Aldrich (USA).

### *2.2. Polymer solution preparation*

For preparation of polymer solution, the polymer mixture of PHA was dissolved in dichloromethane with concentration at 8% (w/v). Then, nanoclay or tricalcium phosphate was blended at 0%, 0.1%, 1.0%, and 10% by weight, respectively, and mixed together with temperature at 60°C for 15 min.

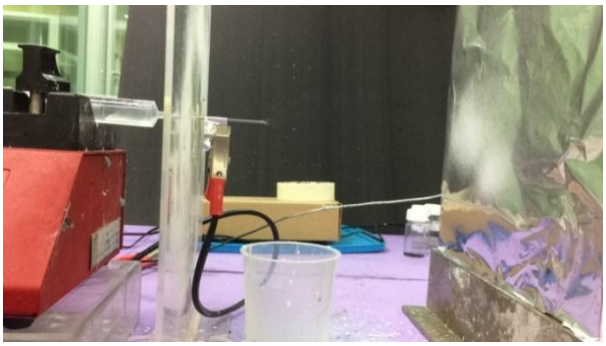

**Figure 1.** Experimental set up during electrospinning process.

*2.3. Fabrication of PHA–composite electrospun scaffolds by electrospinning*

A single nozzle electrospinning setup was used to prepare electrospun nanofibers as shown in figure 1. A high voltage power supply (ES60P-10 W, Gamma High Voltage, Florida, USA) was attached to a metal nozzle to create a voltage difference between the metal nozzle and a grounded collector of 15–25 kV. A charged metal nozzle with 0.57 mm outer diameter was placed 15 cm directly above the center of the 20 × 20 cm aluminum flat grounded collector. A mechanical pump (NE-300, New York, USA) was used to deliver the solution from a plastic syringe to the metal nozzle at a rate of 1 ml/hr.

*2.4. Morphology characterization*

Scanning Electron Microscope (SEM) (JSM-6400, JEOL Ltd., Tokyo, Japan) was used to characterize electrospun fibers characteristic and size. Samples of the electrospun nanofibers were sputter coated with platinum. Images were captured with an accelerating voltage of 5 kV. Image J software (www.imagej.nih.gov) was used for measuring fiber diameter. The average fiber diameter and Standard Deviation (SD) were determined.

*2.5. Mechanical property testing*

The mechanical properties of electrospun nanofibers were evaluated using a texture analyzer (TA.XT plus, Stable Micro Systems, UK) with a 5 kg load cell equipped with tensile grips. Specimens tested had dimensions 0.5 cm x 3 cm, and were tested at a rate of 5.0 mm/min until fracture occurred.

## 3. Results and discussion

*3.1. Morphological property of electrospun scaffolds of PHA–composite with nanoclay and tricalcium phosphate*

Non-woven nanofibers via electrospining were processed by high voltage on the needle and zero voltage on the plate collector (grounded), accumulated electrostatic on the PHA/DCM droplet was pushed by high voltage on the needle to the collector, therefore the droplet became to a solution jet that rapidly elongate the solution into nanosize till the DCM solvent was completely evaporated before the nanosized fiber drop on the collector as shown in figure 1. In this study, we selected 8% PHA solution to be investigated because the 8% PHA nanofiber via electrospinning exhibited a smooth cylindrical, uniformity and diameter is at 0.83 ± 0.14 μm (less than 1 μm) of fibre formation as shown in figures 2A, 3A, and 4.

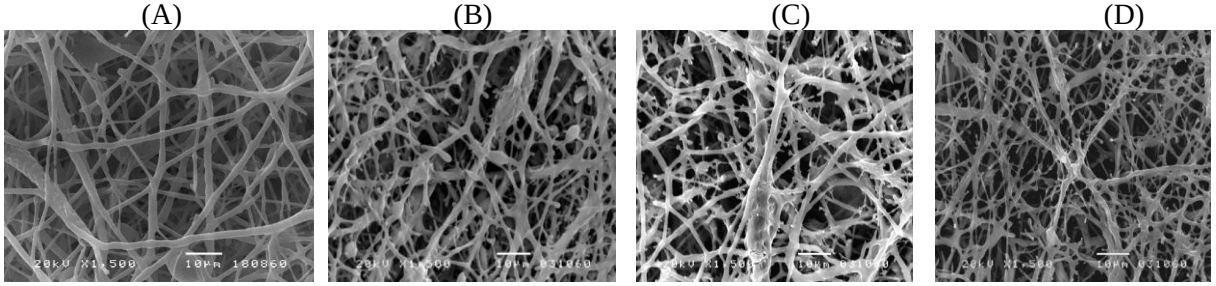


**Figure 2.** SEM morphology of PHA fiber mats composited with nanoclay in concentration by polymer of 0% (A), 0.1% (B), 1.0% (C), and 10% (D) by weight, respectively. All the SEM images were captured at 1500x.

Within 1500x microscale, the morphologies of the fibers obtained through electrospinning of PHA/nanoclay solutions (figure 2) are the same (minuscule nanoclay particles attached to fibers) but fiber diameter changes by increasing from nanometer diameter of pure PHA nanofiber to microfiber

scale (more than 1 μm). The average diameters of the PHA/nanoclay fibers containing 0.1%, 1.0% and 10% nanoclay were at 2.08 ± 0.14, 2.15 ± 0.91 and 1.91 ± 0.88 μm, respectively as shown in figure 4A. From these results, the PHA containing nanoclay of all additive contents exhibited micrometer diameter distributions.

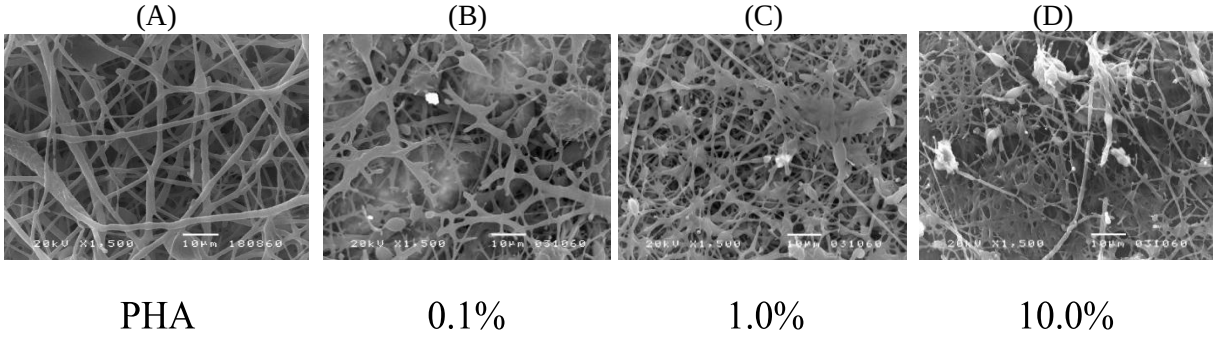


**Figure 3.** SEM morphology of PHA fiber mats composited with tricalcium phosphate in concentration by polymer of 0% (A), 0.1% (B), 1.0% (C), and 10% (D) by weight, respectively. All the SEM images were captured at 1500x.

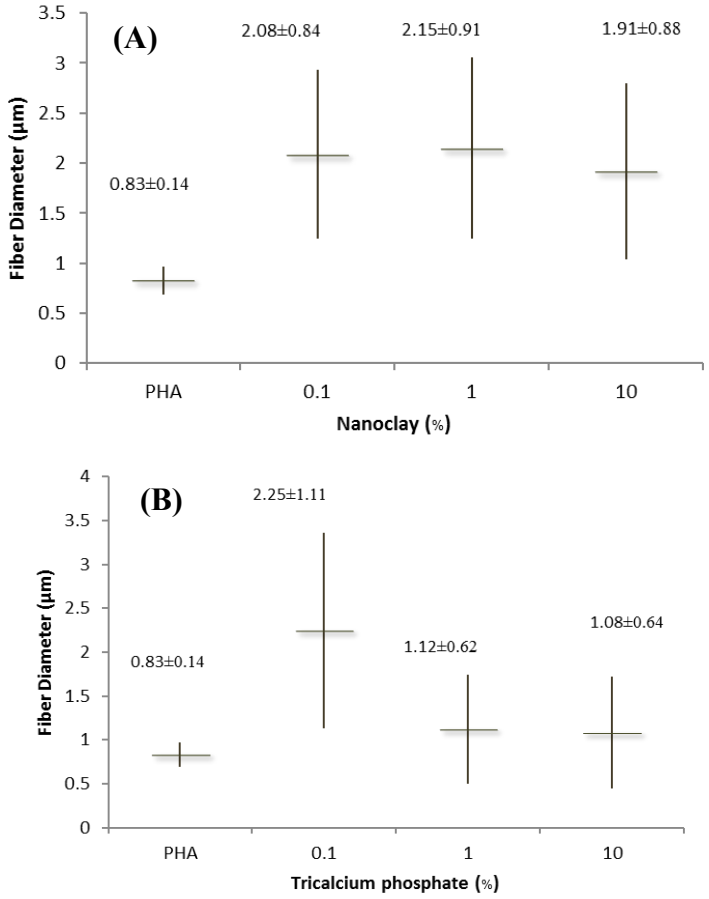


**Figure 4.** The bar graph between fiber diameter of PHA fiber mats composited with nanoclay (A), and with tricalcium phosphate (B) which are dependent on the amount of each additive.

Whereas, the morphologies of the fibers obtained through electrospinning of PHA/tricalcium phosphate solutions (figure 3) bearing an amount of knots and forming some complex particles attached to the fibers are the same, but fiber diameter still increases from nanometer diameter of pure PHA nanofiber to microfiber scale (more than 1 μm) when loading the additive. The average diameters of the PHA/ tricalcium phosphate fibers containing 0.1%, 1.0% and 10% additive were at 2.25 ± 1.11, 1.12 ± 0.62 and 1.08 ± 0.64 μm, respectively as shown in figure 4B.

For tissue engineering work, the ECM matrix should be in nanoscale scaffold for suitable application. As a result, we confirmed that PHA fibers loaded with 1% tricalcium phosphate still had the nano-scale diameter range, and might be the optimum additive load for further nanometer medical applications.

### *3.2. Mechanical property of electrospun PHA fibers containing nanoclay and tricalcium phosphate additives*

A tensile test was performed to determine the effect of nanoclay and tricalcium phosphate contents on the mechanical properties of the electrospun PHA films. The films was performed at room temperature using a tensile test machine with a strain rate of 5 mm/min. Figure 5 shows typical tensile curve depicting relative between tensile force and elongated distance of PHA fiber mats composited with nanoclay and with tricalcium phosphate, which are dependent on the amount of each additive. Table 1 shows a summary of mechanical data of PHA fibers loaded with each additive.

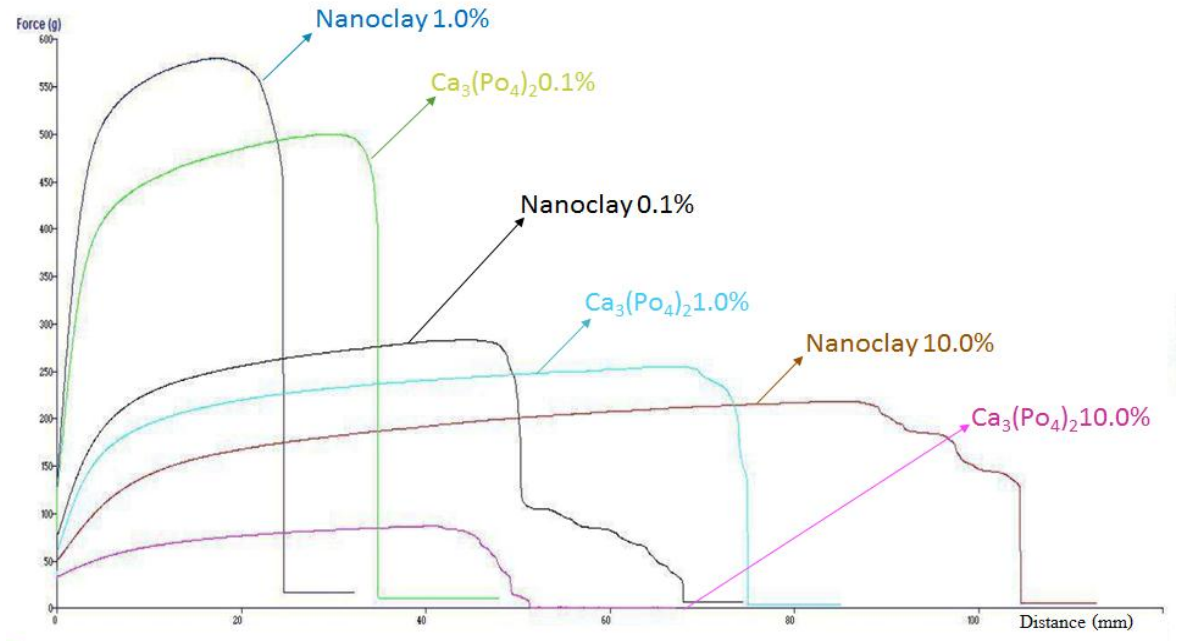


**Figure 5.** The tensile curve depicting relative between tensile force and elongated distance of PHA nanofibers composited with nanoclay and with tricalcium phosphate, which are dependent on the amount of each additive.

The mechanical property results for PHA fibers containg 1.0% nanoclay indicate a Young's modulus of 50 MPa that is higher than 3 folds compare to 0.1% nanoclay loaded. But the elongation at break value is reduced from 70% to 25% as we decreased the amount of nanoclay. These results are consistent with nanoclay components being integrated into the polymer matrix, and subsequent reduction in fiber crystallinity after addition of nanoclay. As a result, it can be confirmed that PHA fiber mat containing 1% nanoclay may have a potential for using as a rigid scaffold which bearing force loading in human organ system.

**Table 1.** Mechanical Properties of PHA fiber mats composited with nanoclay and with tricalcium phosphate, which are dependent on the amount of each additive.

| Sample | Modulus | % Elongation |
|---|---|---|
| Nanoclay 0.1% | 15.31 MPa | 70% |
| Nanoclay 1.0% | 49.40 MPa | 25% |
| Nanoclay 10.0% | 6.55 MPa | 105% |
| Tricalcium phosphate 0.1% | 32.5 MPa | 36% |
| Tricalcium phosphate 1.0% | 11.19 MPa | 76% |
| Tricalcium phosphate 10.0% | 2.74 MPa | 53% |

For tricalcium phosphate loading in PHA fibers, the mechanical property results for PHA fibers containg 1.0% tricalcium phosphate shows a Young's modulus of 11 MPa, while the elongation at break is at 76%. As a result, it can be indicated that PHA fiber mat containing 1% tricalcium phosphate might be employed as a flexible scaffold for biomedical materials application such as suture and mesh.

## 4. Conclusion

In this study, we investigated the morphological and mechanical properties of the fiber mat by using polyhydroxyalkanoate (PHA) added with various amount of nanoclay and tricalcium phosphate at 0.1%, 1% and 10% by weight, and fabricate nanofiber samples by electrospinning technique. The tested results of scanning electron microscope (SEM) morphology show that the fibers have a uniformed pattern. The PHA containing nanoclay of all additive contents exhibited micrometer diameter distributions, while PHA loaded with 1% tricalcium phosphate still had the nano-scale diameter range, and might be the optimum additive load for further nanometer medical applications. A tensile test was performed to determine the effect of nanoclay and tricalcium phosphate contents on the mechanical properties of the electrospun PHA films, and reflect the level of modularity. With nanoclay components being integrated into the polymer matrix, subsequent reduction in fiber crystallinity was occurred after addition of nanoclay with an increase of modulus value. The results confirmed that PHA fiber mat containing 1% nanoclay may have a potential for using as a rigid scaffold which bearing force loading in human organ system. Whereas, it can be indicated that PHA fiber mat containing 1% tricalcium phosphate might be employed as a flexible scaffold for biomedical materials application due to a high elongation at break value.

## Acknowledgment

The authors appreciate financial support from the Research Institute of Rangsit University (contract number 58/2557). We thank National Nanotechnology Center (NANOTEC) for use of the tensile testing equipment and Thailand Institute of Scientific and Technological Research for SEM experiment. We wish to thank Thanawat Saengsunanon, Phimphichaya Sanmanot, Acharawan Panlomso and Stayu Kitmongkolpaisarn from Rangsit University for laboratory assistance in this study.

## References

[1] Hu X, Liu S, Zhou G, Huang Y, Xie Z, *et al* 2014 *J Control Release* **185** 12-21
[2] Ko F K and Wan Y 2014 *Introduction to Nanofiber Materials* (Cambridge University Press)
[3] Sun B, Long Y, Zhang H, Li M, Duvail J, *et al* 2014 *Prog Polym Sci* **39** 862-90
[4] Gaharwar A K, Mukundan S, Karaca E, Dolatshahi-Pirouz A, Patel A, *et al* 2014 *Tissue Eng Part A* **20** 2088-101
[5] Jang J H, Castano O and Kim H W 2009 *Adv Drug Deliv Rev* **61** 1065-83
[6] Agrawal C M and Ray R B 2001 *J Biomed Mater Res* **55** 141-50
[7] Patel A, Gaharwar A K, Iviglia G, Zhang H B, Mukundan S, *et al* 2013 *Biomaterials* **34** 3970-

83
[8] Zhang H B, Patel A, Gaharwar A K, Mihaila S M, Iviglia G, *et al* 2013 *Biomacromolecules* **14** 1299-310
[9] Lenz R W and Marchessault R H 2004 *Biomacromolecules* **6** 1-8
[10] Luzier W D 1992 *Proc Natl Acad Sci* **89** 839-42
[11] Sudesh K, Abe H and Doi Y 2000 *Prog Polym Sci* **25** 1503-55
[12] Chen G-Q 2009 *Chem Soc Rev* **38** 2434-46
[13] Bordes P, Pollet E and Averous L 2009 *Prog Polym Sci* **34** 125-55
[14] Wu C J, Gaharwar A K, Schexnailder P J and Schmidt G 2010 *Materials* **3** 2986-3005
[15] Kang Z, Zhang X, Chen Y, Akram M Y, Nie J, *et al* 2017 *Mater Sci Eng: C* **70** 1125-31
[16] Gaharwar A K, Kishore V, Rivera C, Bullock W, Wu C J, *et al* 2012 *Macromol Biosci* **12** 779-93
[17] Schexnailder P J, Gaharwar A K, Bartlett R L, Seal B L and Schmidt G 2010 *Macromol Biosc* **10** 1416-23
[18] Tanadchangsaeng N and Yu J 2013 *J Appl Polym Sci* **129** 2004-16