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Processing and Characterization of Poly (butylene adipate-co-terephthalate) / Wollastonite Biocomposites for Medical Applications

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Abstract

By melt blending process Poly (butylene adipate-co-terepthalate) (PBAT) based composites were prepared using PBAT with 0 to 7 wt % of wollastonite (W). The prepared PBAT biocomposites were characterized for XRD, FTIR, SEM, TGA, cell viability, swelling studies, and tensile properties. XRD reveals that PBAT was attained crystalline nature after the addition of filler into the polymer matrix. SEM micrographs confirmed that the morphology of the blend has homogeneous dispersion of the filler and after soaking in SBF (stimulated body fluid) for 5 days showed that good formation of HA layer on the PBAT/W composite (3 wt%) . TGA study reveals that the thermal stability slightly increases with filler loading. The tensile strength of PBAT increases by the addition of wollastonite up to 5 wt % and then it tends to decreases. The effect of filler on the PBAT for biocompatibility level was evaluated through in vitro cytotoxicity test (MTT Assay). Cytotoxicity tests showed that good cell viability at 3 wt % filler content at short incubation periods. The potential benefits of the developed biocomposites can be used as new biomaterials in biomedical field.

Keywords: PBAT, biocomposites, wollastonite, cytotoxicity, biomaterials

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1.**Introduction**

Biomaterials for medical applications are prepared from natural or man-made origin is used to direct, supplement, or replaces the functions of living tissues of the human body. Biomaterials used for medical practice utilizes a large number of implants and devices. Biomaterials in the form of implants such as sutures, bone and joint replacement, dental implants, ligaments, vascular grafts, heart valves etc., and devices such as biosensors, pacemakers, artificial heart, etc., are widely used to restore the function of traumatized and or replace organ or regenerated tissues, to assist in healing, to correct abnormalities and to improve the quality of life of the patients. Researchers have coined the words 'biomaterial' and 'biocompatibility' to indicate the biological performance of materials. Materials that are biocompatible are called biomaterials, and the biocompatibility is a descriptive term which indicates the ability of a material to perform with an appropriate host response, in a specific application. All biomaterials intended for use in contact with living systems must meet certain criteria and regulatory requirements such as (i) the materials must be biocompatible such as nontoxic, tissue or blood compatible, non-carcinogenic, etc., (ii) the materials must not leach or release harmful components into the living system, (iii) must have superior mechanical properties such as strength, elasticity, durability, stability, etc., for the intended applications, (iv) must be sterilizable. Researchers also classified materials into several types such as bioinert and bioactive, biostable and biodegradable, etc. Among these materials polymers and composite materials have gained much attention for biomedical applications. Among various polymers, biodegradable polyesters obtained from renewable or fossil sources, or a combination of both, has gaining more interest in research and industrial fields day by day due to the economic and environmental needs. Biodegradable synthetic materials such as polyesters and its copolymers have been proposed as excellent materials for bone engineering materials, medical packaging, implants, etc which are commonly used in biomedical engineering field.

Poly (butylene adipate-co-terepthalate) (PBAT), is an aliphatic-aromatic random co polyester prepared from 1, 4-butanediol, adipic acid and terephthalic acid. PBAT is flexible and has a high elongation at break than most biodegradable polyesters and widely used for packaging applications, but rarely studied for medical applications. PBAT has poor thermal and mechanical properties, which can be overcome by enhancing thermal and mechanical properties through the addition of fillers[1,2]. Biocomposites based on biodegradable polyesters with other polymers or fillers/fibers have been extensively investigated and studied for their potential applications in the various fields due to the environmental protection. PBAT based composites were extensively studied for packaging and industrial applications but for medical purposes it has not been studied extensively. In the recent studies due to the presence of excellent physical properties along with biocompatibility, PBAT have been proposed for use in various biomaterial applications [3-6]. A wide range of bioactive inorganic materials like tri calcium phosphate, bioactive glass, hydroxyapatite, and wollastonite were studied for medical applications [7-10]. Wollastonite (β-$CaSiO_3$) is an inorganic material of calcium-silicate-based ceramic which is known to be bioactivity and degradability; it has been proposed as a potential material for bone tissue regeneration [11]. Wollastonite used as filler in polymers enhances the mechanical and bioactive properties in biocomposites and has been

attracted more attention for its excellent biocompatibility and bioactivity in medical field as biomaterials [12, 13].The aim of this paper is to explore the processing condition by incorporating wollastonite into PBAT polymer matrix to improve the mechanical, thermal and biocompatibility behaviour of the biocomposites which can be used as potential biomaterials in the medical field.

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2. Materials and Methods

2.1 Materials

Poly (butylene adipate-co-terepthalate) PBAT, trade name ECOFLEX F BX 7011, BASF supplied by Natur Tec Pvt Ltd, India. Wollastonite was obtained in powder form from Wolkem India Company, India. MG63 oesteosarcoma cells were obtained from National Centre for Cell Sciene (NCCS), Pune. 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Dulbecco's modified Eagle's medium (DMEM), dimethyl sulfoxide (DMSO) and Phosphate buffered saline (PBS) pH 7.4 was obtained from Sigma Aldrich.

2.2 Preparation of Composites

PBAT pellets and Wollastonite powder were dried at 70 ºC for 6 h before mixing process. The composites were mixed at 140 ºC in an internal mixer (Rheomix-Brabender Plasticorder) for 10 minutes by melt blending method. The mixing level was performed at two different rotor speeds at 30 rpm in the loading step (3 min) and 60 rpm during mixing (7 min). The samples were prepared by adding filler wollastonite to neat PBAT of 50/0, 49/1, 47/3, 45/5 and 43/7 in weight percentage respectively. From the mixing chamber the batch was extracted manually and allowed to cool to room temperature. The sheets were obtained by compression molding using a hot-plate hydraulic press at 150 ºC and allowed to cool to room temperature under the pressure (60kgf/cm^2). All sample characterizations were made on 0.9-1.0 mm thick films.

2.3 Testing of the Composites

X-ray diffractometer (XRD) were recorded at room temperature in the range 1-30º by using (RIGUKU Miniflex II C XRD). Infrared spectra of the samples were obtained using an AFTIR spectrophotometer (AGILENT CARY 630 FTIR). Thermo gravimetric analysis (TGA) of PBAT biocomposites was carried out using TA instrument at 10 ºC/min of heating rate from RT to 800 ºC under nitrogen atmosphere. Hardness tests were performed on the 0.9-1.0 mm thick films by using Shore A HARDNESS DUROMETER as per ASTM D2240. Five readings of each sample were taken for statistical purposes. A mechanical tester (UTM -Tinius Olsen) was used to measure the tensile strength and elongation at the break, in the accordance

with ASTM D638. The cross head speed is 50 mm/min. Three measurements were performed for each sample and the results were averaged to obtain a mean value.

2.4 Evaluation of the in vitro studies on composites

The bioactivity of the composite was evaluated by examining the HA deposition on the surface of the scaffolds in SBF with ion concentrations similar to those in human blood plasma (BP), according to Kokubo solution [14]. The scaffolds were soaked in the SBF solution at 37.5◦C for 5 days in 25ml. After soaking, the scaffolds were removed, washed in distilled water for three times, and finally freeze-dried. The morphologies of the soaked scaffolds were observed by SEM before and after soaking in SBF. The surface morphology of PBAT and its biocomposites were examined by means of scanning electron microscope (SEM-JEOL JSM 850) on the surfaces of 0.9-1.0 mm specimens, previously coated by sputtering with gold.

Biocompatibility of PBAT and its biocomposites was evaluated through an in vitro cytotoxicity test using colorimetric MTT assay. MTT assay measures the reduction of the tetrazolium component MTT by viable cells. The reduction level of MTT into formazan (medium) can reflect the level of cell metabolism used to quantify the cells grown on the membranes. In this study, MG63 Osteosarcoma cells were cultured in DMEM, it was supplemented with 10 % fetal bovine serum (FBS) and 100 U/Ml penicillin-100 g/mL streptomycin, in a humidified 5 % CO_2 balanced air incubator at 37 ºC. Medium was changed every 3 days. All samples were cut into 1x1 cm^2 films and sterilized in an absolute ethanol under UV radiation. These sterilized samples were placed each in 24-well culture plates and make them to immerse in 10ml of DMEM for 24 hrs. They were incubated with cells in individual wells for 24, 72 and 120 hrs, respectively. 5mg of MTT was dissolved in 1ml of PBS and filter sterilized. To the samples, 10 µl MTT solutions (5mg/mL in PBS solution) was added into each well and incubated for 3 h at 37 ºC. After incubation, culture supernatants were aspirated and the insoluble purple MTT product was dissolved in 100 µl of dimethyl sulfoxide (DMSO) for 15 min. the polystyrene (PS) surface of the 24-well culture plates was taken as negative control and 5 % DMSO in DMEM was taken as positive control. The optical density of the solution was measured at a wave length of 570 nm using an Elisa plate reader (Bio-Rad ELISA Plate Reader Colorimeter). Triplicate samples were analyzed for each experiment.

3. Results and Discussion

3.1 X-ray diffraction

X-ray diffraction patterns of neat PBAT, PBAT/W (3 wt %), and wollastonite are shown in Figure 1(a-c). Pure PBAT in Figure 1 (a) exhibited four diffraction peaks at about 17.5º, 20.5º, 22.9ºand 24.5º. Wu et al., confirms the similar 2 θ value for pure PBAT in which 24.5º 2 θ was most intense. The diffraction peaks of neat wollastonite in Figure 1(c) reveals main peaks at about 11.2 º , 23.9 º,25.4 º,27.0 º and 30.0 º 2 θ which has high crystalline peak and the peak at 30.0 º 2 θ was most intense was observed by Zhu et al. A comparison of the diffraction peaks of PBAT/W (3 wt %) with neat PBAT and wollastonite in Figure 1 (b)

showed peak at 11.1º, 23.9 º, 25.4 º 2 θ and the peak at 23.9 º 2 θ suggests that good physical dispersion and interaction of wollastonite throughout the PBAT composites. This indicates that the increase in crystalline structure of PBAT/W biocomposites was due to the addition of wollastonite into the polymer matrix when compared to neat PBAT.

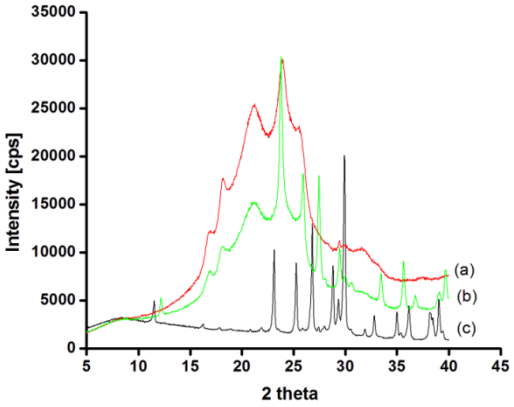

Figure -1 XRD graph of a) neat PBAT b) PBAT/W (3 wt %) c) Wollastonite powder

3.2 FTIR

The FTIR spectra of neat PBAT and their biocomposites are shown in Figure 2. In the spectrum, the characteristic transition peaks of PBAT at around 3000 cm^{-1}representing C-H stretching in aliphatic and aromatic portions. At around 1710 cm^{-1}representing higher absorbance carbonyl groups (C=O) in the ester linkage. In 1267 cm^{-1}represnting C-O in the ester linkage; and a sharp peak at 720 cm^{-1}representing four or more adjacent methylene (-CH_2-) groups. Bending peaks of the benzene substitutes are located at wave numbers between 700 $cm^{-1}$and 900 cm^{-}1,Similar result was observed by Chin-Shan Wu (2012).The characteristic transitions of wollastonite shows the absorption band center at 1075 cm^{-1}, 1033 cm^{-1}, and 948 cm^{-1} attribute to the Si-O stretching vibration mode observed by Zhu et al [15]. After compounding of PBAT with Wollastonite, the FTIR spectra of the composite does not show any obvious new absorption bands that suggests there was no chemical bonding occurred between polymer and filler matrix. This was evident that in the biocomposites the filler was physically blended with the polymer material, but the neat PBAT attains crystallinity which was reported by XRD analysis.

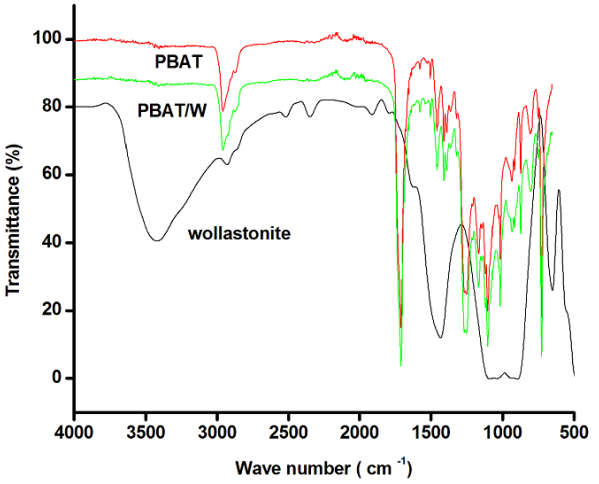

Figure -2 FTIR spectra of PBAT, PBAT/W (3 wt %) and Wollastonite powder

3.3 Mechanical properties

The tensile strength and elongation at break of PBAT and its biocomposites were shown in Figure 3 a & 3 b. Table 1 summarized the mechanical property of PBAT biocomposites i.e., tensile strength, stress at break and elongation at break values. Figure 3a shows the tensile strength increases by the addition of increased wollastonite with PBAT. The tensile strength and Figure 3b shows the elongation at break of pure PBAT is 10.50 MPa and 2800 % respectively. By adding wollastonite into polymer matrix tensile strength and elongation at break increased up to 5 wt % with 11.63 MPa and 6158 % respectively. Further, the increases of wollastonite content into the polymer matrix, the biocomposites showed reduced values of tensile strength and elongation at break. This is due to the absence of chemical bond interaction between PBAT and wollastonite particles which has been proved from FTIR results.

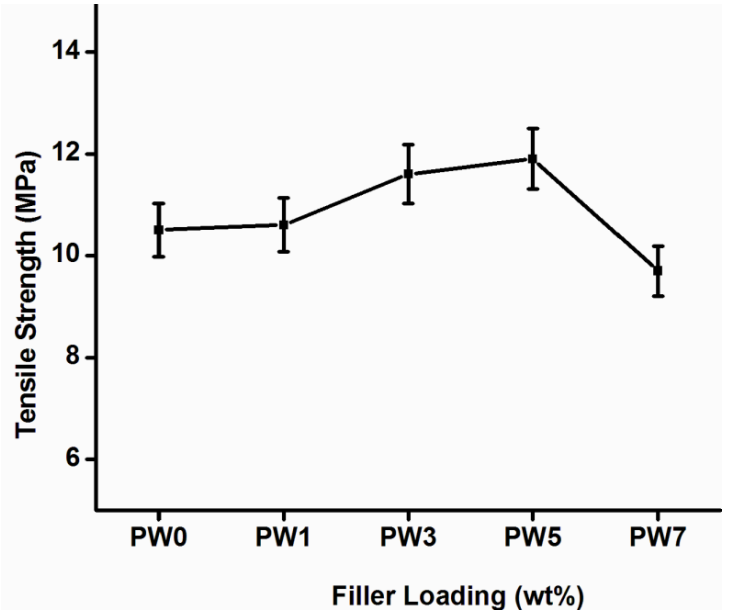

Figure-3a Tensile strength of PBAT and its composites

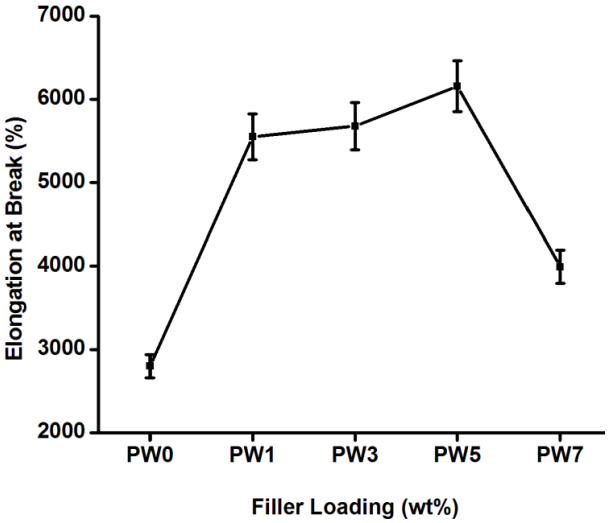

Figure-3b Elongation at break of PBAT and its biocomposites

Table 1: Mechanical property of PBAT biocomposites

Sample	Tensile strength (MPa)	Stress at break (MPa)	Elongation at break (%)
PW0	10.50	8.5	2800
PW1	10.64	12	5550
PW3	11.63	13	5678
PW5	11.9	13.5	6158
PW7	9.76	10	3992

3.4 Hardness testing

The hardness of the biocomposites was tested using a Durometer hardness tester. Table 2 summarized the hardness value for the PBAT and its biocomposites. It is evident that the PBAT/W biocomposites exhibit higher hardness value than the neat PBAT polymer. The increase in hardness can be explained as being associated with increase in the crystallinity of the biocomposites discussed in XRD analysis. The increase in the crystallinity explains that the molecules were more orderly arranged in polymer resulting in less displacement which leads to higher hardness values.

Table 2: Hardness of neat PBAT and PBAT/wollastonite compositions

sample	Hardness value (Shore A)
PW0	54
PW1	57
PW3	59
PW5	60
PW7	53

3.5 Thermal behavior

Figure 4 reports the TGA thermograms for PBAT biocomposites under nitrogen atmosphere. Thermal stability is an important performance of polymeric materials. The influence of the

filler addition content on the thermal stability of filled PBAT composites were measured by TGA thermograms. It is evident that the onset temperature and decomposition temperature of neat PBAT was noted at 391.09 ºC and 416.26 ºC respectively. The T_{onset} and $T_{maximum}$ values of PBAT biocomposites were reported in Table 3.The thermal stability of PBAT under nitrogen atmosphere was slightly improved by increasing the wollastonite weight fraction due to the barrier effect of filler towards polymer decomposition. The maximum decomposition values are non linear, while the amount of filler weight fraction increased. It is generally that the movement of the macromolecular chains of the matrix is blocked by the inorganic filler particles hence thermal stability should be improved slightly correspondingly.

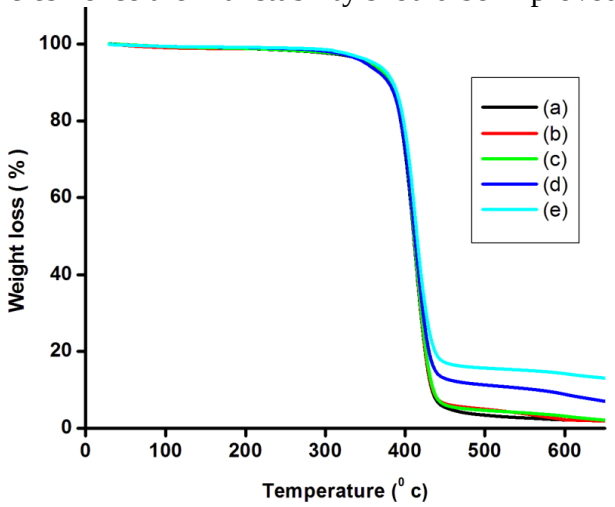

Figure-4 TGA of PBAT biocomposites a) neat PBAT b) PW1 wt % c) PW3 wt % d) PW5 wt % and e) PW7 wt %

Table 3: Thermal behavior of PBAT biocomposites

sample	T_{onset} (ºC)	$T_{maximum}$ (ºC)
PW0	391.09	416.26
PW1	392.49	411.44
PW3	393.40	415.06
PW5	392.86	413.24
PW7	393.48	413.85

3.6 Morphological Studies

Figure 5 shows the scanning electron micrographs of PBAT biocomposites with varying wollastonite content. In Figure 5a, the smooth surfaces with closed pores were observed for neat PBAT. In Figure 5b, the needle shaped (acicular) micrograph was seen for wollastonite powder. From figure 5 c, d, and e, it was seen that the wollastonite particles (1 wt % - 5 wt %) were uniformly dispersed and distributed the wollastonite particles throughout the polymer matrix without any agglomeration showing good interfacial adhesion. When the addition of wollastonite content is increased (7wt %), the dispersion and distribution is good but the wollastonite particles were aggregated in smaller portions on the surface of the polymer

matrix obviously seen in Figure 5f. This was evident that the size of the filler particles exposed to the surface of the matrix explains that there is no further effect of filler loading into polymer matrix. Moreover the interface between the filler particles and the polymer matrix is good which indicates that the compatibility of filler with surface of the matrix is good. Generally the uniform dispersion and distribution of filler particles into the polymer matrix promotes the crystallinity of polymer composite which proved by XRD analysis.

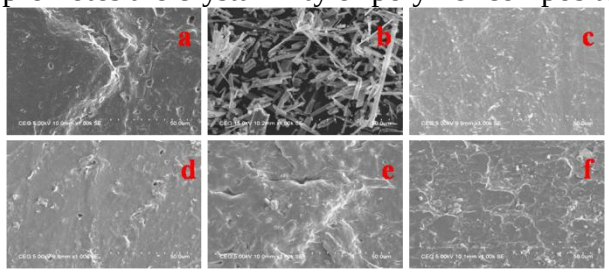

Figure-5 SEM micrographs of PBAT biocomposites a) neat PBAT b) Wollastonite powder c) PW1 wt % d) PW3 wt % e) PW5 wt % and f) PW7 wt %

3.7 Evaluation of in vitro bioactivity in SBF

Figure 6(a-d) shows the SEM micrographs of the PBAT and its composites after soaking in SBF for 5 days. It can be seen that the PBAT composites after soaking in SBF for 5 days explains that Figure 5c showed good formation of HA layer on PBAT/W (3 wt%) composite than neat PBAT and other compositions due to the good tensile strength and interfacial adhesion between PBAT and wollstonite filler. After increasing the wollstonite content, the formation of HA layer reduces which effects the bioactivity of the composites supported by cell viability test explained below.

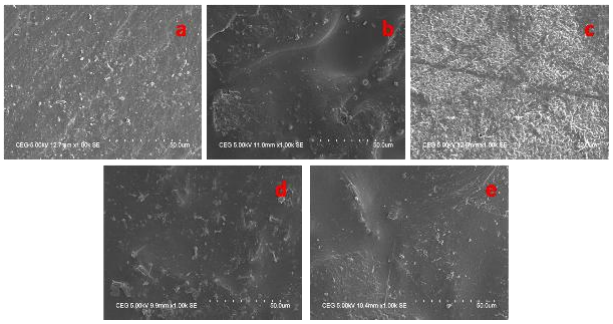

Figure -6 SEM images of PBAT and its composites after soaking in SBF for 5 days a) neat PBAT b) PW1 wt % c) PW3 wt % d) PW5 wt % and e) PW7 wt %

3.8 Cell viability (MTT assay)

MTT assay were conducted to PBAT biocomposites to test their cytotoxicity. This is an indirect method for cell viability and proliferation since the absorbance at 570 nm can be correlated to the number of cells. MG63 cells were co-cultured with PBAT and its composites for 24, 72 and 120 h and assayed for cell viability. The cell viability of PBAT biocomposites were shown in Figure 7. The cell morphology images of PBAT and its composites are shown in Figure 8 obtained by the optical inverted microscopy. The optical density values of biocomposites did not show any decrease of cell growth compared to the negative control, 24

h, 72 h and 120 h incubation periods of the cells. The result suggests that there is no significant toxic leachables in PBAT/W composites compared to neat PBAT. It can be seen that PBAT/W (3 wt %) composites has higher cell viability showing more number of cells at 120 h with same intervals compared to neat PBAT. Cell proliferation studies performed at different tine intervals showed that the cells continued to proliferate with the increase of culture time and cells increased considerably after compared to 24 h (1 day). The bioactive wollastonite plays an important role in the cell proliferation to the polymer composites when compared to neat PBAT. It is presumed that the presence of silica and calcium ions in wollastonite acts as bioactive agent and produce a favorable surface environment for cell growth leads to good compatibility in polymer composite. MTT results supported that the PBAT/W (3 wt %) composites are found to be highly biocompatible and further addition of filler shows reduced cell viability due to the absence of filler effect into polymer matrix leads to the unfavorable condition for cell growth.

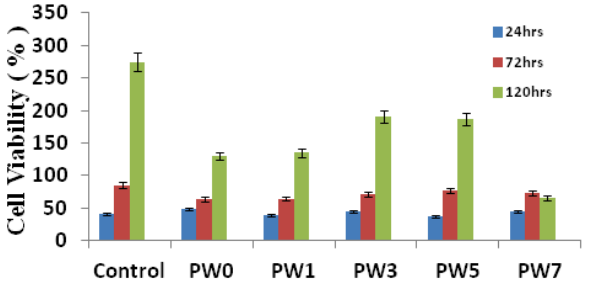

Figure-7 Cell viability for PBAT biocomposites

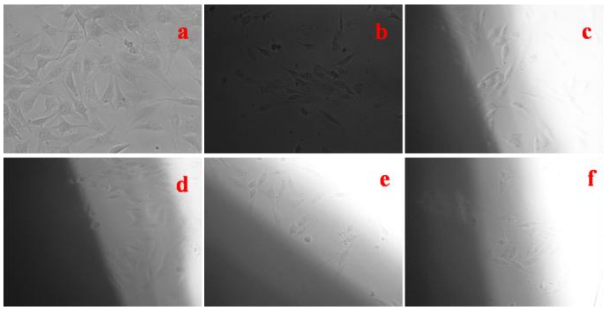

Figure-8 Cell morphology for PBAT biocomposites a) positive control b) neat PBAT c) PW1 wt % d) PW3 wt % e) PW5 wt % and f) PW7 wt %

Conclusion

PBAT/wollastonite biocomposites were developed and evaluated for its thermal, mechanical properties and in vitro cell viability. The results reported in this paper demonstrated that the tensile properties were influenced by the distribution of wollastonite in the PBAT biocomposites. PBAT with 5 wt % wollastonite shows a uniform distribution of particles in PBAT matrix. The highest tensile strength and hardness value was attained at 5 wt % of filler loading compared to neat PBAT. By adding wollastonite filler to the PBAT polymer, it attains crystallinity with homogeneous dispersion throughout the polymer matrix even though it is

physical blending proved by XRD, SEM and FTIR analysis. TGA reveals that the incorporation of wollastonite into neat PBAT slightly increases the thermal stability. SBF immersion results showed that large amount of HA formed on the scaffolds after 5 days of soaking, which suggested that the composite (3 wt%) had good bioactivity by incorporating wollstonite in PBAT matrix . Previous study have shown that wollastonite has excellent bioactivity, and could induce growth of HA when soaking in SBF [16]. The in vitro cell culture test proved that the PBAT/wollastonite composites showed good biocompatibility for the growth of human osteosarcoma cells. However, the optimum value of 3 wt % wollastonite shows good tensile strength with good cell viability in short incubation periods as favorable composite among others. This result indicated that PBAT/W biocomposites without any modifier can be used as potential biomaterial candidates in biomedical field. But for further these biocomposites can be studied by blending with other natural or synthetic polymers in presence of modifiers even to get better properties which can be use in medical applications.

References

[1]K. Fukushima, M.H .Wu, S. Bocchini, A. Rasyida, M. C. Yang, Mater Sci Eng C. 32(2012)1331-1351.
[2] J.H. Chen, C.C Chen, M.C. Yang, J. Polym. Research 18 (2011) 2151-2159.
[3]K. Fukushima, A. Rasyida, M.C. Yang, J. Polym. Res. 20(2013) 302
[4] C.S. Wu, Carbohydrate polymers 87 (2012) 1249-1256.
[5]Y. Someya, K. Naoto, M.Shibata, J. Appl. Polym. Sci. 106 (2007) 730-736.
[6]K. Fukushima, A. Rasyida, M.C. Yang, J.Appl. clay Sci. 80 (2013)291-298.
[7] F.E. Zhang, C.E. Jiang, L.E. Kaili, L. Jianxi, J Mater. Sci. Mater Med (2007)
[8] A. R. Boccaccini, M. Erol, W. J. Stark, D. Mohn, Z. Hong, J.F. Mano, Comp.Sci.Tech. 70 (2010) 1764–1776.
[9]V. M. Correlo, L. F. Boesel, M. Bhattacharya, J. F. Mano, N. M. Neves, R. L.Reis, Macromolecular materials and engineering (2005).
[10] H. Zhu, J. Shen, X. Feng, H. Zhang, Y. Guo, J. Chen, Mat. Sci. Eng. C 30 (2010) 132–140.
[11]X. Li, J. Chang, J.Mat. Sci. 16 (2005) 361– 365.
[12]M. Risbud, D. N. Saheb, J. Jog, R. Bhonde, Biomaterials 22 (2001)1591-1597.
[13] L. Xu, R. Sun, L.F. Zhang, D.J. Yang, Y.F. Tan, C.D. Xiong, Iranian Polym. J. 17 (2008) 407-418.
[14] J. Yang, G. X. Shi, J. Z. Bei, S . G. Wang, Y. Lcao, Q. X. Shang, G. H. Yang ,W. J. Wang, J Biomed. Mater. Res. 62 (2002) 438.
[15] H. Zhu, J. Shen, X. Feng, H. Zhang, Y. Guo, J. Chen, Mat. Sci. Eng. C 30 (2010) 132–140.
[16] P . Siriphannon, S . Hayashi, A. Yasumori, K. Okada, J. Mater. Res. 14 (1999) 529.