# CHAPTER IV
RESULTS AND DISCUSSION

Tissue engineering is a new technique to repair tissue defects and regenerate new tissue which connected with the development of suitable scaffold materials. In bone tissue engineering need to develop a suitable bone scaffold with sufficient porosity and mechanical strength to allow cell growth results in good integration with surrounding tissue. The technique used to create a scaffold for tissue engineering must give a scaffold with an interconnected structure of well distributed pores with appropriate sizes for guide cell attachment, proliferation and tissue regeneration which without affecting the biocompatibility of the material. Solvent casting and particulate leaching is a method for producing the porous structures which can be used for bone tissue engineering (Ciapetti et al., 2003). In the leaching of soluble particulates, porosity can be controlled by variation of the amount of leachable particles and the pore size of the porous structure can be adjusted by using particles of different sizes. Polycaprolactone (PCL) is a type of biodegradable polymer with excellent biocompatibility. It has been tested as scaffolding materials for cartilage (Li et al., 2005) and bone cell (Coombes et al., 2004). PCL is a good candidate to be used in bone scaffolding applications, due to its slow degradation rate and sufficient mechnical properties to serve as a scaffold. Both mechanical and biological properties of the PCL scaffold, such as osteoconduction and osteoinduction can be improved by adding bioactive materials. Hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$) is a bioceramic which has the same chemical composition (Ca/P = 1.67) as bone mineral. A composite containing both, a bioceramic and a biodegradable polymer required both the osteogenic characteristic of the ceramic, and the biodegradable property of the polymer. For better osteoconductivity and osteoinductivity of the composites, some researches investigated the potential application of ipriflavone on the healing process of injured bone. Ipriflavone (7-isopropoxyisoflavone) (IP), a synthetic flavonoid, has important effects on bone metabolism. Some studies have been reported that IP has the effect on the proliferation and differentiation of rat osteoblast like cells and human periodontal

ligament fibroblasts (Kakai *et al.*, 1992). IP can represent a potential therapeutic tool to promote repair of injured perialveolar bone (Martini *et al.*, 1998).

4.1 Synthesis of Hydroxyapatite and Characterization of Particles

Hydroxyapatite (HAp) could be synthesized by DCPD ($CaHPO_4.2H_2O$). The hydrolysis of DCPD is based on the reaction in this equation (Wutticharoenmongkol *el at.*, 2006).

$$10\ CaHPO_4.2H_2O + 18H_2O \longrightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O + 12H^+ + 4PO_4^{3-}$$
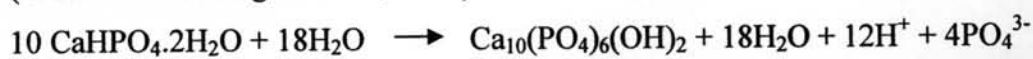
A FTIR spectrum of the as-synthesized HAp powder after annealing at 800°C is shown in Figure 4.1. The two bands at 632 and 3572 cm^{-1} belonged to the vibration of hydroxyl. Those bands at 1045, 1091 and 962 cm^{-1} were the characteristic bands of phosphate stretching vibration, while the bands at 602 and 570 cm^{-1} were due to phosphate bending vibration (Choi *et al.*, 2004).
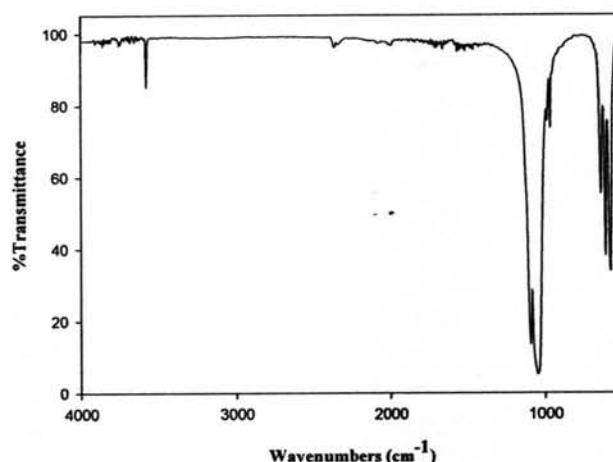

Figure 4.1 FT-IR spectrum of the as synthesized HAp powder.

A WAXD pattern of the as-synthesized HAp powder is shown in Figure 4.2. The peaks at the diffraction angles of about 26, 32, 33, and 53° corresponded to the (002), (300), (211), and (004) refraction planes of HAp. HAp was reported to crystallize in the hexagonal unit cell with axes a=b=9.418 Å and c=6.884 Å in the $P6_3/m$ space group (JCPDS card no. 9-432) (Shin *et al.*, 2004). The presence of other

peaks suggested contamination by other **phosp**hate compounds. From these measurements, the powder was proved to be **hydroxy**apatite.
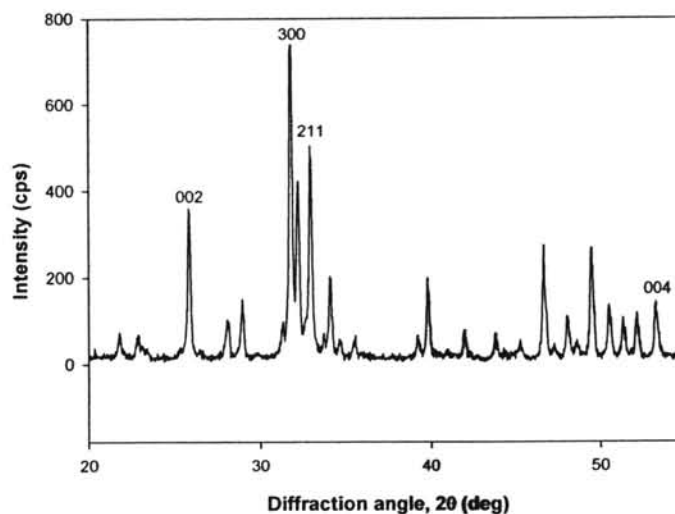

Figure 4.2 WAXD pattern of the as synthesized HAp powder.

The morphology of the obtained HAp **powder** was observed by SEM as shown in figure 4.3. The size of the particles **could** be measured directly from the SEM image and the average value was 10 μm (n=**100**)
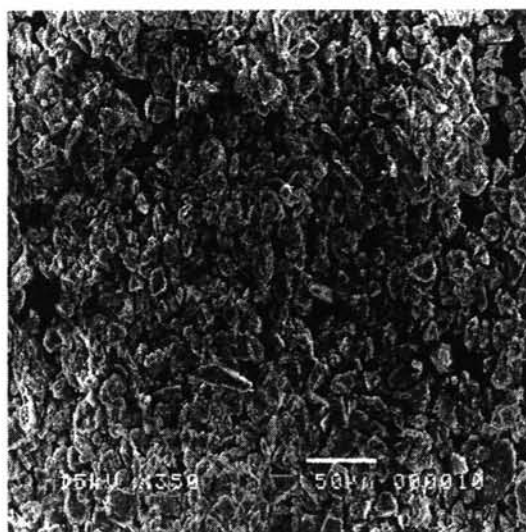

Figure 4.3 SEM image of the as synthesized HAp powder.

4.2 Preparation and Characterization of Composite Scaffolds

Solvent casting and particulate leaching process was used to prepare porous scaffolds for tissue engineering. Sucrose, as the porogen, was used to generate an open pore structure. The mixture of polycaprolactone, hydroxyapatite, ipriflavone and porogen were molded into cylindrical samples and then soaked in water for leaching. Pores were connected via the dissolving of sucrose porogen prior to formation of a three dimensional scaffolds, as shown in figure 4.4. In this work, a number of parameters that effect on the porous scaffolds were studied.
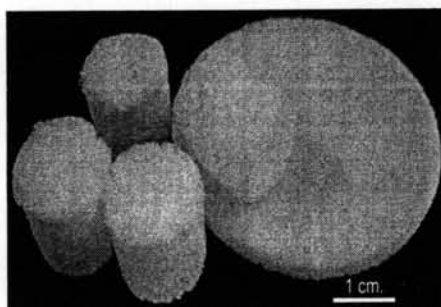

Figure 4.4 The structure of the porous scaffolds.

4.2.1 Effect of PCL to Sucrose Ratios on Porous Scaffolds

The effect of PCL to sucrose ratios on morphological appearance of the porous PCL scaffolds at a given sucrose size (400-500 μm) is shown in figure 4.5. The pores created by leaching away the sucrose particles were uniformly distributed and interconnected with average pore diameters in the range of 400-500 μm. The pore interconnectivity of the scaffold was found to increase with increasing the amount of sucrose.
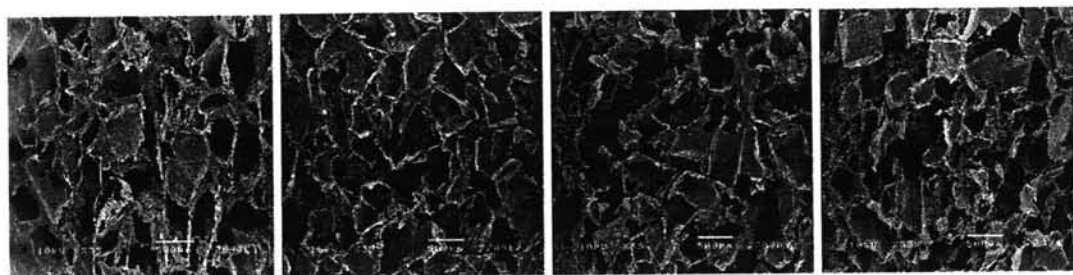
Figure 4.5 Morphological changes in the porous scaffolds at various PCL to sucrose ratios (scale bar = 500 μm and magnification = 35X).

(a)1:5   (b)1:7.5   (c)1:10   (d)1:15

Pore size and porosity were important parameters for synthetic biodegradable scaffolds. These parameters would affect the transport processes through the scaffolds as well as the structural properties of the scaffolds. The porosity of the scaffolds would affect the movement of nutrients and wastes to and from the cells in the scaffolds respectively (Shum *et al.*, 2004). The effect of PCL to sucrose ratios on the porosity and density of the porous scaffold are shown in Figure 4.6 and 4.7, respectively. There was a difference in porosity and density between sucrose loading. The porosity of the scaffolds increased with increasing sucrose loading. Over the sucrose range studied the values of porosity estimated from 80% to 93%. And the density of the porous scaffolds decreased with increasing in sucrose contents, which estimated from 0.17 ± 0.01 g/cm^3 to 0.08 ± 0.02 g/cm^3. Therefore, the PCL to sucrose ratios have influence on the porosity and density of the scaffolds. As reported by Gong *et al.*, prepared zein scaffolds in which the NaCl in size of 150-220 μm has been leached out. The porosity of zein scaffold increased from $76.0 \pm 1.5\%$ when polymer to NaCl ratio was 1:1.4, to $78.4 \pm 1.5\%$ when the ratio increased to 1:2.5. And the density of zein scaffold decreased from 0.292 ± 0.018 to 0.263 ± 0.018 g/cm^3 with increasing of polymer to NaCl ratio of 1:1.4 to 1:2.5.
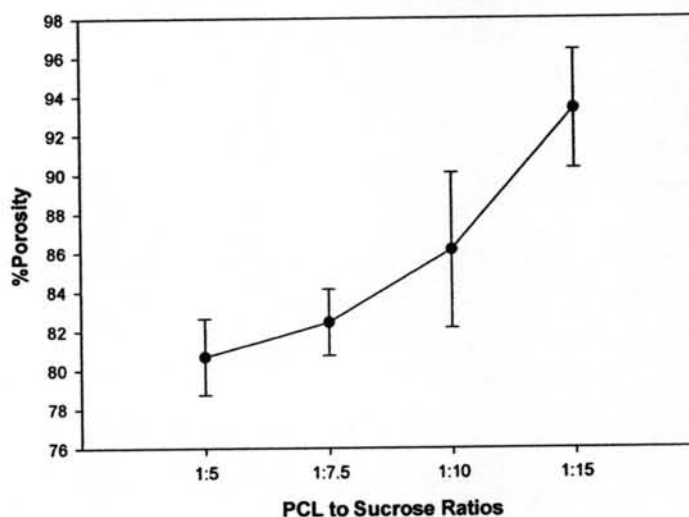
Figure 4.6 Porosity of porous scaffolds at various PCL to sucrose ratios (Raw data are shown in Appendix A).
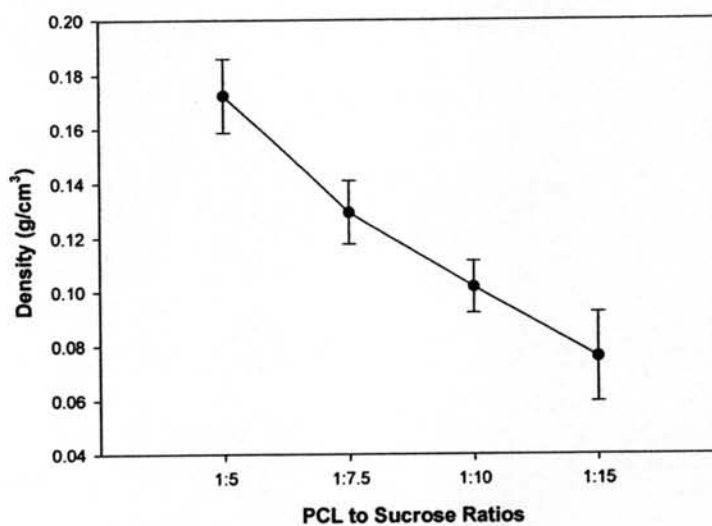

Figure 4.7 Density of porous scaffolds at various PCL to sucrose ratios (Raw data are shown in Appendix A).

The compressive modulus of scaffolds were measured in the dry state after fabrication, which showed that leaching method in this study allowed for the fabrication of porous scaffolds with good mechanical properties. The effect of PCL

to sucrose ratios on compressive modulus of the porous PCL scaffolds is shown in figure 4.8. The highest compressive modulus for the scaffold with 1:5 was 0.9273 ± 0.0576 MPa, and the lowest value for the scaffold with 1:15 was 0.0536 ± 0.0030 MPa. With the increasing of sucrose amount, compressive modulus of porous scaffolds decreased. The higher porosity corresponded to the lower compressive modulus, which was consistent with the inverse tendency between porosity and mechanical properties.
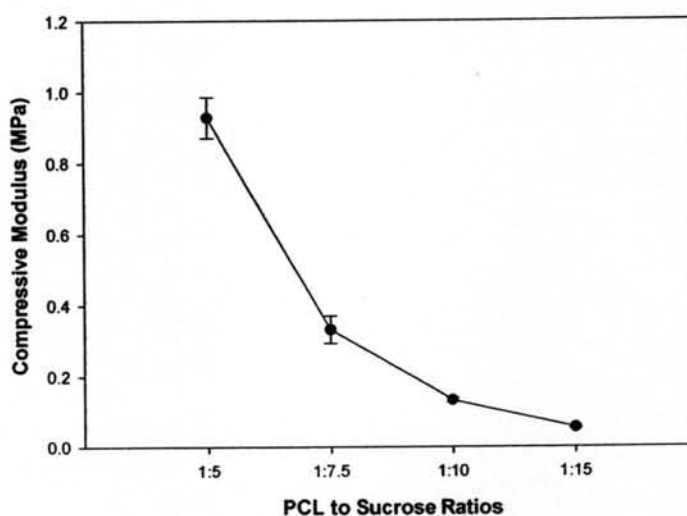

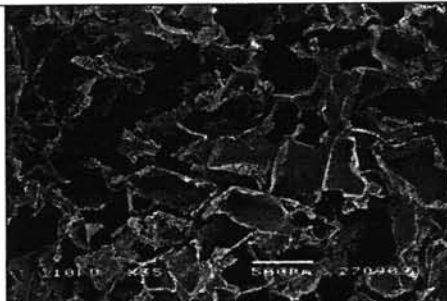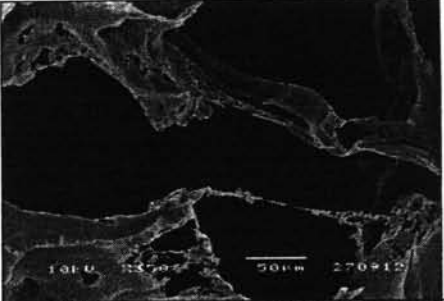**Figure 4.8** Compressive modulus of porous scaffolds at various PCL to sucrose ratio (Raw data are shown in Appendix A).
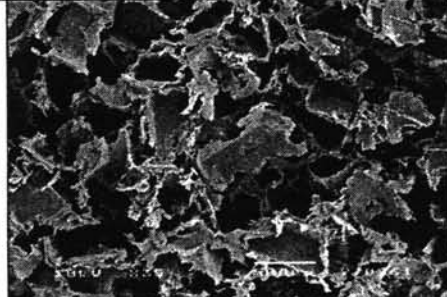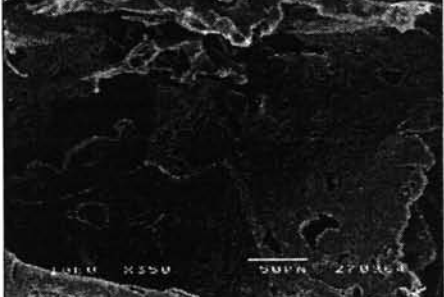
In all of PCL to sucrose ratios investigated, the average pore diameter of the scaffolds ranged of 400-500 μm. 1:10 ratio gave the porous scaffold with compressive modulus of 0.1317 ± 0.0059 MPa. It could be used in soft tissue engineering applications which the compressive modulus was less than 10 MPa (Kothapalli *et al.*, 2005). Murugan *et al.* reported the biomechanical properties of bone. The cancellous bone (spongy bone) has the density of 0.1-1.0 g/cm^3 and compressive strength of 7-10 MPa. Cattaneo *et al.* exhibited that the material properties of periodontal ligament (PDL) should have the elastic modulus ranging from 0.07-100 MPa.
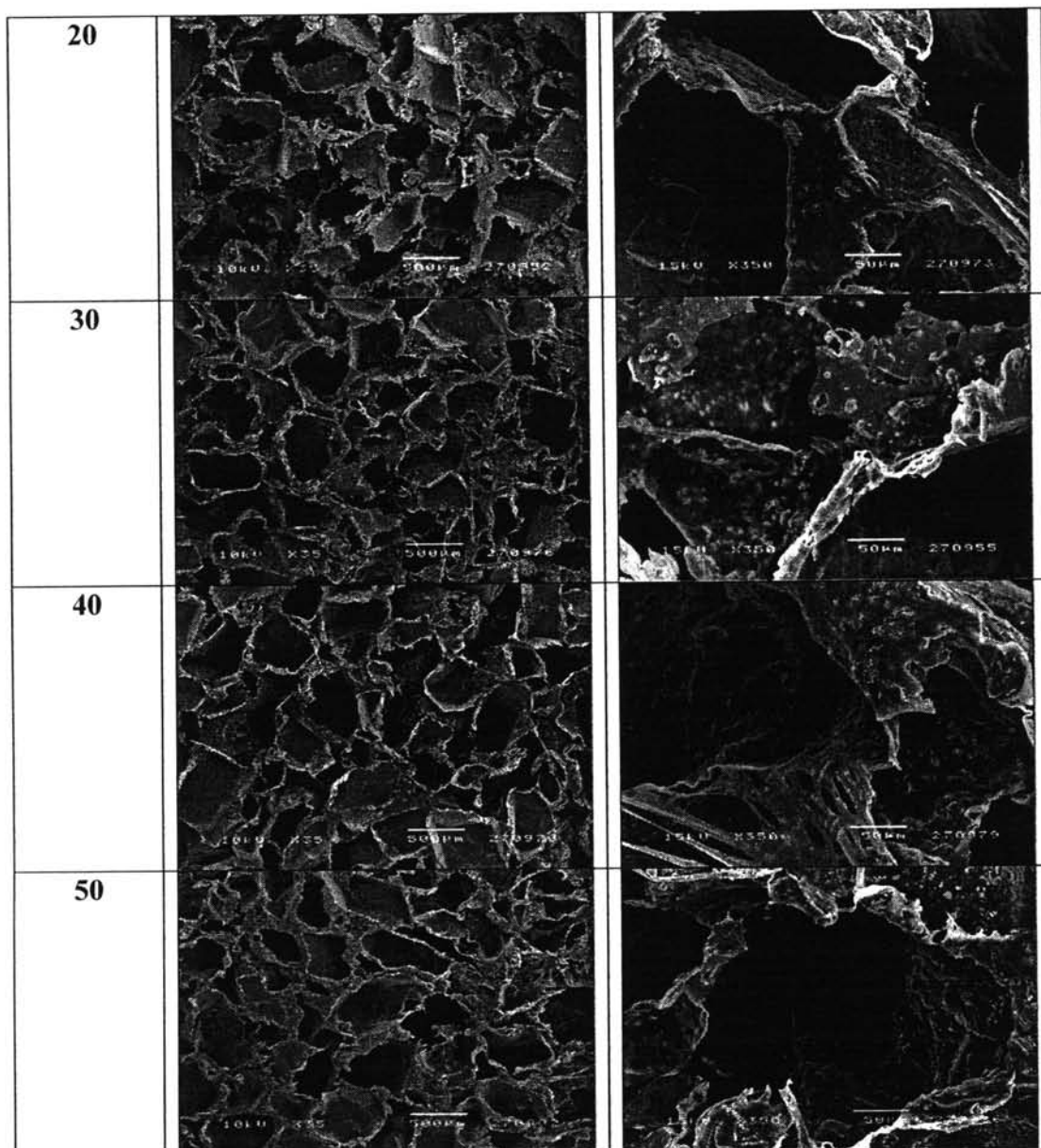
In addition, among the various PCL to sucrose ratios investigated, 1:10 was chosen as the condition to further study due to it suitable morphological and mechanical properties for bone regeneration scaffolds.

4.2.2 Effect of Hydroxyapatite Amount on Porous Scaffolds

Table 4.1 shows the selected SEM images at two different magnifications of the microstructure of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio). Apparently, the open pore structures and well pore interconnectivity were obtained at all HAp composition investigated. The SEM images with the higher magnification showed that the HAp particles 10 µm in size appeared to be uniformly distributed within the polymer matrix. The increasing the HAp contents caused the surface roughness of porous scaffolds increase. Therefore, the PCL scaffolds containing HAp can increase the chances of osteogenic cells to make contact with the bioactive ceramics, which enhances osteoblast differentiation and growth.

Table 4.1 SEM images of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio)

HAp (%wt of PCL)	Magnification	
	35X	**350X**
0		
10		

The adhesion of HAp to the PCL matrix can be expected to the weak hydrogen bonding formed between the –OH groups of HAp and =O sites of the PCL polymer. Figure 4.9 shows the FT-IR spectrum results of HAp, PCL and PCL containing HAp. Table 4.2 shows the characteristic infrared bands of PCL (Elzubair et al., 2006). In PCL containing HAp, the hydroxyl stretching and bending bands were observed not strong at 3572 and 632 cm^{-1}, respectively. This is a strong indicator confirming the formation of the HAp phase. The vibration bands of OH$^-$ at 3572 and 632 cm^{-1} decreased in intensity, showing the decrease in hydroxyl group. Some new bands can be observed in the spectrum. The carbonyl stretching region

1780–1680 cm^{-1} of PCL containing HAp can be due to the hydrogen bonding between HAp and PCL (Choi *et al.*, 2004).
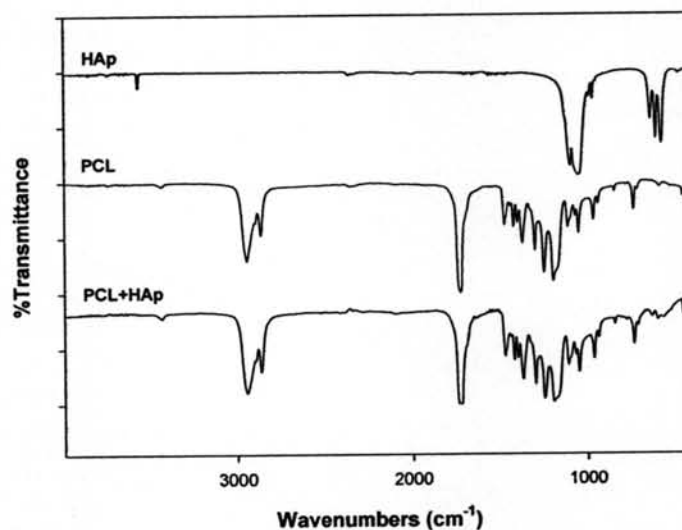

Figure 4.9 FT-IR spectrums of HAp, PCL and PCL scaffolds containing HAp.

Table 4.2 Characteristic infrared bands of PCL (Elzubair *et al.*, *2006*)

Position (cm^{-1})	Vibrator	Abbreviation
2949	Asymmetric CH$_2$ stretching	$v_{as}(CH_2)$
2865	Symmetric CH$_2$ stretching	$v_s(CH_2)$
1727	Carbonyl stretching	$v(C=O)$
1293	C-O and C-C stretching in the crystalline phase	v_{cr}
1240	Asymmetric COC stretching	$v_{as}(COC)$
1190	OC-C stretching	$v(CO\text{-}C)$
1170	Symmetric COC stretching	$v_s(COC)$
1157	C-O and C-C stretching in the amorphous phase	v_{am}

The effect of HAp contents on the porosity and density of the PCL scaffolds containing HAp is shown in Figure 4.10 and 4.11. There was a difference in density, but there was not a difference in porosity. The porosity of the PCL scaffolds containing HAp was about 85% to 87%. The density of the scaffolds

increased with increasing HAp contents which estimated from 0.10 ± 0.01 g/cm^3 to 0.13 ± 0.01 g/cm^3. For comparative studies, Kothapalli *et al.* prepared PLA-HAp scaffold by using solvent casting and salt leaching technique. Density of the PLA scaffold was increased from 0.8 to 1.3 g/cm^3 with increasing in HAp content from 0 to 50 wt%. The porosity of the PLA scaffold incorporated with 0-50wt%HAp exhibited from the trend line fell from 92% to 86%.
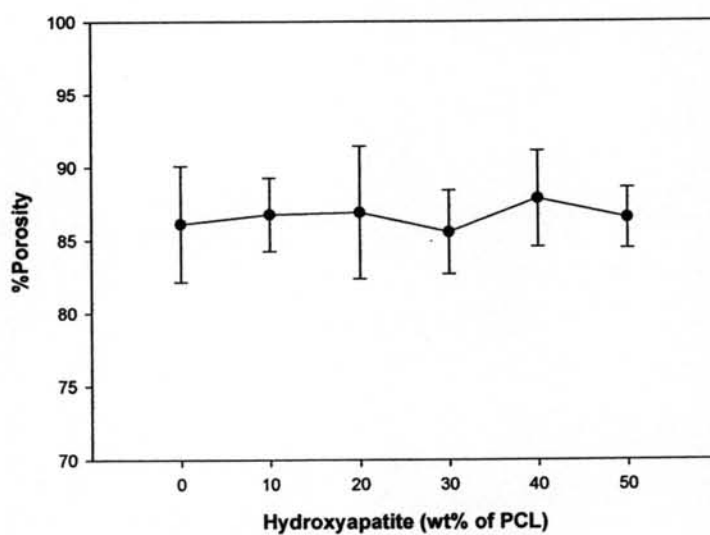

Figure 4.10 Porosity of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio) (Raw data are shown in Appendix B).
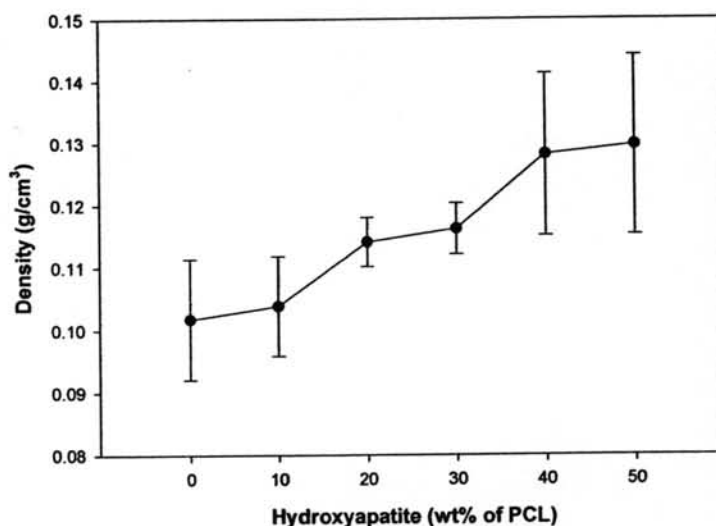
Figure 4.11 Density of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio) (Raw data are shown in Appendix B).

The mechanical properties of the scaffolds depend on the interactive parameters such as pore size, porosity, processing methodology used, particle size of the HAp and distribution of the molecular weight of the PCL matrix (Baji *et al.*, 2006). The mechanical testing results for the scaffolds with varying HAp contents are summarized in figure 4.12 and raw data are shown in Appendix B. When certain HAp particles disperse in the PCL matrix, it obstructed the molecular movement of the matrix. This explains why the compressive modulus of the PCL composite containing HAp was higher than for the pure PCL (Chen *et al.*, 2005). The increase in mechanical properties of the scaffolds with increasing HAp contents might due to interaction between the matrix and filler. The sample without HAp has the lowest compressive modulus of 0.1314 ± 0.0143 MPa, and the highest value for the scaffold with 50%wt HAp was 0.3194 ± 0.0255 MPa. The measured compressive modulus of the prepared scaffolds is rather low compared to that of human bone (cortical bone of 7-30 GPa). This might due to the highly porous structure of the fabricated scaffolds and poor mechanical properties of the PCL itself, even after reinforcement with HAp. Therefore, the fabricate scaffolds could be applied in non load bearing parts. There are numerous reports describing the improvement of the mechanical properties

of polymers by adding inorganic ceramic, such as hydroxyapatite. Kothapalli *et al.* reinforced porous PLA scaffold with HAp and found that the compressive modulus of the scaffold were increased from 4.7 to 9.8 MPa with increasing HAp contents in the range of 0-50 wt% in the scaffold.
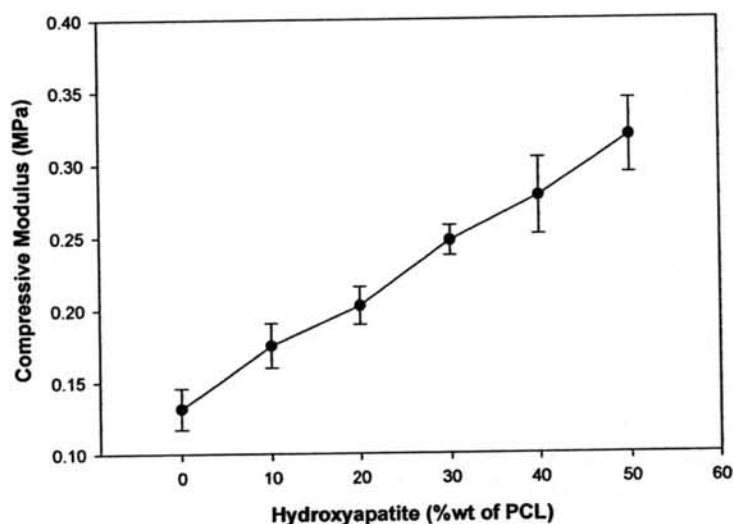

Figure 4.12 Compressive modulus of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio) (Raw data are shown in Appendix B).

The yield of the HAp in the composite containing 10, 20, 30, 40 and 50 wt% were analyzed by TGA as shown in figure 4.13. The endothermic peak at 66°C was the melting of PCL. The endothermic peak at 420°C was the decomposition of PCL. The actual HAp contents can be obtained by taking a ratio of the weight at 750°C to the composite weight at 220°C (Choi *et al.*, 2004). The actual content of the HAp in the composite corresponding to a theoretical composition of are shown in figure 4.14. The actual contents of HAp in PCL containing 40wt%HAp was 29.19%. This indicated that some HAp can be removed from the PCL scaffold in preparation process. For comparative studies, Choi *et al.* synthesized the HAp and PCL composite by a colloidal non aqueous precipitation technique and found that the yield of the reaction was approximately 86%. Base on this, the actual amount of HAp in the composition of 40 wt% was 34.5wt%.
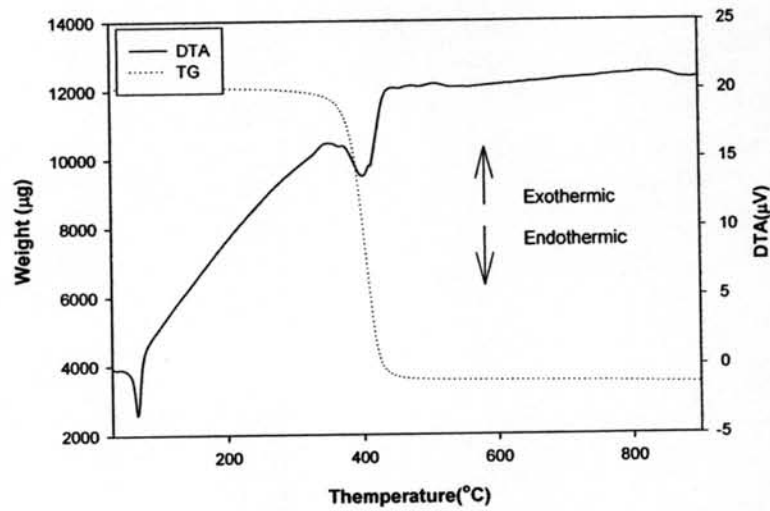
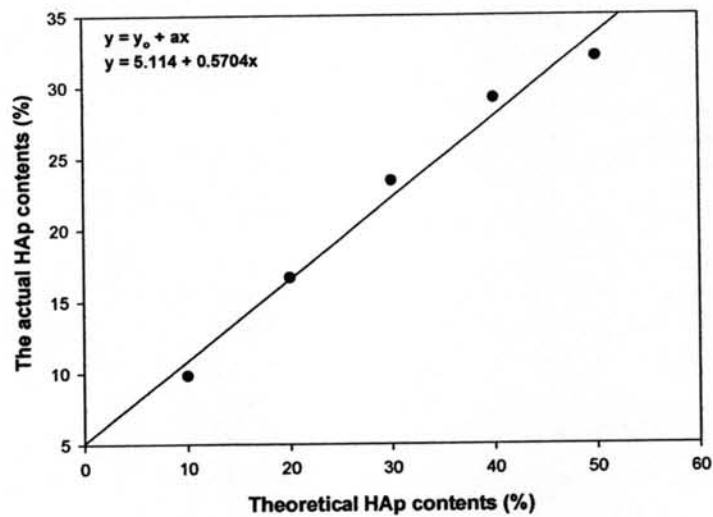**Figure 4.13** TGA and DTA thermographs of the porous PCL scaffolds containing HAp.

Figure 4.14 The actual and theoretical HAp contents in the composite from TGA thermographs.

Thermal properties of the PCL scaffolds containing HAp were investigated by DSC. In DSC, the objective for each cooling or heating run is that first heating is to observe the melting behavior of the original crystalline entity of the ·

sample, cooling is to observe the ability of the sample to crystallize when it was subjected to a constant cooling scan and second heating is to observe the melting behavior of the crystalline entity of the sample which was formed during the cooling scan. PCL is a semi-crystalline polymer which reveals three thermal transitions. The first order transition at about 60°C correspond to a melting endothermic, the second order glass transition at -60°C and the exothermic crystallization peak around 25°C. The thermal properties of PCL and DSC thermographs exhibit in figure 4.15 to 4.17. From the thermographs, the melting temperature of the original crystalline ($T_{m,o}$) was about 62°C. With regards to the crystallinity, the apparent enthalpy of fusion (ΔH_f) as obtained from first heating was used to calculate the apparent degree of crystallinity ($\chi_{c,PCL}^{DSC}$). The degree of crystallinity of PCL scaffold was increased by the presence of HAp. In the subsequent cooling, the crystallization peak temperature ($T_{m,c}$) of PCL scaffold was increased by the presence of HAp, suggesting modest interaction between PCL and HAp. Clearly, the presence of the HAp was effect to the crystallization of PCL during cooling appreciably. In comparative studies, Chen *et al.* studied the interfacial interaction of PCL and HAp by using DSC. They found that the melting temperature (T_m) and crystallization temperature (T_c) of PCL were 58.4°C and 36.8°C, respectively. With 20wt%HAp, T_m and T_c of PCL were 61.1 °C and 41.8 °C, respectively. The increased in T_c may suggest that HAp facilitates the crystallization of PCL under cooling, acting as a nucleating agent. Therefore, the introduction of HAp into PCL chains influenced the crystallization of PCL. In the second heating, the melting temperature of scaffold was about 55°C. All values of the DSC analyses are summarized in table 4.3.
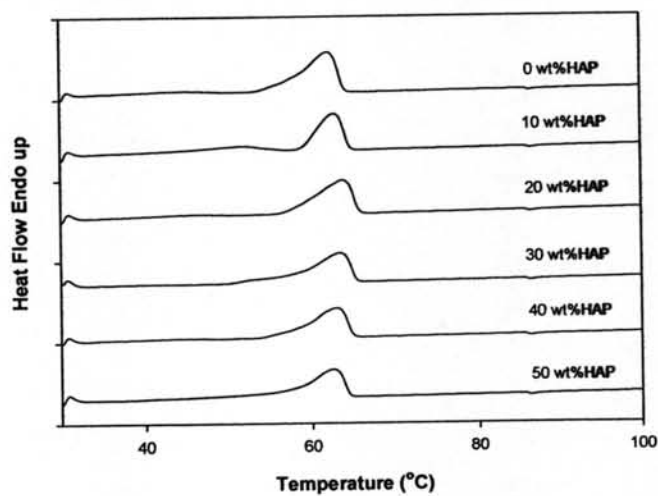
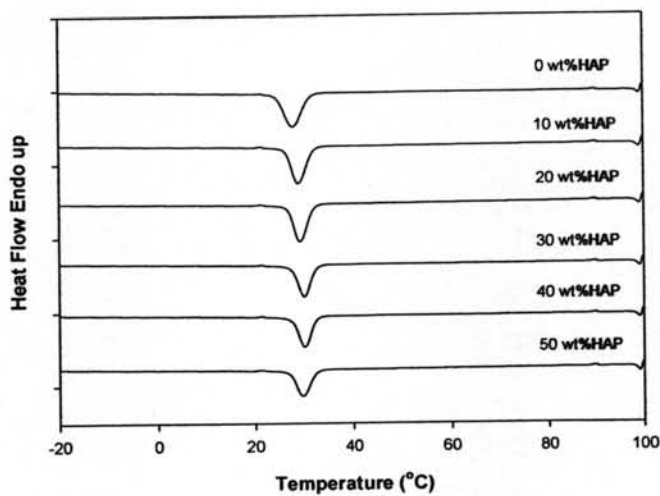**Figure 4.15** DSC thermographs of the porous PCL scaffolds containing 0-50 wt% HAp during first heating.

Figure 4.16 DSC thermographs of the porous PCL scaffolds containing 0-50 wt% HAp during cooling.
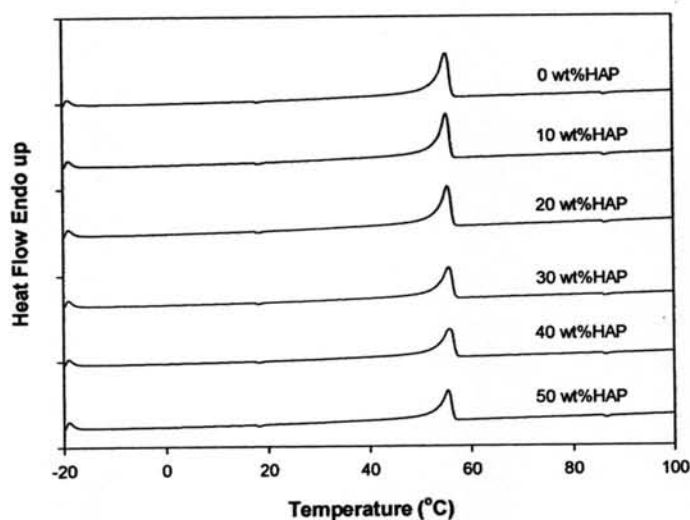
Figure 4.17 DSC thermographs of the porous PCL scaffolds containing 0-50 wt% HAp during second heating.

Table 4.3 T_m, T_c and %crystalline of the porous PCL scaffolds containing 0-50 wt% HAp

Theoretical HAp (% wt of PCL)	Actual HAp (% wt of PCL)	$T_{m,o}^a$ (°C)	$T_{m,s}^a$ (°C)	$T_{m,c}^a$ (°C)	ΔH_f^a (J.g^{-1})	$\chi_{c,PCL}^{DSC}$ [b]
0	0.00	62.17	55.37	27.80	56.82	40.79
10	9.80	62.83	55.37	28.80	52.05	41.42
20	16.60	63.83	55.53	29.13	48.33	41.60
30	23.40	63.33	55.70	29.97	50.83	47.63
40	29.20	63.00	55.70	29.97	44.47	45.09
50	32.10	62.50	55.37	29.47	44.02	46.55

[a] These data were determined by DSC: $T_{m,o}$, apparent melting peak temperature of the original samples, obtained from first heating; $T_{m,c}$, apparent crystallization peak temperature of the samples, obtained from cooling; $T_{m,s}$, apparent melting peak temperature of the samples subjected to second heating, obtained from second heating and ΔH_f, apparent enthalpy of fusion, obtained from first heating.

[b] $\chi_{c,PCL}^{DSC}$, apparent degree of crystallinity, calculated from $\dfrac{\Delta H_f / \Delta H_f^\circ}{\%wtPCL}$. ΔH_f^0 is the ΔH_f value of the polymer crystal. The ΔH_f^0 values of PCL are reported to be 139.3 J/g (Jenkins *et al.*, 2003). %wt of PCL is the actual weight of PCL in the composites.

WAXD patterns of PCL composites containing 10, 20, 30, 40 and 50 wt% HAp are shown in figure 4.18. The WAXD pattern of PCL showed the strong peak at 21.4 and 23.8° corresponding to crystalline or highly ordered chain folding characteristic of PCL, but in the patterns of 10-50 wt% HAp shows sharper peaks at 26, 32 and 33° corresponding to crystalline HAp. The intensity of HAp peaks at 26, 32 and 33° increased with increasing HAp content, indicating a high crystalline HAp phase or high crystallinity of the composite. Therefore, the crystalline structure of the composite corresponded to HAp contents.
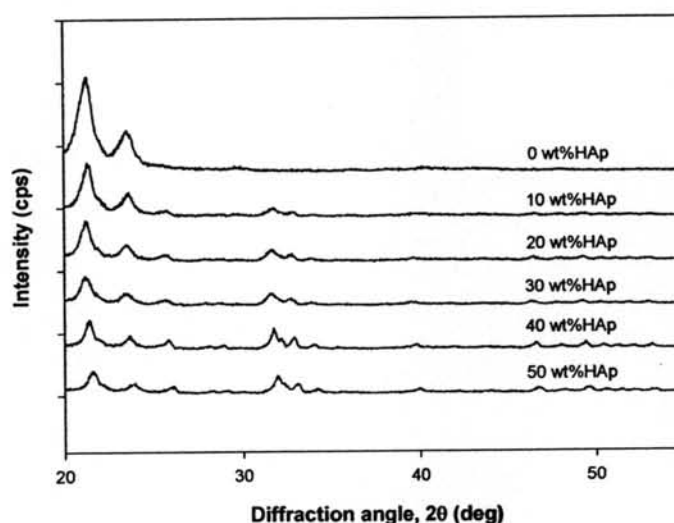
Figure 4.18 WAXD patterns of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio).

Water absorption was measured as the assessment of the wettability of PCL scaffolds containing HAp. Being aliphatic polyester, PCL is highly hydrophobic polymer. Immersion of the scaffolds in water resulted in an increase in mass within 1, 3 and 5 min as shown in Figure 4.19. It was observed that there was a mass increase of $231.08 \pm 9.52\%$ for pure PCL scaffolds while the mass of the scaffolds with 50 wt% HAp increased by $174.14 \pm 23.52\%$ at 5 min. In studies on PLA-HAp scaffold, Kothapalli *et al.* immersed the scaffold in water for 24h. They found that there was the mass of water in the scaffold decreased with increasing the HAp

contents in the scaffold. In pure PLA scaffold, the mass increase was 540%. With 50 wt% HAp, the mass increase was 274%.
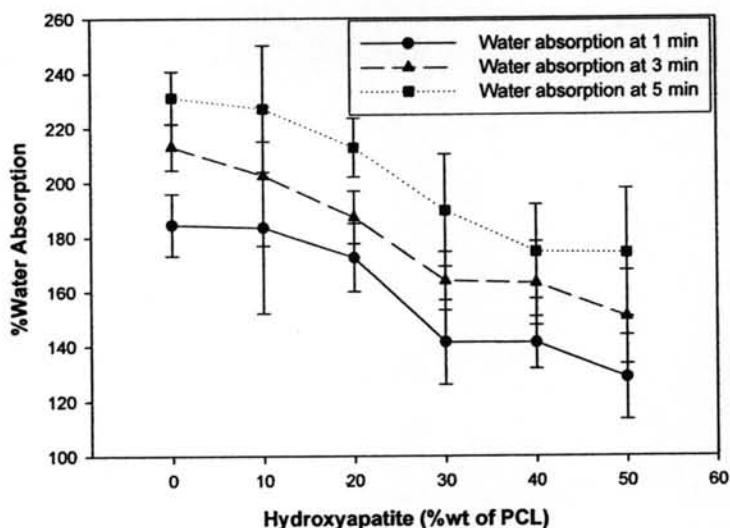

Figure 4.19 Water absorption of the porous PCL scaffolds containing 0-50 wt% HAp with 1:10 (PCL to sucrose ratio) (Raw data are shown in Appendix B).

4.2.3 Effect of NaOH Concentrations on Porous Scaffolds

In the application, bone tissue engineering needed the hydrophilic polymer to form the scaffolds. One possible approach to improve hydrophilicity to PCL was hydrolysis reaction by alkali treated PCL. In this work, the porous PCL scaffolds with 1:10 (PCL to sucrose ratio) were immersed in NaOH solution. When the NaOH concentrations were increased, the porous PCL scaffolds with higher water absorption were obtained (figure 4.20). This evidence can be explained by the increased in NaOH concentrations caused the polymer chain disturbed which increased the density of hydrophilic terminal carboxyl and hydroxyl groups.
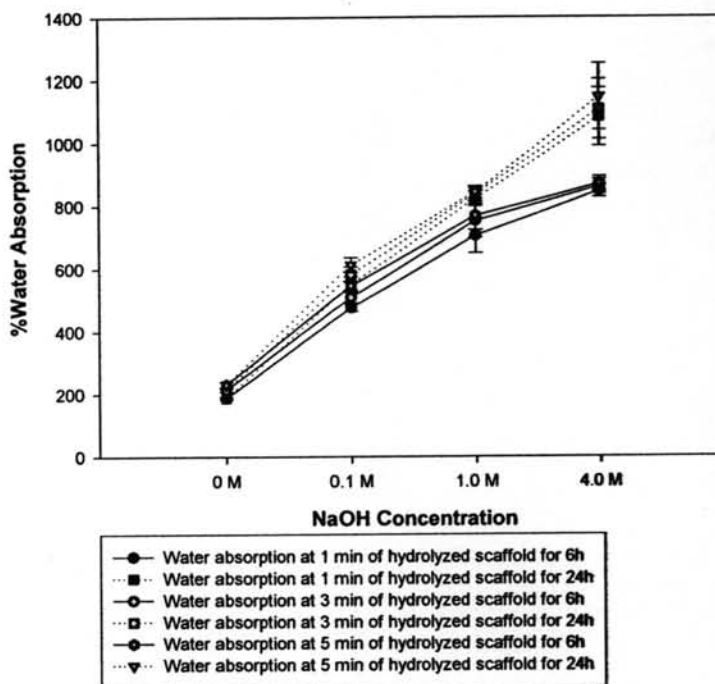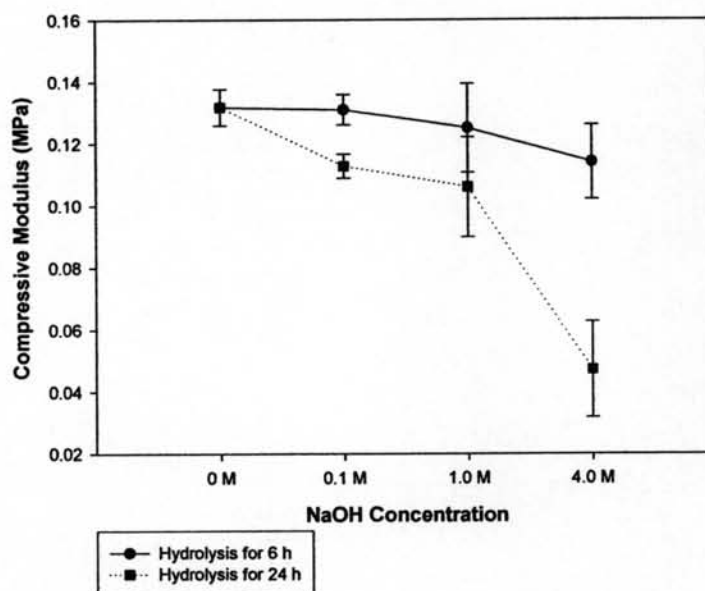
Figure 4.20 Water absorption of the porous scaffolds with 1:10 (PCL to sucrose ratio) at various NaOH concentrations (Raw data are shown in Appendix C).

In figure 4.21 showed the morphology of porous scaffolds with alkali treatment. High NaOH concentrations caused the formation of cracks and pores on the porous scaffolds, which induced dramatic decrease in its mechanical properties and increase in its weight loss after alkali hydrolysis. The values of mechanical properties and weight loss are shown in figure 4.22 and 4.23, respectively. The compressive modulus of PCL scaffold was 0.1317 ± 0.0059 MPa. In hydrolysis for 6h, these values were decreased to 0.1309 ± 0.0049, 0.1250 ± 0.0143 and 0.1140 ± 0.0120 MPa when the PCL scaffolds were treated with 0.1, 1.0 and 4.0M NaOH, respectively. The weight loss values of PCL scaffold were $0.37 \pm 0.10\%$, $0.88 \pm 0.16\%$ and $3.58 \pm 1.34\%$ after treatment with 0.1, 1.0 and 4.0M NaOH, respectively. In hydrolysis for 24h, the compressive modulus of PCL scaffold were decreased to 0.1127 ± 0.0039, 0.1061 ± 0.0161 and 0.0472 ± 0.0155 MPa when the PCL scaffolds were treated with 0.1, 1.0 and 4.0M NaOH, respectively. The weight loss values of PCL scaffold were $0.60 \pm 0.03\%$, $2.56 \pm 0.23\%$ and $34.77 \pm 6.12\%$ after treatment

with 0.1, 1.0 and 4.0M NaOH, respectively. The hydrolyzed scaffolds with 4.0M NaOH have the lowest compressive modulus and highest weight loss that were not suitable used for hydrolysis bone scaffold.

As reported by Tsuji *et al.*, the alkali treatment of PCL film was performed in 0.1 and 1.0 N NaOH solution. They found that the high alkali concentration induced the decreasing in mechanical properties of PCL. The tensile strength of PCL films were 0.88 kg/mm^2. But these values were decreased to 0.68 and 0.53 kg/mm^2 when PCL film was treated with 0.1 and 1.0 N NaOH, respectively. And the weight loss of PCL film after alkali treatment increased with increasing NaOH concentration. The weight loss values of PCL film were 0.1% and 0.8% after treatment with 0.1 and 1.0 N NaOH, respectively. Evidently, the contact angle of PCL was monotonous decreased with increasing NaOH concentration. Therefore, the alkali treatment enhanced the hydrophilicity of the PCL.
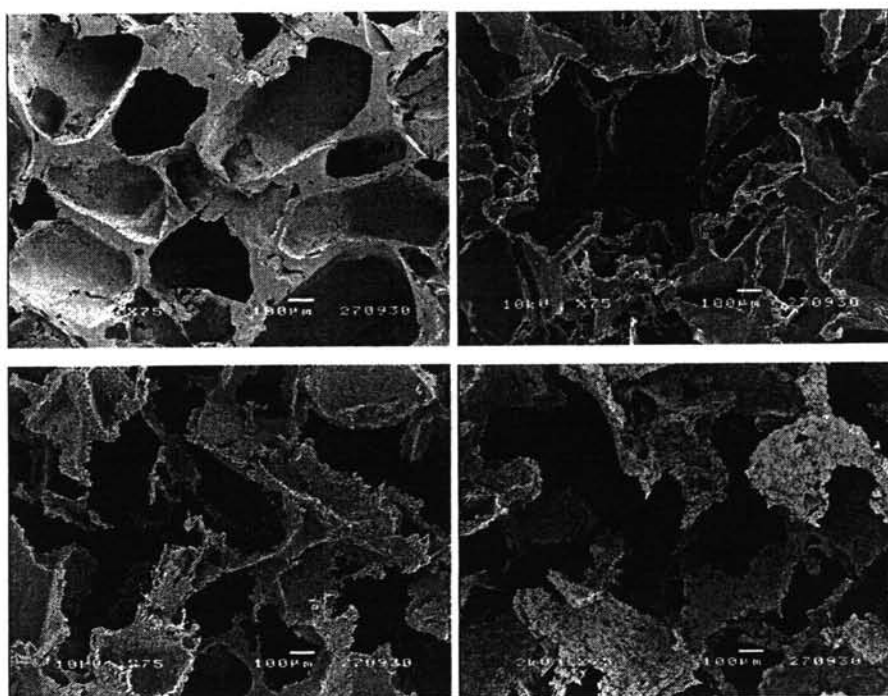
Figure 4.21 Morphological of porous scaffolds with 1:10 (PCL to sucrose ratio) at various NaOH concentrations (scale bar = 100 μm and magnification = 75X).

(a) 0M NaOH   (b) 0.1M NaOH   (c) 1.0M NaOH   (d) 4.0M NaOH

Figure 4.22 The compressive modulus of porous scaffolds with 1:10 (PCL to sucrose ratio) at various NaOH concentrations (Raw data are shown in Appendix C).
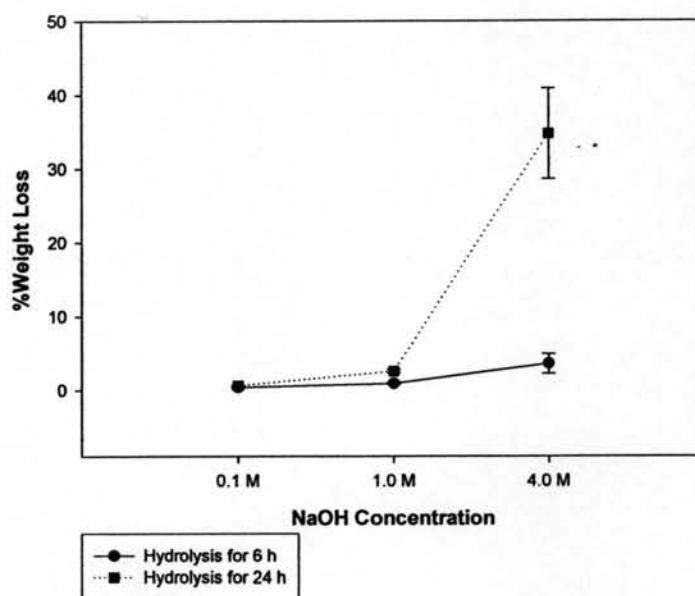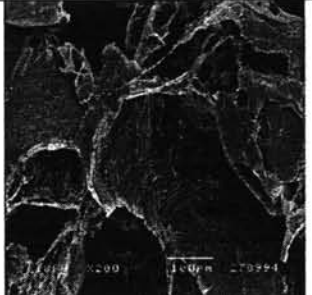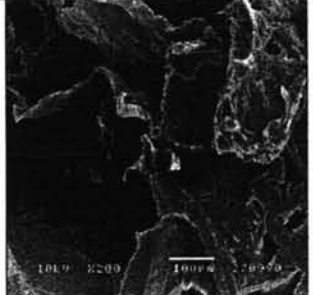

Figure 4.23 Weight loss of porous scaffolds with 1:10 (PCL to sucrose ratio) at various NaOH concentrations (Raw data are shown in Appendix C).

In case of 1.0M NaOH solution gave the porous scaffolds with the suitable water absorption and compressive modulus. 1.0M NaOH gave the porous scaffolds with higher water absorption than the lower NaOH concentrations. But the compressive modulus of these porous scaffolds was not more reduced which they were close to the compressive modulus of nonhydrolyzed scaffolds. In case of 4.0M NaOH gave the porous scaffold with lowest compressive modulus and highest weight loss. Thus, the optimum condition used to further study was alkali treatment with 1.0M NaOH.

The porous PCL scaffolds and their composites containing 20 and 40 wt% HAp were used to determine the morphology, water absorption and compressive modulus after hydrolysis with 1.0M NaOH.

Table 4.4 shows the selected SEM images of the structure and porosity of the PCL scaffolds containing 0, 20 and 40 wt% HAp after hydrolysis with 1.0M NaOH. The results showed that the porous scaffolds retained their porous structure even after submersion in the NaOH solution for as long as 24 h. As observed from table 4.4, HAp was remained into the matrix after hydrolysis.

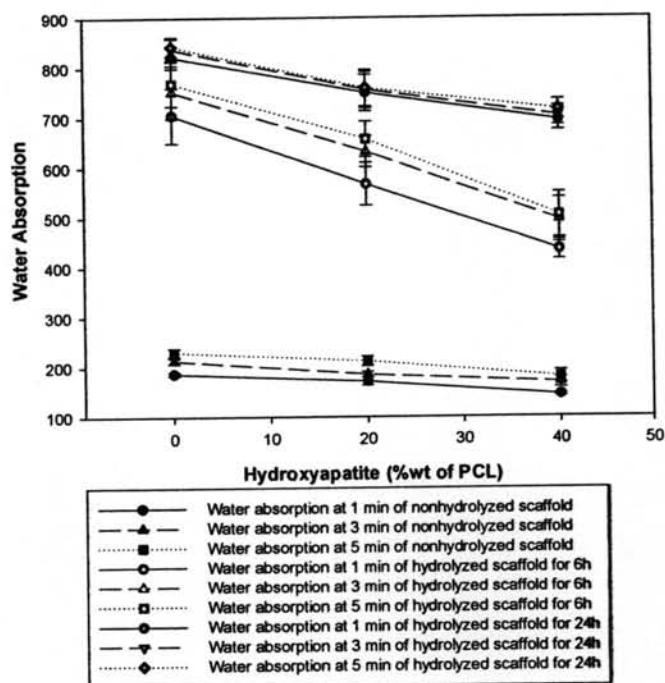**Table 4.4** Selected SEM images morphological of porous scaffolds with 1:10 (PCL to sucrose ratio) at various hydroxyapatite contents after hydrolysis with 1.0M NaOH

HAp (%wt of PCL)	Before hydrolysis	After hydrolysis for 6h	After hydrolysis for 24h
0			
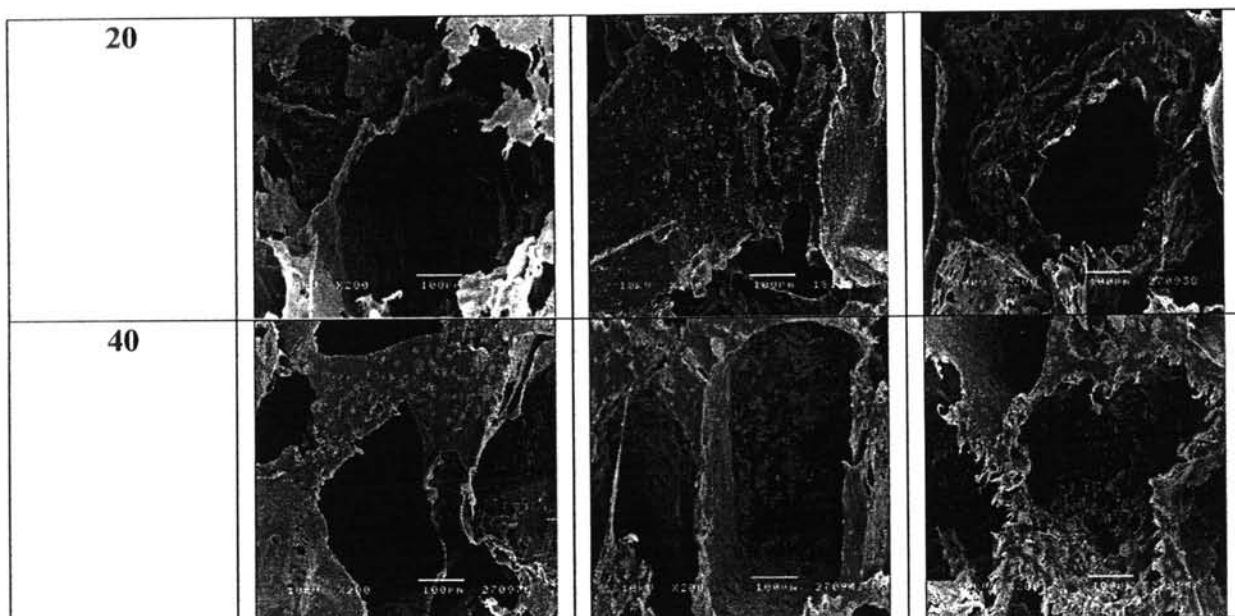
From TGA analysis, the actual amount of HAp in the composition of 40 wt% before hydrolysis was 29.19wt%. The actual amount of HAp after hydrolysis for 6h was 28.25wt%, and for 24h was 27.82wt%. They could prove that the most of HAp still remained on the porous scaffolds after hydrolysis with 1.0M NaOH. Some HAp could be removed from porous PCL scaffold with increasing the hydrolysis times.

The water absorption of hydrolyzed PCL scaffolds containing HAp were higher than the non hydrolyzed scaffolds due to increasing the density of hydrolytic terminal groups carbonyl and hydroxyl groups. The water absorption of the porous scaffolds exhibited a downward trend with HAp loading in the matrix, as shown in figure 4.24. The hydrolysis times were the parameters which effect to water absorption and mechanical properties of the porous scaffolds. The water absorption of hydrolyzed scaffolds for 24 h were higher than the for 6 h. Long hydrolysis times caused high the formation of pores and the destruction of the polymer chains on the porous scaffolds. Therefore, water can easily penetrated into the porous scaffolds and bonded with hydrophilic terminal groups of polymer chain.
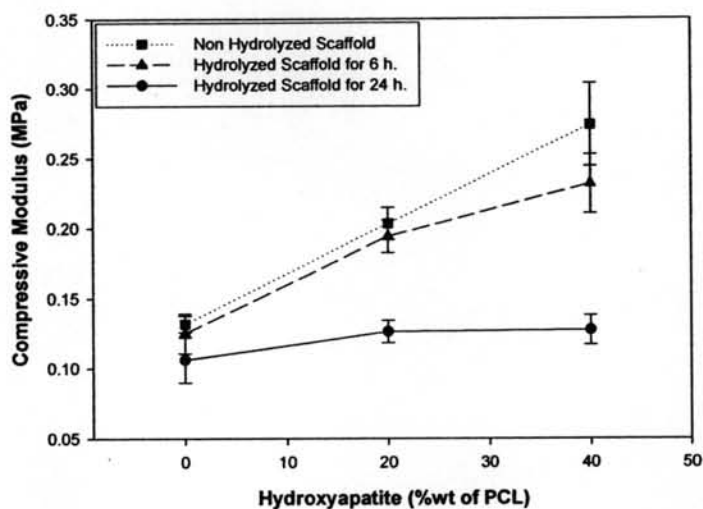
Figure 4.24 Water absorption of the porous scaffolds with 1:10 (PCL to sucrose ratio) at various hydroxyapatite contents after hydrolysis with 1.0M NaOH (Raw data are shown in Appendix C).

The compressive modulus obtained for hydrolyzed PCL scaffolds containing 0, 20 and 40 wt% HAp are displayed in figure 4.25. The mechanical test showed a decreased in compressive modulus when the porous scaffolds were hydrolyzed. And the compressive modulus increase with increasing hydroxyapatite contents. The lower compressive modulus might due to micro pore forming during hydrolysis. Micro pore structures in the scaffolds caused the decrease in the density of the scaffolds leading to the reduction in mechanical properties. The hydrolyzed scaffolds for 24 h have the lowest compressive modulus due to its highest destroyed polymer chains.

Figure 4.25 The compressive modulus of porous scaffolds with 1:10 (PCL to sucrose ratio) at various hydroxyapatite contents after hydrolysis with 1.0M NaOH (Raw data are shown in Appendix C).

4.2.4 Effect of Ipriflavone on Porous Scaffolds

The PCL scaffolds containing HAp can be increased the biological properties by adding the additive into the porous scaffolds. Ipriflavone is the substrate which can increase the proliferation and differentiation of the osteoblast. Hence, the PCL scaffolds containing HAp can be treated with ipriflavone to give the better properties. When the scaffolds were incorporated with ipriflavone, FT-IR spectrum is shown in figure 4.26.
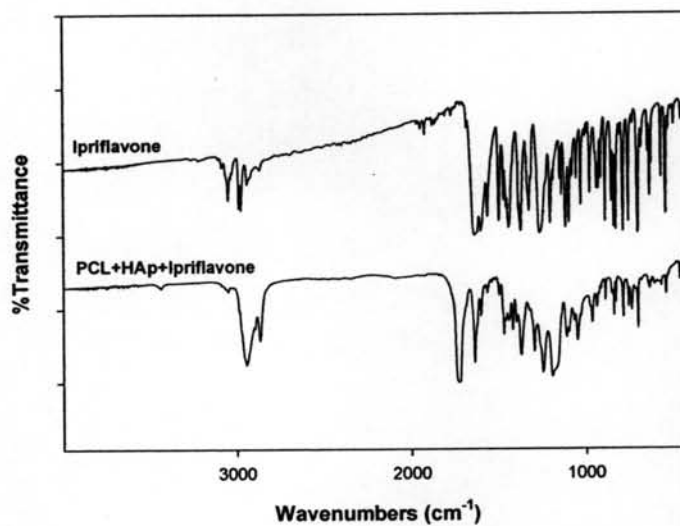
Figure 4.26 FT-IR spectrum of PCL containing HAp and IP.

The compressive modulus of PCL scaffolds containing HAp and IP are displayed in figure 4.27. The mechanical test showed that the IP concentration not effect to the compressive modulus of the nonhydrolyzed scaffolds. But in hydrolyzed scaffolds, the compressive modulus decreased with increasing IP concentrations.
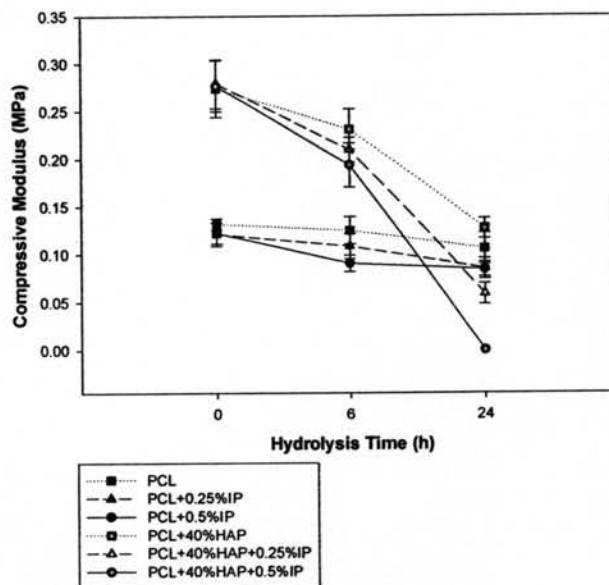
Figure 4.27 The compressive modulus of porous scaffolds at various IP concentrations (Raw data are shown in Appendix D).

The degree of crystalline of the PCL scaffolds containing HAp were lower than PCL scaffolds when they were incorporated with IP, as shown in table 4.5.

Table 4.5 Degree of crystallinity of the porous scaffold containing IP

Conditions	Degree of crystallinity of the porous scaffold		
	0h	**6h**	**24h**
PCL	40.616	52.311	52.960
PCL+0.25wt%IP	53.775	52.739	54.030
PCL+0.50wt%IP	53.273	50.277	53.429
PCL+40wt%HAp	45.087	54.800	61.453
PCL+40wt%HAp+0.25wt%IP	33.826	27.093	29.307
PCL+40wt%HAp+0.50wt%IP	45.087	48.335	39.876

4.2.5 Cell Culture on the Porous Scaffolds

The cells seeded onto osteoconductive polymers for bone regeneration is a technique in orthopedic surgery and biomedical engineering. The goal is the achievement of a structure that promoting viable cells to lay down new bone. Examination of cell seeded constructs by scanning electron microscopy provided the visual confirmation of interaction between cells and scaffold. From figure 4.28 shows images of mouse osteoblasts (MC3T3-E1) cells that were culture on the PCL porous scaffold at 48 h. Cell morphology were slightly spindle shape cells on PCL scaffold. The cells were more expanded and easily observed on PCL scaffold containing 40 wt%HAp and PCL scaffold containing both HAp and IP. Therefore, the addition of HAp particles and IP has proven useful to promote bone formation by cells covering HAp and IP added PCL samples.

From comparative studies, Ciapetti et al., investigated the ability of porous PCL and PCL containing HAp to support osteoblast like SaOS2 cell growth and mineral formation. In the short term culture (3-7 days) of SaOS2 on polymers, it was found that the presence of HAp moderately improved the number of cell on the surfaces and showed the higher metabolic activity of the cells compared to pure PCL scaffold. In the long term (3-4 weeks), it was found that the HAp added polymers obtained the best colonization by cells. Therefore HAp can improved osteoblast activity. Civitelli et al., studied the human osteoblast differentiation in vitro model. They found that IP can stimulate the expression of bone sialoprotein, type I collagen and facilitate the deposition of mineralized matrix. And in vivo, they added the drug orally to adult male rats for 1 month. IP could increase the bone density and improve the biomechanical properties of adult male rat bones without altering mineral composition or bone crystallinity. Kakai et al., concluded that IP can modulates osteogenic cell differentiation of rat osteoblast like cells and human periodontal ligament fibroblasts cells by using alkaline phosphatase (ALP) as a marker. Martini et al., found that IP can stimulated the perialveolar bone formation in vivo studies.
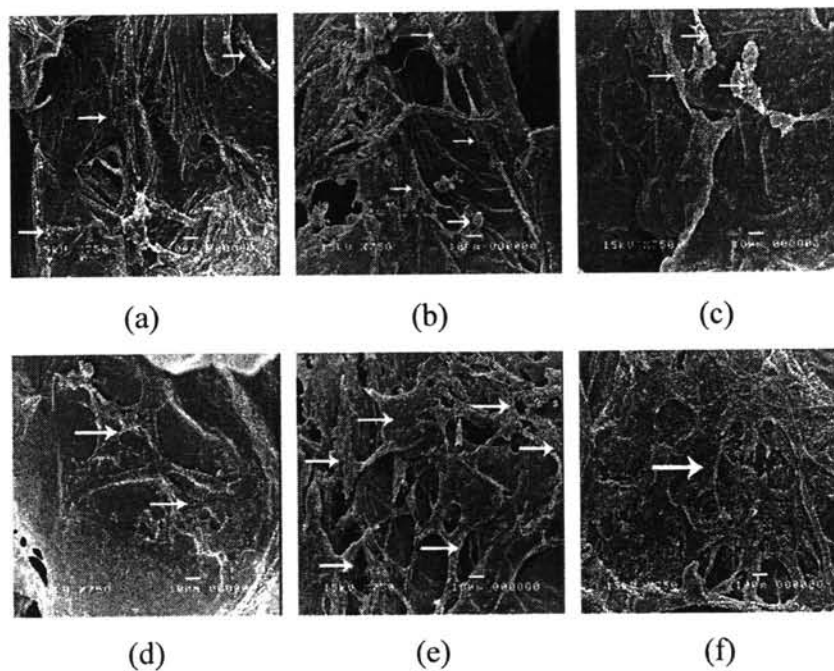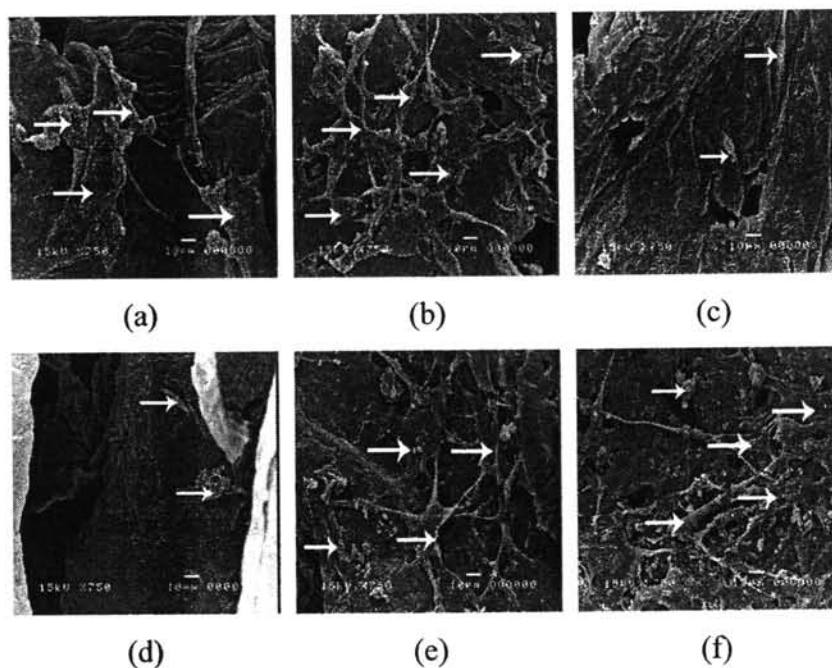
Figure 4.28 The SEM images of MC3T3-E1 cells cultured on the various types of scaffolds that were hydrolyzed with 1.0M NaOH for 6 h (1:10).

(a) PCL
(b) PCL+40wt% HAp
(c) PCL+0.25wt%IP
(d) PCL+0.5wt%IP
(e) PCL+40wt% HAp+0.25wt%IP
(f) PCL+40wt%HAp+0.5wt%IP

Figure 4.29 shows the effect of hydrolysis times on the cell behavior on the porous scaffold. The cell can attached and spread on the surface of porous scaffold which were hydrolyzed for 24 h. Long hydrolysis times caused the higher water absorption of the scaffold. Serrano *et al.*, found that the treatment of PCL film with NaOH can increased the surface hydrophilicity. It can improved fibroblast cells adhesion and proliferation. Therefore, the cells can expand to cover the surface of the scaffold and some cells can go to inside the scaffold.

Figure 4.29 The SEM images of MC3T3-E1 cells cultured on the various types of scaffolds that were hydrolyzed with 1.0M NaOH for 24 h (1:10).

(a) PCL   (b) PCL+40wt% HAp

(c) PCL+0.25wt%IP   (d) PCL+0.5wt%IP

(e) PCL+40wt% HAp+0.25wt%IP   (f) PCL+40wt%HAp+0.5wt%IP

4.2.6 Biodegradation in *Vitro*

The in *vitro* degradation of PCL and PCL scaffolds containing 40wt%HAp were assessed in two different environments under PBS and *Pseudomonas* lipase conditions. In this study, the variation in HAp composition and hydrolysis times were examined to observe a change in degradation rate. Weight loss, morphological and mechanical properties change were evaluated. Figure 4.30 and 4.31 indicated the weight loss of the composite in PBS and lipase solution.

In comparative studies, Chawla *et al.* studied biodegradation in the presence and absence of *Pseudomonas* lipase in phosphate buffer saline (PBS, pH7.4) at 37°C and found that the degradation of PCL was enhanced by the presence of lipase. In the presence of 45 units/l lipase, PCL nanoparticles were degraded 100% after 36h, whereas only around 8% of the polymer degraded in the absence of lipase.

The surface area of the polymer was the parameter which effect to degradation rate such as nanoparticles, enhanced the rate of degradation. Because the solution was difficult diffuse into a hydrophobic polymer cause to the degradation at the surface of the polymer.
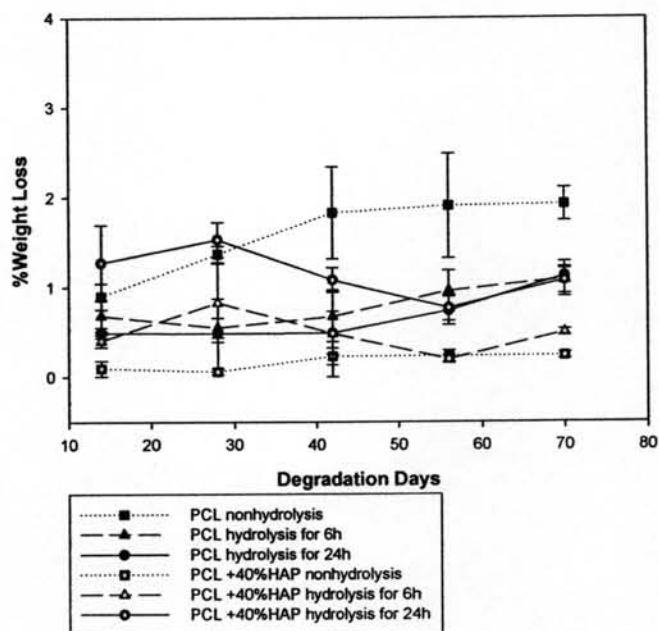
Figure 4.30 The weight loss of the PCL and PCL scaffolds containing 40wt%HAp in PBS solution after hydrolysis with 1.0M NaOH (1:10) (Raw data are shown in Appendix E).
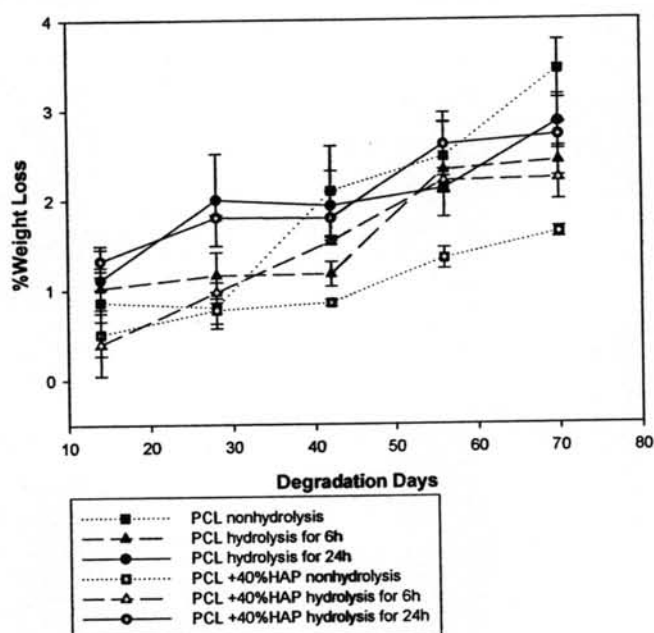
Figure 4.31 The weight loss of the PCL and PCL scaffolds containing 40wt%HAp in lipase solution after hydrolysis with 1.0M NaOH (1:10) (Raw data are shown in Appendix E).

In PCL and PCL scaffolds containing 40 wt% HAp exhibited a mean weight loss of $1.92 \pm 0.18\%$ and $0.24 \pm 0.03\%$, respectively in PBS solution and $3.44 \pm 0.32\%$ and $1.62 \pm 0.06\%$, respectively in lipase solution. In PCL and PCL scaffolds containing 40 wt% HAp with hydrolysis for 6h exhibited a mean weight loss of $1.09 \pm 0.19\%$ and $0.50 \pm 0.04\%$, respectively in PBS solution and $2.42 \pm 0.43\%$ and $2.22 \pm 0.03\%$, respectively in lipase solution. In PCL and PCL scaffolds containing 40 wt% HAp with hydrolysis for 24h exhibited a mean weight loss of $1.13 \pm 0.08\%$ and $1.08 \pm 0.15\%$, respectively in PBS solution and $2.85 \pm 0.30\%$ and $2.71 \pm 0.13\%$, respectively in lipase solution. From the results, the presence of lipase enhanced the degradation rate of the polymer more as compared with degradation in PBS alone, and the weight loss of PCL and PCL scaffolds containing 40wt% HAp were around 1-3% after degradation 70 days.

Table 4.6 Selected SEM images morphological of porous scaffolds at various degradation times (1:10)
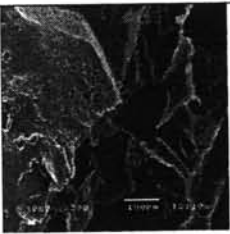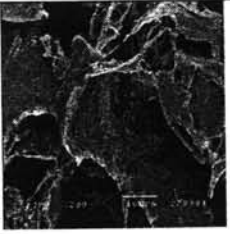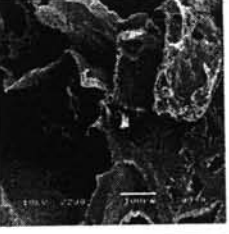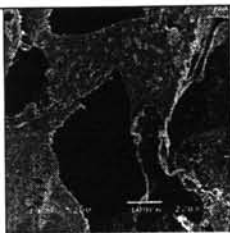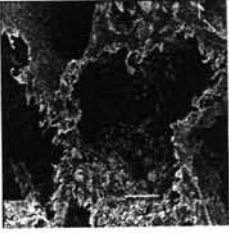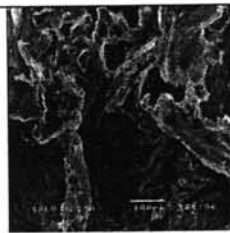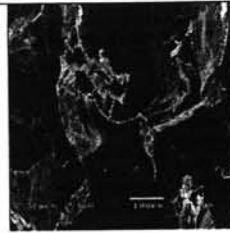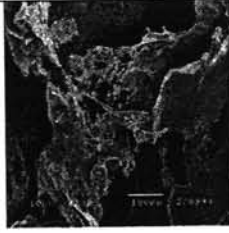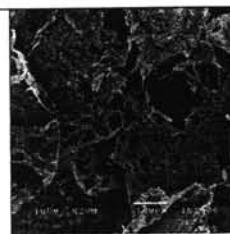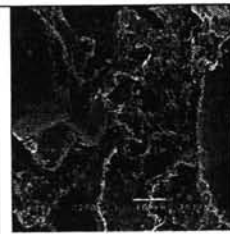
	Nonhydrolysis	1.0M NaOH, 6h	1.0M NaOH, 24h
Before degradation			
PCL			
PCL+40wt%HAp			
After degradation 70 days			
PCL			
PCL+40wt%HAp			

Table 4.6 shows that the morphological of the scaffolds were remain unchanged after incubation of samples for 70 days in PBS and lipase solution at 37°C (raw data are shown in Appendix E).

WAXD patterns of the degraded PCL scaffolds are showed in figure 4.32 and 4.33. The patterns confirm the crystalline which remained in the PCL scaffolds after degradation.
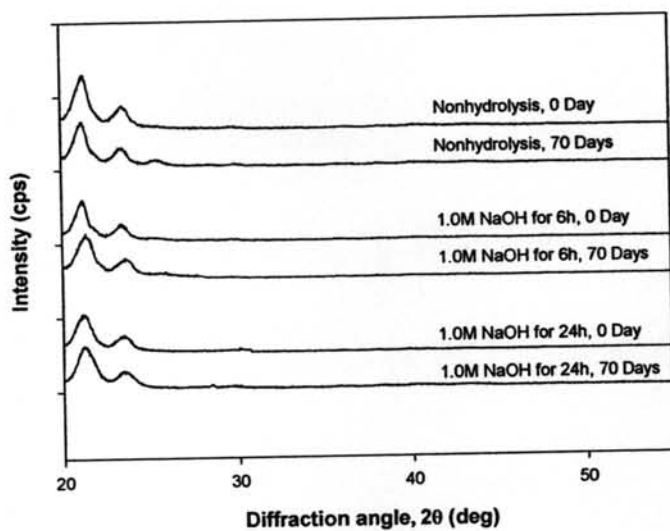
Figure 4.32 WAXD patterns of degraded PCL scaffolds in lipase solution (1:10).
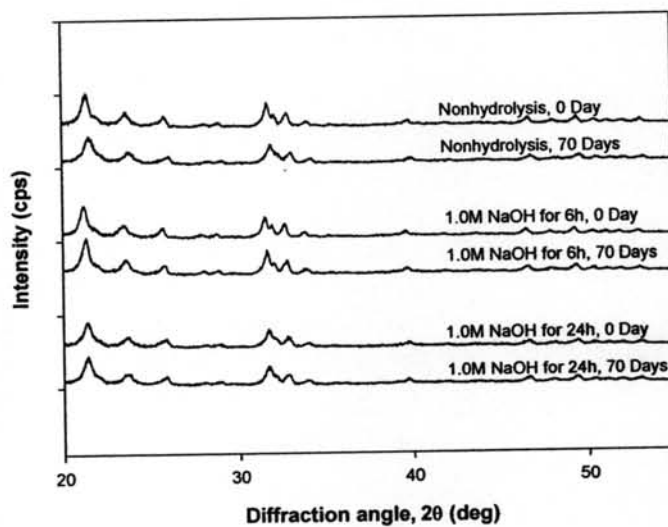

Figure 4.33 WAXD patterns of degraded PCL scaffolds containing 40wt%HAp in lipase solution (1:10).
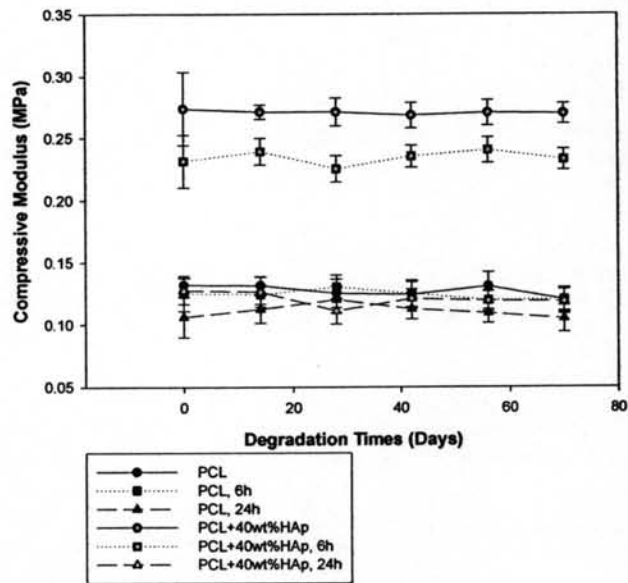
Figure 4.34 The compressive modulus of the PCL and PCL scaffolds containing 40wt%HAp in PBS solution after hydrolysis with 1.0M NaOH (Raw data are shown in Appendix E).
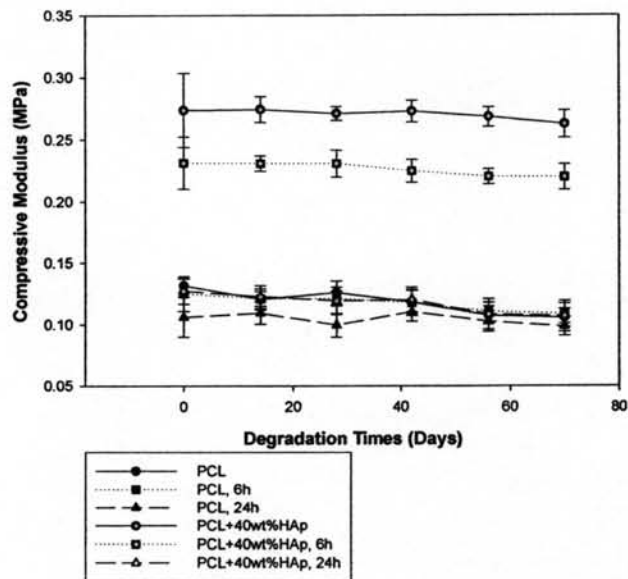

Figure 4.35 The compressive modulus of the PCL and PCL scaffolds containing 40wt%HAp in lipase solution after hydrolysis with 1.0M NaOH (Raw data are shown in Appendix E).

The compressive modulus of degraded PCL and PCL scaffolds containing 40 wt% HAp are shown in figure 4.34 and 4.35. Because the structure of the scaffolds were remain unchanged and weight loss of the scaffolds were only 1-3% after incubation in PBS and lipase for 70 days at 37°C. Hence, the compressive modulus of the degraded scaffold was unchanged with increasing the degradation days.