CHAPTER 4

Result and Discussion

This research study aims to develop Chitosan/Fibroin/Hydroxyapatite scaffold for bone filling application. In this part, the results of the study of Chitosan/Fibroin/ Hydroxyapatite porous scaffold are shown and discussed.

The discussion will be divided into three main parts with material characterization, scaffold fabricationand scaffold characterization.

4.1 Material Characterization

4.1.1 X-ray Diffraction Analysis

X-ray diffraction (XRD) analysis was used to investigate the synthetic material's powder phase. The XRD spectra of HA from mollusk shell were compared with JCPDS-ICDD Card no. 9 – 43283.Figure 4.1 show that the peak of the HA powder synthesized in the study was similar to the peak of HA from JCPDS – ICDD with approximately matching rate 80% or higher. From the results can confirm that HA could be synthesis by sintering mollusk shell at 700°C and mixed with $NH_4H_2PO_4$.

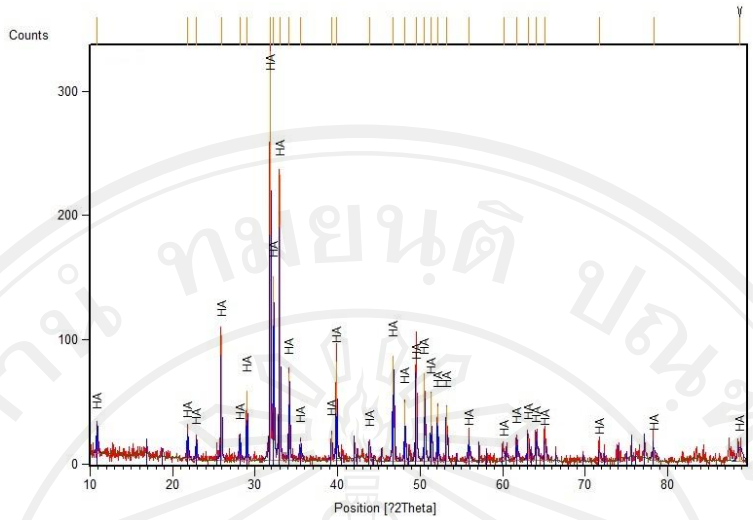


Figure 4.1 XRD pattern of powder synthesized from mollusk shell compared with database

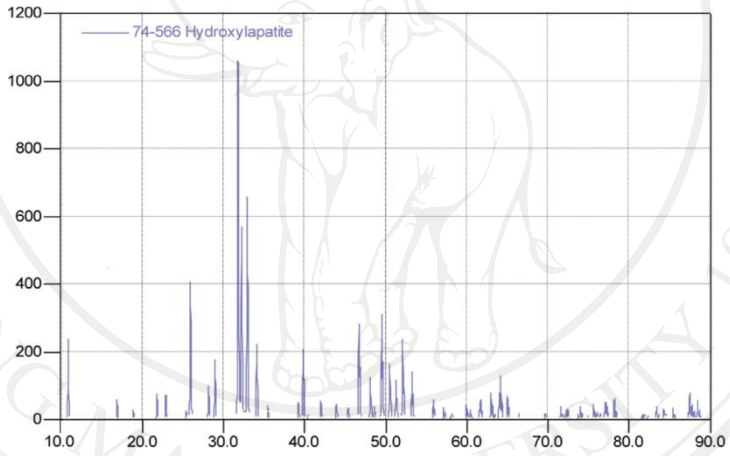


Figure 4.2 XRD pattern of hydroxyapatite from database

4.1.2 SDS-PAGE Analysis

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrelamide Gel Electrophoresis) was used to investigate the powder extract from silk cocoon. Figure 4.3 shows thatthe molecular weight of the extracted powder is higher than 250 kDa which has high possibility that the extracted powder is silk fibroin powder which has molecular weight 450kDa as reported in Hee Jung et al [26].

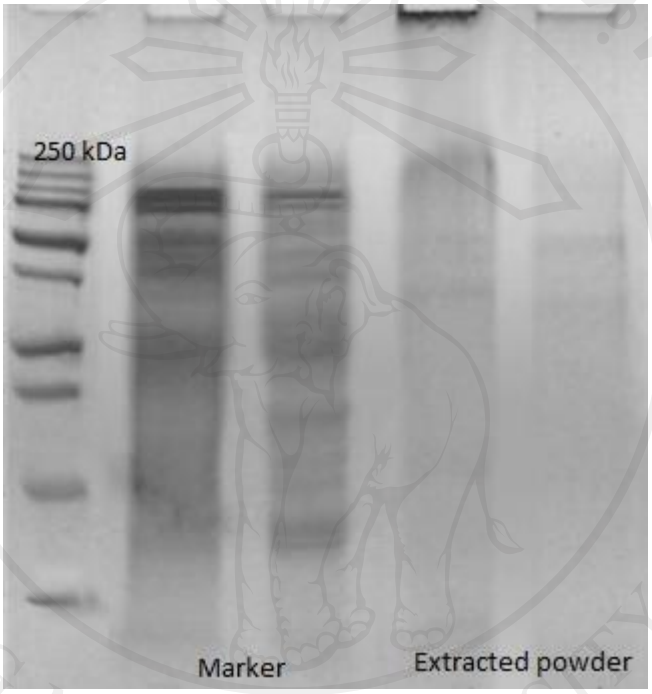


Figure 4.3 SDS-PAGE results of powder extract from silk cocoon

4.1.2 Scaffold Fabrication

The porous scaffold with variety HA:Fibroin ratio was successfully fabricated by freeze drying method.By using pre-freezing temperature at -20°C and freeze drying temperature at -40°C. The porous scaffold were cross-linked by glutaraldehyde and were neutralized by NaOH solution.

4.1.3 Scaffold Characterization

4.1.3.1 Mechanical Property

The compression test was used to test the mechanical property of the porous scaffold in this research. The scaffolds with the thickness of 12 mm and diameter 14mm were pressed until the thickness become 1mm.After remove the stress on the scaffold the dimensional change were not returned to normal. Table 4.1-4.5 show the Young's modulus of the porous scaffold obtained from compression test (raw data can be found in Appendix D).

Table 4.1 Young's Modulus of porous scaffold with 100:0 HA:Fibroin ratio

Extension (mm)	Load (N)	Stress (kPa)	Strain
0	0	0	0
1	1.333	8.654	0.083
2	2.023	13.136	0.167
3	2.681	17.413	0.25
4	3.330	21.623	0.333
5	4.067	26.410	0.417
6	4.956	32.182	0.5
7	6.2149	40.382	0.583
8	8.209	53.303	0.667
9	11.743	76.253	0.75
10	19.280	125.195	0.833
11	40.057	260.110	0.916

Table 4.2 Young's Modulus of porous scaffold with 75:25 HA:Fibroin ratio

Extension (mm)	Load (N)	Stress (kPa)	Strain
0	0	0	0
1	1.721	11.177	0.083
2	2.836	18.415	0.167
3	3.495	22.696	0.25
4	4.105	26.658	0.333
5	4.760	30.910	0.416
6	5.624	36.518	0.5
7	6.822	44.298	0.583
8	8.678	56.351	0.667
9	12.082	78.454	0.75
10	19.015	123.475	0.833
11	36.782	238.845	0.916

Table 4.3 Young's Modulus of porous scaffold with 50:50 HA:Fibroin ratio

Extension (mm)	Load (N)	Stress (kPa)	Strain
0	0	0	0
1	1.376	8.934	0.083
2	2.003	13.006	0.167
3	2.448	15.898	0.25
4	2.807	18.228	0.333
5	3.226	20.949	0.416
6	3.760	24.416	0.5
7	4.527	29.401	0.583
8	5.821	37.798	0.667
9	8.167	53.033	0.75
10	13.100	85.067	0.833
11	26.027	169.004	0.916

Table 4.4 Young's Modulus of porous scaffold with 25:75 HA:Fibroin ratio

Extension (mm)	Load (N)	Stress (kPa)	Strain
0	0	0	0
1	1.186	7.700	0.083
2	1.987	12.902	0.167
3	2.521	16.371	0.25
4	3.031	19.682	0.333
5	3.582	23.260	0.416
6	4.232	27.480	0.5
7	5.154	33.468	0.583
8	6.585	42.761	0.667
9	8.986	58.356	0.75
10	13.695	88.928	0.833
11	25.636	166.469	0.916

Table 4.5 Young's Modulus of porous scaffold with 0:100 HA:Fibroin ratio

Extension (mm)	Load (N)	Stress (kPa)	Strain
0	0	0	0
1	1.875	12.175	0.083
2	2.472	16.053	0.167
3	3.337	21.670	0.25
4	3.791	24.616	0.333
5	4.296	27.897	0.416
6	4.894	31.778	0.5
7	5.332	34.625	0.583
8	6.855	44.512	0.667
9	8.592	55.793	0.75
10	12.671	82.3279	0.833
11	23.379	151.813	0.916

From Table 4.1 – 4.5 the stress-strain curves of each ratio can be plotted in Figure 4.4 According to this plot, the Young's modulus can be evaluated from the stress-strain relationship in the elastic region. The linear relationship was observed in a range of strain between 0.167- 0.583. Figure 4.5 illustrate the stress-strain relationship of HA:fibroin at each ratio within elastic condition corresponding to fitted linear equation. From this graph, it can show that the Young's modulus (slope of the graph) were maximum at 100:0 HA:fibroin ratio and lowest at 50:50 ratio. The Young's modulus of each ratio were plotted in Figure 4.6

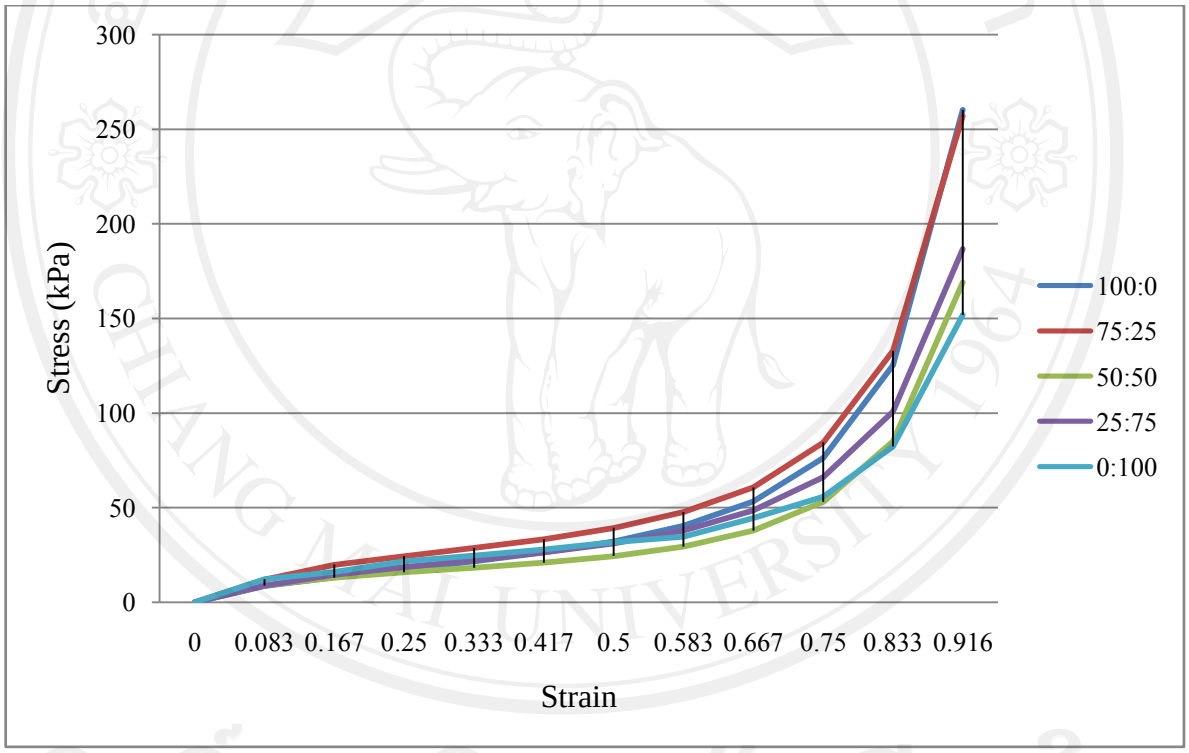


Figure 4.4 Stress-Strain curves of porous scaffolds with different HA:fibroin ratios

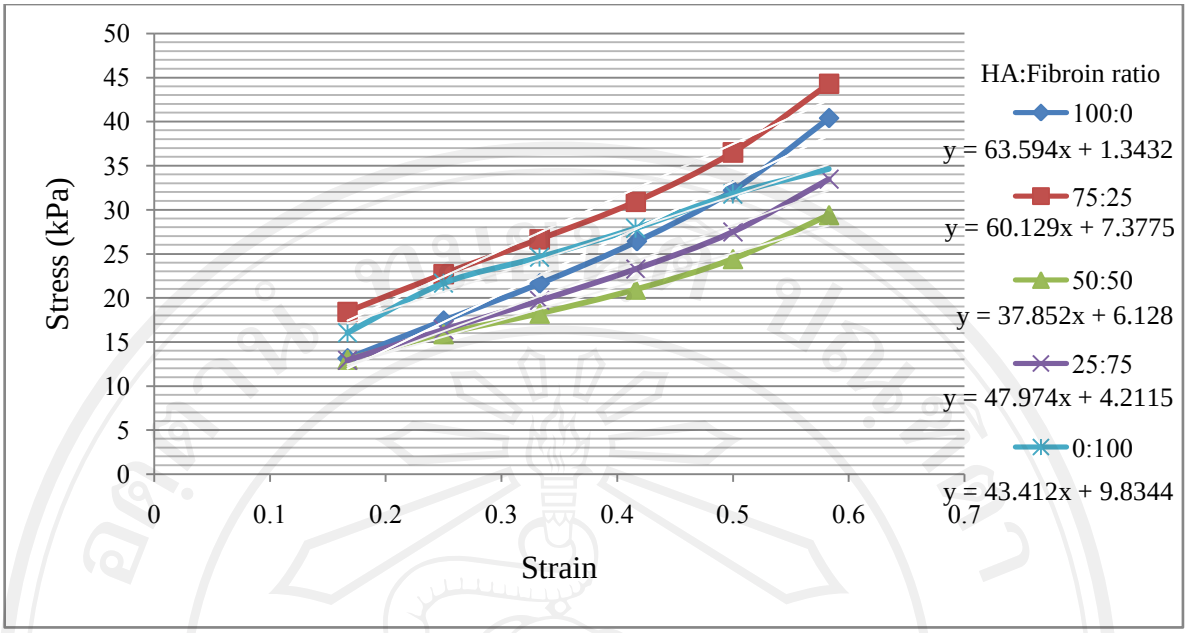


Figure 4.5 Stress-Strain curves of porous scaffold in elastic deformation region

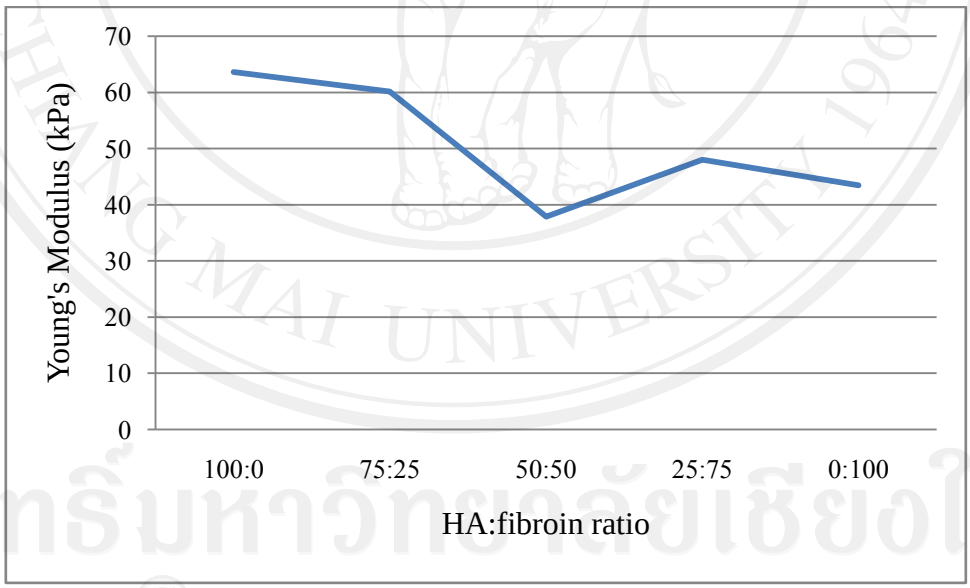


Figure 4.6 Relationship between Young's Modulus and HA:fibroin ratio

4.1.3.2 Porosity

The porosity of porous scaffold with each HA:Fibroin was tested and shown in the Figure 4.7 (raw data in Appendix B). While the porosity appropriate for the porous bone scaffold is 90.5% and the porosity of trabecular bone was around 50-90% [6,7,8]. The porosity of fabricated scaffold was obtained by liquid replacement method are 93.56±0.1%, 94.57±0.17%, 95.66±0.41%, 95.09±0.2%, 94.26±0.23%From the results it can be concluded that the porosity of every ratio was surpassed the minimum porosity requirement.

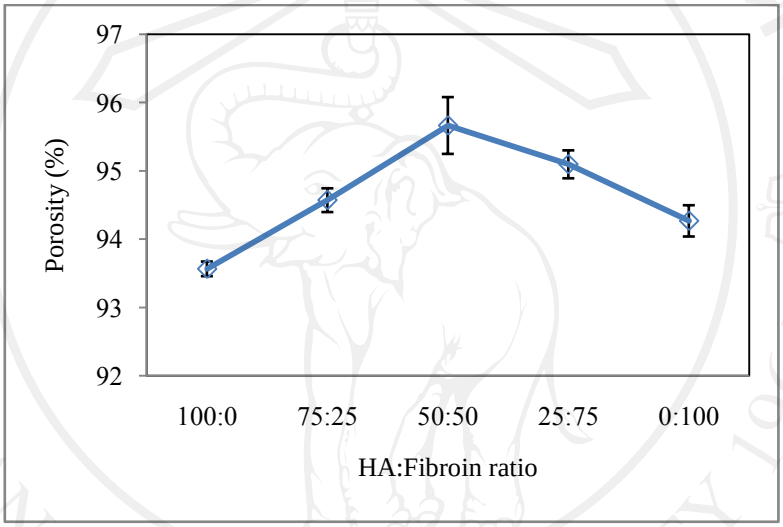


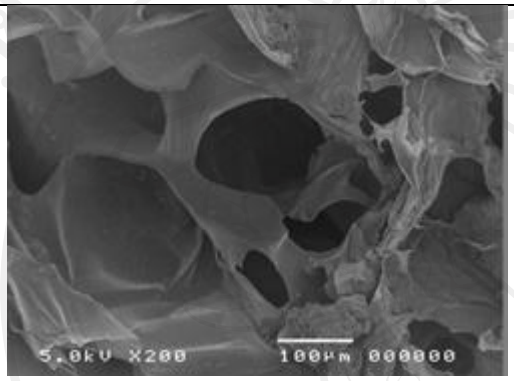
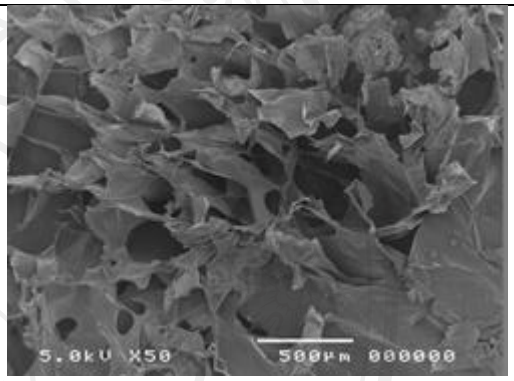
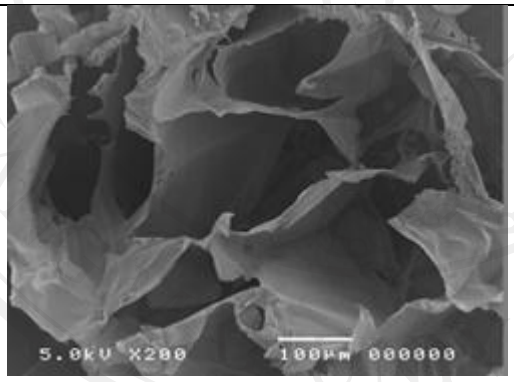
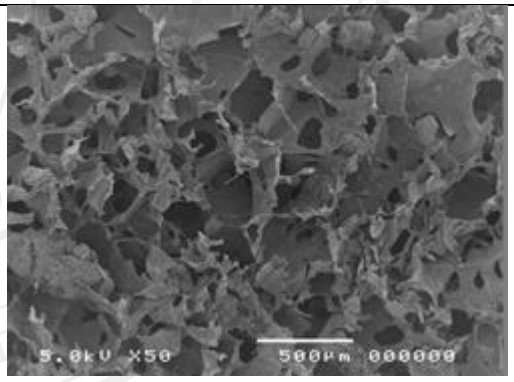
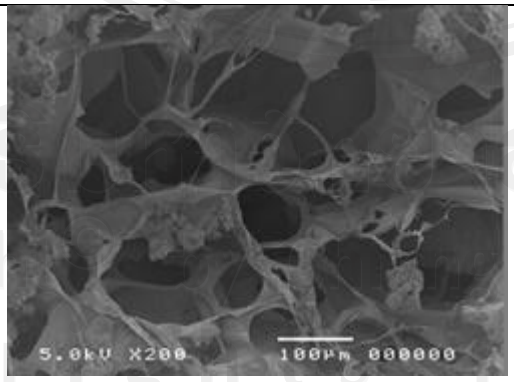
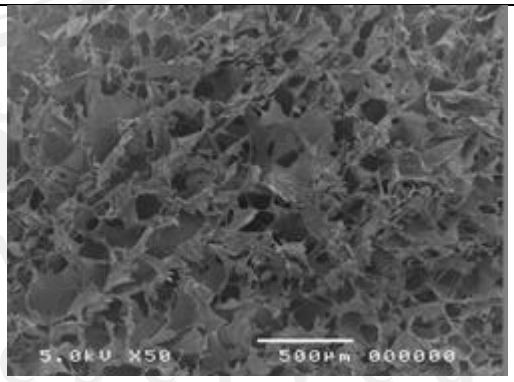
Figure 4.7 Porosity of porous scaffold with diferentHA:fibroin ratio

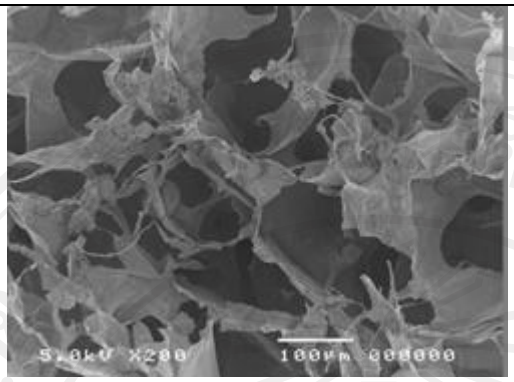
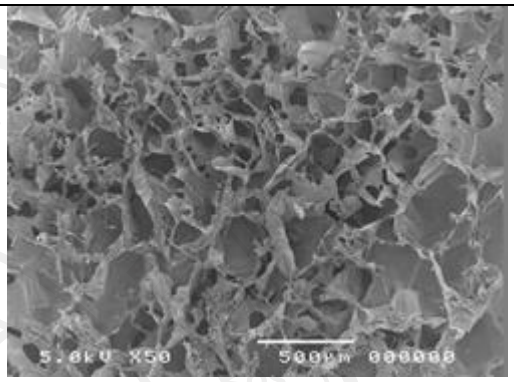
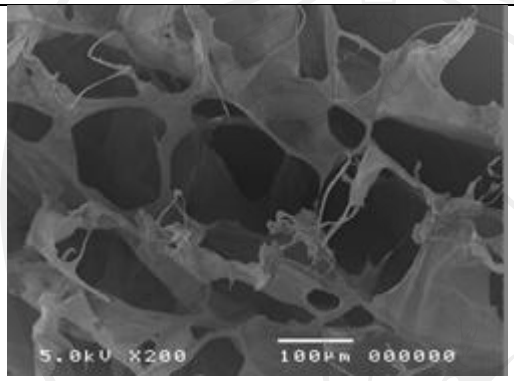
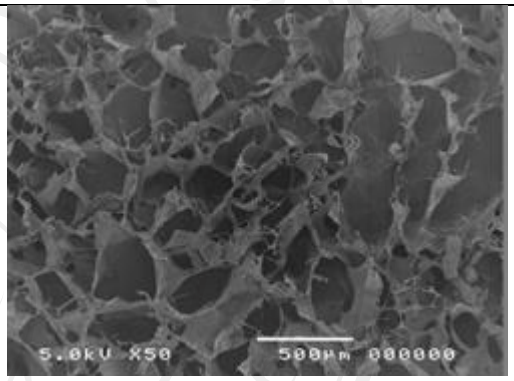
The scaffold with 50:50 HA:fibroin ratio has highest porositywhich is correspond to the results of mechanical property. The porosity of scaffolds has an effect on mechanical property of the scaffold. The scaffold with higher porosity will be decreased in mechanical property.

4.1.3.3 Pore Morphology

Pore morphology of the porous scaffold was examined by scanning electron microscope (SEM) as shown in the following table.

Table 4.6 Pore morphology of porous scaffold

HA:Fibroin ratio	SEM image at x200	SEM image at x50
100:0		
75:25		
50:50		

HA:Fibroin ratio	SEM image at x200	SEM image at x50
25:75		
0:100		

SEM images of each ratio of HA:Fibroin shown that the structure of scaffold are porous with interconnected pore with the average pore size around 150-200μmwhich are efficient for the osteoblast cells(average size 20-25μm) to permeate. The scaffold pore structure was found to be micropore and macropore which are surpass the minimum pore size requirement to support the tissue ingrowth and blood supply.

4.1.3.4 Swelling Property

Swelling ratio of porous scaffold was tested and the result was shown in Table 4.7.

Table 4.7 Swelling Ability(%) of porous scaffold

	Test#1	Test#2	Test#3	Average
100:0	43.55	45.12	42.34	43.67
75:25	39.64	42.69	42.43	41.59
50:50	60.46	59.33	61.12	60.30
25:75	59.42	59.70	58.84	59.32
0:100	66.85	65.98	64.49	65.77

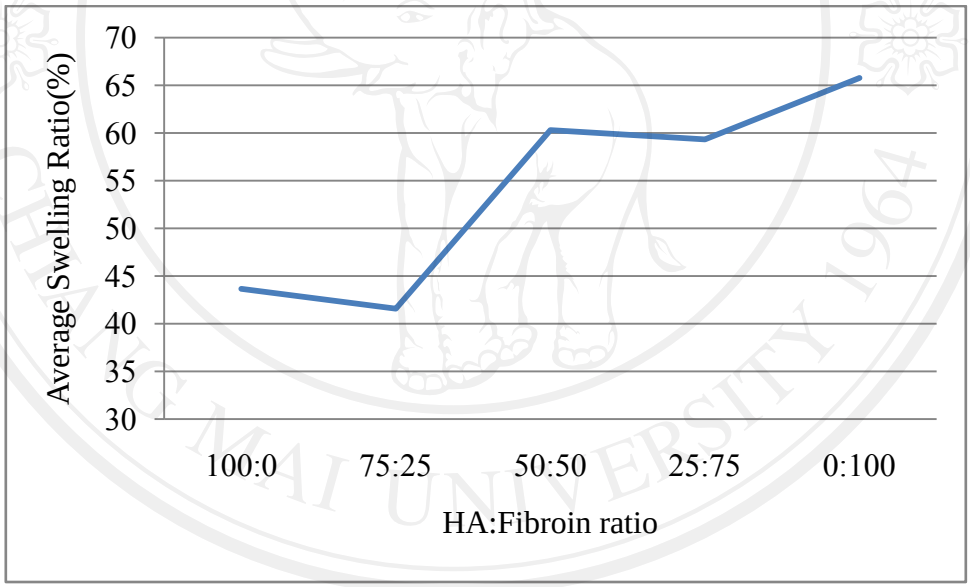


Figure 4.8 Swelling ability(%) of porous scaffold

From the Figure 4.8the scaffold with highest swelling ability was fabricated by 0:100 HA:Fibroin ratio while the scaffold with 100:0 ratio have lower swelling rate. The result can conclude that increasing fibroin ratio can increase the swelling property of scaffold. Thus, the swelling ability of scaffold can be controlled by varying the amount of fibroin and HA.

4.1.3.5 Biocompatibility

Biocompatibility of the scaffold was tested by cell culture. Lymphocytes of white blood cells or so called peripheral blood mononuclear cell (PBMC) were used to prove that the material was non-cytotoxicity. XTT assay were used to evaluate cell viability as shown in Figure 4.9 three experiments were performed and analyzed with a Microplate reader (Sunrise™ TECAN). Optical Density (OD) value was used to calculate cell viability percentage. After 24 hours of incubation, the percentage of cell viability was evaluated from the OD value of the XTT solution with porous scaffold and control cell without porous scaffold.

Table 4.8 Optical Density (OD) Value and percentage of cell viability obtain from XTT assay

	Sample#1	Sample#2	Sample#3
Control	0.12	0.115	0.121
With scaffold	0.118	0.113	0.119
Cell Viability(%)	98.33	98.26	98.35

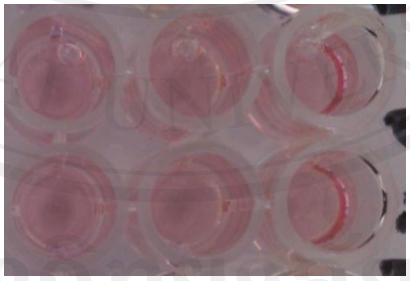


Figure 4.9 Sample were analyzed in Microplate Reader after perform XTT assay

From the results shown in Table 4.8, cell viability of the solution with scaffold was over 98% which can prove that the porous scaffold were non-cytotoxicity to living cells.

4.1.3.6 Biodegradability

Biodegradation rate of the scaffold was tested by soak the scaffold in PBS containing lysozyme. After 7days the dry weight of scaffold were measured and biodegradation rate were calculated as shown in Figure 4.10 (raw data can be found in Appendix A).

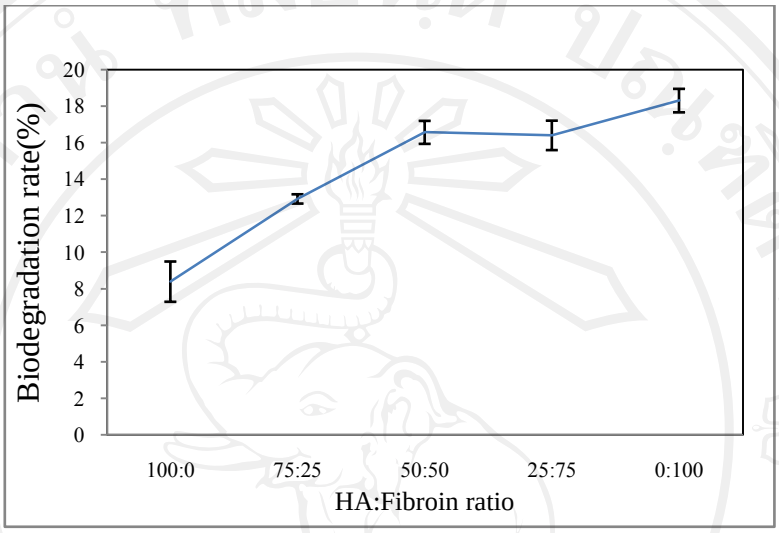


Figure 4.10 Average degradation rate of porous scaffold at 7 days

The results had shown that the scaffold with higher HA ratio can degrade slower than scaffold with higher fibroin ratio which corresponds to the property of HA and fibroin. Thus, this result can proof that the biodegradation rate of the scaffold can be controlled by varying the amount of HA and fibroin