APPENDICES

APPENDIX A

BIODEGRADABILITY

Table A Raw data of percentage of biodegradability

HA:Fibroin Ratio	Biodegradability (%)
100:0	7.62
100:0	9.18
100:0	8.02
100:0	8.87
75:25	13.11
75:25	12.75
75:25	13.32
75:25	12.54
50:50	17.02
50:50	16.13
50:50	16.44
50:50	16.71
25:75	16.98
25:75	15.84
25:75	16.50
25:75	16.32
0:100	18.77
0:100	17.86
0:100	17.52
0:100	19.11

APPENDIX B

POROSITY

Table B Raw data of percentage of porosity

HA:Fibroin Ratio	Porosity (%)
100:0	93.66
100:0	93.45
100:0	93.59
75:25	94.38
75:25	94.63
75:25	94.71
50:50	96.03
50:50	95.21
50:50	95.76
25:75	95.01
25:75	94.95
25:75	95.33
0:100	94.32
0:100	94.02
0:100	94.47

APPENDIX C

SWELLING ABILITY

Table C Raw data of percentage of swelling ability

HA:Fibroin Ratio	Swelling Ratio (%)
100:0	43.55
100:0	45.12
100:0	42.34
75:25	39.64
75:25	42.69
75:25	42.43
50:50	60.46
50:50	59.33
50:50	61.12
25:75	59.42
25:75	59.70
25:75	58.84
0:100	66.85
0:100	65.98
0:100	64.49

APPENDIX D

MECHANICAL PROPERTIES

Table D-1 Raw data of mechanical properties of scaffold with 100:0 HA:Fibroin ratio

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
0	0	0.004632	0.000154	30.08978	0
1	0.0833	0.254872	0.000154	1655.012	0.006942
2	0.166713	0.458636	0.000154	2978.157	0.013893
3	0.249787	0.583863	0.000154	3791.318	0.020816
4	0.333257	0.686559	0.000154	4458.176	0.027771
5	0.416784	0.788754	0.000154	5121.778	0.034732
6	0.499857	0.870755	0.000154	5654.255	0.041655
7	0.583214	0.962445	0.000154	6249.641	0.048601
8	0.666684	1.043831	0.000154	6778.123	0.055557
9	0.749984	1.125331	0.000154	7307.344	0.062499
10	0.833171	1.179563	0.000154	7659.5	0.069431
11	0.916697	1.274589	0.000154	8276.552	0.076391
12	1.000111	1.332792	0.000154	8654.494	0.083343
13	1.083241	1.39634	0.000154	9067.143	0.09027
14	1.166484	1.465199	0.000154	9514.279	0.097207
15	1.250011	1.520948	0.000154	9876.286	0.104168
16	1.333311	1.582248	0.000154	10274.34	0.111109
17	1.416668	1.641962	0.000154	10662.09	0.118056
18	1.499854	1.70349	0.000154	11061.62	0.124988
19	1.583438	1.754748	0.000154	11394.47	0.131953
20	1.666624	1.814525	0.000154	11782.63	0.138885
21	1.749868	1.857507	0.000154	12061.73	0.145822
22	1.833281	1.912487	0.000154	12418.75	0.152773
23	1.916694	1.968524	0.000154	12782.62	0.159725
24	1.999938	2.022967	0.000154	13136.15	0.166662
25	2.083125	2.076659	0.000154	13484.8	0.173594
26	2.166765	2.130777	0.000154	13836.21	0.180564
27	2.250008	2.185323	0.000154	14190.41	0.187501
28	2.333251	2.245651	0.000154	14582.15	0.194438
29	2.416552	2.292591	0.000154	14886.95	0.201379
30	2.500135	2.355463	0.000154	15295.21	0.208345
31	2.583152	2.407732	0.000154	15634.62	0.215263

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
32	2.666622	2.458755	0.000154	15965.94	0.222219
33	2.750035	2.515301	0.000154	16333.12	0.22917
34	2.833335	2.575828	0.000154	16726.16	0.236111
35	2.916578	2.624167	0.000154	17040.05	0.243048
36	2.999992	2.681672	0.000154	17413.45	0.249999
37	3.083349	2.737286	0.000154	17774.58	0.256946
38	3.166648	2.792213	0.000154	18131.25	0.263887
39	3.249892	2.850211	0.000154	18507.86	0.270824
40	3.333249	2.909347	0.000154	18891.86	0.277771
41	3.416832	2.95928	0.000154	19216.1	0.284736
42	3.499849	3.004159	0.000154	19507.53	0.291654
43	3.583206	3.059488	0.000154	19866.81	0.298601
44	3.666675	3.108048	0.000154	20182.13	0.305556
45	3.750089	3.168097	0.000154	20572.06	0.312507
46	3.833049	3.215831	0.000154	20882.02	0.319421
47	3.916632	3.272457	0.000154	21249.72	0.326386
48	4.000046	3.329984	0.000154	21623.27	0.333337
49	4.083176	3.378903	0.000154	21940.93	0.340265
50	4.166533	3.443076	0.000154	22357.64	0.347211
51	4.250116	3.498697	0.000154	22718.81	0.354176
52	4.333246	3.554139	0.000154	23078.82	0.361104
53	4.416546	3.62438	0.000154	23534.94	0.368046
54	4.49996	3.681694	0.000154	23907.1	0.374997
55	4.583373	3.740781	0.000154	24290.79	0.381948
56	4.666616	3.807539	0.000154	24724.28	0.388885
57	4.749916	3.872714	0.000154	25147.49	0.395826
58	4.83333	3.937039	0.000154	25565.19	0.402778
59	4.916686	3.99835	0.000154	25963.31	0.409724
60	4.99993	4.067219	0.000154	26410.51	0.416661
61	5.083173	4.142013	0.000154	26896.19	0.423598
62	5.166813	4.20949	0.000154	27334.35	0.430568
63	5.249887	4.275783	0.000154	27764.82	0.437491
64	5.333243	4.348238	0.000154	28235.31	0.444437
65	5.416657	4.415925	0.000154	28674.84	0.451388
66	5.500127	4.488342	0.000154	29145.08	0.458344
67	5.583143	4.563064	0.000154	29630.29	0.465262
68	5.666557	4.63667	0.000154	30108.25	0.472213
69	5.750027	4.710985	0.000154	30590.81	0.479169
70	5.83327	4.784427	0.000154	31067.71	0.486106

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
71	5.91657	4.868574	0.000154	31614.12	0.493048
72	5.999927	4.956047	0.000154	32182.12	0.499994
73	6.08334	5.041741	0.000154	32738.58	0.506945
74	6.166641	5.133758	0.000154	33336.09	0.513887
75	6.249883	5.229358	0.000154	33956.87	0.520824
76	6.333354	5.327101	0.000154	34591.56	0.52778
77	6.416711	5.422214	0.000154	35209.18	0.534726
78	6.499841	5.530572	0.000154	35912.81	0.541653
79	6.583254	5.639365	0.000154	36619.25	0.548605
80	6.666781	5.748327	0.000154	37326.8	0.555565
81	6.749968	5.853323	0.000154	38008.59	0.562497
82	6.833097	5.969922	0.000154	38765.73	0.569425
83	6.916738	6.091732	0.000154	39556.7	0.576395
84	7.000038	6.218843	0.000154	40382.1	0.583337
85	7.083167	6.342124	0.000154	41182.62	0.590264
86	7.166581	6.476612	0.000154	42055.92	0.597215
87	7.250165	6.626685	0.000154	43030.42	0.60418
88	7.333237	6.768347	0.000154	43950.31	0.611103
89	7.416538	6.926782	0.000154	44979.1	0.618045
90	7.500008	7.09133	0.000154	46047.6	0.625001
91	7.583308	7.26636	0.000154	47184.16	0.631942
92	7.666608	7.426804	0.000154	48226	0.638884
93	7.749851	7.614542	0.000154	49445.08	0.645821
94	7.833378	7.817393	0.000154	50762.29	0.652782
95	7.916621	8.022367	0.000154	52093.29	0.659718
96	7.999978	8.2088	0.000154	53303.9	0.666665
97	8.083109	8.450528	0.000154	54873.56	0.673592
98	8.166749	8.685464	0.000154	56399.12	0.680562
99	8.249879	8.92749	0.000154	57970.71	0.68749
100	8.333178	9.16969	0.000154	59543.44	0.694432
101	8.416706	9.430375	0.000154	61236.2	0.701392
102	8.500062	9.689448	0.000154	62918.49	0.708339
103	8.583136	9.984176	0.000154	64832.31	0.715261
104	8.666606	10.29925	0.000154	66878.25	0.722217
105	8.750075	10.62088	0.000154	68966.75	0.729173
106	8.833205	10.9731	0.000154	71253.9	0.7361
107	8.916562	11.35284	0.000154	73719.74	0.743047
108	8.999975	11.743	0.000154	76253.25	0.749998
109	9.083389	12.1561	0.000154	78935.71	0.756949

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
110	9.166576	12.60746	0.000154	81866.62	0.763881
111	9.249876	13.07862	0.000154	84926.1	0.770823
112	9.333289	13.58573	0.000154	88219.03	0.777774
113	9.416702	14.11611	0.000154	91663.05	0.784725
114	9.499833	14.68038	0.000154	95327.14	0.791653
115	9.583302	15.3321	0.000154	99559.09	0.798609
116	9.666716	15.9951	0.000154	103864.3	0.80556
117	9.749902	16.72972	0.000154	108634.5	0.812492
118	9.83309	17.53034	0.000154	113833.4	0.819424
119	9.916786	18.41014	0.000154	119546.4	0.826399
120	9.999915	19.28008	0.000154	125195.3	0.833326
121	10.08322	20.27187	0.000154	131635.5	0.840268
122	10.16657	21.36858	0.000154	138757	0.847214
123	10.25004	22.52242	0.000154	146249.5	0.85417
124	10.33323	23.76476	0.000154	154316.6	0.861103
124.894	10.40775	25.01617	0.000154	162442.7	0.867313
125.716	10.47643	26.27069	0.000154	170588.9	0.873036
126.488	10.5408	27.52288	0.000154	178720	0.8784
127.214	10.60109	28.77558	0.000154	186854.4	0.883424
127.896	10.65793	30.03006	0.000154	195000.4	0.888161
128.52	10.70984	31.28022	0.000154	203118.3	0.892487
129.098	10.75817	32.53499	0.000154	211266.2	0.896514
129.654	10.80441	33.78601	0.000154	219389.7	0.900368
130.18	10.84822	35.03659	0.000154	227510.3	0.904018
130.658	10.88828	36.29013	0.000154	235650.2	0.907357
131.148	10.92902	37.54702	0.000154	243811.8	0.910752
131.584	10.96523	38.80051	0.000154	251951.4	0.913769
132.032	11.00263	40.05694	0.000154	260110	0.916886
132.218	11.01816	40.69725	0.000154	264267.9	0.91818

Table D-2 Raw data of mechanical properties of scaffold with 75:25 HA:Fibroin ratio

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
0	0	0	0.000154	0	0
1	0.083243	0.154318	0.000154	1002.065	0.006937
2	0.1666	0.318824	0.000154	2070.286	0.013883
3	0.250127	0.443126	0.000154	2877.442	0.020844
4	0.3332	0.551238	0.000154	3579.468	0.027767
5	0.416557	0.671315	0.000154	4359.188	0.034713
6	0.500027	0.821567	0.000154	5334.851	0.041669
7	0.583384	0.961023	0.000154	6240.409	0.048615
8	0.666457	1.148483	0.000154	7457.682	0.055538
9	0.749984	1.321785	0.000154	8583.019	0.062499
10	0.833397	1.476512	0.000154	9587.74	0.06945
11	0.916584	1.608237	0.000154	10443.1	0.076382
12	0.999884	1.721279	0.000154	11177.14	0.083324
13	1.083354	1.794413	0.000154	11652.04	0.09028
14	1.166711	1.870655	0.000154	12147.11	0.097226
15	1.249897	1.950136	0.000154	12663.22	0.104158
16	1.333311	2.032995	0.000154	13201.26	0.111109
17	1.416724	2.119374	0.000154	13762.17	0.11806
18	1.500024	2.209422	0.000154	14346.9	0.125002
19	1.583211	2.303297	0.000154	14956.48	0.131934
20	1.666738	2.401161	0.000154	15591.95	0.138895
21	1.749981	2.503182	0.000154	16254.43	0.145832
22	1.833281	2.609539	0.000154	16945.06	0.152773
23	1.916468	2.720414	0.000154	17665.03	0.159706
24	2.000165	2.835991	0.000154	18415.53	0.16668
25	2.083295	2.885816	0.000154	18739.07	0.173608
26	2.166538	2.936516	0.000154	19068.29	0.180545
27	2.249951	2.988108	0.000154	19403.3	0.187496
28	2.333478	3.040605	0.000154	19744.19	0.194457
29	2.416552	3.094025	0.000154	20091.07	0.201379
30	2.499908	3.148384	0.000154	20444.05	0.208326
31	2.583435	3.203697	0.000154	20803.23	0.215286
32	2.666622	3.259982	0.000154	21168.72	0.222219
33	2.749978	3.317256	0.000154	21540.63	0.229165
34	2.833278	3.375537	0.000154	21919.07	0.236107
35	2.916692	3.434841	0.000154	22304.16	0.243058
36	2.999935	3.495187	0.000154	22696.02	0.249995
37	3.083292	3.54237	0.000154	23002.4	0.256941

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
38	3.166648	3.590189	0.000154	23312.92	0.263887
39	3.250062	3.638654	0.000154	23627.62	0.270839
40	3.333249	3.687773	0.000154	23946.58	0.277771
41	3.416549	3.737555	0.000154	24269.84	0.284712
42	3.500076	3.78801	0.000154	24597.47	0.291673
43	3.583376	3.839145	0.000154	24929.51	0.298615
44	3.666506	3.890971	0.000154	25266.05	0.305542
45	3.749976	3.943496	0.000154	25607.12	0.312498
46	3.833446	3.996731	0.000154	25952.8	0.319454
47	3.916519	4.050684	0.000154	26303.14	0.326377
48	3.999933	4.105365	0.000154	26658.21	0.333328
49	4.083459	4.156308	0.000154	26989.01	0.340288
50	4.166703	4.207883	0.000154	27323.91	0.347225
51	4.249832	4.260097	0.000154	27662.97	0.354153
52	4.33336	4.31296	0.000154	28006.24	0.361113
53	4.416659	4.366479	0.000154	28353.76	0.368055
54	4.49996	4.420662	0.000154	28705.6	0.374997
55	4.583203	4.475517	0.000154	29061.8	0.381934
56	4.666673	4.531053	0.000154	29422.42	0.388889
57	4.749973	4.587278	0.000154	29787.52	0.395831
58	4.833216	4.644201	0.000154	30157.15	0.402768
59	4.916573	4.70183	0.000154	30531.36	0.409714
60	5.0001	4.760174	0.000154	30910.22	0.416675
61	5.08323	4.826777	0.000154	31342.71	0.423603
62	5.16653	4.894312	0.000154	31781.25	0.430544
63	5.250057	4.962792	0.000154	32225.93	0.437505
64	5.333413	5.032231	0.000154	32676.82	0.444451
65	5.416543	5.10264	0.000154	33134.03	0.451379
66	5.4999	5.174035	0.000154	33597.63	0.458325
67	5.583484	5.246429	0.000154	34067.72	0.46529
68	5.666501	5.319836	0.000154	34544.39	0.472208
69	5.749913	5.39427	0.000154	35027.73	0.479159
70	5.833327	5.469745	0.000154	35517.83	0.486111
71	5.916797	5.546277	0.000154	36014.78	0.493066
72	5.99987	5.623879	0.000154	36518.69	0.499989
73	6.08334	5.715118	0.000154	37111.16	0.506945
74	6.166697	5.807837	0.000154	37713.23	0.513891
75	6.250054	5.902061	0.000154	38325.07	0.520838
76	6.333241	5.997813	0.000154	38946.84	0.52777

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
77	6.416541	6.095119	0.000154	39578.69	0.534712
78	6.500067	6.194003	0.000154	40220.8	0.541672
79	6.58331	6.294491	0.000154	40873.32	0.548609
80	6.666497	6.39661	0.000154	41536.43	0.555541
81	6.750024	6.500385	0.000154	42210.29	0.562502
82	6.833494	6.605844	0.000154	42895.09	0.569458
83	6.916511	6.713014	0.000154	43591	0.576376
84	6.999981	6.821923	0.000154	44298.2	0.583332
85	7.083451	6.960118	0.000154	45195.57	0.590288
86	7.166637	7.101112	0.000154	46111.11	0.59722
87	7.249881	7.244962	0.000154	47045.21	0.604157
88	7.333464	7.391726	0.000154	47998.22	0.611122
89	7.416538	7.541464	0.000154	48970.54	0.618045
90	7.499951	7.694234	0.000154	49962.56	0.624996
91	7.583251	7.8501	0.000154	50974.67	0.631938
92	7.666721	8.009123	0.000154	52007.29	0.638893
93	7.749965	8.171367	0.000154	53060.82	0.64583
94	7.833265	8.336898	0.000154	54135.7	0.652772
95	7.916565	8.505782	0.000154	55232.35	0.659714
96	8.000148	8.678087	0.000154	56351.21	0.666679
97	8.083335	8.920729	0.000154	57926.81	0.673611
98	8.166521	9.170155	0.000154	59546.46	0.680543
99	8.250105	9.426555	0.000154	61211.4	0.687509
100	8.333349	9.690124	0.000154	62922.88	0.694446
101	8.416535	9.961063	0.000154	64682.23	0.701378
102	8.499948	10.23958	0.000154	66490.76	0.708329
103	8.583532	10.52588	0.000154	68349.86	0.715294
104	8.666492	10.82018	0.000154	70260.94	0.722208
105	8.749962	11.12272	0.000154	72225.46	0.729164
106	8.833376	11.43371	0.000154	74244.9	0.736115
107	8.916676	11.7534	0.000154	76320.81	0.743056
108	8.999862	12.08203	0.000154	78454.76	0.749989
109	9.083275	12.54739	0.000154	81476.56	0.75694
110	9.166689	13.03067	0.000154	84614.75	0.763891
111	9.249989	13.53257	0.000154	87873.82	0.770832
112	9.333175	14.05379	0.000154	91258.41	0.777765
113	9.416589	14.5951	0.000154	94773.36	0.784716
114	9.500059	15.15725	0.000154	98423.7	0.791672
115	9.583246	15.74105	0.000154	102214.6	0.798604

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
116	9.666489	16.34734	0.000154	106151.6	0.805541
117	9.750072	16.97699	0.000154	110240.2	0.812506
118	9.83343	17.63088	0.000154	114486.2	0.819453
119	9.916503	18.30996	0.000154	118895.8	0.826375
120	10.00003	19.0152	0.000154	123475.3	0.833336
121	10.08339	20.00516	0.000154	129903.7	0.840283
122	10.16663	21.04667	0.000154	136666.7	0.847219
123	10.24982	22.14241	0.000154	143781.9	0.854152
124	10.33346	23.29518	0.000154	151267.4	0.861122
125	10.41664	24.50798	0.000154	159142.7	0.868053
126	10.49989	25.78391	0.000154	167428	0.874991
127	10.58324	27.12627	0.000154	176144.6	0.881937
128	10.66683	28.53852	0.000154	185315.1	0.888903
129	10.7499	30.02429	0.000154	194962.9	0.895825
129.976	10.83127	31.58742	0.000154	205113.1	0.902606
130.924	10.91021	33.23192	0.000154	215791.7	0.909184
131.752	10.97934	34.96204	0.000154	227026.2	0.914945
132.236	11.01975	36.78223	0.000154	238845.7	0.918313

Table D-3 Raw data of mechanical properties of scaffold with 50:50 HA:Fibroin ratio

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
0	0.000113	0.043787	0.000154	284.4431	0
1	0.083527	0.179959	0.000154	1168.568	0.006961
2	0.166657	0.320168	0.000154	2079.013	0.013888
3	0.25007	0.421806	0.000154	2739.001	0.020839
4	0.33354	0.520584	0.000154	3380.416	0.027795
5	0.416784	0.632424	0.000154	4106.647	0.034732
6	0.49997	0.748362	0.000154	4859.494	0.041664
7	0.583497	0.869147	0.000154	5643.813	0.048625
8	0.666854	0.986516	0.000154	6405.947	0.055571
9	0.75004	1.098389	0.000154	7132.396	0.062503
10	0.833341	1.196928	0.000154	7772.26	0.069445
11	0.916924	1.288692	0.000154	8368.13	0.07641
12	1.000111	1.375887	0.000154	8934.331	0.083343
13	1.083411	1.44416	0.000154	9377.662	0.090284
14	1.166711	1.514548	0.000154	9834.727	0.097226
15	1.250237	1.585434	0.000154	10295.03	0.104186
16	1.333424	1.655414	0.000154	10749.44	0.111119
17	1.416724	1.722749	0.000154	11186.68	0.11806
18	1.500138	1.768645	0.000154	11484.71	0.125012
19	1.583551	1.83905	0.000154	11941.88	0.131963
20	1.666738	1.829946	0.000154	11882.77	0.138895
21	1.749981	1.908605	0.000154	12393.54	0.145832
22	1.833621	1.95356	0.000154	12685.45	0.152802
23	1.916694	1.985552	0.000154	12893.19	0.159725
24	2.000051	2.002982	0.000154	13006.38	0.166671
25	2.083465	2.047344	0.000154	13294.44	0.173622
26	2.166878	2.099184	0.000154	13631.06	0.180573
27	2.250008	2.119463	0.000154	13762.75	0.187501
28	2.333421	2.155718	0.000154	13998.17	0.194452
29	2.416835	2.201044	0.000154	14292.49	0.201403
30	2.500192	2.229236	0.000154	14475.56	0.208349
31	2.583322	2.270728	0.000154	14744.99	0.215277
32	2.666735	2.303677	0.000154	14958.94	0.222228
33	2.750205	2.338059	0.000154	15182.2	0.229184
34	2.833392	2.370681	0.000154	15394.03	0.236116
35	2.916692	2.406522	0.000154	15626.77	0.243058
36	3.000105	2.4484	0.000154	15898.7	0.250009
37	3.083519	2.471616	0.000154	16049.45	0.25696

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
38	3.166648	2.492804	0.000154	16187.04	0.263887
39	3.250062	2.527764	0.000154	16414.05	0.270839
40	3.333532	2.558975	0.000154	16616.72	0.277794
41	3.416775	2.593298	0.000154	16839.6	0.284731
42	3.499906	2.620519	0.000154	17016.36	0.291659
43	3.583546	2.65388	0.000154	17232.99	0.298629
44	3.666732	2.680621	0.000154	17406.63	0.305561
45	3.750032	2.709053	0.000154	17591.25	0.312503
46	3.833332	2.745368	0.000154	17827.06	0.319444
47	3.916916	2.769959	0.000154	17986.75	0.32641
48	4.000046	2.807262	0.000154	18228.97	0.333337
49	4.083346	2.84185	0.000154	18453.57	0.340279
50	4.166759	2.871008	0.000154	18642.91	0.34723
51	4.250116	2.903668	0.000154	18854.99	0.354176
52	4.333416	2.943778	0.000154	19115.44	0.361118
53	4.416659	2.972074	0.000154	19299.18	0.368055
54	4.500243	3.007851	0.000154	19531.5	0.37502
55	4.583486	3.033598	0.000154	19698.69	0.381957
56	4.666673	3.074568	0.000154	19964.73	0.388889
57	4.750029	3.096335	0.000154	20106.07	0.395836
58	4.83367	3.152702	0.000154	20472.09	0.402806
59	4.916686	3.18172	0.000154	20660.52	0.409724
60	5.000043	3.226262	0.000154	20949.75	0.41667
61	5.083457	3.261836	0.000154	21180.75	0.423621
62	5.16687	3.302736	0.000154	21446.34	0.430573
63	5.249943	3.344819	0.000154	21719.6	0.437495
64	5.33347	3.390407	0.000154	22015.63	0.444456
65	5.416827	3.433441	0.000154	22295.07	0.451402
66	5.50007	3.477607	0.000154	22581.86	0.458339
67	5.58337	3.525771	0.000154	22894.62	0.465281
68	5.666784	3.574353	0.000154	23210.08	0.472232
69	5.75014	3.611775	0.000154	23453.08	0.479178
70	5.833384	3.658114	0.000154	23753.99	0.486115
71	5.916684	3.712859	0.000154	24109.47	0.493057
72	6.000153	3.760081	0.000154	24416.11	0.500013
73	6.083454	3.813134	0.000154	24760.61	0.506955
74	6.166641	3.873104	0.000154	25150.03	0.513887
75	6.25011	3.921527	0.000154	25464.46	0.520843
76	6.333524	3.978482	0.000154	25834.3	0.527794

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
77	6.416711	4.034842	0.000154	26200.27	0.534726
78	6.499954	4.106217	0.000154	26663.75	0.541663
79	6.583594	4.164559	0.000154	27042.59	0.548633
80	6.666781	4.223297	0.000154	27424.01	0.555565
81	6.750024	4.305849	0.000154	27960.06	0.562502
82	6.833381	4.377798	0.000154	28427.26	0.569448
83	6.916908	4.449308	0.000154	28891.61	0.576409
84	7.000038	4.527756	0.000154	29401.01	0.583337
85	7.083394	4.60978	0.000154	29933.64	0.590283
86	7.166864	4.695769	0.000154	30492.01	0.597239
87	7.250051	4.789435	0.000154	31100.23	0.604171
88	7.333464	4.882711	0.000154	31705.92	0.611122
89	7.416651	4.981152	0.000154	32345.14	0.618054
90	7.500235	5.090381	0.000154	33054.42	0.62502
91	7.583421	5.195277	0.000154	33735.56	0.631952
92	7.666721	5.309791	0.000154	34479.16	0.638893
93	7.750078	5.430509	0.000154	35263.05	0.64584
94	7.833604	5.553244	0.000154	36060.03	0.6528
95	7.916621	5.682532	0.000154	36899.56	0.659718
96	8.000035	5.820966	0.000154	37798.48	0.66667
97	8.083505	5.96321	0.000154	38722.14	0.673625
98	8.166805	6.114282	0.000154	39703.13	0.680567
99	8.249935	6.272	0.000154	40727.27	0.687495
100	8.333405	6.443256	0.000154	41839.32	0.69445
101	8.416875	6.614828	0.000154	42953.43	0.701406
102	8.500062	6.807657	0.000154	44205.56	0.708339
103	8.583362	7.001696	0.000154	45465.56	0.71528
104	8.666832	7.208465	0.000154	46808.21	0.722236
105	8.750189	7.429357	0.000154	48242.58	0.729182
106	8.833376	7.664758	0.000154	49771.16	0.736115
107	8.916731	7.906931	0.000154	51343.71	0.743061
108	9.000145	8.167211	0.000154	53033.84	0.750012
109	9.083502	8.436529	0.000154	54782.66	0.756959
110	9.166633	8.723039	0.000154	56643.11	0.763886
111	9.250159	9.055601	0.000154	58802.6	0.770847
112	9.333459	9.39145	0.000154	60983.44	0.777788
113	9.416702	9.750975	0.000154	63318.02	0.784725
114	9.499945	10.14124	0.000154	65852.21	0.791662
115	9.583586	10.55433	0.000154	68534.61	0.798632

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
116	9.666716	10.98145	0.000154	71308.12	0.80556
117	9.749959	11.45481	0.000154	74381.88	0.812497
118	9.83343	11.98215	0.000154	77806.17	0.819453
119	9.916843	12.52365	0.000154	81322.4	0.826404
120	9.999973	13.10037	0.000154	85067.34	0.833331
121	10.08333	13.73394	0.000154	89181.43	0.840278
122	10.16686	14.43225	0.000154	93715.91	0.847238
123	10.2501	15.15741	0.000154	98424.74	0.854175
124	10.33334	15.96721	0.000154	103683.2	0.861112
125	10.4167	16.84147	0.000154	109360.2	0.868058
126	10.50028	17.79759	0.000154	115568.8	0.875023
127	10.58336	18.81969	0.000154	122205.8	0.881947
128	10.66666	19.97248	0.000154	129691.4	0.888888
128.992	10.7495	21.22428	0.000154	137820	0.895792
129.896	10.82487	22.47616	0.000154	145949.1	0.902073
130.752	10.89616	23.72816	0.000154	154079	0.908013
131.528	10.96076	24.97879	0.000154	162199.9	0.913397
132.108	11.00926	26.02672	0.000154	169004.7	0.917438

Table D-4 Raw data of mechanical properties of scaffold with 25:75 HA:Fibroin ratio

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
0	0	0	0.000154	0	0
1	0.083413	0.089626	0.000154	581.987	0.006951
2	0.16677	0.195165	0.000154	1267.305	0.013898
3	0.249957	0.263187	0.000154	1709.006	0.02083
4	0.333427	0.331893	0.000154	2155.149	0.027786
5	0.41684	0.424841	0.000154	2758.708	0.034737
6	0.500027	0.526988	0.000154	3422	0.041669
7	0.58327	0.582313	0.000154	3781.253	0.048606
8	0.66691	0.674156	0.000154	4377.636	0.055576
9	0.75004	0.742316	0.000154	4820.234	0.062503
10	0.833341	0.851319	0.000154	5528.045	0.069445
11	0.916641	0.941238	0.000154	6111.935	0.076387
12	1.000167	1.185899	0.000154	7700.643	0.083347
13	1.083354	1.263482	0.000154	8204.429	0.09028
14	1.166711	1.351021	0.000154	8772.864	0.097226
15	1.250067	1.404156	0.000154	9117.894	0.104172
16	1.333481	1.45938	0.000154	9476.495	0.111123
17	1.416724	1.516777	0.000154	9849.199	0.11806
18	1.499968	1.576431	0.000154	10236.56	0.124997
19	1.583494	1.63843	0.000154	10639.16	0.131958
20	1.666738	1.702869	0.000154	11057.59	0.138895
21	1.749981	1.769842	0.000154	11492.48	0.145832
22	1.833338	1.839448	0.000154	11944.47	0.152778
23	1.916921	1.911792	0.000154	12414.24	0.159743
24	1.999938	1.986982	0.000154	12902.48	0.166662
25	2.083351	2.028734	0.000154	13173.6	0.173613
26	2.166821	2.071364	0.000154	13450.41	0.180568
27	2.250065	2.114889	0.000154	13733.05	0.187505
28	2.333195	2.159329	0.000154	14021.62	0.194433
29	2.416778	2.204703	0.000154	14316.25	0.201398
30	2.500078	2.25103	0.000154	14617.08	0.20834
31	2.583378	2.293955	0.000154	14895.81	0.215282
32	2.666622	2.337699	0.000154	15179.86	0.222219
33	2.750148	2.382277	0.000154	15469.33	0.229179
34	2.833448	2.427705	0.000154	15764.32	0.236121
35	2.916692	2.473999	0.000154	16064.93	0.243058

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
36	2.999992	2.521176	0.000154	16371.27	0.249999
37	3.083462	2.562699	0.000154	16640.9	0.256955
38	3.166818	2.604906	0.000154	16914.98	0.263902
39	3.249948	2.647809	0.000154	17193.56	0.270829
40	3.333475	2.691418	0.000154	17476.74	0.27779
41	3.416775	2.735745	0.000154	17764.58	0.284731
42	3.500019	2.780802	0.000154	18057.15	0.291668
43	3.583262	2.826601	0.000154	18354.55	0.298605
44	3.666902	2.866365	0.000154	18612.76	0.305575
45	3.750032	2.906689	0.000154	18874.6	0.312503
46	3.833276	2.94758	0.000154	19140.13	0.31944
47	3.916689	2.989046	0.000154	19409.39	0.326391
48	4.000216	3.031095	0.000154	19682.44	0.333351
49	4.083289	3.073585	0.000154	19958.35	0.340274
50	4.166646	3.116671	0.000154	20238.13	0.347221
51	4.250172	3.160361	0.000154	20521.83	0.354181
52	4.33336	3.204664	0.000154	20809.5	0.361113
53	4.416716	3.249587	0.000154	21101.21	0.36806
54	4.500016	3.29514	0.000154	21397.01	0.375001
55	4.583542	3.341332	0.000154	21696.96	0.381962
56	4.666673	3.388171	0.000154	22001.11	0.388889
57	4.750029	3.435667	0.000154	22309.53	0.395836
58	4.833386	3.483829	0.000154	22622.26	0.402782
59	4.916913	3.532666	0.000154	22939.39	0.409743
60	4.99993	3.582187	0.000154	23260.95	0.416661
61	5.083343	3.632295	0.000154	23586.33	0.423612
62	5.166756	3.683105	0.000154	23916.26	0.430563
63	5.250057	3.734625	0.000154	24250.81	0.437505
64	5.333187	3.786865	0.000154	24590.03	0.444432
65	5.416827	3.839837	0.000154	24934.01	0.451402
66	5.50007	3.893549	0.000154	25282.79	0.458339
67	5.583313	3.948013	0.000154	25636.45	0.465276
68	5.666671	4.003239	0.000154	25995.06	0.472223
69	5.75014	4.059237	0.000154	26358.68	0.479178
70	5.833384	4.116018	0.000154	26727.39	0.486115
71	5.916684	4.173594	0.000154	27101.26	0.493057
72	6.00004	4.231975	0.000154	27480.36	0.500003
73	6.083454	4.305266	0.000154	27956.28	0.506955
74	6.166697	4.379827	0.000154	28440.44	0.513891

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
75	6.249883	4.455679	0.000154	28932.98	0.520824
76	6.333524	4.532845	0.000154	29434.06	0.527794
77	6.416711	4.611346	0.000154	29943.81	0.534726
78	6.499954	4.691208	0.000154	30462.39	0.541663
79	6.58331	4.765362	0.000154	30943.91	0.548609
80	6.666894	4.840688	0.000154	31433.04	0.555575
81	6.750024	4.917204	0.000154	31929.9	0.562502
82	6.833267	4.99493	0.000154	32434.61	0.569439
83	6.916738	5.073885	0.000154	32947.31	0.576395
84	7.000151	5.154088	0.000154	33468.1	0.583346
85	7.083281	5.257056	0.000154	34136.72	0.590273
86	7.166694	5.36208	0.000154	34818.7	0.597225
87	7.250221	5.469203	0.000154	35514.31	0.604185
88	7.333294	5.578466	0.000154	36223.81	0.611108
89	7.416708	5.689912	0.000154	36947.48	0.618059
90	7.500008	5.803584	0.000154	37685.61	0.625001
91	7.583534	5.927088	0.000154	38487.59	0.631961
92	7.666664	6.053221	0.000154	39306.63	0.638889
93	7.750021	6.182037	0.000154	40143.1	0.645835
94	7.833435	6.313595	0.000154	40997.37	0.652786
95	7.916791	6.447953	0.000154	41869.83	0.659733
96	7.999865	6.58517	0.000154	42760.84	0.666655
97	8.083392	6.758037	0.000154	43883.36	0.673616
98	8.166805	6.935441	0.000154	45035.33	0.680567
99	8.250048	7.117503	0.000154	46217.55	0.687504
100	8.333292	7.304344	0.000154	47430.81	0.694441
101	8.416875	7.49609	0.000154	48675.91	0.701406
102	8.500118	7.692869	0.000154	49953.69	0.708343
103	8.583305	7.894814	0.000154	51265.02	0.715275
104	8.666719	8.10206	0.000154	52610.78	0.722227
105	8.750132	8.314746	0.000154	53991.86	0.729178
106	8.833376	8.533016	0.000154	55409.19	0.736115
107	8.916676	8.757015	0.000154	56863.74	0.743056
108	9.000145	8.986895	0.000154	58356.46	0.750012
109	9.083389	9.303311	0.000154	60411.11	0.756949
110	9.166745	9.630868	0.000154	62538.11	0.763895
111	9.249932	9.969958	0.000154	64739.99	0.770828
112	9.333515	10.32099	0.000154	67019.4	0.777793
113	9.416702	10.68437	0.000154	69379.06	0.784725

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
114	9.499945	11.06056	0.000154	71821.8	0.791662
115	9.583359	11.44998	0.000154	74350.55	0.798613
116	9.666886	11.85312	0.000154	76968.33	0.805574
117	9.749959	12.28896	0.000154	79798.45	0.812497
118	9.833316	12.74083	0.000154	82732.64	0.819443
119	9.916843	13.20931	0.000154	85774.72	0.826404
120	10.00009	13.69501	0.000154	88928.65	0.833341
121	10.08327	14.28473	0.000154	92758.01	0.840273
122	10.16669	14.89985	0.000154	96752.26	0.847224
123	10.25021	15.54145	0.000154	100918.5	0.854184
124	10.33334	16.21068	0.000154	105264.2	0.861112
124.98	10.41511	16.90873	0.000154	109796.9	0.867926
125.952	10.49603	17.63683	0.000154	114524.9	0.874669
126.818	10.56811	18.39629	0.000154	119456.5	0.880676
127.628	10.63566	19.18846	0.000154	124600.4	0.886305
128.386	10.69884	20.01473	0.000154	129965.8	0.89157
129.088	10.75755	20.85774	0.000154	135439.9	0.896463
129.758	10.81331	21.73625	0.000154	141144.5	0.901109
130.376	10.86488	22.65177	0.000154	147089.4	0.905407
130.964	10.91367	23.60585	0.000154	153284.8	0.909473
131.522	10.96025	24.60012	0.000154	159741	0.913354
132.048	11.00405	25.63626	0.000154	166469.2	0.917004
132.224	11.01867	27.12054	0.000154	176107.4	0.918223

Table D-5 Raw data of mechanical properties of scaffold with 100:0 HA:Fibroin ratio

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
0	0	0	0.000154	0	0
1	0.083243	0.135423	0.000154	879.3701	0.006937
2	0.166827	0.306115	0.000154	1987.76	0.013902
3	0.249957	0.467728	0.000154	3037.195	0.02083
4	0.3332	0.639125	0.000154	4150.162	0.027767
5	0.416784	0.863218	0.000154	5605.312	0.034732
6	0.500084	1.023514	0.000154	6646.195	0.041674
7	0.583214	1.195153	0.000154	7760.734	0.048601
8	0.66674	1.389361	0.000154	9021.825	0.055562
9	0.75004	1.569845	0.000154	10193.8	0.062503
10	0.833397	1.688412	0.000154	10963.71	0.06945
11	0.916527	1.77342	0.000154	11515.71	0.076377
12	1.000054	1.875024	0.000154	12175.48	0.083338
13	1.083354	1.965212	0.000154	12761.12	0.09028
14	1.166654	2.058631	0.000154	13367.73	0.097221
15	1.249897	2.096156	0.000154	13611.4	0.104158
16	1.333424	2.141283	0.000154	13904.44	0.111119
17	1.416668	2.176315	0.000154	14131.92	0.118056
18	1.499911	2.201451	0.000154	14295.14	0.124993
19	1.583381	2.258463	0.000154	14665.34	0.131948
20	1.666794	2.321458	0.000154	15074.4	0.1389
21	1.749924	2.364412	0.000154	15353.32	0.145827
22	1.833224	2.401849	0.000154	15596.42	0.152769
23	1.916865	2.434849	0.000154	15810.71	0.159739
24	1.999994	2.472232	0.000154	16053.45	0.166666
25	2.083238	2.534545	0.000154	16458.08	0.173603
26	2.166651	2.602152	0.000154	16897.09	0.180554
27	2.250121	2.682123	0.000154	17416.38	0.18751
28	2.333251	2.765162	0.000154	17955.6	0.194438
29	2.416665	2.884512	0.000154	18730.6	0.201389
30	2.499965	2.971233	0.000154	19293.72	0.20833
31	2.583378	3.091523	0.000154	20074.82	0.215282
32	2.666565	3.154813	0.000154	20485.8	0.222214
33	2.749922	3.201518	0.000154	20789.08	0.22916
34	2.833392	3.251411	0.000154	21113.06	0.236116
35	2.916692	3.291516	0.000154	21373.48	0.243058
36	2.999878	3.337234	0.000154	21670.35	0.24999
37	3.083235	3.361231	0.000154	21826.18	0.256936

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
38	3.166818	3.398451	0.000154	22067.86	0.263902
39	3.249948	3.442123	0.000154	22351.45	0.270829
40	3.333249	3.501234	0.000154	22735.29	0.277771
41	3.416719	3.541231	0.000154	22995.01	0.284727
42	3.500019	3.591561	0.000154	23321.82	0.291668
43	3.583149	3.631315	0.000154	23579.97	0.298596
44	3.666732	3.687894	0.000154	23947.36	0.305561
45	3.750032	3.711235	0.000154	24098.93	0.312503
46	3.833219	3.738792	0.000154	24277.87	0.319435
47	3.916576	3.761208	0.000154	24423.43	0.326381
48	4.000159	3.790934	0.000154	24616.45	0.333347
49	4.083289	3.841024	0.000154	24941.71	0.340274
50	4.166646	3.884152	0.000154	25221.77	0.347221
51	4.249946	3.931218	0.000154	25527.39	0.354162
52	4.333416	3.965487	0.000154	25749.92	0.361118
53	4.416659	4.004889	0.000154	26005.77	0.368055
54	4.499846	4.058972	0.000154	26356.96	0.374987
55	4.58343	4.098945	0.000154	26616.53	0.381953
56	4.66673	4.134869	0.000154	26849.8	0.388894
57	4.749973	4.179643	0.000154	27140.54	0.395831
58	4.833216	4.202316	0.000154	27287.77	0.402768
59	4.916856	4.249954	0.000154	27597.1	0.409738
60	4.99993	4.296154	0.000154	27897.1	0.416661
61	5.08323	4.345971	0.000154	28220.59	0.423603
62	5.166644	4.395787	0.000154	28544.07	0.430554
63	5.250113	4.445604	0.000154	28867.56	0.437509
64	5.333243	4.495421	0.000154	29191.04	0.444437
65	5.4166	4.545237	0.000154	29514.53	0.451383
66	5.500013	4.595054	0.000154	29838.01	0.458334
67	5.58337	4.644871	0.000154	30161.5	0.465281
68	5.666557	4.694687	0.000154	30484.98	0.472213
69	5.74997	4.744504	0.000154	30808.47	0.479164
70	5.83344	4.794321	0.000154	31131.95	0.48612
71	5.916627	4.844137	0.000154	31455.44	0.493052
72	5.999927	4.893954	0.000154	31778.92	0.499994
73	6.083397	4.934215	0.000154	32040.36	0.50695
74	6.166697	4.994875	0.000154	32434.25	0.513891
75	6.24994	5.034815	0.000154	32693.6	0.520828
76	6.333354	5.089784	0.000154	33050.55	0.52778

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
77	6.416767	5.123697	0.000154	33270.76	0.534731
78	6.50001	5.169831	0.000154	33570.33	0.541668
79	6.58314	5.200658	0.000154	33770.51	0.548595
80	6.666724	5.234684	0.000154	33991.45	0.55556
81	6.750024	5.276991	0.000154	34266.18	0.562502
82	6.833211	5.296415	0.000154	34392.31	0.569434
83	6.916624	5.318942	0.000154	34538.58	0.576385
84	7.000151	5.332264	0.000154	34625.09	0.583346
85	7.083224	5.445064	0.000154	35357.56	0.590269
86	7.166637	5.560249	0.000154	36105.52	0.59722
87	7.249995	5.677872	0.000154	36869.3	0.604166
88	7.333351	5.797983	0.000154	37649.24	0.611113
89	7.416651	5.920634	0.000154	38445.68	0.618054
90	7.499838	6.04588	0.000154	39258.96	0.624987
91	7.583421	6.173776	0.000154	40089.45	0.631952
92	7.666664	6.304377	0.000154	40937.51	0.638889
93	7.749965	6.437741	0.000154	41803.51	0.64583
94	7.833265	6.573926	0.000154	42687.83	0.652772
95	7.916848	6.712992	0.000154	43590.86	0.659737
96	7.999865	6.855	0.000154	44512.99	0.666655
97	8.083221	6.98524	0.000154	45358.7	0.673602
98	8.166692	7.117955	0.000154	46220.49	0.680558
99	8.250105	7.253192	0.000154	47098.65	0.687509
100	8.333122	7.390998	0.000154	47993.49	0.694427
101	8.416648	7.531422	0.000154	48905.34	0.701387
102	8.500118	7.674514	0.000154	49834.5	0.708343
103	8.583305	7.820324	0.000154	50781.33	0.715275
104	8.666606	7.968905	0.000154	51746.14	0.722217
105	8.750018	8.120309	0.000154	52729.28	0.729168
106	8.833376	8.27459	0.000154	53731.1	0.736115
107	8.916562	8.431801	0.000154	54751.96	0.743047
108	8.99992	8.592128	0.000154	55793.04	0.749993
109	9.083389	8.874827	0.000154	57628.75	0.756949
110	9.166745	9.166828	0.000154	59524.86	0.763895
111	9.249819	9.468436	0.000154	61483.35	0.770818
112	9.333346	9.779967	0.000154	63506.28	0.777779
113	9.416702	10.10175	0.000154	65595.77	0.784725
114	9.49989	10.43412	0.000154	67754.01	0.791658
115	9.583132	10.77742	0.000154	69983.26	0.798594

Time (sec)	Extension (mm)	Load (N)	Surface area (m^2)	Stress (Pa)	Strain
116	9.666829	11.13202	0.000154	72285.86	0.805569
117	9.749959	11.49829	0.000154	74664.21	0.812497
118	9.833203	11.87661	0.000154	77120.83	0.819434
119	9.916673	12.26737	0.000154	79658.26	0.826389
120	10.00009	12.671	0.000154	82279.19	0.833341
121	10.08327	13.33457	0.000154	86588.14	0.840273
122	10.16657	14.0329	0.000154	91122.74	0.847214
123	10.2501	14.7678	0.000154	95894.83	0.854175
124	10.33334	15.54119	0.000154	100916.8	0.861112
125	10.41664	16.35508	0.000154	106201.8	0.868053
126	10.49989	17.21159	0.000154	111763.6	0.874991
127	10.58347	18.11296	0.000154	117616.6	0.881956
128	10.6666	19.06154	0.000154	123776.2	0.888883
129	10.74996	20.05979	0.000154	130258.3	0.89583
130	10.83326	21.11031	0.000154	137080	0.902772
131	10.91678	22.21586	0.000154	144258.8	0.909732
132	10.99991	23.3793	0.000154	151813.6	0.916659
132.28	11.02332	24.76988	0.000154	160843.4	0.91861

APPENDIX E

INTERNATIONAL CONFERENCE

ORAL PRESENTATION

Tarin Sukhachiradet and Wassanai Wattanutchariya*

"Preparation and Characterization of Fibroin/Chitosan/Hydroxyapatite Porous Scaffold", 2013 International Conference on Advances and Trends in Engineering Material and their Applications (ATEMA 2013), 11 – 12 October 2013, Holiday Inn Singapore.

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Preparation and Characterization of Fibroin/Chitosan/Hydroxyapatite porous scaffold for bone filling

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Abstract. Autograft is a general method used in orthopedic surgery for a bone replacement. However, the disadvantage of this method is the amount of risk factor to the donor sites. Currently, bone tissue engineering is another technique that could be implemented to solve this problem. Artificial bone scaffold generated by bone tissue engineering can be employed in order to accelerate damaged bone regeneration. In fact, this scaffold can be fabricated from synthetic contents such as bioceramics, biopolymers or composite. Three types of biomaterials: Chitosan, Hydroxyapatite(HA) and Fibroin were used to form porous scaffold. This research investigated the preparation of Hydroxyapatite and Fibroin from natural materials. Hydroxyapatite was synthesized from Mollusk shell by wet chemical precipitation method. While, Silk Fibroin was extracted from silk worms cocoons. Simultaneously, Freeze drying method was selected to fabricate this composite porous scaffold. A variety of mixing ratio between HA and Fibroin was studied to evaluate biodegradability, biocompatibility, porosity and pore structure of the output scaffolds. The output scaffolds have an interconnected porous structure with a pore size around 150-200μm and average porosity 94.26%. While the average degradation rate of the scaffold in lysozyme was between 9-11% at 7 days. Moreover, from biocompatibility test shown that the scaffold were non-cytotoxicity. Which, could be good for bone filling application in the future.

Introduction

Bone tissue is one of the most parts that need for replacement or repair material because of the bone defects from accident and bone related diseases. To solve this problem Autograft is a general method used in orthopedic surgery for bone replacement. Its advantages include good biocompatibility and no immunological response. However the disadvantage of this method is the amount of risk factor to donor site, the pain and

infection from surgery and the failure of the integration into the defect site after implantation. Therefore bone tissue engineering is trying to provide another technique that could be implemented to solve this problem.

Bone tissue engineering is a kind of science that applies the principle of biology and engineering together to study and develop substitutes to repair or replace the damaged bone tissue [1]. Usually, Methods to repair the damaged bone tissue include autograft, allograft and metallic replacement. Recently, there is a new popular approach for bone tissue engineering which is seeding 3D highly porous biodegradable matrices with cells and culture the cells to let the cells to proliferate in the 3D matrices. After that, implant to the defect area to induce bone tissue regeneration. Scaffold is a kind of biomedical device that uses to implant to the damaged tissue to help tissue regeneration. Scaffold is a 3D porous matrix that can be fabricated by three main materials including natural polymer, synthetic polymers, or ceramics. Ideally, scaffold should have the following characteristics: (1) three-dimensional and highly porous with interconnected pore network (2) biodegradability or bioresorbability (3) suitable surface chemistry for cell attachment, proliferation and differentiation (4) mechanical properties to match the tissue at transplantation sites (5) versatility to be processed into a variety of shape and size [1]. There are many ways to fabricate the scaffold such as freeze drying method, particulate leaching method, solid free form fabrication and polymer/ceramic fiber composite foam each method has their own advantages. Current research areas in biomaterials include the synthesis of bioactive/biodegradable materials from natural materials. For example, calcium carbonate and HA can be synthesized from mollusk shell [2, 3], and bovine bone [4]; fibroin can be extracted from cocoon silk [5], spider silk [6], and red ant nets [7]. These biomaterials are not only produced with cheaper cost, but also have sufficient performance to apply in bone tissue engineering such as biocompatibility, osteo-conductivity, and reasonable mechanical and physical properties.

Methodology

Fibroin Preparation

Silk Cocoons will be cut in to small pieces and peel the inner membrane off before degummed in 0.5% Na_2CO_3 solution. The degummed fiber will be washed and filtrated with water before dried at room temperature. The degummed fibers will then be put in Ajisawa's solution and boiled at 75ºC, prior to filtration and dialyzation for 5-6 days. Next, the solution is solidified by placing in a freezer. Afterward, the solid - mixture will be cleaned in methanol and put in lyophilizer for 12 hours for freeze drying before grinding the specimen into fibroin powders. After that the fibroin powder will be obtained.

Hydroxyapatite Preparation

Hydroxyapatite will be prepared by wet chemical precipitation method. Mollusk Shell will be used as a raw material for HA preparation. First, Mollusk shell will be cleaned and soaked in Hydrogen peroxide. After that put cleaned mollusk shell into oven for calcinations at 650°C. The mollusk shell will be grinded with ball mills for 2 hrs to obtain $CaCO_3$ powders. Next, the achieved $CaCO_3$ will be mixed with $NH_4H_2PO_4$ using the mol ratio Ca/P = 1/0.5 and dissolved in DI water while control the pH in range of 8-9 and stir continuously for 2 hrs. Then the solution was left at the room temperature for 12 hrs for chemical reaction and precipitation before filtering with Whatman No.45 filter paper. Next, the slurries will be oven dried at 110°C. Finally, Hydroxyapatite powder will be obtained after calcinations the specimen in an oven at 700°C. After that the HA powder will be obtained and grind in ball mills. Finally, the obtained HA powder will be characterized byX-ray diffraction (XRD) for phase identification.

Scaffold Fabrication

Porous Scaffold was prepared by soak chitosan flakes (97% deacetylation degree) in 2%(w/v) acetic acid solution (CH_3COOH) until they are completely dissolved. After that the same amount of hydroxyapatite and fibroin powder were put into chitosan solution and mixed together until they become homogeneous solution. Then the slurry pH was adjusted to 7 and transferred into mould and put in the -20^{o}C freezer for overnight for pre-freezing. Finally, the mould was freeze dried by lyophilizer for 48 hrs to form porous scaffold.

Scaffold Characterization

Porous scaffold will be characterized in the following parameters

Porosity

Porosity of fabricated porous scaffold was measured by liquid displacement method. Dimension of scaffold were measured and the scaffold were immersed in a known volume (V_1)of hexane for 5 minutes. The residual hexane volume after removal of scaffold were recorded as V_2. The volume of scaffold were calculated and porosity of scaffold was obtained by Eq.1

$$Porosity(\%) = \frac{V_1 - V_2}{V_{scaffold}} \times 100 \quad (1)$$

Pore Structure

The pore structure of porous scaffold was examined by scanning electron microscope (SEM) after the scaffold was coated with gold. The diameters of the pore was measured by SEM images.

Biodegradability

Biodegradability of the porous scaffold was determined by weight loss of scaffold after incubated in 1 mL PBS containing 1.6µg/mL of lysozyme from egg white at 37°C. After incubated for 7days and 15days, the scaffold were washed by Deionized (DI) water and freeze-dried. Finally, the weight different of the dry scaffold before and after incubated were used to calculate.

Biocompatibility Test

Biocompatibility was tested in order to prove that prepared scaffolds were not toxic or harmful to living cells. This procedure was performed by investigating cell viability percentage of peripheral blood mononuclear cells (PBMC).

Biocompatibility was conducted using PBMC maintained in RPMI – 1640. Prior to cell seeding, scaffolds were sterilized in an autoclave for 90 minutes. Cell was then seeded into scaffolds ($5x10^5$ cells/ml of medium/scaffold) in 12 – well plate. The well plate was kept at 37 °C in a humidified incubator for 24 hours under standard culturing conditions. Viability of cells grown on the scaffolds was determined using XTT assay

XTT assay is a technique based on the conversion of the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reagent to an orange formazan product by actively respiring cells. For this assay, XTT solution was mixed with Activator reagent. 250 µl of XTT was then added to cell – seeded scaffolds in each well. The well plate was incubated in an incubator with standard culturing conditions for four hours. The Microplate reader (SUNRISE, TACAN) at 450 nm wavelength was used measure absorbance in samples as shown in Figure

Optical density (OD) values were obtained after the Microplate reader read the absorbance. Percentage of cell viability could be calculated by using Eq. 2

$$Percentage\ of\ Cell\ viability = \frac{OD\ of\ treated\ cell}{OD\ of\ control\ cell} \times 100 \qquad (2)$$

Results and Discussion

Morphology of HA

The XRD spectra of synthesized HA powder were compared with JCPDS-ICDD Card no. 9 – 432[83] . Figure 1 shows that all significant peak of HA powder matched JCPDS-ICDD.

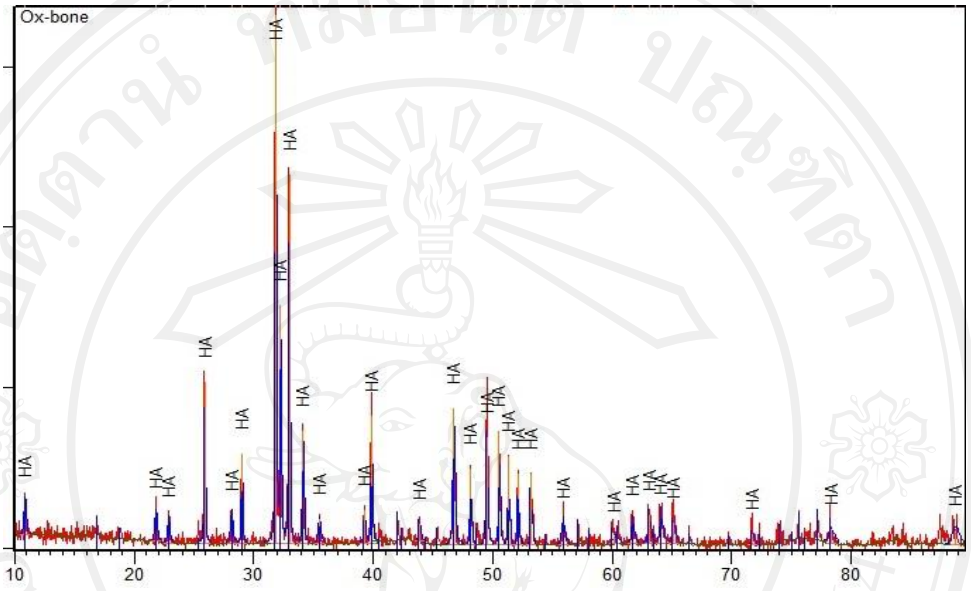

Figure 1. XRD analysis of hydroxyapatite

Porosity

The porosity of porous scaffold fabricated in this research was examined and shown that the porosity of scaffold surpass the minimum requirement for bone ingrowth 90% as shown in figure 2.

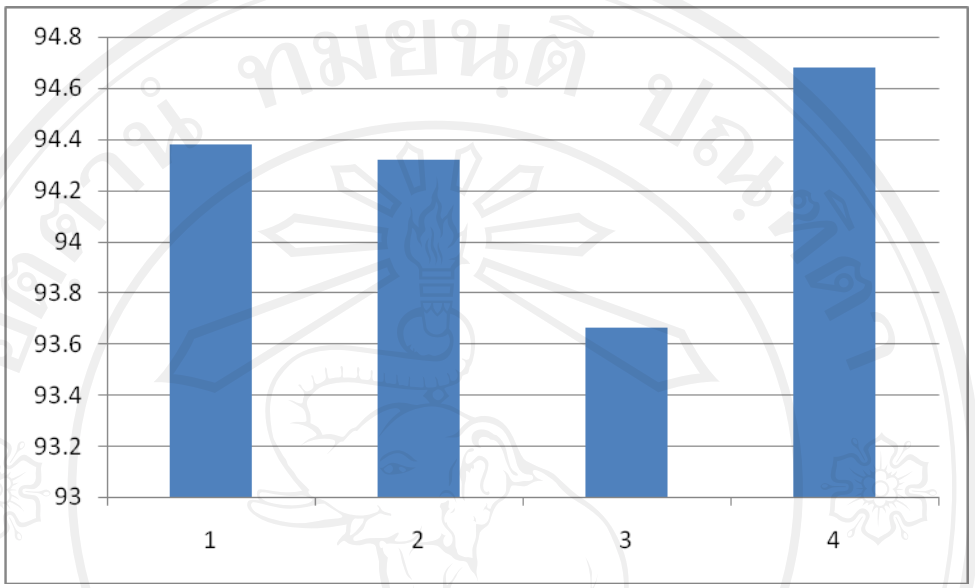

Figure 2 Porosity percentage of porous scaffold

Pore Structure

Figure 3 showed that fabricated porous scaffold structure is porous and share similarities with human trabecular bone. The pore size was found to be around 150-200 µm range. Which, surpass the minimum requirement considered to support tissue ingrowth, 100µm.

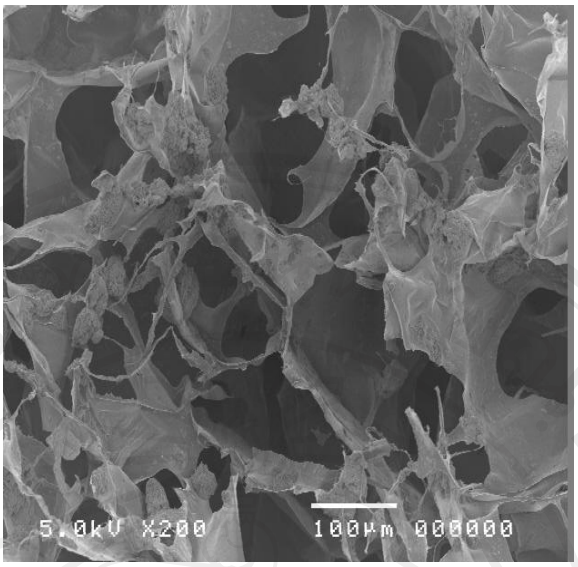

Figure 3. SEM image of fabricated scaffold

Biodegradation Test

Biodegradability of porous scaffold was tested 4 times by soak scaffolds in PBS containing lysozyme and incubated at 37°C for 7days. The results was shown in figure 4

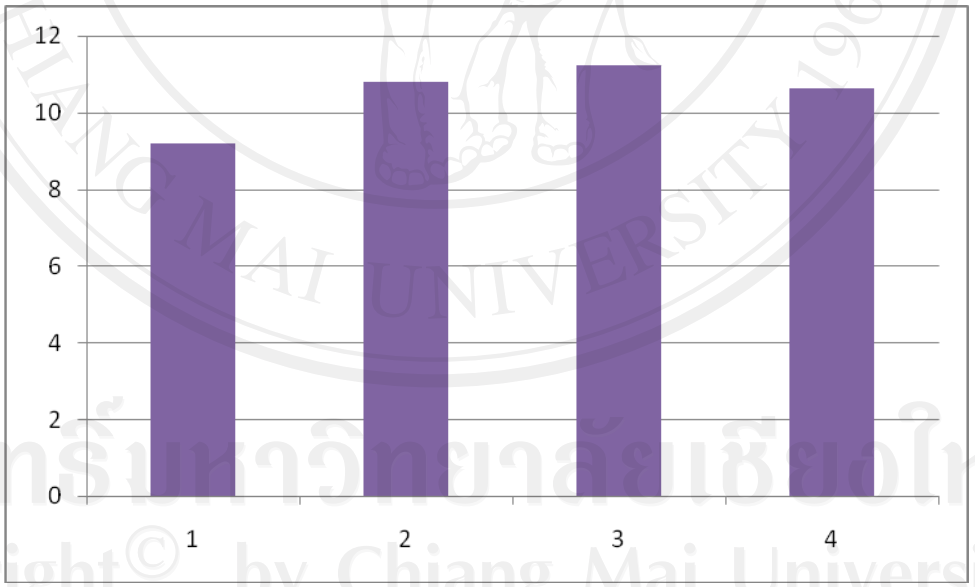

Figure 4 Degradability percentage of porous scaffold

In Vitro Biocompatibility Test

Optical density(OD) was used to calculate cell viability percentage as shown in table 1.

Treatment	Control	XTT assay	Cell viability[%]
1	0.148	0.144	97.29
2	0.145	0.139	95.86
3	0.15	0.143	95.33
4	0.145	0.141	97.24
Average			96.43

Table 1 Optical density and Cell viability percentage of XTT assay

Conclusion

The porous Fibroin/Chitosan/Hydroxyapatite porous scaffold are successfully fabricated with interconnected pore structure, suitable degradation and porosity. These Fibroin/Chitosan/Hydroxyapatite blended scaffold also non-cytotoxicity and combined the advantage of chitosan, fibroin and hydroxyapatite which can be promising candidates for various bone tissue engineering application in the future.

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