APPENDIX

APPENDIX A

STANDARD CURVE AND CALCULATION OF TOTAL PROTEIN OF ISOLATED COLLAGEN

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1. Standard curve

BSA was dissolved in distilled water at various concentration (0.2, 0.6, 1.0, 1.2 and 1.5 mg/ml) and then 5 μl of BSA solution was pipetted into a 96-well plate. 25 μl of reagent A and 200 μl of reagent B, were added and mixed by microplate reader for 5 seconds followed by incubation at room temperature for 15 min before measuring the absorbance at 750 nm. The result was taken to plot between the values of absorbance and their concentrations.

Table 42 Absorbance of standard protein.

BSA Concentration (mg/ml)	Absorbance (750 nm)			Average ± SD
	1	2	3	
1.5	0.266	0.269	0.265	0.267±0.002
1.2	0.228	0.241	0.228	0.232±0.008
1.0	0.193	00.208	0.200	0.200±0.008
0.6	0.153	0.159	0.158	0.157±0.003
0.2	0.097	0.103	0.101	0.100±0.003

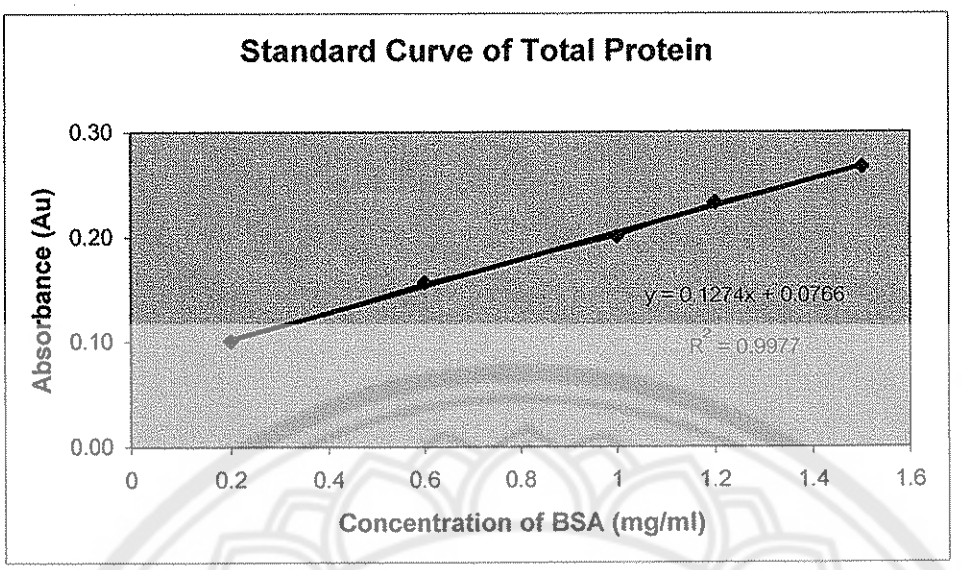

Figure 36 Standard curve for total protein calculation.

2. Sample preparation

Isolated collagen was dissolved in 0.5 M acetic acid at the concentration of 4 mg/ml followed by dilution of collagen solution into 2, 1, 0.5 and 0.25 mg/ml. The solution (5 μl) was pipetted into a 96-wells plate. 25 μl of reagent A and 200 μl of reagent B were added and mixed by microplate reader for 5 seconds followed by incubation at room temperature for 15 min before measuring the absorbance at 750 nm. The result was ploted compared with the standard curve.

2.1 Example of total protein calculation

The equation of standard curve is

$$y = 0.1274x + 0.0766$$

Whereas y is the absorbance of the sample and x is the sample concentration.

The absorbance of total protein from 4 mg/ml of the isolated collagen was 0.443. Such absorbance was replaced into above equation as shown below.

$$y = 0.1274x + 0.0766$$

$$0.443 = 0.1274x + 0.0766$$

$$x = \frac{(0.443 - 0.0766)}{0.1274}$$

$$x = 2.876$$

Total protein of the isolated collagen was calculated as follow.

$$\frac{(2.876 \times 100)}{4} = 71.90\%$$

APPENDIX B

PERCENT REMAINING WEIGHT

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Table 43 Percent remaining weight of the matrix after 1 month of degradation by collagenase.

Matrix	Initial weight (mg)	Final weight (mg)	% Remaining weight	Average±SD
1. Collagen/Shrimp MW 30,000 (8:2) + 0.15% GA				
1	6.66	2.73	40.99	
2	6.45	2.80	43.41	43.58±2.673
3	5.72	2.65	46.33	
2. Collagen/Shrimp MW 100,000 + 0.15% GA				
8:2				
1	6.57	3.51	53.42	
2	7.04	4.09	58.10	52.48±6.148
3	7.58	3.48	45.91	
7:3				
1	8.45	2.52	29.82	
2	7.53	4.79	63.61	54.99±22.150
3	7.27	5.20	71.53	
3. Collagen/Crab MW 100,000 - 1,000,000 (7:3) + 0.1% GA				
1	7.53	2.79	37.05	
2	7.80	1.92	24.62	30.45±6.254
3	7.75	2.30	29.68	
4. Collagen/Shrimp MW 30,000 (8:2) + 0.5% GP				
1	5.65	0	0	
2	5.70	0	0	-
3	5.70	0	0	

Table 43 (cont.).

Matrix	Initial weight (mg)	Final weight (mg)	% Remaining weight	Average±SD
5. Collagen/Shrimp MW 100,000 + 1% GP				
8:2				
1	2.90	0.78	26.90	
2	2.80	0.84	30.00	27.59±2.153
3	2.90	0.75	25.86	
7:3				
1	2.90	0.59	20.34	
2	3.00	0.62	20.67	20.81±0.556
3	2.80	0.60	21.43	
6. Collagen/Crab MW 100,000 - 1,000,000 (7:3) + 0.5% GP				
1	7.45	0	0	
2	7.50	0	0	-
3	7.32	0	0	
7. Collagen/PVA MW 72,000 (8:2)				
1	6.52	0	0.00	
2	6.88	0	0.00	-
3	6.75	0	0.00	
8. Collagen/PVA MW 145,000 (8:2)				
1	9.08	1.64	18.06	
2	7.09	1.38	19.46	18.70±0.708
3	8.93	1.66	18.59	

Table 43 (cont.).

Matrix	Initial weight (mg)	Final weight (mg)	% Remaining weight	Average±SD
9. Collagen/Shrimp chitosan MW 100,000				
8:2				
1	6.08	0	0	
2	6.00	0	0	-
3	6.00	0	0	
7:3				
1	6.00	0	0	
2	6.80	0	0	-
3	6.08	0	0	

Note: the formula 4 and 6 were completely degraded within 28 days, 7 and 9 were completely degraded within 14 days

APPENDIX C

STANDARD CURVE AND CALCULATION OF RELEASED HYDROXYPROLINE

APPENDIX C

STANDARD CURVE AND CALCULATION OF RELEASE HYDROXYPROLINE

1. Standard curve

HyP was dissolved in phosphate buffer saline (pH 7.4) in various concentrations, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml. The absorbance of HyP was scanned for the optimum wavelength (the optimum wavelength is 202 nm). The standard solution was analysed for HyP absorbance at the 202 nm.

Table 44 Absorbance of standard protein.

HyP Concentration (mg/ml)	Absorbance (202 nm)			Average ± SD
	1	2	3	
0.10	0.0425	0.0996	0.0506	0.0642±0.0309
0.20	0.1420	0.1479	0.1040	0.1313±0.0238
0.40	0.2720	0.2371	0.2944	0.2678±0.0289
0.80	0.4842	0.4980	0.5258	0.5027±0.0212
1.60	0.9463	1.0029	0.9846	0.9846±0.0332

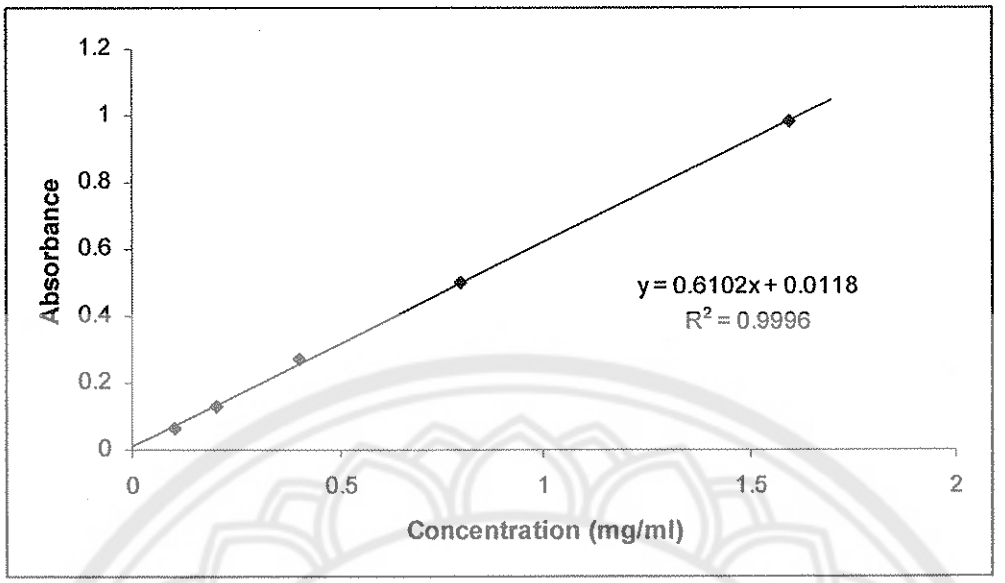


Figure 37 Standard curve of standard hydroxyproline.

Table 45 Absorbance of HyP released from the matrix degradation.

Time (hr)	Absorbance (202 nm)			Average±SD
	1	2	3	
1. Collagen/Shrimp chitosan MW 30,000 (8:2) + 0.15% GA				
2	2.159	2.151	-	2.155±0.006
4	1.810	1.870	-	1.840±0.043
6	1.615	1.502	-	1.558±0.079
16	1.787	1.577	-	1.682±0.148
24	1.809	0.512	-	1.161±0.917
48	1.536	1.084	-	1.310±0.319
168	1.354	1.455	-	1.404±0.071
2. Collagen/Shrimp chitosan MW 100,000 (8:2) + 0.15% GA				
2	1.692	1.779	1.808	1.760±0.061
4	1.442	1.407	1.492	1.447±0.043
6	1.153	1.054	1.122	1.109±0.051
16	1.136	1.072	1.098	1.102±0.032
24	1.005	1.038	1.076	1.040±0.036
48	0.765	0.626	0.657	0.683±0.073
168	0.499	0.748	0.846	0.698±0.179

Table 45 (cont.).

Time (hr)	Absorbance (202 nm)			Average±SD
	1	2	3	
3. Collagen/Shrimp chitosan MW 100,000 (7:3) + 0.15% GA				
2	1.335	1.263	1.232	1.277±0.053
4	1.054	0.945	0.934	0.977±0.067
6	0.855	0.757	0.718	0.776±0.071
16	0.831	0.721	0.705	0.753±0.069
24	0.816	0.758	0.791	0.788±0.029
48	0.687	0.580	0.615	0.627±0.055
168	0.863	0.653	0.560	0.692±0.155
4. Collagen/Crab chitosan MW 100,000 - 1,000,000 (7:3) + 0.1% GA				
2	2.225	3.461	2.922	2.869±0.619
4	2.577	2.259	1.679	2.172±0.455
6	1.407	2.190	2.195	1.931±0.453
16	1.486	2.148	2.157	1.931±0.385
24	1.610	2.168	2.170	1.983±0.323
48	1.095	2.155	2.196	1.815±0.624
168	1.477	2.593	2.813	2.294±0.716
5. Collagen/Shrimp chitosan MW 30,000 (8:2) + 0.5% GP				
2	1.410	1.490	1.490	1.470±0.046
4	1.430	1.410	1.410	1.420±0.014
6	1.420	1.460	1.440	1.440±0.018
16	1.340	1.380	1.450	1.390±0.055
24	1.270	1.240	1.270	1.260±0.018
48	1.320	1.350	1.260	1.310±0.045
168	0.620	0.630	0.620	0.620±0.003

Table 45 (cont.).

Time (hr)	Absorbance (202 nm)			Average±SD
	1	2	3	
6. Collagen/Shrimp chitosan MW 100,000 (8:2) + 1% GP				
2	1.150	1.150	1.140	1.150±0.007
4	1.160	1.140	1.160	1.150±0.012
6	1.030	1.180	1.180	1.130±.087
16	1.190	1.250	1.330	1.260±0.070
24	1.230	1.210	1.210	1.220±0.012
48	1.120	1.250	1.080	1.150±0.089
168	1.040	1.390	1.060	1.160±0.197
7. Collagen/Shrimp chitosan MW 100,000 (7:3) + 1% GP				
2	1.540	1.600	1.660	1.600±0.060
4	1.030	0.990	0.800	0.940±0.123
6	0.950	1.270	1.450	1.220±0.253
16	0.560	1.050	1.090	0.900±0.295
24	0.450	0.680	0.540	0.560±0.116
48	0.770	1.350	1.450	1.190±0.367
168	1.720	1.300	1.640	1.550±0.223
8. Collagen/Crab chitosan MW 100,000 - 1,000,000 (7:3) + 0.5% GP				
2	1.560	1.570	-	1.570±0.007
4	1.780	1.420	1.400	1.530±0.216
6	1.330	1.360	1.290	1.330±0.034
16	1.380	1.370	1.360	1.370±0.008
24	1.390	1.360	1.330	1.360±0.027
48	1.280	1.290	1.330	1.300±0.027
168	0.550	0.560	0.570	0.560±0.004

Table 45 (cont.).

Time (hr)	Absorbance (202 nm)			Average±SD
	1	2	3	
9. Collagen/PVA MW 72,000 (8:2)				
2	2.273	2.283	2.299	2.176±0.013
4	2.030	2.061	2.113	1.860±0.042
6	1.620	1.649	1.685	1.530±0.032
16	1.485	1.560	1.178	1.357±0.202
24	1.315	1.463	1.140	1.081±0.162
48	0.831	0.996	0.742	0.761±0.129
168	0.729	0.511	0.754	0.665±0.134
10. Collagen/PVA MW 145,000 (8:2)				
2	2.029	1.743	1.943	1.905±0.147
4	1.704	1.383	1.651	1.580±0.172
6	1.295	1.099	1.327	1.240±0.124
16	1.006	1.137	1.135	1.092±0.075
24	1.001	0.873	1.032	0.969±0.084
48	0.683	0.832	0.552	0.689±0.140
168	0.889	0.715	0.772	0.792±0.089
11. Collagen/ Shrimp chitosan MW 100,000 (8:2)				
2	2.819	2.819	2.794	2.811±0.014
4	2.843	2.794	2.843	2.827±0.028
6	2.563	2.892	2.892	2.782±0.190
16	2.916	3.063	3.258	3.079±0.172
24	3.014	2.965	2.965	2.981±0.028
48	2.745	3.063	2.648	2.819±0.217
168	2.550	3.405	2.599	2.851±0.480

2. Calculation of HyP concentration from the standard curve

Example of total protein calculation: collagen/shrimp MW 30,000 (8/2) + 0.15%GA.

Equation of the standard curve is

$$y = 0.6102x + 0.0118$$

Whereas y is the absorbance of the sample and x is the sample concentration.

The average absorbance of sample at hour 2 is 2.155. Such absorbance is replaced into above equation as shown below.

$$2.155 = 0.6102x + 0.0188$$

$$x = \frac{(2.155 - 0.0188)}{0.6102}$$

$$x = 3.5$$

∴ HyP concentration is 3.5 mg/ml

Table 46 Amounts of released HyP from matrix degradation.

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
1. Collagen/Shrimp chitosan MW 30,000 (8:2)+ 0.15% GA				
Hour 2	1	3.48	208.80	
	2	3.46	207.60	208.20±0.849
	3	-	-	
Hour 4	1	2.91	174.60	
	2	3.01	180.60	177.60±4.243
	3	-	-	
Hour 6	1	2.60	156.00	
	2	2.42	145.20	150.60±7.637
	3	-	-	
Hour 16	1	2.88	172.80	
	2	2.54	152.40	162.60±14.425
	3	-	-	
Hour 24	1	2.91	174.60	
	2	2.71	162.60	168.60±8.485
	3	-	-	
Hour 48	1	2.47	148.20	
	2	1.75	105.00	126.60±30.547
	3	-	-	
Day 7	1	2.18	130.80	
	2	2.34	140.40	135.60±6.788
	3	-	49.20	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
2. Collagen/Shrimp chitosan MW 100,000 (8:2)+ 0.15% GA				
Hour 2	1	2.72	163.20	
	2	2.87	172.20	170.00±6.010
	3	2.91	174.60	
Hour 4	1	2.32	139.20	
	2	2.27	136.20	139.80±3.934
	3	2.40	144.00	
Hour 6	1	1.86	111.60	
	2	1.70	102.00	107.40±4.911
	3	1.81	108.60	
Hour 16	1	1.83	109.80	
	2	1.73	103.80	106.60±3.020
	3	1.77	106.20	
Hour 24	1	1.62	97.20	
	2	1.67	100.20	100.40±3.305
	3	1.73	103.80	
Hour 48	1	1.23	73.80	
	2	1.01	60.60	66.00±6.920
	3	1.06	63.60	
Day 7	1	0.80	48.00	
	2	1.20	72.00	67.20±17.307
	3	1.36	81.60	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
3. Collagen/Shrimp chitosan MW 100,000 (7:3) + 0.15% GA				
Hour 2	1	2.15	129.00	123.20±5.242
	2	2.03	121.80	
	3	1.98	118.80	
Hour 4	1	1.70	102.00	94.40±6.609
	2	1.52	91.20	
	3	1.50	90.00	
Hour 6	1	1.38	82.80	75.20±6.823
	2	1.22	73.20	
	3	1.16	69.60	
Hour 16	1	1.34	80.40	72.80±6.609
	2	1.16	69.60	
	3	1.14	68.40	
Hour 24	1	1.31	78.60	76.00±2.706
	2	1.22	73.20	
	3	1.27	76.20	
Hour 48	1	1.11	66.60	60.60±5.499
	2	0.93	55.80	
	3	0.99	59.40	
Day 7	1	1.39	83.40	66.80±15.064
	2	1.05	63.00	
	3	0.90	54.00	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
4. Collagen/ Crab chitosan MW 100,000 – 1,000,000 (7:3) + 0.1% GA				
Hour 2	1	3.58	99.78	84.12±13.760
	2	5.57	78.61	
	3	4.71	73.97	
Hour 4	1	4.15	115.67	69.81±39.963
	2	3.64	51.37	
	3	2.70	42.40	
Hour 6	1	2.27	63.27	56.17±6.755
	2	3.53	49.82	
	3	3.53	55.44	
Hour 16	1	2.39	66.61	56.65±9.084
	2	3.46	48.83	
	3	3.47	54.49	
Hour 24	1	2.59	72.19	58.80±11.938
	2	3.49	49.26	
	3	3.50	54.96	
Hour 48	1	1.76	49.05	51.21±3.799
	2	3.47	48.97	
	3	3.54	55.59	
Day 7	1	2.38	66.33	65.49±6.117
	2	4.18	58.99	
	3	4.53	71.14	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
5. Collagen/Shrimp chitosan MW 30,000 (8:2) + 0.5% GP				
Hour 2	1	1.41	84.80	88.00±2.771
	2	1.49	89.60	
	3	1.49	89.60	
Hour 4	1	1.43	86.00	85.07±0.833
	2	1.41	84.80	
	3	1.41	84.40	
Hour 6	1	1.42	85.40	86.53±1.102
	2	1.46	87.60	
	3	1.44	86.60	
Hour 16	1	1.34	80.20	83.33±3.313
	2	1.38	83.00	
	3	1.45	86.80	
Hour 24	1	1.27	76.20	75.67±1.102
	2	1.24	74.40	
	3	1.27	76.40	
Hour 48	1	1.32	79.40	78.60±2.691
	2	1.35	80.80	
	3	1.26	75.60	
Day 7	1	0.62	37.40	37.40±0.200
	2	0.63	37.60	
	3	0.62	37.20	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
6. Collagen/Shrimp chitosan MW 100,000 (8:2) + 1% GP				
Hour 2	1	1.15	28.84	
	2	0.99	24.82	26.84±2.006
	3	1.08	26.85	
Hour 4	1	0.40	10.03	
	2	1.02	25.58	17.25±7.831
	3	0.65	16.16	
Hour 6	1	1.41	35.36	
	2	1.42	35.61	27.96±13.023
	3	0.52	12.93	
Hour 16	1	1.17	29.34	
	2	1.09	27.33	21.38±12.095
	3	0.30	7.46	
Hour 24	1	0.43	10.78	
	2	0.33	8.27	9.75±1.311
	3	0.41	10.19	
Hour 48	1	1.29	32.35	
	2	0.97	24.32	23.86±8.725
	3	0.60	14.91	
Day 7	1	0.64	16.05	
	2	1.02	25.58	20.34±4.835
	3	0.78	19.39	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
7. Collagen/Shrimp chitosan MW 100,000 (7:3) + 1% GP				
Hour 2	1	1.15	80.78	80.36±0.505
	2	1.15	80.50	
	3	1.14	79.80	
Hour 4	1	1.16	81.20	80.73±0.808
	2	1.14	79.80	
	3	1.16	81.20	
Hour 6	1	1.03	61.80	67.80±5.196
	2	1.18	70.80	
	3	1.18	70.80	
Hour 16	1	1.19	71.40	75.40±4.214
	2	1.25	75.00	
	3	1.33	79.80	
Hour 24	1	1.23	73.80	73.00±0.693
	2	1.21	72.60	
	3	1.21	72.60	
Hour 48	1	1.12	67.20	69.00±5.333
	2	1.25	75.00	
	3	1.08	64.80	
Day 7	1	1.04	62.40	69.80±11.793
	2	1.39	83.40	
	3	1.06	63.60	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
8. Collagen/Crab chitosan MW 100,000 – 1,000,000 (7:3) + 0.5% GP				
Hour 2	1	1.56	93.60	93.90±0.424
	2	1.57	94.20	
	3	-	-	
Hour 4	1	1.78	107.00	92.07±12.957
	2	1.42	85.40	
	3	1.40	83.80	
Hour 6	1	1.33	80.00	79.73±2.013
	2	1.36	81.60	
	3	1.29	77.60	
Hour 16	1	1.38	82.80	82.27±0.503
	2	1.37	82.20	
	3	1.36	81.80	
Hour 24	1	1.39	83.20	81.60±1.600
	2	1.36	81.60	
	3	1.33	80.00	
Hour 48	1	1.28	77.00	78.13±1.629
	2	1.29	77.40	
	3	1.33	80.00	
Day 7	1	0.55	32.80	33.53±0.643
	2	0.56	33.80	
	3	0.57	34.00	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
9. Collagen /PVA MW 72,000 (8:2)				
Hour 2	1	3.66	219.60	
	2	3.68	220.80	220.80±1.200
	3	3.70	222.00	
Hour 4	1	3.27	196.20	
	2	3.32	199.20	199.80±3.934
	3	3.40	204.00	
Hour 6	1	2.61	156.60	
	2	2.66	159.60	159.60±3.000
	3	2.71	162.60	
Hour 16	1	2.39	143.40	
	2	2.57	154.20	137.20±20.805
	3	1.90	114.00	
Hour 24	1	2.12	127.20	
	2	2.36	141.60	126.40±15.615
	3	1.84	110.40	
Hour 48	1	1.34	80.40	
	2	1.60	96.00	82.80±12.179
	3	1.20	72.00	
Day 7	1	1.17	70.20	
	2	0.82	49.20	64.20±13.077
	3	1.22	73.20	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
10. Collagen /PVA MW 145,000 (8:2)				
Hour 2	1	3.27	196.20	
	2	2.81	168.60	184.20±14.148
	3	3.13	187.80	
Hour 4	1	2.74	164.40	
	2	2.23	133.80	152.60±16.457
	3	2.66	159.60	
Hour 6	1	2.09	125.40	
	2	1.77	106.20	120.00±12.045
	3	2.14	128.40	
Hour 16	1	1.62	97.20	
	2	1.83	109.80	105.60±7.275
	3	1.83	109.80	
Hour 24	1	1.61	96.60	
	2	1.41	84.60	93.60±7.937
	3	1.66	99.60	
Hour 48	1	1.10	66.00	
	2	1.34	80.40	66.60±13.510
	3	0.89	53.40	
Day 7	1	1.43	85.80	
	2	1.15	69.00	76.40±8.577
	3	1.24	74.40	

Table 46 (cont.).

The marix		HyP concentration (mg/ml)	Amount of HyP (mg)	Average±SD (mg)
11. Collagen/Shrimp chitosan MW 100,000 (8:2)				
Hour 2	1	4.60	322.00	
	2	4.60	322.00	321.07±1.617
	3	4.56	319.20	
Hour 4	1	4.64	324.80	
	2	4.56	319.20	322.93±3.233
	3	4.64	324.80	
Hour 6	1	4.12	247.20	
	2	4.72	283.20	271.20±20.785
	3	4.72	283.20	
Hour 16	1	4.76	285.60	
	2	5.00	300.00	301.60±16.857
	3	5.32	319.20	
Hour 24	1	4.92	295.20	
	2	4.84	290.40	292.00±2.771
	3	4.84	290.40	
Hour 48	1	4.48	268.80	
	2	5.00	300.00	276.00±21.332
	3	4.32	259.20	
Day 7	1	4.16	249.60	
	2	5.56	333.60	279.20±47.173
	3	4.24	254.40	

3. **Amount of HyP calculation**

Example of total protein calculation: collagen/shrimp MW 30,000 (8/2) + 0.15%GA.

According to the concentration of HyP in the collected sample (10 ml), the total amount of HyP is calculated as follow.

Amount of HyP = Concentration of HyP x dilution factor x total volume

= 3.48 (mg/ml) x 6 x 10 (ml)

= 208.80 mg

The total amount of HyP is: 208.80 mg

APPENDIX D

CALCULATION OF PERCENT CELL ADHESION ON THE MATRIX

APPENDIX D

CALCULATION OF PERCENT CELL ADHESION ON THE MATRIX

1. XTT assay protocol

After culture (from the part of adhesion test), the XTT solution was added into each culture well in an amount of 50 µl/well. The incubation was incubated at 37°C for 4 hr then followed by measurement of the absorbance at 490 nm. The result of absorbance (Abs.) is shown in table 45.

2. Calculation example

This is the calculation of percent of cell adhesion on the matrix prepared from collagen/shrimp chitosan MW 30,000 (8:2) crosslinked with 0.15% GA.

The average of the absorbance of cell adhesion on scaffold was subtracted by the absorbance of matrix without cell seeding.

Abs. of cell on matrix = Abs. of cell & matrix – Abs. of matrix without cell

= 1.377- 0.980

= 0.397

Percent of cell adhesion on the matrix compared with the control (100%) was calculated as below.

$$\% \text{ cell adhesion} = \frac{(\text{Abs. of cell on matrix} \times 100\%)}{\text{Abs. of cell on plastic plate}}$$

= (0.397x100)/0.429

= 92.54%

Table 47 Result of an absorbance at 490 nm of cell adhesion on the matrix after 3 hr of incubation

Matrix	Absorbance of matrix with cells				Absorbance of matrix without cells	% Adhesion
	1	2	3	Average± SD		
Control	0.429			0.429	-	100
1. Collagen/Shrimp MW 30,000 (8:2) + 0.15% GA	1.425	1.329	-	1.377±0.068	0.980	92.54
2. Collagen/Shrimp MW 100,000 (8:2) + 0.15% GA	0.764	0.953	0.720	0.773±0.124	0.559	69.12
3. Collagen/Shrimp MW 100,000 (7:3) + 0.15% GA	0.852	0.752	0.715	0.812±0.071	0.630	39.06
4. Collagen/Crab MW 100,000-1,000,000 (7:3) + 0.1% GA	0.992	0.931	-	0.962±0.043	0.629	77.62
5. Collagen/PVA MW 145,000 (8:2)	1.207	0.982	-	1.005±0.0.32	0.689	77.43

APPENDIX E

CALCULATION OF PERCENT CELL PROLIFERATION ON THE MATRIX

APPENDIX E

CALCULATION OF PERCENT CELL PROLIFERATION ON THE MATRIX

1. XTT assay protocol

After culture (from the part of proliferation test), the XTT solution was added into each culture well in an amount of 50 µl/well. The incubation was calculated at 37°C for 4 hr then followed by measurement of the absorbance at 490 nm. The absorbance results of are shown in table 46.

2. Calculation

The calculation was performed similarly to the calculation of percent cell adhesion (Appendix D).

Table 48 Result of an absorbance at 490 nm of cell proliferation on the matrix after 5 days of incubation.

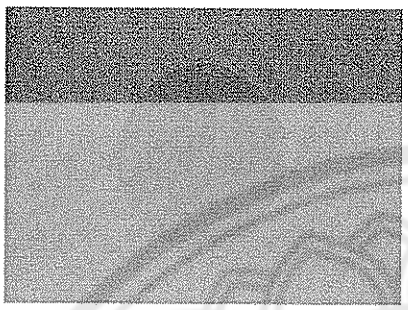
Matrix	Absorbance of matrix + cell				Absorbance of matrix	% Proliferation
	1	2	3	Average ± SD		
Control	0.780			0.780		100
1.Collagen/ Shrimp MW 30,000 (8:2) + GA 0.15%	1.480	1.720	1.768	1.656±0.154	0.980	86.63
2.Collagen/ Shrimp MW 100,000 (8:2) + GA0.15%	1.418	1.232	1.246	1.299±0.104	0.678	89.61
3.Collagen/ Shrimp MW 100,000 (7:3) + GA 0.15%	1.233	1.232	1.298	1.254±0.038	0.644	88.07
4.Collagen/ Crab MW 100,000-1,000,000 (7:3) + GA 0.1%	1.418	1.937	1.654	1.670±0.260	0.629	133.36
5.Collagen/ PVA MW 145,000 (8:2)	1.247	0989	0.975	1.070±0.153	0.689	48.87

APPENDIX F

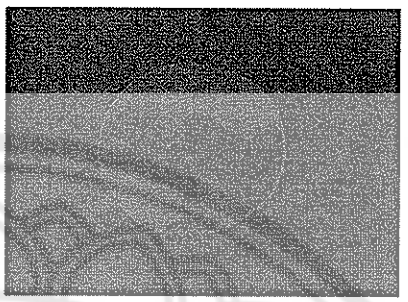
EXAMPLES OF MATRIX

APPENDIX F

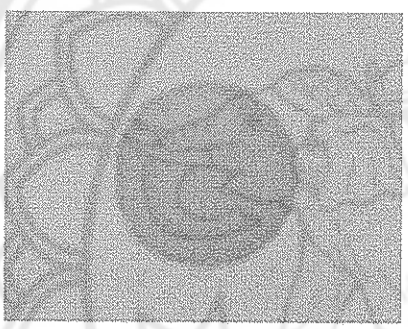
EXAMPLES OF MATRIX



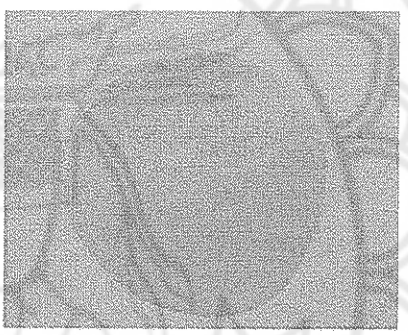
Chitosan matrix



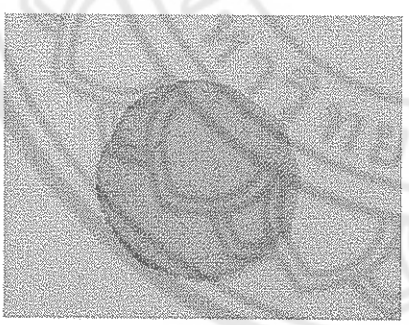
PVA matrix



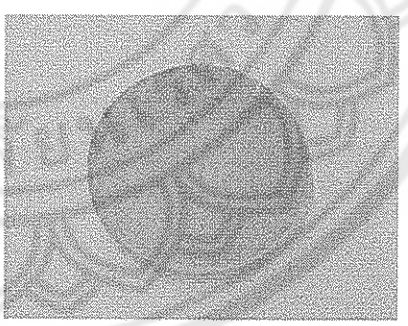
Collagen/Chitosan matrix



Collagen/PVA matrix



Collagen/Chitosan matrix crosslinked with GA



Collagen/Chitosan matrix crosslinked with GP

APPENDIX G

CALCULATION OF CELL PROLIFERATION RATE ON DEVELOPED MATRIX

APPENDIX G

CALCULATION OF CELL PROLIFERATION RATE ON DEVELOPED MATRIX

Rate of cell proliferation on the matrix was calculated as follow.

$$\text{Proliferation rate} = \frac{\text{Abs. at day } i - \text{Abs. At day } j}{\text{Incubation time (day)}}$$

When *i* and *j* represented the time of incubation (day).

Example: Proliferation rate on the matrix at day 0 - 2

Proliferation rate at day 0 - 2 = (0.388 – 0) / 2

= 0.194 Abs./day