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APPENDICES

Appendix A Determination of Molecular Weight of Chitosan

Table A1 Running time of solvent and 1st treated chitosan solution

Concentration (g/100ml)	Time (Second)			
	1	2	3	Average
0.0000	99.38	99.13	99.03	99.18
0.0125	116.68	116.77	116.78	116.74
0.0250	136.25	136.25	136.24	136.25
0.0500	180.85	180.73	181.85	181.14
0.0750	232.38	232.47	232.53	232.46
0.1000	294.7	294.58	294.62	294.63

Table A2 Data of relative viscosity (η_{rel}), specific viscosity (η_{sp}), and reduced viscosity (η_{red}) of chitosan solution with various concentrations

Concentration (g/100ml)	η_{rel}	η_{sp}	η_{red}	$\ln[\eta_{rel}]/c$
0.0000	1.00	0.00	-	-
0.0125	1.18	0.18	14.63	13.43
0.0250	1.38	0.38	15.22	12.90
0.0500	1.84	0.84	16.71	12.14
0.0750	2.36	1.36	18.07	11.42
0.1000	2.99	1.99	19.85	10.94

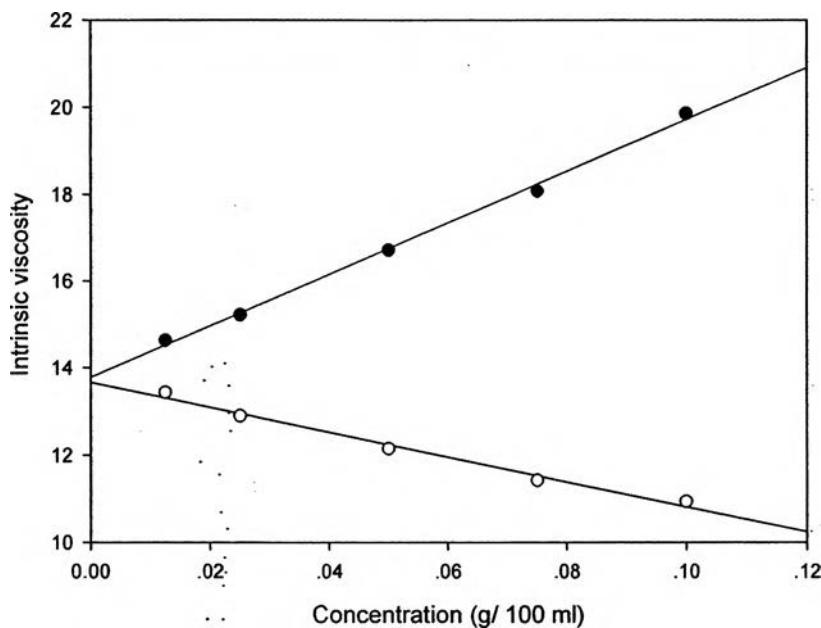


Figure A1 Plot of reduced viscosity (η_{sp}/c) and $\ln((\eta_{rel})/c)$ versus concentration of chitosan solution: ● = (η_{sp}/c) and ○ = $\ln((\eta_{rel})/c)$.

The viscosity-average molecular weight (M_v) of chitosan was determined base on Mark-Houwink equation. The K and a values were according to Wang *et al.* (1997).

$$[\eta] = (6.59 \times 10^{-5}) M^{0.88}$$

where $[\eta]$ = intrincsic viscosity

M = viscosity-average molecular weight

Interception: $[\eta] = 13.70$

From calculation; $M^{0.88} = (13.70)/6.59 \times 10^{-5} = 2.08 \times 10^5$

$$0.88 \log M = \log[2.08 \times 10^5]$$

$$\log M = 6.04$$

$$M = 1.10 \times 10^6$$

The viscosity-average molecular weight of chitosan after 1st deacetylation obtained from calculation was 1.10×10^6 g/mol.

Table A3 Running time of solvent and 2nd treated chitosan solution

Concentration (g/100ml)	Time (Second)			
	1	2	3	Average
0.0000	98.78	98.70	98.61	98.70
0.0125	114.34	114.72	114.60	114.55
0.0250	131.62	131.24	131.67	131.51
0.0500	168.48	168.23	168.23	168.31
0.0750	210.74	210.77	210.68	210.73
0.1000	260.37	260.43	259.56	260.12

Table A4 Data of relative viscosity (η_{rel}), specific viscosity (η_{sp}), and reduced viscosity (η_{red}) of chitosan solution with various concentrations

Concentration (g/100ml)	η_{rel}	η_{sp}	η_{red}	$\ln[\eta_{\text{rel}}]/c$
0.0000	1.00	0.00	-	-
0.0125	1.16	0.16	12.85	11.92
0.0250	1.33	0.33	13.30	11.48
0.0500	1.71	0.71	14.11	10.68
0.0750	2.14	1.14	15.14	10.11
0.1000	2.64	1.64	16.36	9.69

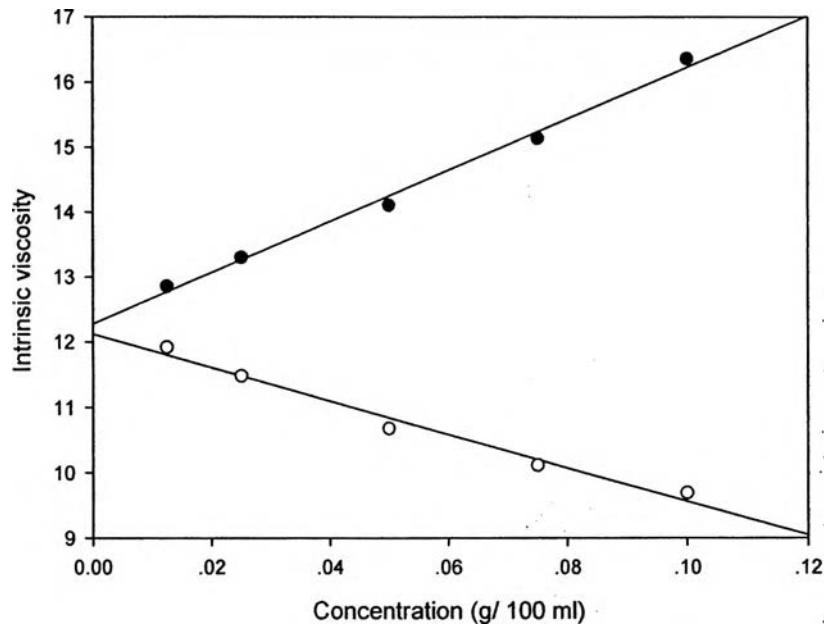


Figure A1 Plot of reduced viscosity (η_{sp}/c) and $\ln((\eta_{rel})/c)$ versus concentration of chitosan solution: ● = (η_{sp}/c) and ○ = $\ln((\eta_{rel})/c)$.

The viscosity-average molecular weight (M_v) of chitosan was determined base on Mark-Houwink equation. The K and a values were according to Wang *et al.* (1997).

$$[\eta] = (6.59 \times 10^{-5}) M^{0.88}$$

where $[\eta]$ = intrinsic viscosity

M = viscosity-average molecular weight

Interception: $[\eta] = 12.20$

From calculation; $M^{0.88} = (12.20)/6.59 \times 10^{-5} = 1.85 \times 10^5$

$$0.88 \log M = \log[1.85 \times 10^5]$$

$$\log M = 5.99$$

$$M = 9.68 \times 10^5$$

The viscosity-average molecular weight of chitosan after 2nd deacetylation obtained from calculation was 9.68×10^5 g/mol.

Table A5 Running time of solvent and 3rd treated chitosan solution

Concentration (g/100ml)	Time (Second)			
	1	2	3	Average
0.0000	98.78	98.70	98.61	98.70
0.0125	112.12	112.05	112.08	112.08
0.0250	126.56	126.59	127.02	126.72
0.0500	159.37	159.18	159.21	159.25
0.0750	194.90	194.72	194.95	194.86
0.1000	236.55	236.37	236.66	236.53

Table A6 Data of relative viscosity (η_{rel}), specific viscosity (η_{sp}), and reduced viscosity (η_{red}) of chitosan solution with various concentrations

Concentration (g/100ml)	η_{rel}	η_{sp}	η_{red}	$\ln[\eta_{\text{rel}}]/c$
0.0000	1.00	0.00	-	-
0.0125	1.14	0.14	10.85	10.18
0.0250	1.28	0.28	11.36	10.00
0.0500	1.61	0.61	12.27	9.57
0.0750	1.97	0.97	12.99	9.07
0.1000	2.40	1.40	13.97	8.74

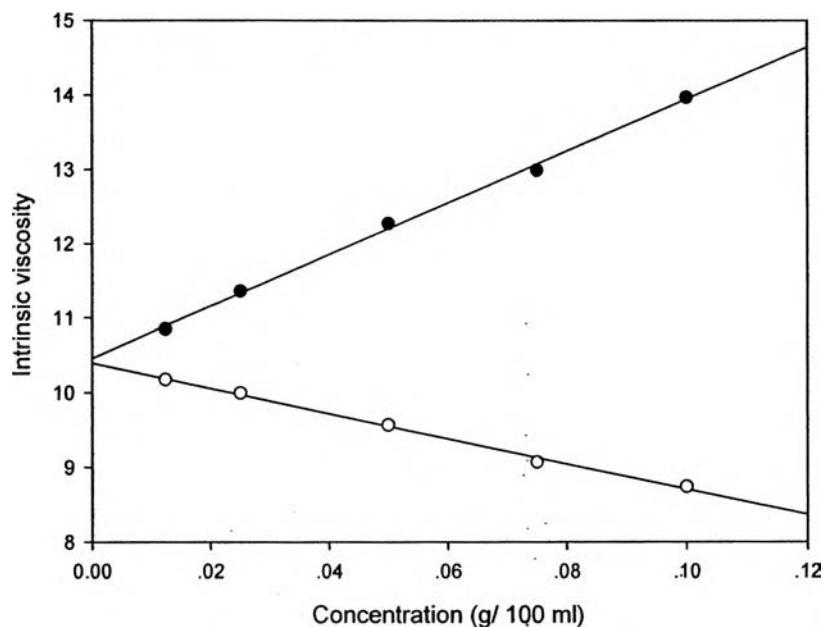


Figure A1 Plot of reduced viscosity (η_{sp}/c) and $\ln((\eta_{rel})/c)$ versus concentration of chitosan solution: ● = (η_{sp}/c) and ○ = $\ln((\eta_{rel})/c)$.

The viscosity-average molecular weight (M_v) of chitosan was determined base on Mark-Houwink equation. The K and a values were according to Wang *et al.* (1997).

$$[\eta] = (6.59 \times 10^{-5}) M^{0.88}$$

where $[\eta]$ = intrinsic viscosity

M = viscosity-average molecular weight

Interception: $[\eta] = 10.40$

From calculation; $M^{0.88} = (10.40)/6.59 \times 10^{-5} = 1.58 \times 10^5$

$$0.88 \log M = \log[1.58 \times 10^5]$$

$$\log M = 5.91$$

$$M = 8.07 \times 10^5$$

The viscosity-average molecular weight of chitosan after 3rd deacetylation obtained from calculation was 8.07×10^5 g/mol.

Table A7 Running time of solvent and 4th treated chitosan solution

Concentration (g/100ml)	Time (Second)			
	1	2	3	Average
0.0000	98.78	98.70	98.61	98.70
0.0125	110.60	110.59	110.54	110.58
0.0250	123.18	123.04	123.08	123.10
0.0500	151.48	151.84	151.68	151.67
0.0750	181.82	181.81	181.87	181.83
0.1000	217.86	217.52	217.98	217.79

Table A8 Data of relative viscosity (η_{rel}), specific viscosity (η_{sp}), and reduced viscosity (η_{red}) of chitosan solution with various concentrations

Concentration (g/100ml)	η_{rel}	η_{sp}	η_{red}	$\ln[\eta_{\text{rel}}]/c$
0.0000	1.00	0.00	-	-
0.0125	1.12	0.12	9.63	9.09
0.0250	1.25	0.25	9.89	8.84
0.0500	1.54	0.54	10.73	8.59
0.0750	1.84	0.84	11.23	8.15
0.1000	2.21	1.21	12.07	7.91

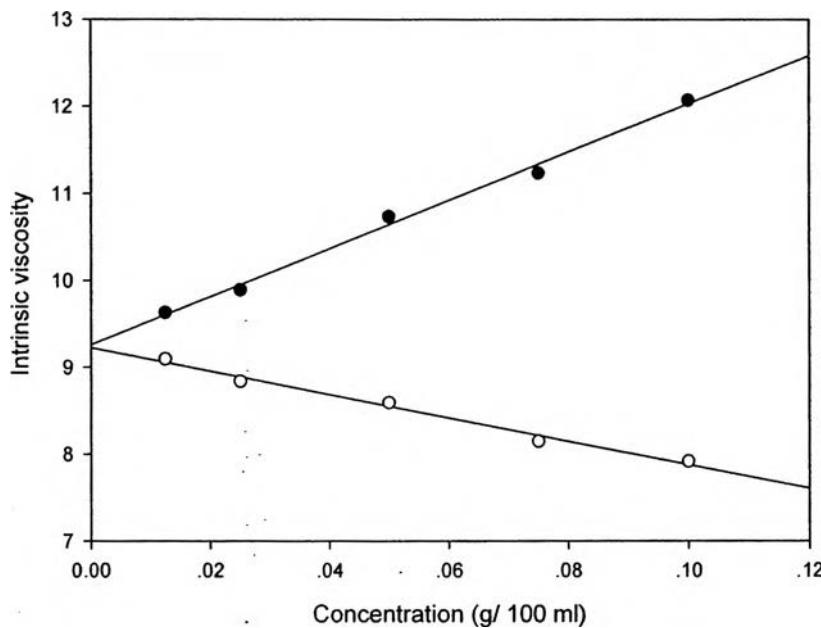


Figure A1 Plot of reduced viscosity (η_{sp}/c) and $\ln((\eta_{rel})/c)$ versus concentration of chitosan solution: ● = (η_{sp}/c) and ○ = $\ln((\eta_{rel})/c)$.

The viscosity-average molecular weight (M_v) of chitosan was determined base on Mark-Houwink equation. The K and a values were according to Wang *et al.* (1997).

$$[\eta] = (6.59 \times 10^{-5}) M^{0.88}$$

where $[\eta]$ = intrinsic viscosity

M = viscosity-average molecular weight

Interception: $[\eta] = 9.22$

From calculation; $M^{0.88} = (9.22)/6.59 \times 10^{-5} = 1.40 \times 10^5$

$$0.88 \log M = \log[1.40 \times 10^5]$$

$$\log M = 5.85$$

$$M = 7.04 \times 10^5$$

The viscosity-average molecular weight of chitosan after 4th deacetylation obtained from calculation was 7.04×10^5 g/mol.

Appendix B Characterization of Bacterial Cellulose Pellicle

Table B1 Thickness and diameter of bacterial cellulose pellicle

Sample No.	1	2	3	Average	S.D
Thickness (mm)	0.42	0.56	0.34	0.44	0.11
Diameter (mm)	10.43	10.49	10.14	10.35	0.19

Table B2 Weight of bacterial cellulose pellicle before and after freeze-drying

Sample No.	Weight of hydrated pellicle (g)	Weight of freeze-dried pellicle (g)
1	44.56	0.69
2	45.67	0.78
3	40.80	0.71
Average	43.68	0.72
S.D	2.55	0.05

$$\text{From calculation; water content} = (43.68 - 0.72) / 43.68 \\ = 0.9835$$

$$\% \text{ water content} = 98.35$$

$$\% \text{ solid content} = 100 - 98.35 \\ = 1.65 \%$$

Appendix C Swelling Ratios of Bacterial Cellulose and Chitosan-Coated Bacterial Cellulose With and Without Plasma Treatment

Table C1 Swelling ratios of bacterial cellulose and chitosan-coated bacterial cellulose without plasma treatment

- Non-plasma treated bacterial cellulose

Time (min)	Swelling Ratio				
	No.1	No.2	No.3	Average	SD
0.1	10.61	10.07	19.91	13.53	5.53
15	11.21	14.58	22.43	16.07	5.76
60	16.04	14.54	21.08	17.22	3.43
120	17.58	16.17	24.97	19.58	4.73
180	20.13	18.00	21.73	19.95	1.87
240	19.87	18.32	23.77	20.65	2.81
360	19.43	19.14	20.54	19.70	0.74
540	21.10	20.78	21.24	21.04	0.24

- Non-plasma treated bacterial cellulose coated with 0.75 % chitosan

Time (min)	Swelling Ratio				
	No.1	No.2	No.3	Average	SD
0.1	7.09	8.97	16.53	10.86	5.00
15	18.72	23.17	26.39	22.76	3.85
60	27.04	26.30	30.43	27.92	2.20
120	28.61	26.96	31.45	29.01	2.27
180	29.89	27.44	31.07	29.46	1.85
240	31.27	28.86	34.30	31.48	2.73
360	30.03	29.46	32.87	30.79	1.83
540	30.52	29.58	32.17	30.76	1.31

- Non-plasma treated bacterial cellulose coated with 1.00 % chitosan

Time (min)	Swelling Ratio				
	No.1	No.2	No.3	Average	SD
0.1	9.61	14.45	13.21	12.42	2.05
15	19.71	24.19	21.87	21.92	1.83
60	28.86	34.29	27.46	30.20	2.95
120	30.59	33.93	30.72	31.75	1.54
180	30.51	34.59	32.71	32.60	1.67
240	30.60	33.84	35.73	33.39	2.12
360	30.45	32.94	38.38	33.93	3.31
540	29.41	31.76	40.01	33.73	4.55

Table C2 Swelling ratios of bacterial cellulose and chitosan-coated bacterial cellulose with plasma treatment

- Plasma treated bacterial cellulose

Time (min)	Swelling Ratio				
	No.1	No.2	No.3	Average	SD
0.1	11.27	12.25	28.22	17.25	9.51
15	18.26	16.58	29.48	21.44	7.01
60	19.78	20.48	29.89	23.38	5.65
120	24.70	24.64	36.24	28.52	6.68
180	24.98	27.06	43.13	31.72	9.93
240	26.09	27.12	41.77	31.66	8.77
360	25.66	25.06	41.71	30.81	9.44
540	24.74	27.56	41.76	31.35	9.12

- Plasma treated bacterial cellulose coated with 0.75 % chitosan

Time (min)	Swelling Ratio				
	No.1	No.2	No.3	Average	SD
0.1	8.41	13.24	23.81	15.16	7.88
15	18.92	24.95	42.35	28.74	12.17
60	30.20	33.05	51.49	38.25	11.56
120	34.02	37.27	53.93	41.74	10.68
180	34.81	39.76	55.55	43.37	10.83
240	34.62	39.02	55.34	43.00	10.92
360	34.60	40.10	56.20	43.63	11.22
540	35.70	40.62	54.42	43.58	9.70

- Plasma treated bacterial cellulose coated with 1.00 % chitosan

Time (min)	Swelling Ratio				
	No.1	No.2	No.3	Average	SD
0.1	11.27	10.06	26.27	15.87	7.38
15	24.61	21.59	43.29	29.83	9.60
60	34.68	32.81	51.05	39.51	8.20
120	37.37	34.45	56.88	42.90	9.96
180	36.43	33.92	54.14	41.49	9.00
240	37.03	33.37	56.71	42.37	10.25
360	35.93	33.19	62.73	43.95	13.33
540	35.81	31.80	67.35	44.99	15.90

Appendix D Dye Absorbsion Capacity of Bacterial Cellulose and Chitosan-Coated Bacterial Cellulose Without Plasma Treatment

Table D1 The percentage concentration of amino black 10B (mg/ 100 ml distilled water) absorbed by samples with different chitosan concentrations

Time (h)	Chitosan concentration (g/100 ml)				
	0.00	0.25	0.50	0.75	1.00
0.5	0.1896	0.5069	0.5243	0.5354	0.4815
1.0	0.2461	0.5469	0.5317	0.6152	0.5539
1.5	0.1991	0.5736	0.5442	0.6007	0.7325
2.0	0.2075	0.5316	0.5854	0.5875	0.5655
3.0	0.2034	0.5360	0.6498	0.6354	0.6783
4.0	0.1893	0.5730	0.6009	0.6224	0.7341
6.0	0.1729	0.5639	0.6077	0.6540	0.8291
8.0	0.1550	0.5634	0.6299	0.7450	0.8640
10.0	0.1898	0.6124	0.7140	0.8170	0.9007
15.0	0.1719	0.6750	0.7562	0.7738	1.0128
24.0	0.1770	0.7404	0.8702	0.8929	1.1184

Appendix E Amount of Chitosan Coated on Non-plasma Treated and Plasma Treated Bacterial Cellulose

Table E1 Amount of chitosan coated on non-plasma treated bacterial cellulose

Chitosan (g/100ml)	Sample No.	Sample Weight (g)	Mole of NH_3 (or N)	CS* (mg)	CS (mg)/ Sample (g)	Averag e	S.D
0.00	1	0.0706	0.000001	0.1006	1.43	0.68	0.66
	2	0.0685	0.000000	0.0124	0.18		
	3	0.0671	0.000000	0.0300	0.45		
0.25	1	0.0831	0.000005	0.7361	8.86	17.89	8.34
	2	0.0668	0.000010	1.6893	25.29		
	3	0.0684	0.000008	1.3363	19.54		
0.50	1	0.0947	0.000042	6.8084	71.89	83.02	9.70
	2	0.0621	0.000035	5.5727	89.74		
	3	0.0698	0.000038	6.1023	87.43		
0.75	1	0.0654	0.000080	12.9865	198.57	185.13	13.10
	2	0.0697	0.000074	12.0157	172.39		
	3	0.0685	0.000078	12.6335	184.43		
1.00	1	0.0668	0.000088	14.1339	211.59	211.76	7.41
	2	0.0705	0.000096	15.4578	219.26		
	3	0.0687	0.000087	14.0457	204.45		
2.00	1	0.0624	0.000085	13.7809	220.85	209.37	12.35
	2	0.0670	0.000088	14.1339	210.95		
	3	0.0765	0.000093	15.0165	196.29		

*CS = Chitosan

Table E2 Amount of chitosan coated on plasma treated bacterial cellulose

Chitosan (g/100ml)	Sample No.	Sample Weight (g)	Mole of NH_3 (or N)	CS* (mg)	CS (mg)/ Sample (g)	Averag e	S.D
0.00	1	0.0919	0.000004	0.6302	6.86	1.94	2.35
	2	0.0650	-0.000001	-0.1642	-2.53		
	3	0.0675	0.000001	0.1006	1.49		
0.25	1	0.0823	0.000018	2.8367	34.47	30.98	1.55
	2	0.0684	0.000012	1.9541	28.57		
	3	0.0624	0.000012	1.8658	29.90		
0.50	1	0.0928	0.000067	10.7800	116.16	149.22	15.28
	2	0.0644	0.000062	9.9857	155.06		
	3	0.0631	0.000069	11.1331	176.44		
0.75	1	0.0973	0.000100	16.0756	165.22	207.21	18.94
	2	0.0690	0.000093	15.0165	217.63		
	3	0.0688	0.000102	16.4287	238.79		
1.00	1	0.0975	0.000129	20.8417	213.76	259.70	19.90
	2	0.0665	0.000116	18.7234	281.56		
	3	0.0638	0.000112	18.1056	283.79		
2.00	1	0.0629	0.000105	16.9582	269.61	261.74	6.88
	2	0.0679	0.000103	16.6934	245.85		
	3	0.0645	0.000108	17.3995	269.76		

*CS = Chitosan

Appendix F Releasing Profiles of Chitosan from Non-plasma Treated and Plasma Treated Bacterial Cellulose

Table F1 Cumulative amounts of the chitosan (%based on weight of chitosan in sample) released from non-plasma treated bacterial cellulose coated with 1.0% chitosan into acetate buffer solution (pH = 5.5) at 37°C, 50 rpm (n=3)

Time (h)	Cumulative release of chitosan (%)			Average	S.D
	No.1	No.2	No.3		
1	5.30	2.48	2.64	3.47	1.59
2	7.74	5.03	5.37	6.05	1.48
3	10.25	7.67	8.10	8.68	1.38
4	12.76	10.29	10.90	11.32	1.28
5	15.08	12.72	13.50	13.77	1.20
7	17.47	15.21	16.15	16.28	1.13
9	19.94	17.79	18.91	18.88	1.07
11	22.31	20.27	21.55	21.37	1.03
13	24.69	22.76	24.20	23.88	1.00
15	27.12	25.30	26.90	26.44	0.99
18	29.88	28.19	29.99	29.35	1.01
21	32.06	30.72	32.80	31.86	1.05
27	34.41	33.18	35.42	34.34	1.12
33	36.73	35.60	38.00	36.78	1.20
51	39.17	38.16	40.73	39.36	1.30
72	41.93	40.67	43.31	41.97	1.32

Tabla F2 Cumulative amounts of the chitosan (%based on weight of chitosan in sample) released from non-plasma treated bacterial cellulose coated with 1.0% chitosan into phosphate buffered saline (pH = 7.4) at 37°C, 50 rpm (n=3)

Time (h)	Cumulative release of chitosan (%)			Average	S.D
	No.1	No.2	No.3		
1	4.81	2.49	2.35	3.22	1.38
2	6.85	4.88	4.61	5.45	1.23
3	8.57	6.88	6.49	7.31	1.11
4	10.27	8.87	8.36	9.17	0.98
5	11.94	10.83	10.21	10.99	0.88
7	13.63	12.79	12.06	12.83	0.79
9	15.30	14.74	13.90	14.65	0.70
11	17.00	16.71	15.77	16.49	0.64
13	18.71	18.70	17.64	18.35	0.61
15	20.90	21.37	20.18	20.81	0.60
18	23.15	24.00	22.66	23.27	0.68
21	25.64	26.90	25.38	25.97	0.81
27	27.78	29.40	27.74	28.31	0.95
33	29.88	31.84	30.04	30.59	1.09
51	32.09	34.42	32.47	32.99	1.25
72	34.23	37.02	34.63	35.29	1.51

Table F3 Cumulative amounts of the chitosan (%based on weight of chitosan in sample) released from plasma treated bacterial cellulose coated with 1.0% chitosan into acetate buffer solution (pH = 5.5) at 37°C, 50 rpm (n=3)

Time (h)	Cumulative release of chitosan (%)			Average	S.D
	No.1	No.2	No.3		
1	2.03	1.87	1.62	1.84	0.21
2	4.08	3.74	3.25	3.69	0.42
3	6.49	5.96	5.17	5.88	0.67
4	8.82	8.10	7.03	7.98	0.90
5	11.30	10.38	9.00	10.23	1.16
7	13.29	12.21	10.77	12.09	1.26
9	15.28	14.04	12.55	13.96	1.37
11	17.24	15.84	14.29	15.79	1.47
13	19.25	17.68	16.08	17.67	1.58
15	21.01	19.30	17.64	19.32	1.69
18	22.85	20.99	19.27	21.04	1.79
21	24.84	22.81	21.04	22.90	1.90
27	26.55	24.39	22.58	24.51	1.99
33	28.35	26.04	24.18	26.19	2.09
51	30.17	27.73	25.82	27.91	2.18
72	31.93	29.41	27.27	29.54	2.33

Tabla F4 Cumulative amounts of the chitosan (%based on weight of chitosan in sample) released from plasma treated bacterial cellulose coated with 1.0% chitosan into phosphate buffered saline (pH = 7.4) at 37°C, 50 rpm (n=3)

Time (h)	Cumulative release of chitosan (%)			Average	S.D
	No.1	No.2	No.3		
1	3.99	1.82	1.99	2.60	1.21
2	5.77	3.64	3.98	4.46	1.15
3	7.61	5.52	6.03	6.39	1.09
4	9.43	7.37	8.06	8.29	1.05
5	11.26	9.23	10.10	10.20	1.02
7	13.09	11.09	12.13	12.10	1.00
9	14.82	12.86	14.06	13.92	0.99
11	16.58	14.65	16.02	15.75	0.99
13	18.32	16.42	17.96	17.57	1.01
15	19.98	18.10	19.86	19.31	1.05
18	22.06	20.32	22.14	21.51	1.03
21	23.78	22.09	24.09	23.32	1.07
27	25.55	23.88	26.04	25.16	1.13
33	27.14	25.70	28.04	26.96	1.18
51	29.02	27.61	30.14	28.92	1.26
72	31.00	29.51	32.19	30.90	1.34

Appendix G Colony Forming Unit Counts (cfu/ml) of Non-plasma Treated Bacterial Cellulose and Plasma Treated Bacterial Cellulose against *E. coli* and *S.aureus*

Tabla G1 Colony forming unit counts (cfu/ml) of non-plasma treated bacterial cellulose and plasma treated bacterial cellulose against *E. coli*

Sample		Plate No.			Average	S.D
		1	2	3		
Blank control	Non-plasma treatment	280	291	254	275.00	19.00
	Plasma treatment	280	291	254	275.00	19.00
BC*	Non-plasma treatment	378	361	330	356.33	24.34
	Plasma treatment	285	279	279	281.00	3.46
BC with 0.75 % CS**	Non-plasma treatment	13	12	16	13.67	2.08
	Plasma treatment	3	5	1	3.00	2.00
BC with 1.00 % CS	Non-plasma treatment	1	0	1	0.67	0.58
	Plasma treatment	0	0	0	0.00	0.00

*BC = Bacterial cellulose

**CS = Chitosan

Tabla G2 Colony forming unit counts (cfu/ml) of non-plasma treated bacterial cellulose and plasma treated bacterial cellulose against *S.aureus*

Sample		Plate No.			Average	S.D
		1	2	3		
Blank control	Non-plasma treatment	303	295	282	293.33	10.60
	Plasma treatment	303	295	282	293.33	10.60
BC	Non-plasma treatment	375	390	380	381.67	7.64
	Plasma treatment	295	304	310	303.00	7.55
BC with 0.75 % CS**	Non-plasma treatment	7	12	11	10.00	2.65
	Plasma treatment	3	1	2	2.00	1.00
BC with 1.00 % CS	Non-plasma treatment	1	2	0	1.00	1.00
	Plasma treatment	0	0	0	0.00	0.00

*BC = Bacterial cellulose

**CS = Chitosan

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