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APPENDICES

APPENDIX A

Determination of *%DQ* of *N,N,N*-trimethylammonium chitosan chloride (TMC)

The *%DQ* of TMCs were determined from the relative ratios between the signal at 3.10 ppm can be assigned to three methyl groups of quaternary ammonium group and the signal of H–1 (δ 4.38-5.34 ppm), H–2 (δ 2.95 ppm), H–2′,3,4,5,6,6′ (δ 3.25-4.30 ppm), H–2,2′,3,4,5,6,6′ (δ 2.95 ppm and 3.25-4.30 ppm), or $-NHCOC\underline{H}_3$ (δ 1.84 ppm) of chitosan analyzed by ^{1}H NMR. The position of those signals in the TMC spectra is shown in Figure A-1. $\%DQ$, $\%DS_{N(CH_3)_2}$, $\%DS_{NHCH_3}$, and $\%DS\,(total)$ are quaternization, *N,N*-dimethylation, *N*-methylation, and a total degree of *N*-methylation of chitosan were determined from ^{1}H NMR as shown in Tables A-1 to A-5.

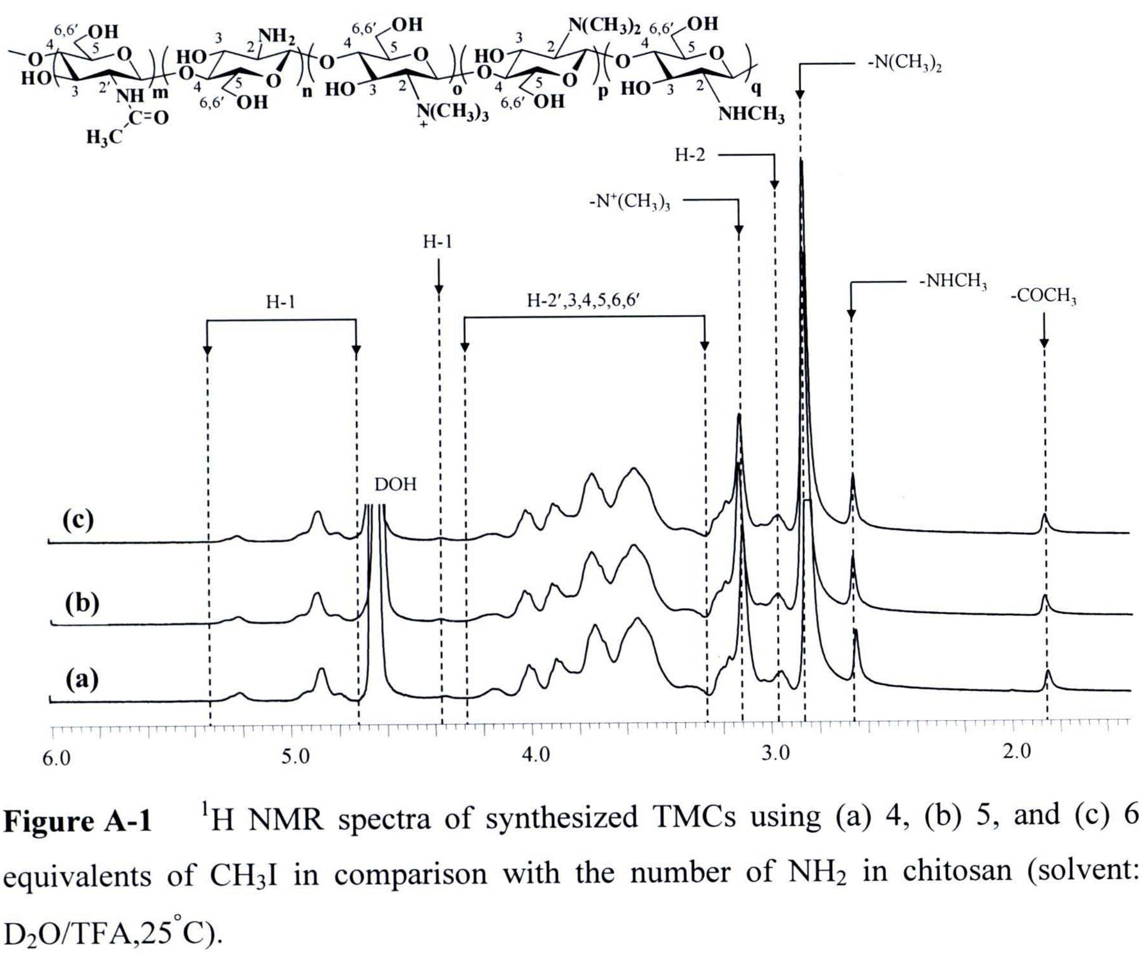

Figure A-1 ^{1}H NMR spectra of synthesized TMCs using (a) 4, (b) 5, and (c) 6 equivalents of CH_3I in comparison with the number of NH_2 in chitosan (solvent: D_2O/TFA,25°C).

Table A-1 Degree of quaternization (%DQ^{a}) was determined from the relative ratio between $-N^{+}(C\underline{H}_3)_3$ and H–1.

Sample	n	Integration				%DQ^{a}	average ± SD	%$DS_{N(CH_3)_2}$	average ± SD	%DS_{NHCH_3}	average ± SD	%$DS\ (total)$	average ± SD
		$N^{+}(CH_3)_3$	$N(CH_3)_2$	$NHCH_3$	H-1	(1)		(2)		(3)		(1)+(2)+(3)	
	1	100	220.94	44.82	36.30	30.6		101.4		13.7		145.8	
TMC_{CH_314eq}	2	100	215.98	42.04	60.39	18.4	22.6 ± 6.9	59.6	73.0 ± 24.7	7.7	9.5 ± 3.6	85.7	105.1 ± 35.2
	3	100	205.65	38.3	59.25	18.8		57.8		7.2		83.8	
	1	100	228.75	50.12	49.42	22.5		77.1		11.3		110.9	
TMC_{CH_315eq}	2	100	245.20	51.72	33.29	33.4	28.3 ± 5.5	122.8	100.5 ± 22.8	17.3	16.4 ± 4.7	173.4	145.1 ± 31.7
	3	100	233.79	70.90	38.36	29.0		101.6		20.5		151.1	
	1	100	253.62	7.38	77.74	14.3		54.4		1.1		69.7	
TMC_{CH_316eq}	2	100	265.82	19.50	66.85	16.6	24.6 ± 15.8	66.3	107.9 ± 82.6	3.2	11.6 ± 16.3	86.1	144.0 ± 114.7
	3	100	316.40	71.07	25.98	42.8		203.0		30.4		276.1	

[a] $Degree\ of\ quaternization = \%DQ = \left[\frac{\int N^{+}(CH_3)_3/9}{\left(\int H-1\right)\times DD}\right]\times 100$

Table A-2 Degree of quaternization ($\%DQ^{a}$) was determined from the relative ratio between $-N^{+}(C\underline{H}_3)_3$ and H–2.

Sample	n	Integration				$\%DQ^{a}$	average ± SD	$\%DS_{N(CH_3)_2}$	average ± SD	$\%DS_{NHCH_3}$	average ± SD	$\%DS\,(total)$	average ± SD
		$N^+(CH_3)_3$	$N(CH_3)_2$	$NHCH_3$	H-2	(1)		(2)		(3)		(1)+(2)+(3)	
	1	100	220.94	44.82	21.59	51.5		170.6		69.2		291.2	
TMC_{CH_3I4eq}	2	100	215.98	42.04	26.33	42.2	46.3 ± 4.7	136.7	148.8 ± 18.9	53.2	58.1 ± 9.7	232.1	253.1 ± 33.1
	3	100	205.65	38.3	24.64	45.1		139.1		51.8		236.0	
	1	100	228.75	50.12	29.02	38.3		131.4		57.6		227.2	
TMC_{CH_3I5eq}	2	100	245.20	51.72	29.43	37.8	40.9 ± 5.1	138.9	144.8 ± 17.2	58.6	71.9 ± 24.0	235.2	257.6 ± 45.9
	3	100	233.79	70.90	23.74	46.8		164.1		99.6		310.5	
	1	100	253.62	7.38	32.91	33.8		128.4		7.5		169.7	
TMC_{CH_3I6eq}	2	100	265.82	19.50	30.71	36.2	34.3 ± 1.7	144.3	142.8 ± 13.7	21.2	32.9 ± 32.8	201.6	209.9 ± 45.0
	3	100	316.40	71.07	33.86	32.8		155.7		70.0		258.5	

[a] $$Degree\ of\ quaternization = \%DQ = \left[\frac{\int N^{+}(CH_3)_3/9}{\left(\int H-2\right)\times DD}\right]\times 100$$

Table A-3 Degree of quaternization ($\%DQ^{a}$) was determined from the relative ratio between $-N^{+}(C\underline{H}_3)_3$ and H–2′,3,4,5,6,6′.

Sample	n	Integration				$\%DQ^{a}$	average ± SD	$\%DS_{N(CH_3)_2}$	average ± SD	$\%DS_{NHCH_3}$	average ± SD	$\%DS\ (total)$	average ± SD
		$N^{+}(CH_3)_3$	$N(CH_3)_2$	$NHCH_3$	H-2',3,4,5,6,6'	(1)		(2)		(3)		(1)+(2)+(3)	
	1	100	240.15	21.65	360.98	21.7		78.3		14.1		114.1	
$TMC_{CH_3 14eq}$	2	100	212.24	13.25	364.34	21.5	21.7 ± 0.1	68.5	71.6 ± 5.7	8.6	10.2 ± 3.4	98.6	103.5 ± 9.2
	3	100	208.83	12.00	360.69	21.7		68.1		7.8		97.7	
	1	100	229.78	36.43	365.82	21.4		73.9		23.4		118.8	
$TMC_{CH_3 15eq}$	2	100	218.17	38.11	371.18	21.1	21.1 ± 0.4	69.1	70.3 ± 3.1	24.2	24.6 ± 1.4	114.4	116.0 ± 2.4
	3	100	218.53	42.09	378.28	20.7		68.0		26.2		114.9	
	1	100	264.66	12.72	442.90	17.7		70.3		6.8		94.8	
$TMC_{CH_3 16eq}$	2	100	261.50	21.66	439.56	17.8	17.8 ± 0.1	70.0	70.1 ± 0.2	11.6	14.0 ± 8.7	99.4	101.9 ± 8.6
	3	100	260.75	43.92	438.09	17.9		70.0		23.6		111.5	

[a] $$Degree\ of\ quaternization = \%DQ = \left[\frac{\int N^{+}(CH_3)_3/9}{\left(\int H-2',3,4,5,6,6'/6\right)\times DD}\right]\times 100$$

Table A-4 Degree of quaternization ($\%DQ^{a}$) was determined from the relative ratio between $-N^{+}(C\underline{H}_3)_3$ and H–2,2′,3,4,5,6,6′.

Sample	n	Integration $N^+(CH_3)_3$	Integration $N(CH_3)_2$	Integration $NHCH_3$	Integration H-2,2',3,4,5,6,6'	$\%DQ^{a}$ (1)	average ± SD	$\%DS_{N(CH_3)_2}$ (2)	average ± SD	$\%DS_{NHCH_3}$ (3)	average ± SD	$\%DS\ (total)$ (1)+(2)+(3)	average ± SD
	1	100	220.94	44.82	360.22	25.4		56.1		11.4		92.9	
TMC_{CH_3I4eq}	2	100	215.98	42.04	398.4	23.0	24.1 ± 1.2	49.6	51.7 ± 3.9	9.7	10.1 ± 1.2	82.2	85.8 ± 6.1
	3	100	205.65	38.3	381.98	24.0		49.3		9.2		82.4	
	1	100	228.75	50.12	385.53	23.7		54.3		11.9		89.9	
TMC_{CH_3I5eq}	2	100	245.20	51.72	412.69	22.2	23.3 ± 0.9	54.4	54.8 ± 0.9	11.5	13.4 ± 3.0	88.0	91.5 ± 4.6
	3	100	233.79	70.90	383.02	23.9		55.9		16.9		96.7	
	1	100	253.62	7.38	464.39	19.7		50.0		1.5		71.1	
TMC_{CH_3I6eq}	2	100	265.82	19.50	478.10	19.1	19.0 ± 0.8	50.9	52.8 ± 4.1	3.7	6.0 ± 6.1	73.7	77.8 ± 9.4
	3	100	316.40	71.07	503.89	18.2		57.5		12.9		88.5	

[a] $$Degree\ of\ quaternization = \%DQ = \left[\frac{\int N^{+}(CH_3)_3/9}{\left(\int H-2,2',3,4,5,6,6'/6\right)\times DD}\right]\times 100$$

Table A-5 Degree of quaternization ($\%DQ^{a}$) was determined from the relative ratio between $-N^{+}(C\underline{H}_3)_3$ and $-NHCOC\underline{H}_3$.

Sample	n	Integration $N^{+}(C\underline{H}_3)_3$	Integration $N(C\underline{H}_3)_2$	Integration $NHC\underline{H}_3$	Integration $-NHCOC\underline{H}_3$	$\%DQ^{a}$ (1)	average ± SD	$\%DS_{N(CH_3)_2}$ (2)	average ± SD	$\%DS_{NHCH_3}$ (3)	average ± SD	$\%DS\ (total)$ (1)+(2)+(3)	average ± SD
	1	100	220.94	44.82	29.73	19.8		65.6		26.6		112.0	
TMC_{CH_3I4eq}	2	100	215.98	42.04	1.87	314.6	216.9 ± 170.7	1019.1	686.7 ± 538.4	396.7	262.2 ± 204.7	1730.4	1165.9 ± 913.5
	3	100	205.65	38.3	1.86	316.3		975.6		363.4		1655.2	
	1	100	228.75	50.12	18.79	31.3		107.4		47.1		185.8	
TMC_{CH_3I5eq}	2	100	245.20	51.72	18.74	31.4	27.9 ± 5.9	115.4	98.9 ± 22.1	48.7	46.9 ± 2.0	195.5	173.7 ± 29.8
	3	100	233.79	70.90	27.94	21.1		73.8		44.8		139.7	
	1	100	253.62	7.38	1.82	323.2		1229.6		71.6		1624.3	
TMC_{CH_3I6eq}	2	100	265.82	19.50	4.67	126.0	155.5 ± 155.0	502.2	604.8 ± 580.3	73.7	60.8 ± 20.5	701.9	821.1 ± 750.7
	3	100	316.40	71.07	33.78	17.4		82.6		37.1		137.2	

$$^{a}\ Degree\ of\ quaternization = \%DQ = \left[\frac{\int N^{+}(CH_3)_3 / 9}{\left(\int NHCOC\underline{H}_3 / 3\right) \times DD}\right] \times 100$$

APPENDIX B

Determination of IC_{50} of *In vitro* cytotoxicity on HaCaT cells line

The IC_{50} values were calculated from a logarithmic regression ($y = a \cdot log(x) + b$ in Table B-1) which obtained from the graph of the concentration (x axis) and the cell viability (y axis) as shown in Figure B-1.

Table B-1 The IC_{50} values (*in vitro* cytotoxicity) of chitosan MW 45000, TMCs, and HTACCs on HaCaT cells line as determined by the MTT assay at pH 6.0.

Sample	$y = a \cdot log(x) + b$			y = 50%	
	a	b	r^2	log (x)	x = IC_{50}
Chitosan 85%*DD*	3.6454	91.712	0.2057	-	>5000[a]
TMC 22%*DQ*	-13.370	93.279	0.6161	3.237	1725.9
TMC 21%*DQ*	-28.129	94.633	0.8147	1.587	38.6
TMC 18%*DQ*	-25.034	78.806	0.9003	1.151	14.1
HTACC 24%*DQ*	4.8118	91.227	0.4580	-	>1000[a]
HTACC 84%*DQ*	-25.316	107.21	0.8141	2.260	181.9
HTACC 139%*DQ*	-29.271	115.60	0.6599	2.241	174.2

[a]The IC_{50} value could not certainly calculated due to the log (x) value is negative.

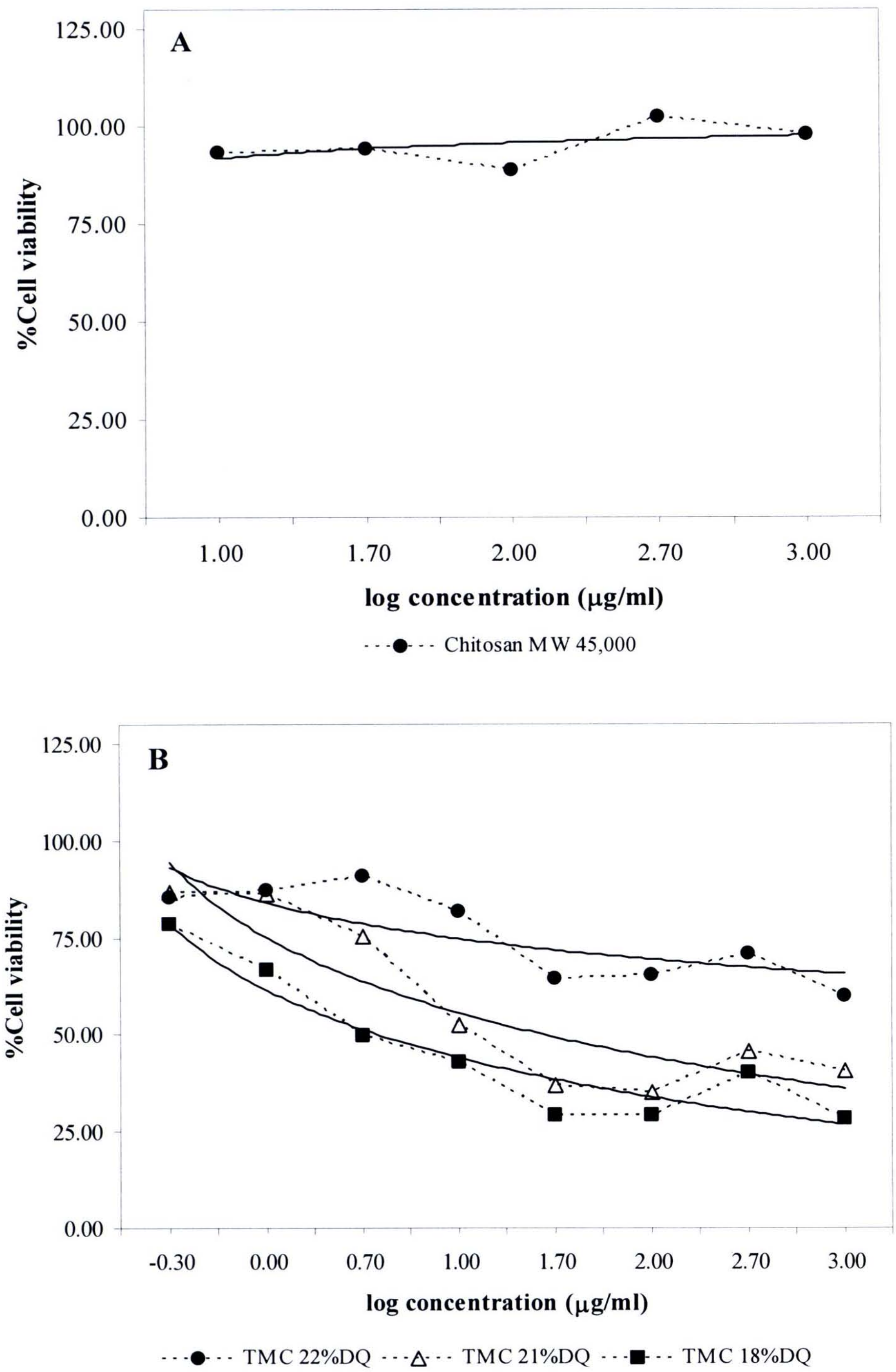

Figure B-1 Relationship between the log concentration (μg/ml) and the cell viability (%): (A) chitosan MW 45,000, (B) TMCs, and (C) HTACCs. Logarithmic trendline (solid line) and original trendline (dotted line).

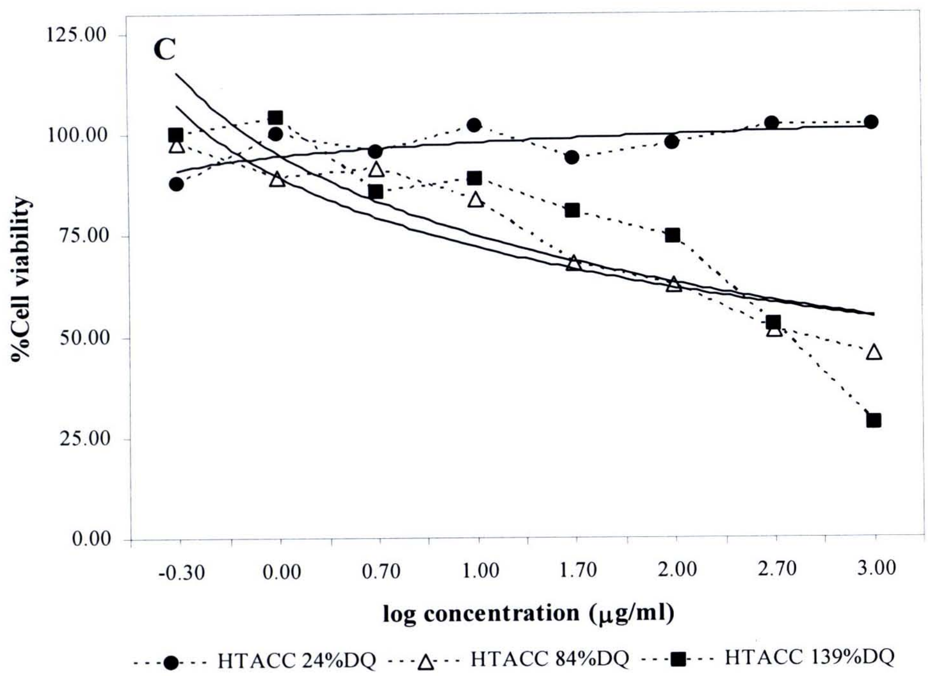

Figure B-1 *continued*

APPENDIX C

The fracture patterns for five types of hair

As a result, virgin, straightened and UV-damaged hairs are observed with the step pattern as shown in Figure C-1. Waved and dyed hairs are observed with the smooth and splitting pattern, respectively. Additionally, if the hair and its cuticle are near the root end, a smooth break tends to occur. As the fiber becomes dryer, below 90%RH, step fractures are the most commonly observed fracture pattern. Fibrillation and splitting describe a distinct cortical fracturing pattern and tend to occur more with twisted or kinky fibers or when the relative humidity is low.

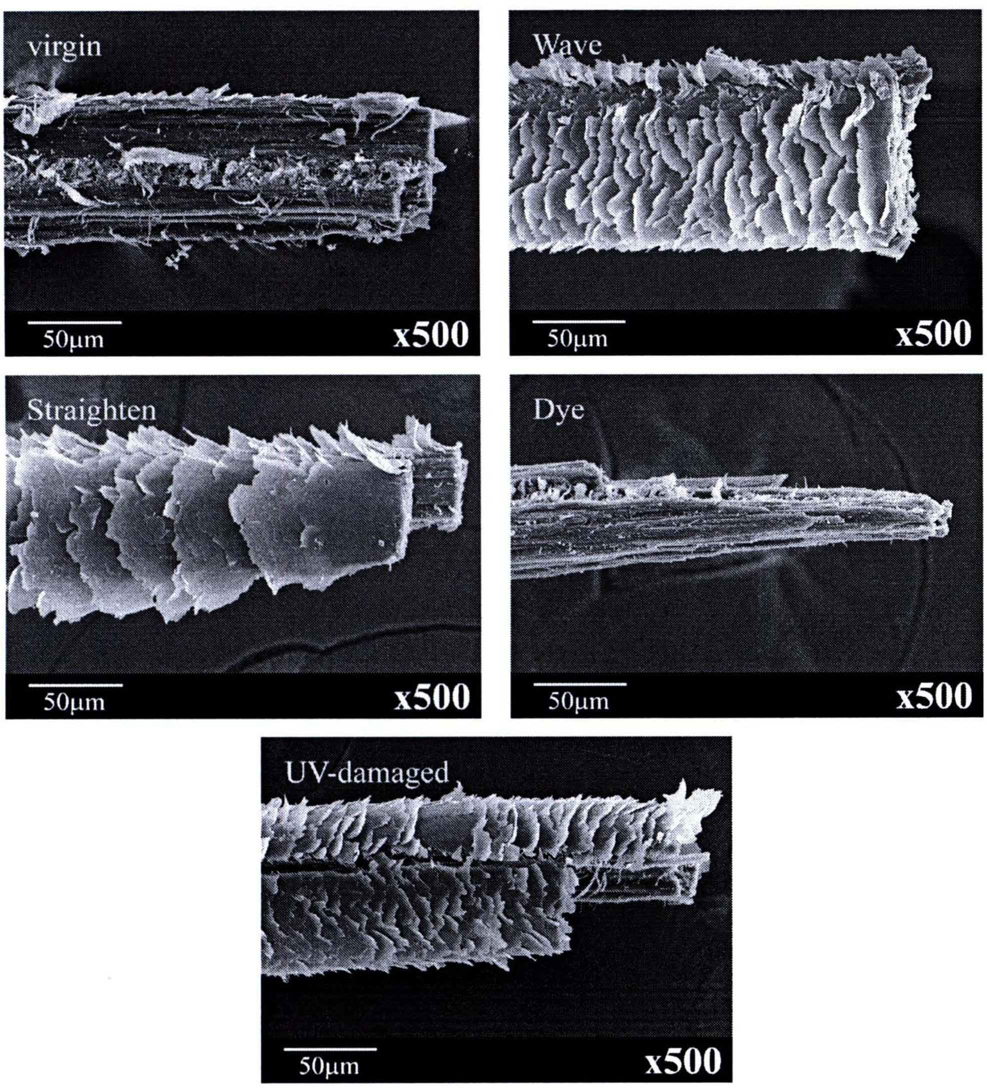

Figure C-1 The fracture patterns for five types of hair.

VITAE

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September 2008	The 5th Thailand Materials Science and Technology Conference, Miracle Grand Convention Hotel, Bangkok, Thailand. Best Poster Award for Student in Polymer Session
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Publication: "Sustained Release of Amoxicillin from Chitosan Tablets" *Archives of Pharmacal Research* 30(4) (2007): 526-531.