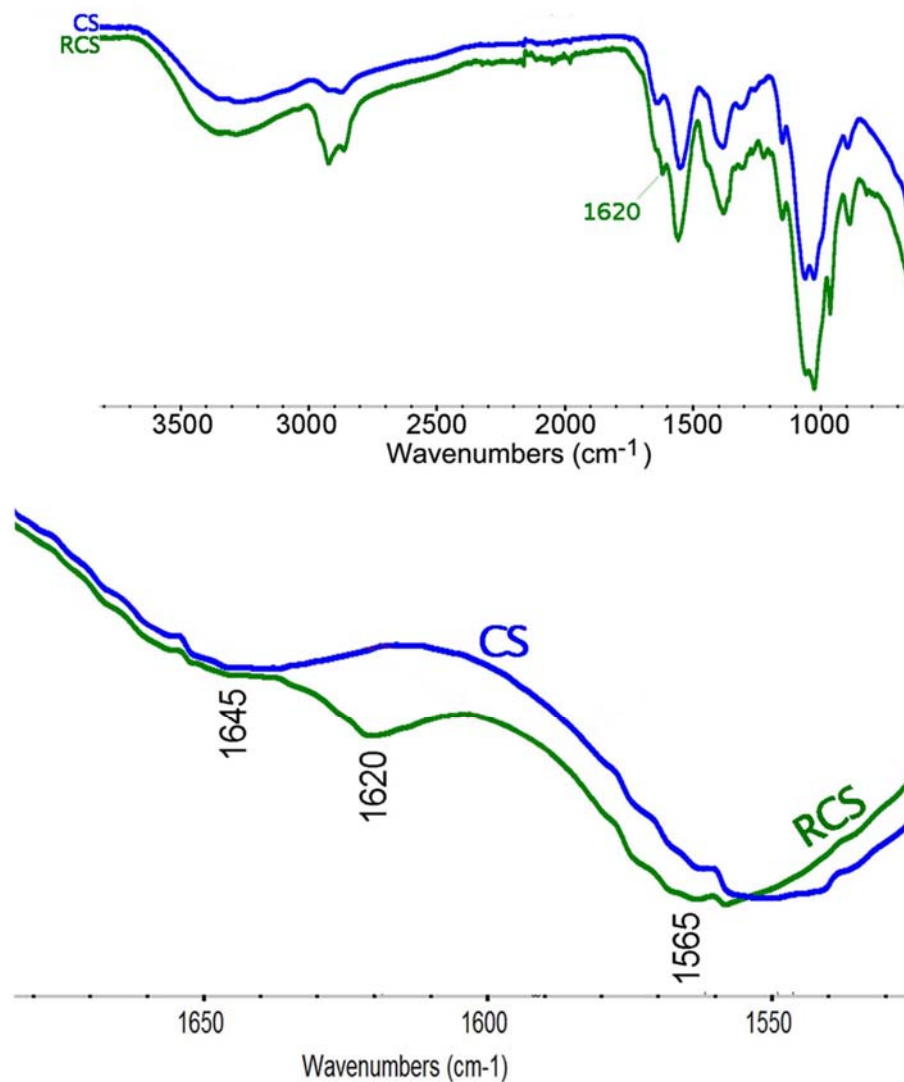**Supplementary materials**

Figure S1 FTIR spectra of chitosan (CS) and retinilidenechitosan (RCS): the whole spectra (top), and the expansion showing the stretching of imine bond at 1620 cm^{-1} in the PRN (bottom).
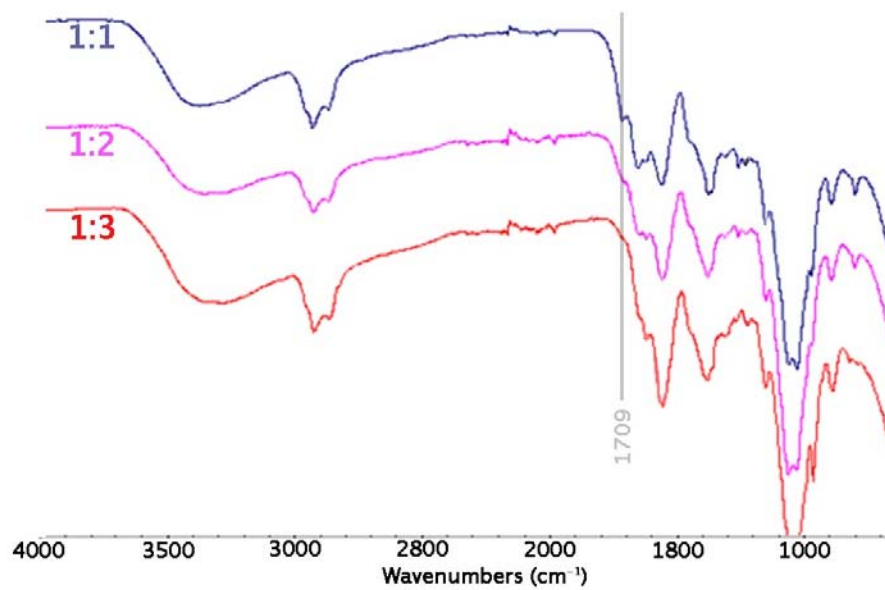
Figure S2 FTIR spectra of the reaction mixtures obtained from the reactions conducted at the mole ratios between retinal: CS monomeric unit of 1:1, 1:2 and 1:3, showing disappearance of C=O stretching from aldehyde functionality at 1709 cm^{-1} in the 1:3 mixture.
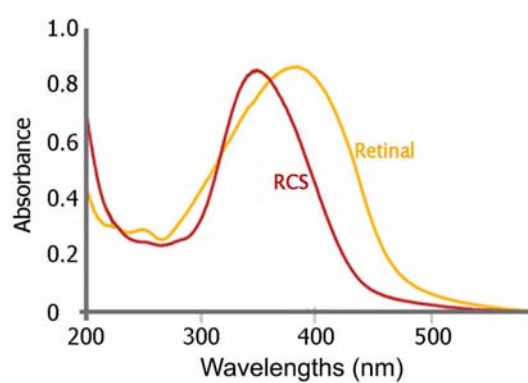

Figure S3 UV-visible absorption profiles of retinal and retinilidenechitosan (RCS).
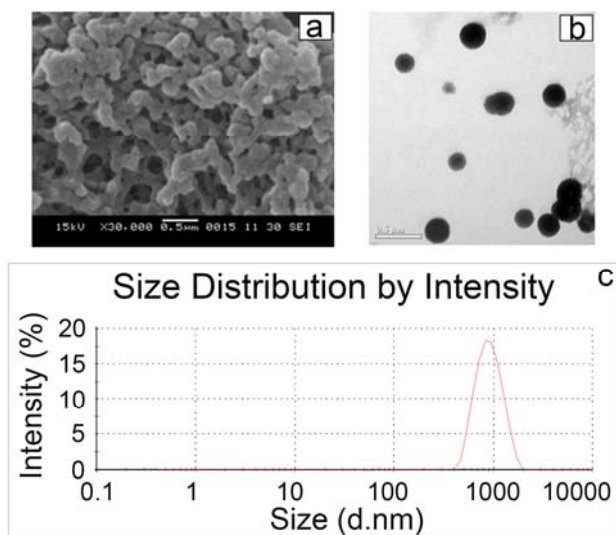
Figure S4 Scanning electron microscopic or SEM image (a) and transmission electron microscopic or TEM image (b), and size distribution graph from dynamic light scattering analysis (c) of the pro-retinal nanoparticles (PRN).

Table S1 Characteristics of volunteers in the single patch test.

Total numbers of subjects	20
Age (years)	
15 – 25	15%
26 – 45	70%
46 – 65	15%
Gender	
Male	20%
Female	80%

Table S2 Characteristics of volunteers participating in the anti-aging efficacy test.

Number of subjects	30
Mean age (S.D.) in years	42.33 (8.121)
Median age (years)	41
Min age (years)	30
Max age (years)	62
Skin type[a]	
III	33.3%
IV	56.7%
V	10.0%

[a]Phototype according to the classification of Fitzpatrick

Table S3 Tolerance study on rat skin; showing the scale of irritation against various samples (rat groups) at day 14 of the daily application of *Water group:* 200 μL distilled water, *Retinoic acid group:* 3.33 mM (0.1% w/v) retinoic acid solution; Retinal group) 3.33 mM retinal solution; *Pro-retinal nanoparticle group:* PRN suspension (at the concentration corresponded to 3.33 mM retinoid).

Rat groups	Irritation scales (Numbers of rats)[a]			
	0	1	2	3
Water	5	-	-	-
Retinoic acid	-	-	-	5
Retinal	-	-	3	2
PRN	5	-	-	-

[a] Tolerance was graded on the following 0 to 3 scale:
      0: no sign
      1: mild stinging and no objective sign
      2: stinging burning and visible erythema with subsequent fine scaling
      3: frank burning and erythema with edema

Table S4 Skin irritation responses of various test materials in volunteers at various times post the removal of the 24 h close patch of the test samples.

Test material	Time (h)	N (%) Grade of skin reaction (n = 20)					Mean ± SD of severity grade
		Negative	Positive	Positive Grades			
				0.5+	1+	2+	
Distilled Water	1	20 (100)	-	-	-	-	-
2.5% SDS	1	9 (45)	11 (55)	8 (40)	1 (5)	2 (10)	0.82±0.60
	24	3 (15)	17 (85)	8 (40)	4 (20)	5 (25)	1.06±0.66
	48	4 (20)	16 (80)	8 (40)	4 (20)	4 (20)	1.00±0.63
PRN	1	19 (95)	1 (5)	1 (5)	-	-	0.50
	24	20 (100)	-	-	-	-	-
	48	20 (100)	-	-	-	-	-

Table S5 Irritant responses in volunteers (shown as numbers of volunteers) against PRN as compared to distilled water and 2.5% SDS, at 1 h post the opening of the 24 h close patch of the test samples.

Irritation potential of PRN compared to controls	N (%) Skin reaction (n = 20)				p-value[a]
	No irritation with control and PRN	Irritation with control	Irritation with PRN	Irritation with control and PRN	
Distilled Water (Negative control)	19 (95)	-	1 (5)	-	>0.005
2.5% SDS (Positive control)	9 (45)	10 (50)	-	1 (5)	<0.005

[a] McNemar test

Table S6 Changes (%) in the surface, volume and texture parameters (energy, entropy, homogeneity, contrast and variance) of volunteers at day 84 post the twice-daily application of samples (retinoic acid (RA) as control, at ≈ 4.17 µg retinoid/cm^2 for each application).

Parameter	Expected change if treatment effective	PRN				RA			
		T0	T0 + 12 weeks	% change	P-value[a]	T0	T0 + 12 weeks	% change	P-value[a]
Surface (%)	decrease	583.953	511.177	-12.5	< 0.05	568.703	528.060	-7.1	< 0.05
Volume (mm^3)	decrease	89.900	81.667	-9.2	not significant	90.833	86.233	-5.1	not significant
Energy (pixels)	increase	0.023	0.032	42.9	< 0.05	0.022	0.031	41.9	< 0.05
Entropy (pixels)	increase	1.390	1.467	5.5	< 0.05	1.389	1.461	5.1	< 0.05
Homogeneity (pixels)	increase	1.303	1.363	4.6	< 0.05	1.305	1.342	2.9	< 0.05
Contrast (pixels)	decrease	2.123	1.561	-26.5	< 0.05	2.095	1.673	-20.2	< 0.05
Variance (pixels)	decrease	6.318	5.465	-13.5	< 0.05	6.278	5.710	-9.1	< 0.05

[a] two-tailed paired Student t-test (normality of distributions checked) or the Wilcoxon test (normality of the distributions rejected)