

Optimizing the preparation of silk fibroin nanoparticles and their loading with polyphenols: towards a more efficient anti-inflammatory effect on macrophages

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Table S1. Calibration equations used for polyphenols quantification.

PPh	Wavelength (nm)	Equation	R ²
CUR	495	$Abs = 3,6724 \cdot [CUR]^a + 0,0059$	0.9986
Q	405	$Abs = 11.278 \cdot [Q]^a - 0.0094$	0.9924
RES	355	$Abs = 2,319 \cdot [RES]^a + 0,0461$	0,9949

^aConcentration of polyphenols expressed in mg·mL⁻¹.

Table S2. Statistic parameters of silk fibroin nanoparticles precipitation reproducibility and repeatability of the precipitation.

Parameter	Z _{ave} (d.nm)	PdI	ζ (mV)
Number of values	18	18	18
Minimum	139.4	0.07300	-31.20
Maximum	149.3	0.14600	-23.60
Range	9.9	0.07300	7.60
10% Percentile	141.6	0.08020	-29.94
90% Percentile	148.9	0.13970	-24.23
Mean	145.1	0.11060	-27.71
Std. Deviation	2.8	0.01872	1.98
Std. Error of Mean	0.6	0.00441	0.46
Lower 95% CI of mean	143.7	0.10120	-28.70
Upper 95% CI of mean	146.5	0.11990	-26.73

Table S3. Effect of irradiated SFN-PPh, non-irradiated SFN-PPh, and free compounds on cell viability of the human macrophage HL-60 differentiated cells.

PPh	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	Irradiated SFN-PPh (%)	Non-irradiated SFN-PPh (%)	Free PPh (%)	p-value
CUR	16	106.00 \pm 9.00	79.00 \pm 3.00	84.00 \pm 4.00	N.S.
	80	83.00 \pm 3.00	67.00 \pm 3.00	64.00 \pm 3.00	N.S.
Q	16	106.00 \pm 11.00	110.00 \pm 4.00	109.00 \pm 6.00	N.S.
	80	106.00 \pm 7.00	114.00 \pm 8.00	91.00 \pm 4.00	N.S.
RES	16	109.00 \pm 7.00	129.00 \pm 2.00	87.00 \pm 13.00	N.S.
	80	33.00 \pm 6.00	23.00 \pm 2.00	23.00 \pm 2.00	N.S.

Data are presented as % of cell viability \pm SE of LPS-stimulated ($0.1 \mu\text{g}\cdot\text{mL}^{-1}$) HL-60 differentiated cells in the presence of irradiated or non-irradiated SFN-PPh (-CUR, -Q, and -RES) and free compounds (CUR, Q and RES) at different concentrations (16 and $80 \mu\text{g}\cdot\text{mL}^{-1}$) measured by MTT assays ($n=3$, three independent experiments performed in triplicate). Statistical differences were calculated using the two-way ANOVA test (considering $p<0.05$ significant), followed by a Dunnet's post hoc analysis for multiple comparisons. All data presented are normalized compared to untreated cells (100% viability). LPS, lipopolysaccharide; SFN, Silk fibroin nanoparticles; PPh, polyphenol; SFN-CUR, SFN-curcumin loaded; SFN-Q, SFN-quercetin loaded; SFN-RES, SFN-resveratrol loaded; CUR, curcumin; Q, quercetin; RES, resveratrol; N.S., not significant.

Table S4. Effect of irradiated SFN-PPh, non-irradiated SFN-PPh, and free compounds on cytokine release (IL-6 and TNF- α) of the human macrophage HL-60 differentiated cells measured by ELISA.

	IL-6				TNF- α			
	Irradiated SFN-PPh	Non-irradiated SFN-PPh	Free PPh	p-value	Irradiated SFN-PPh	Non-irradiated SFN-PPh	Free PPh	p-value
CUR	0.26 \pm 0.04	0.15 \pm 0.028	0.61 \pm 0.13	N.S.	0.87 \pm 0.03	0.73 \pm 0.07	1.07 \pm 0.11	N.S.
Q	0.17 \pm 0.04	0.17 \pm 0.04	0.18 \pm 0.04	N.S.	1.08 \pm 0.11	1.17 \pm 0.09	0.88 \pm 0.12	N.S.
RES	0.74 \pm 0.05 ^{a,b}	0.34 \pm 0.02 ^b	0.95 \pm 0.10 ^a	0.012	0.80 \pm 0.18	0.58 \pm 0.02	0.64 \pm 0.06	N.S.

Values are expressed as fold-change compared to the untreated cells. Data are presented as mean \pm SE of LPS-stimulated ($0.1 \mu\text{g}\cdot\text{mL}^{-1}$) HL-60 differentiated cells in the presence of irradiated or non-irradiated SFN-PPh (-CUR, -Q, and -RES) and free compounds (CUR, Q and RES) at $16 \mu\text{g}\cdot\text{mL}^{-1}$ measured by ELISA assays ($n=3$, three independent experiments performed in triplicate). Statistical differences were calculated using the two-way ANOVA test (considering $p<0.05$ significant), followed by Dunnet's post hoc analysis for multiple comparisons. LPS, Lipopolysaccharide; IL-6, Interleukin 6; TNF- α , tumor necrosis factor alpha; SFN, Silk fibroin nanoparticles; PPh, polyphenols; SFN-CUR, SFN-curcumin loaded; SFN-Q, SFN-quercetin loaded; SFN-RES, SFN-resveratrol loaded; CUR, curcumin; Q, quercetin; RES, resveratrol; N.S., not significant.

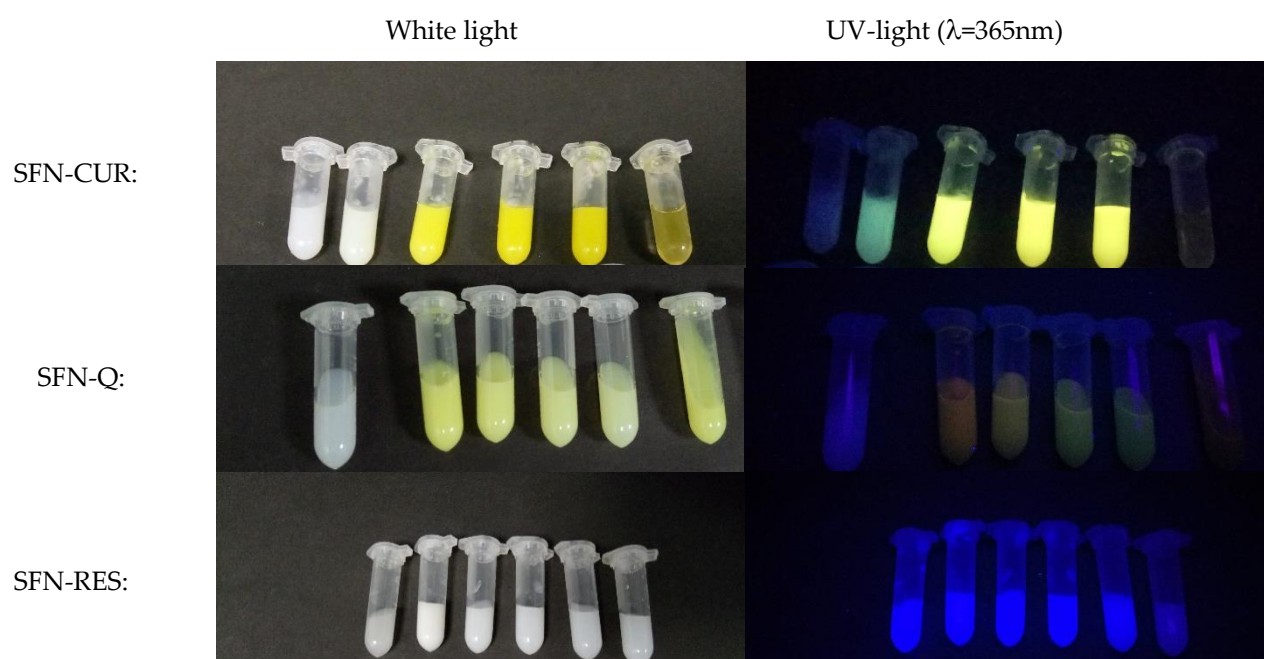


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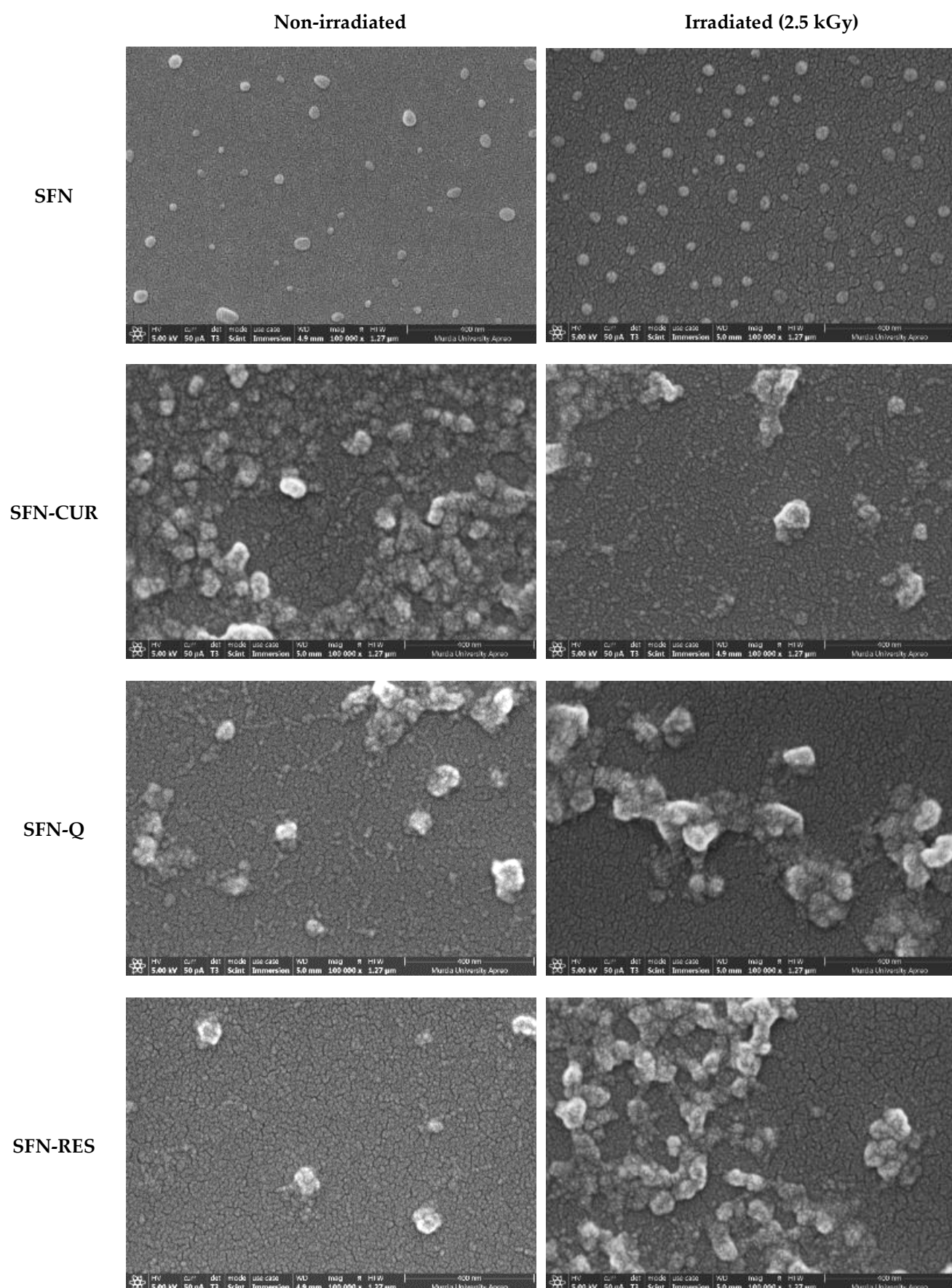


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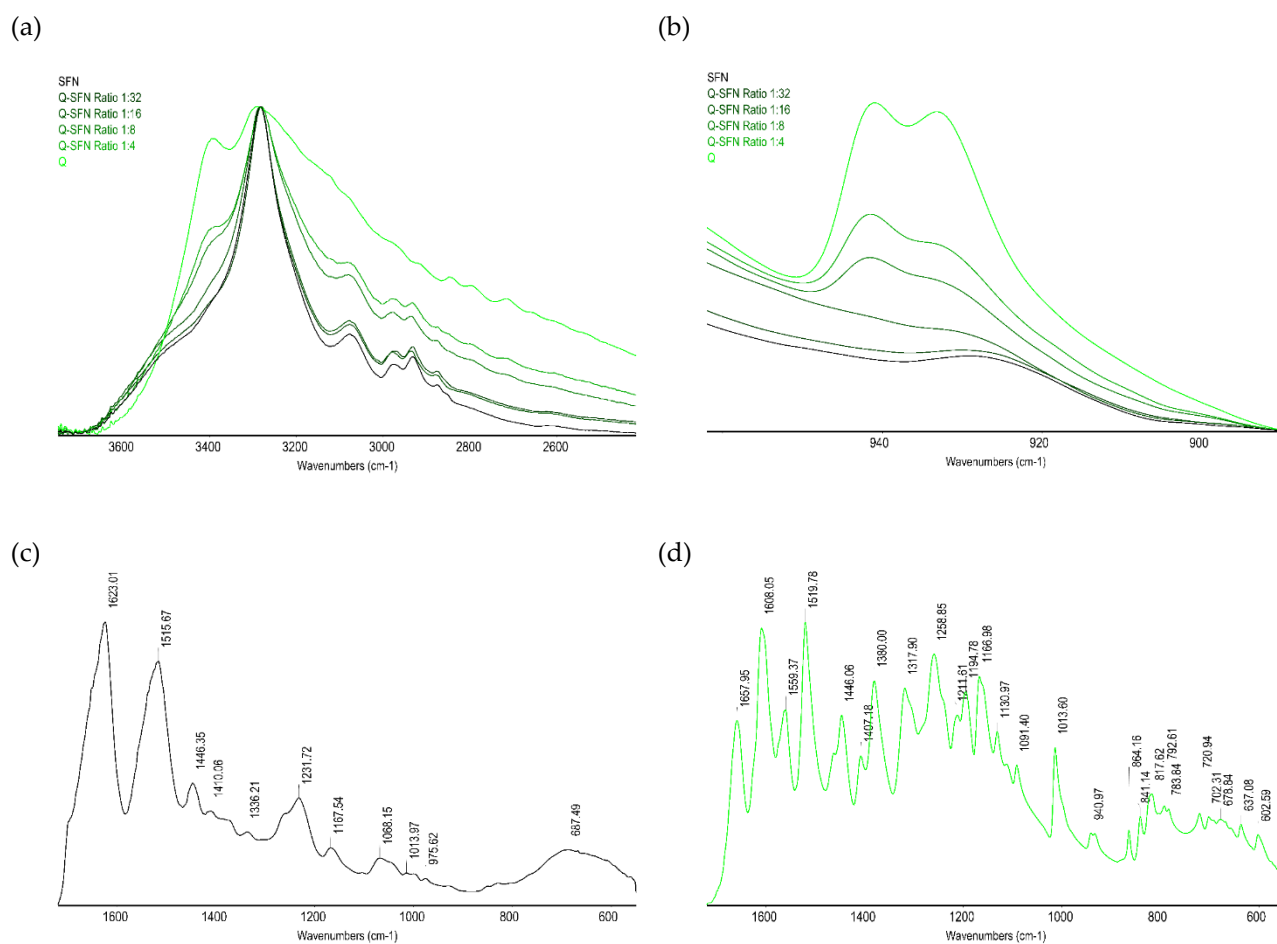


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