Supplementary Information (SI) for Journal of Materials Chemistry B.

Engineering hemin-loaded hyaluronan needle-like microparticles with photoprotective properties against UV-induced tissue damage

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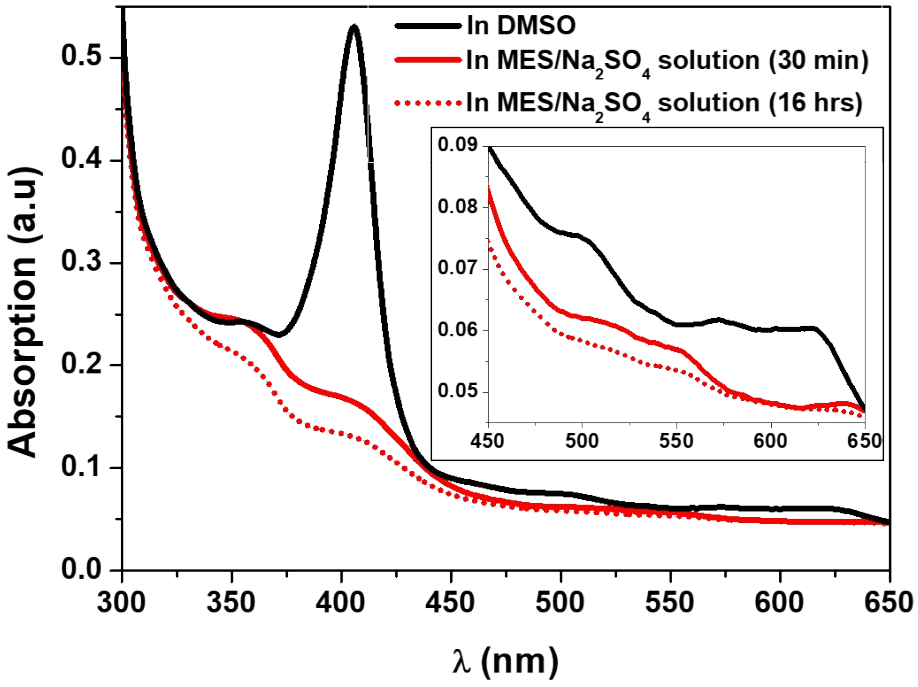


Fig. S1. UV-Vis spectra of hemin in 100% DMSO (black curve), and after dilution in Na_2SO_4-containing MES-buffer (pH 6, 0.1 M) and incubation for 30 minutes **(solid red curve)** and for 16 hours **(dotted red curve).** Results are presented as mean absorption values, n = 3. Insets: the spectra within the range of 450-650 nm.

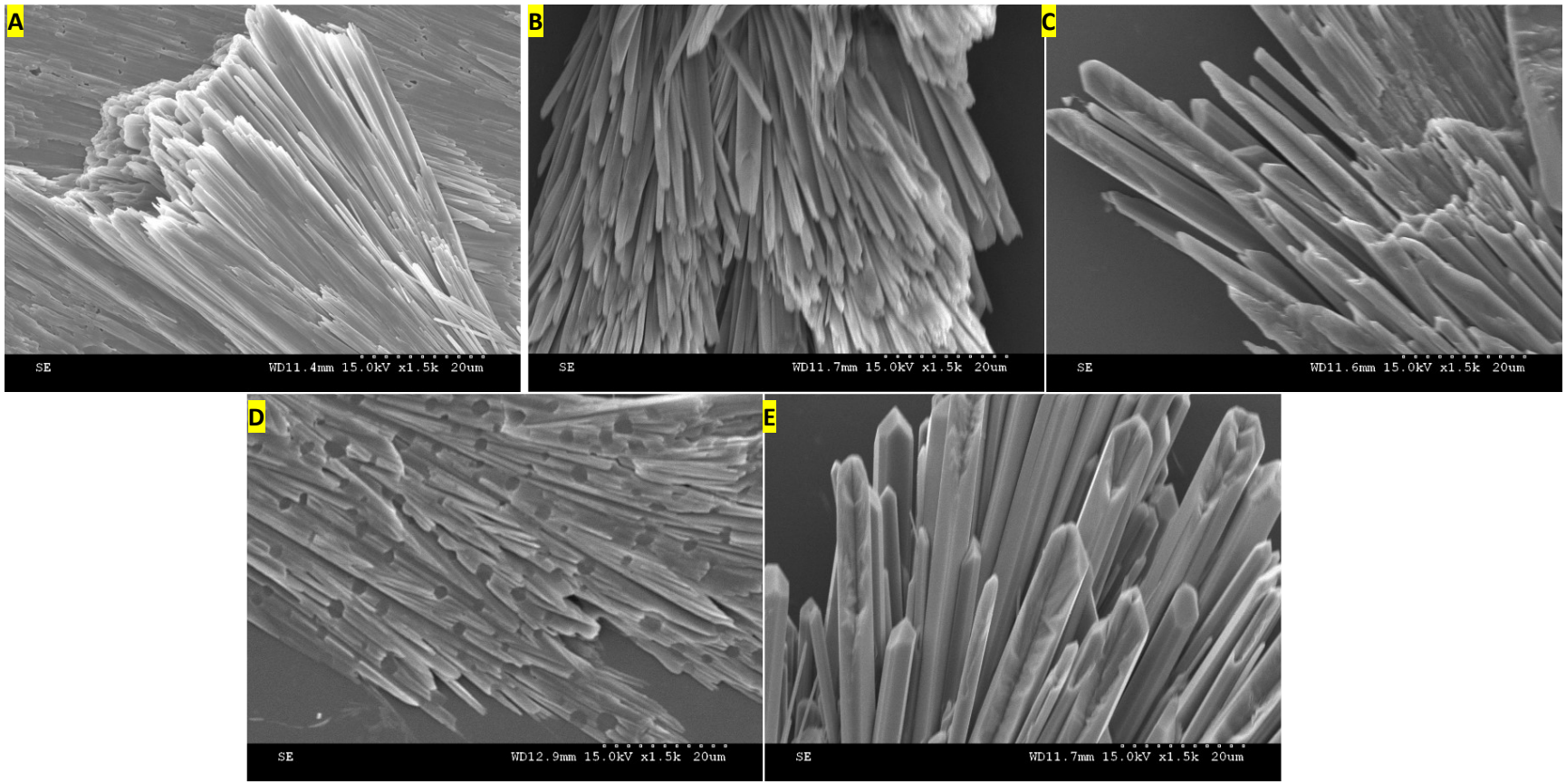


Fig. S2. SEM images of unloaded **A**-**(A)**, **B**-**(B)**, **C**-**(C)**, **D**-**(D)**, and **E**-**(E)** microparticle formulation. Scale bar: 20 µm, indicated by dotted line .

Table S1. Detailed calculations of the concentration and number of moles of hemin in: **(1)** the premixed solution with HA, **(2)** in the washing solutions after complete purification, **(3)** estimation of the total number of moles of hemin loaded to the MPs of **A**-, **C**-, **E**-, **F**- and **I**-formulation, and **(4)** the corresponding hemin entrapment efficiency (%). Hemin was mixed with HA solution at a final concentration of 240 μM, equivalent to 3 μmol.

Formulation	Initial concentration (μM)	Concentration in collected washing solutions (μM)	Initial number of moles (μmol)	Unloaded hemin (μmol)	Loaded hemin (μmol)	Entrapment efficiency (%)
A(H)		16.69		0.33	2.67	88.87
C(H)		17.98		0.36	2.64	88.01
E(H)	240	15.79	3	0.32	2.68	89.47
F(H)		16.01		0.32	2.68	89.33
I(H)		12.67		0.25	2.75	91.56

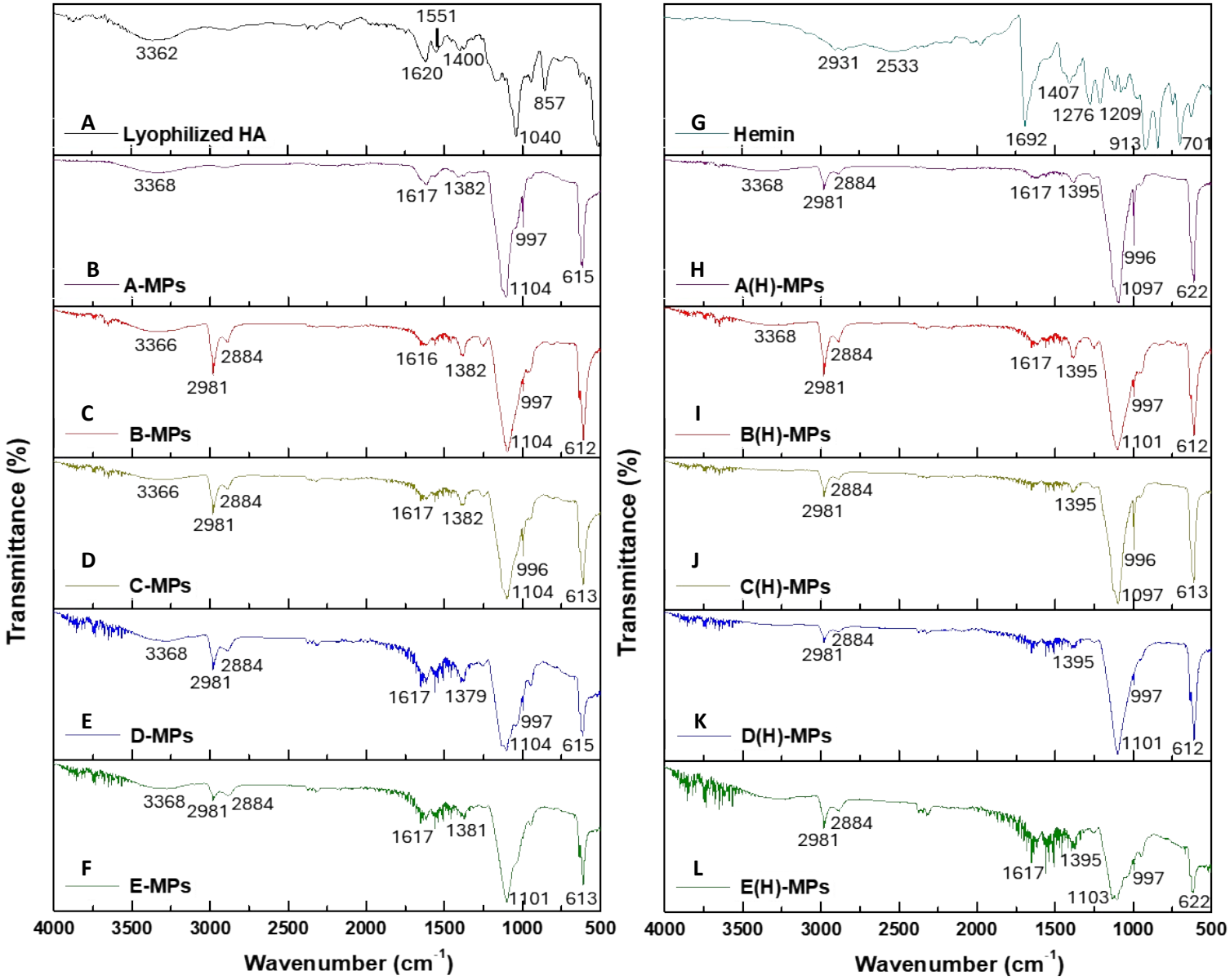

A
Lyophilized HA
3362
1551
1620
1400
857
1040
B
A-MPs
3368
1617
1382
997
1104
615
C
B-MPs
3366
2884
2981
1616
1382
997
1104
612
D
C-MPs
3366
2884
2981
1617
1382
996
1104
613
E
D-MPs
3368
2884
2981
1617
1379
997
1104
615
F
E-MPs
3368
2981
2884
1617
1381
1101
613
G
Hemin
2931
2533
1407
1276
1209
1692
913
701
H
A(H)-MPs
3368
2884
2981
1617
1395
996
1097
622
I
B(H)-MPs
3368
2884
2981
1617
1395
997
1101
612
J
C(H)-MPs
2884
2981
1395
996
1097
613
K
D(H)-MPs
2884
2981
1395
997
1101
612
L
E(H)-MPs
2884
2981
1617
1395
1103
997
622
Transmittance (%)
Transmittance (%)
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)
Wavenumber (cm-1)

Fig. S3 FTIR spectra of lyophilized HA **(A)**, unloaded MPs belonging to the **A**- **(B)**, **B**- **(C)**, **C**- **(D)**, **D**-**(E)** and **E**-formulation **(F)**, hemin **(G)**, and the hemin-loaded MPs: **A(H)**-**(H)**, **B(H)**-**(I)**, **C(H)**-**(J)**, **D(H)**-**(K)** and **E(H)**-formulation **(L).**

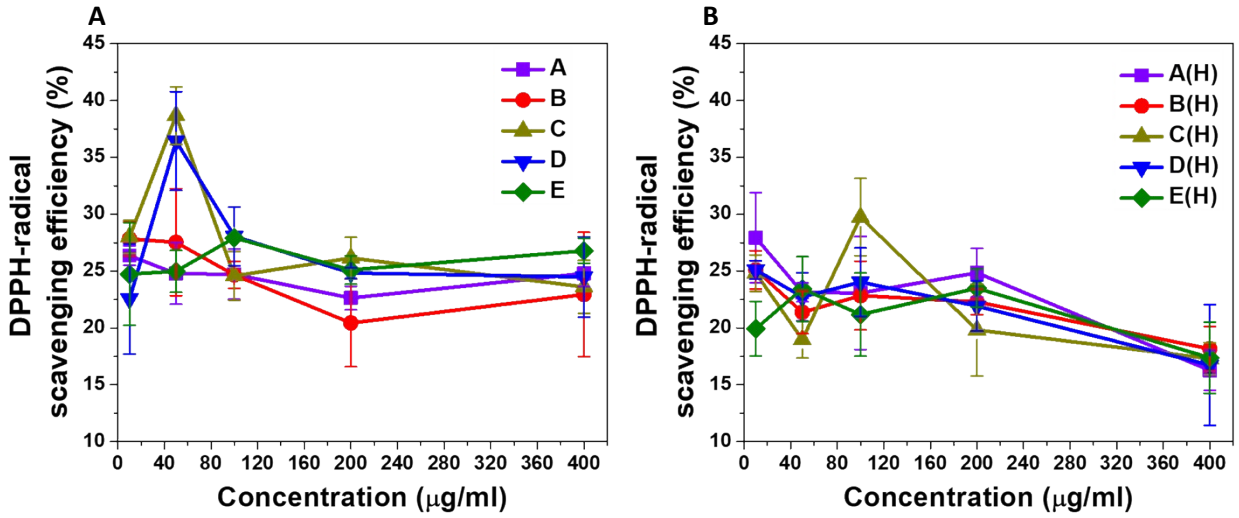


Fig. S4 The DPPH radical scavenging activities of the unloaded **(A)**, and hemin-loaded **A**-, **B**-, **C**-, **D**-, **E**-MPs **(B)** in methanol. All MPs were incubated at R.T for 60 minutes with 50 μM DPPH, followed by measuring the absorbance at 517 nm. Results are presented as mean values ± SD, n = 3.

Table S2. Detailed calculations of the concentration and number of moles of hemin in: **(1)** the premixed solution with HA, **(2)** in the washing solutions after complete purification, **(3)** estimation of the total number of moles of hemin loaded to the MPs of **A**-formulation, and **(4)** the corresponding hemin entrapment efficiency (%).

Sample No.	Initial concentration (μM)	Concentration in collected washing solutions (μM)	Initial number of moles (μmol)	Unloaded hemin (μmol)	Loaded hemin (μmol)	Entrapment efficiency (%)
A1	2	0.99	0.024	0.02	0.01	17.61
A2	8	1.77	0.096	0.02	0.07	76.92
A3	16	2.00	0.188	0.02	0.16	86.68
A4	30	5.62	0.375	0.07	0.30	81.27
A5	60	10.88	0.75	0.14	0.61	81.87
A6	120	22.54	1.5	0.28	1.22	81.21
A7	240	34.71	3	0.43	2.57	85.54
A8	480	45.58	6	0.57	5.43	90.50

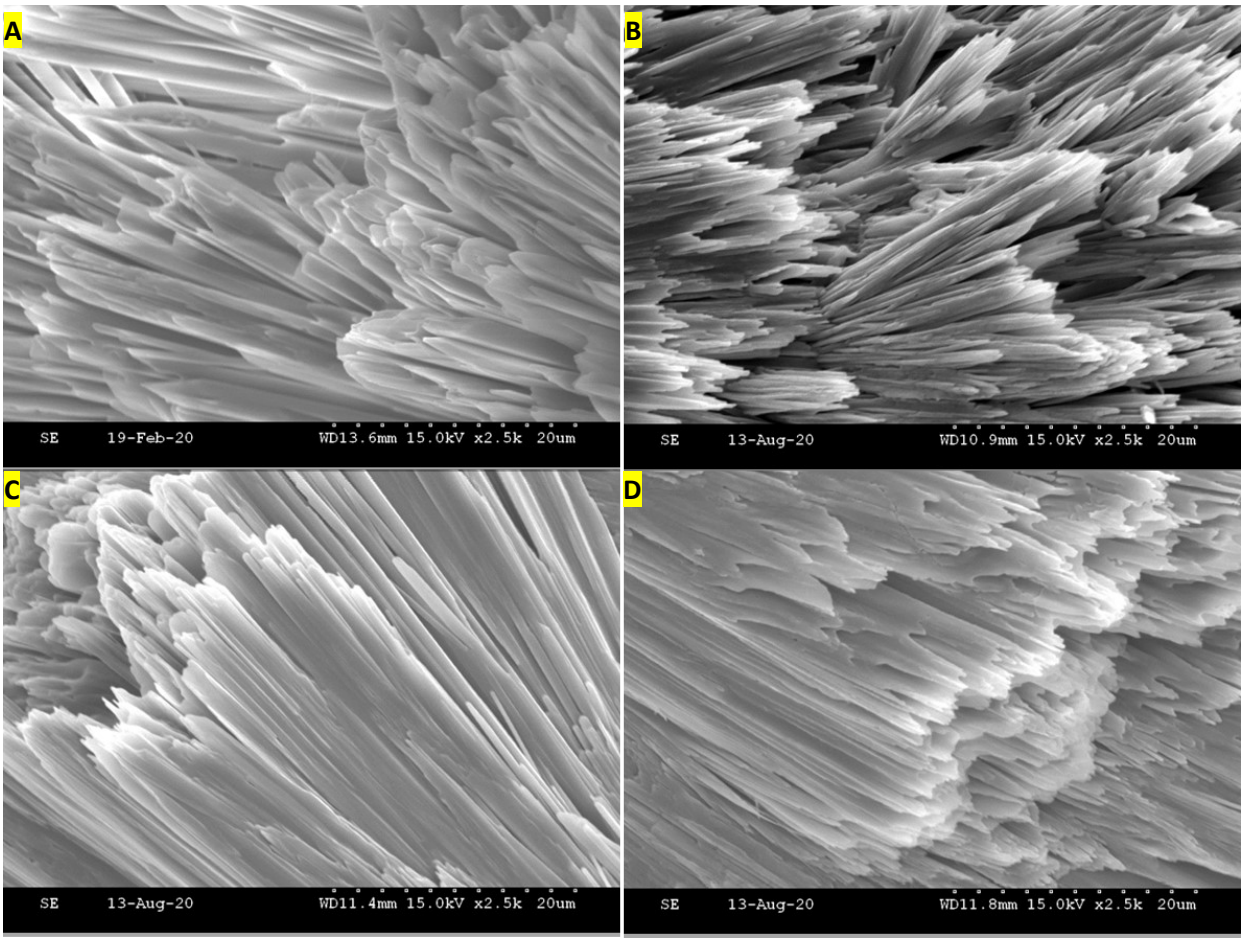


Fig. S5 SEM images of unloaded **(A)**, and hemin-loaded microparticles of A1 **(B),** A5 **(C)** and A7 **(D)**-formulation. Scale bar: 20 µm, indicated by dotted line .

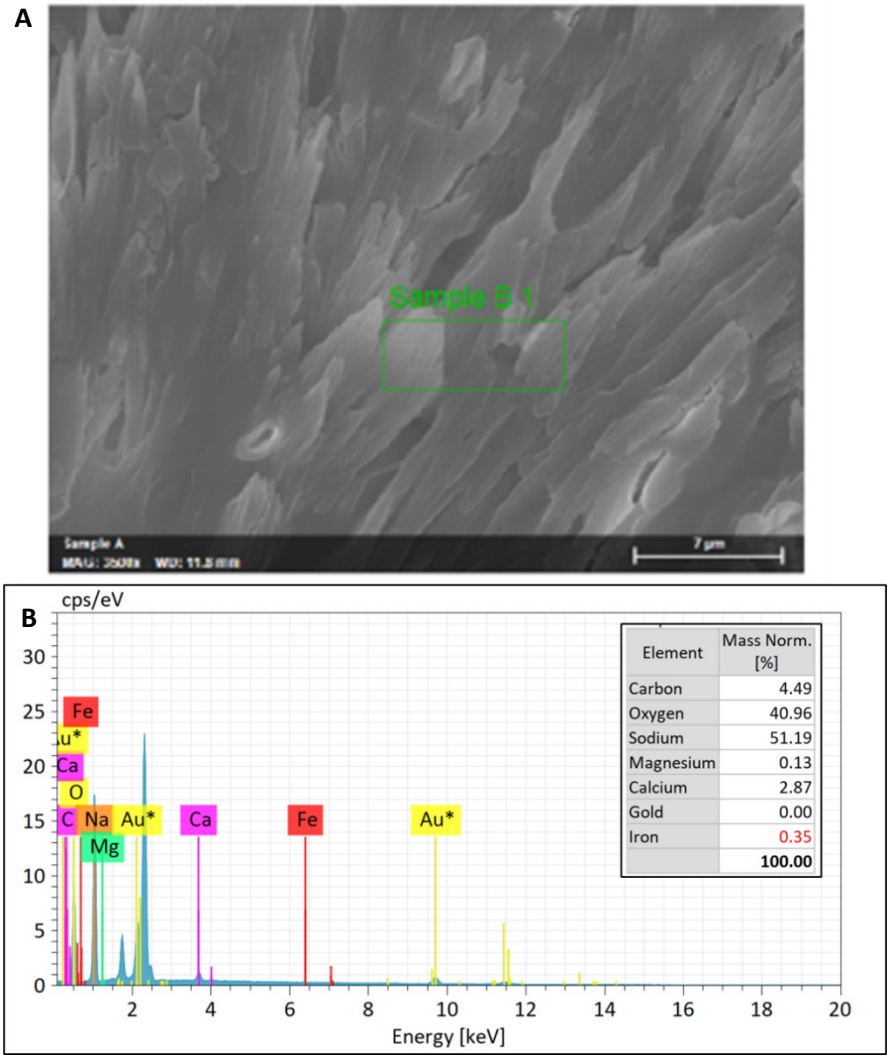


Fig. S6. Characterization of hemin-loaded **B**-MPs. **(A)** SEM image, with the analysed area indicated by a rectangle. **(B)** Energy-dispersive X-ray spectroscopy (EDX) analysis, and the respective weight percentage of the major elements (inset). Scale bar: 7 µm.

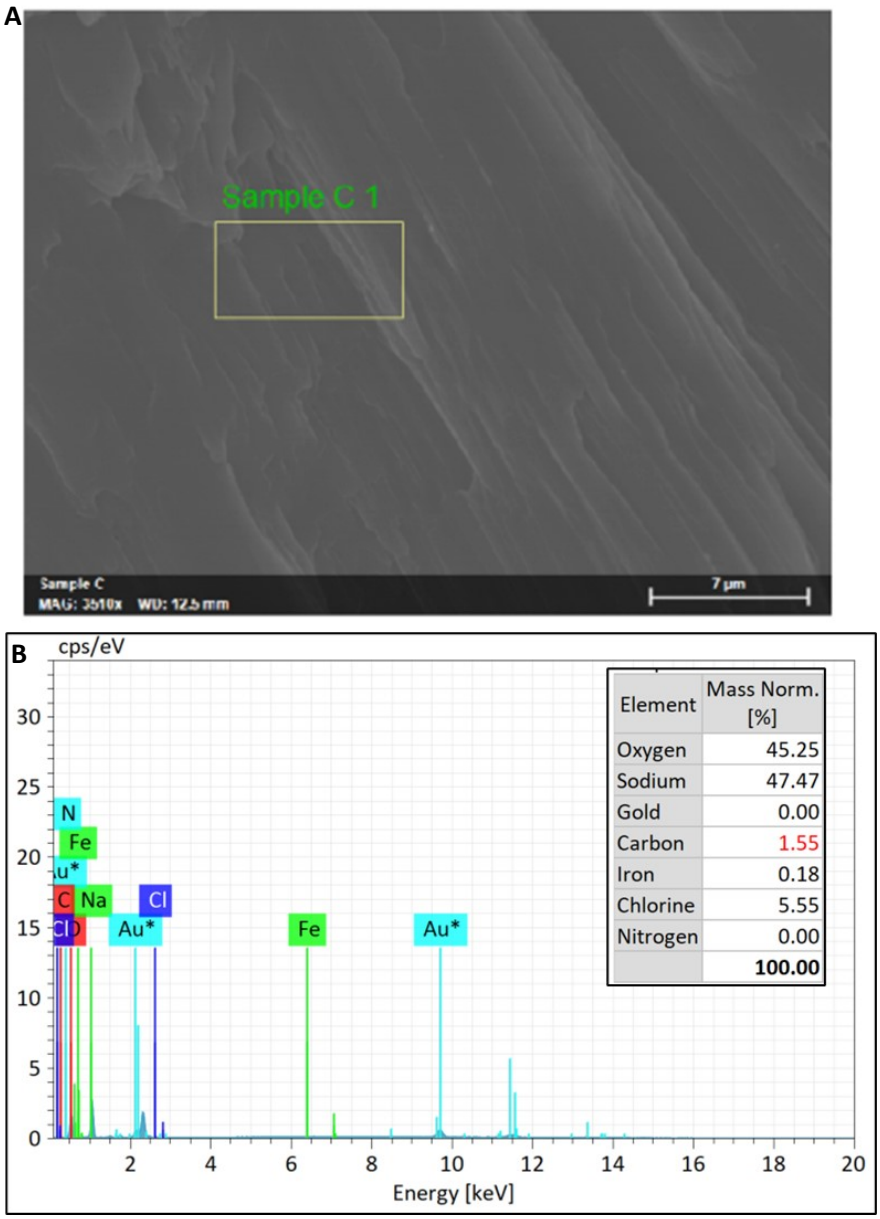


Fig. S7. Characterization of hemin-loaded **C**-MPs. **(A)** SEM image, with the analysed area indicated by a rectangle. **(B)** EDX analysis, and the respective weight percentage of the major elements (inset). Scale bar: 7 µm.

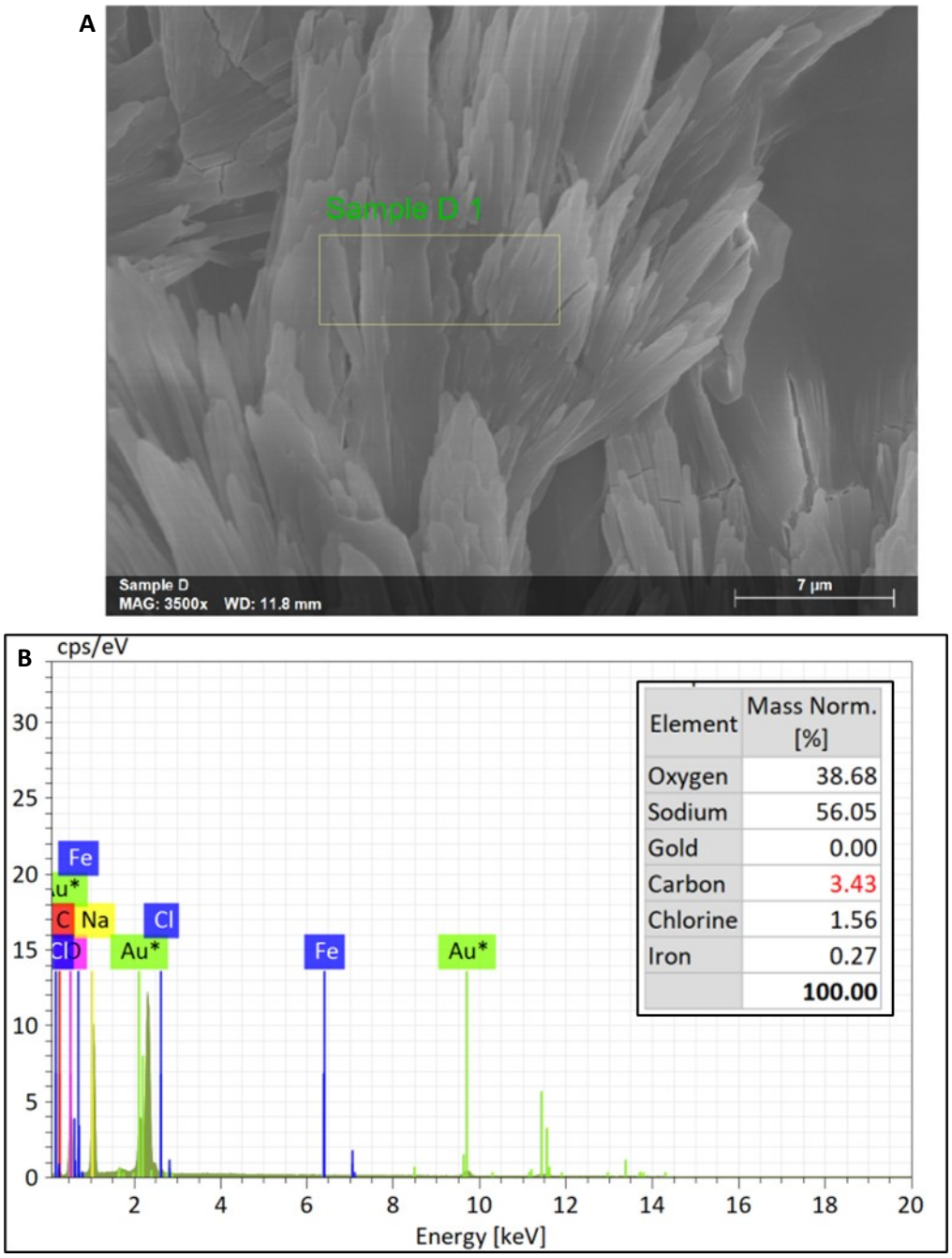


Fig. S8. Characterization of hemin-loaded **D**-MPs. **(A)** SEM image, with the analysed area indicated by a rectangle. **(B)** EDX analysis, and the respective weight percentage of the major elements (inset). Scale bar: 7 µm.

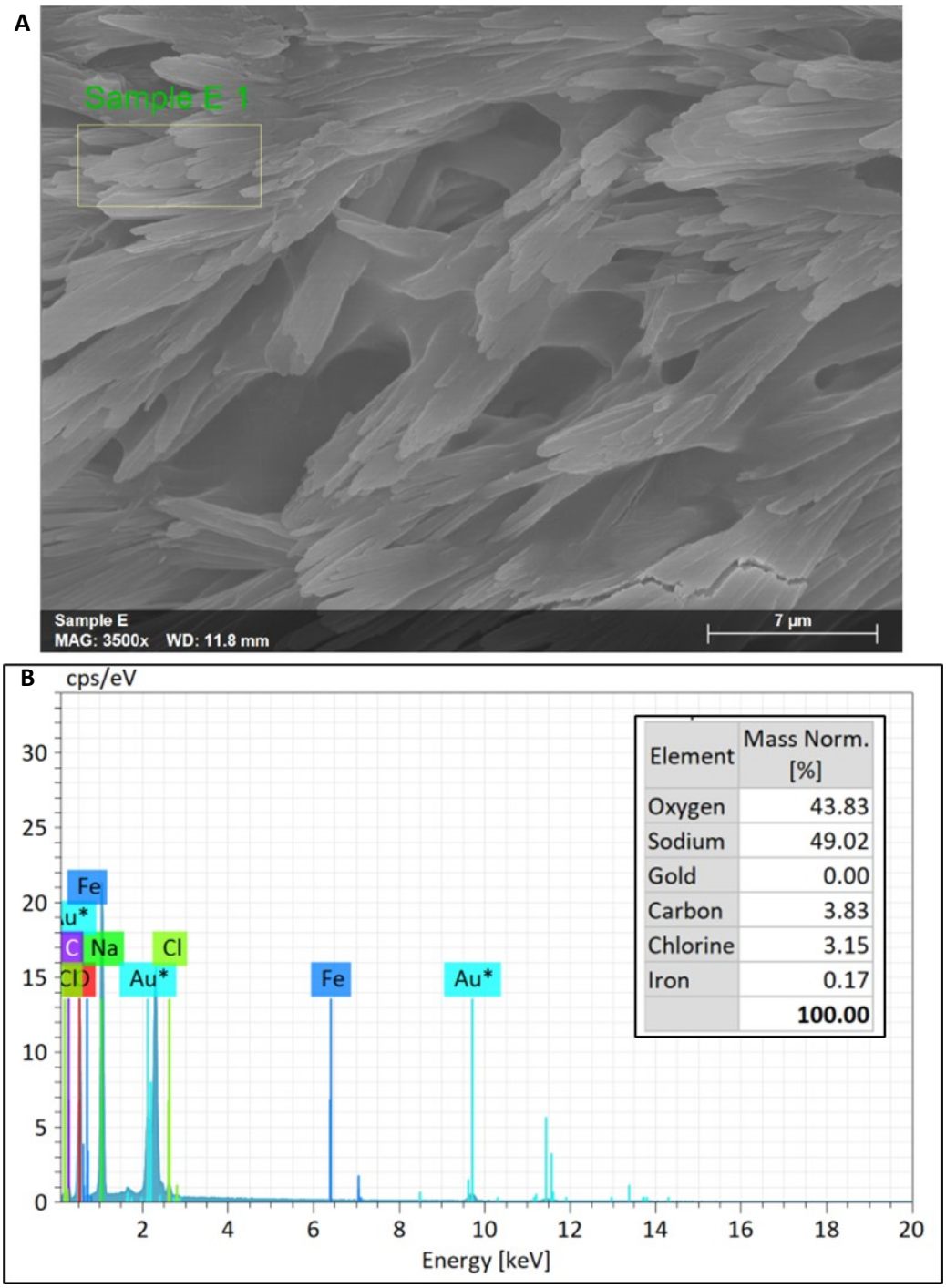


Fig. S9. Characterization of hemin-loaded **E**-MPs. **(A)** SEM image, with the analysed area indicated by a rectangle. **(B)** EDX analysis, and the respective weight percentage of the major elements (inset). Scale bar: 7 µm.

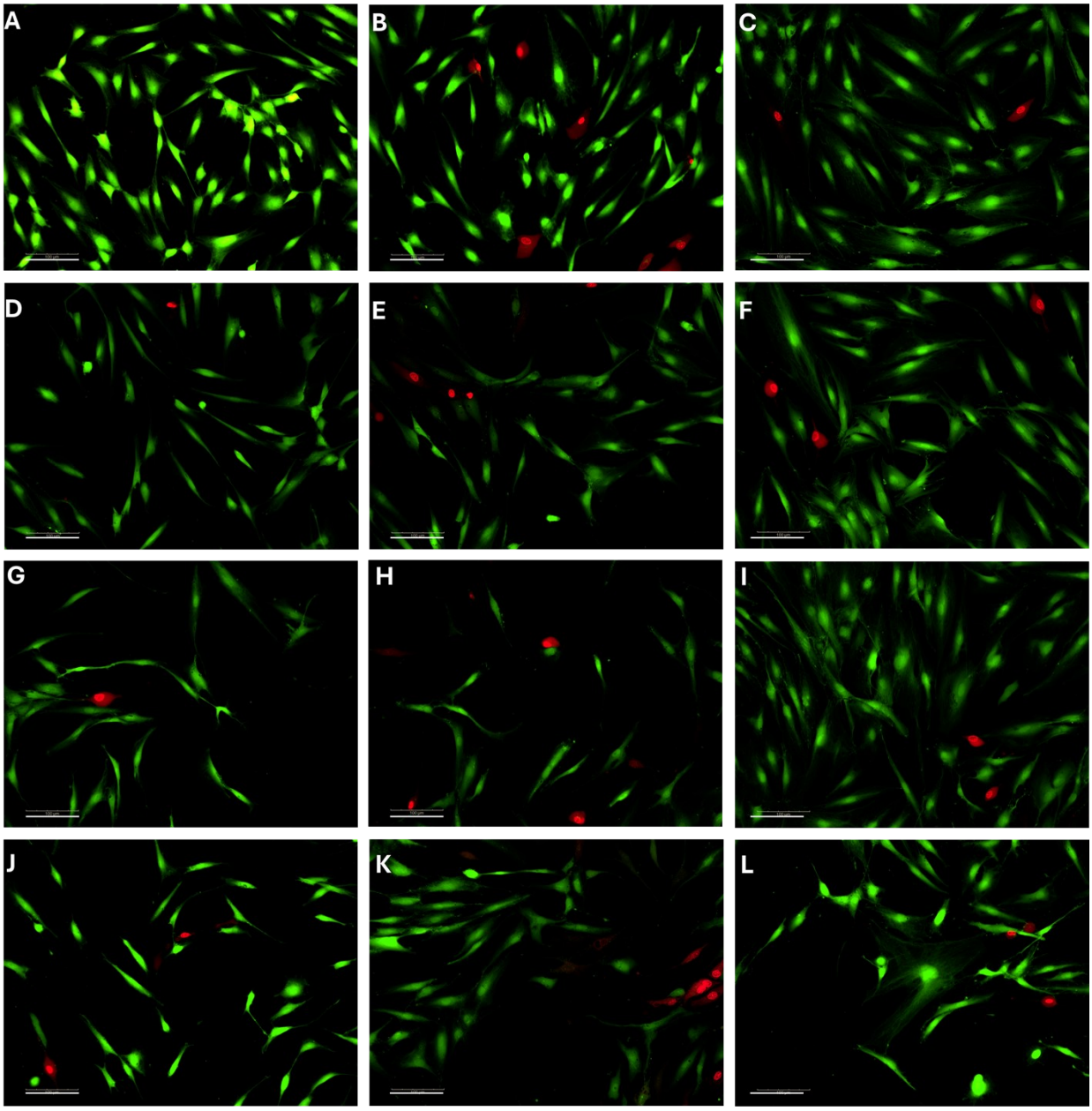


Fig. S10 Calcein AM and EthD-1 live/dead staining of **(A)** untreated HDFs; **(B)** HDFs treated with UV irradiation for 10 seconds; **(C, F, I, L)** HDFs treated with 2, 4, 8 and 16 µM hemin, respectively, followed by UV irradiation; **(D, G, J)** HDFs treated with 100, 200 and 400 µg/mL unloaded MPs **(A0)**, respectively, followed by UV irradiation; **(E, H, K)** HDFs treated with 100, 200 and 400 µg/mL hemin-loaded MPs **(A7)**, respectively, followed by UV irradiation. Scale bar: 100 µm.

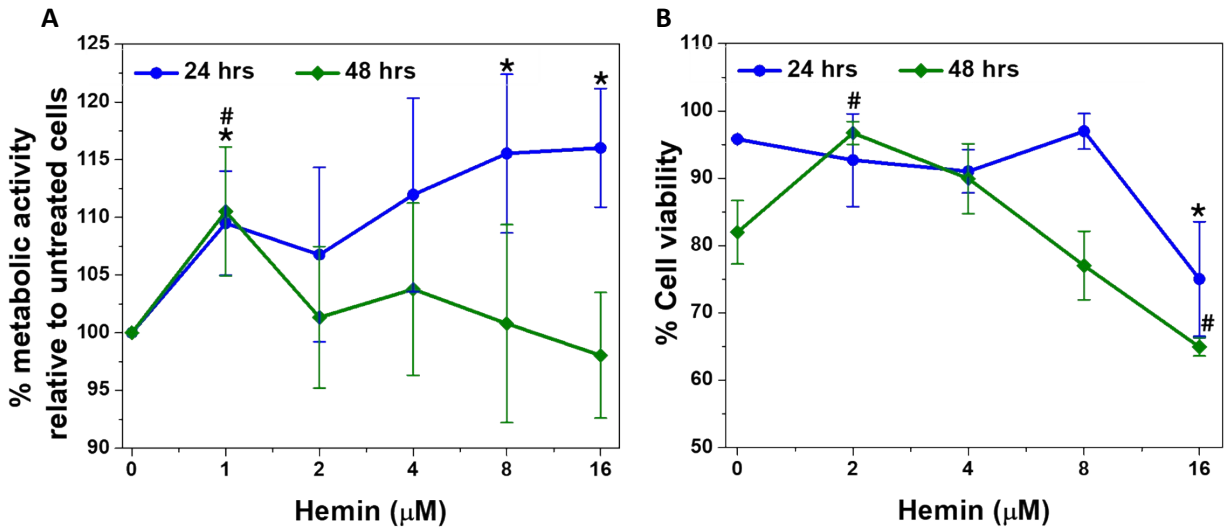


Fig. S11 The influence of hemin on **(A)** the metabolic activity and **(B)** viability of HDFs after culturing for 24 and 48 hours. Results are presented as mean values ± SD, n = 3. *,#, $P < 0.05$ for cells treated for 24 and 48 hours, respectively versus the untreated cells. Results are presented as mean values ± SD, n = 3.

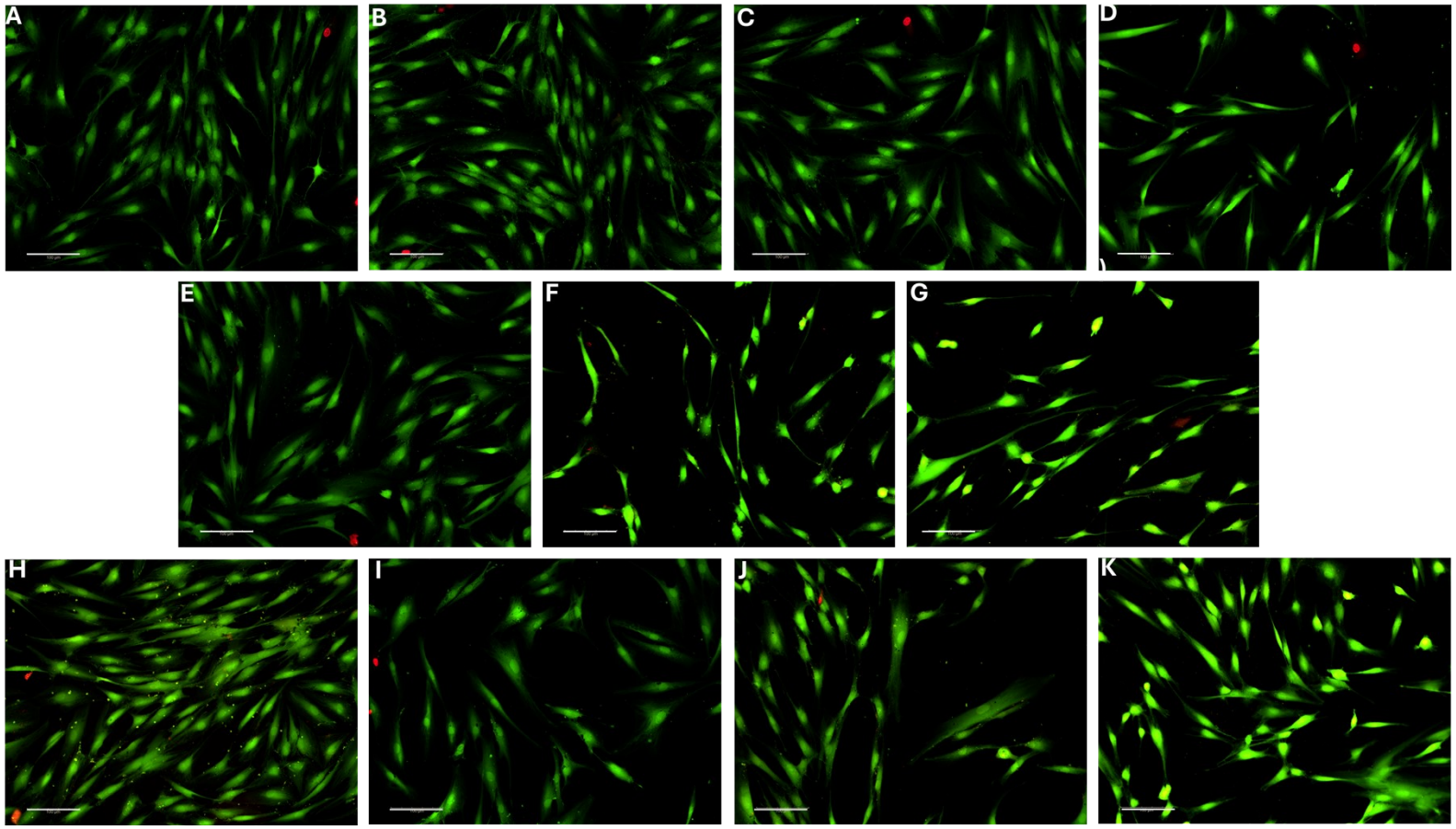


Fig. S12 Calcein AM and EthD-1 live/dead staining of **(A, B, C, D)** HDFs treated with 2, 4, 8 and 16 µM hemin only, respectively; **(E, F, G)** HDFs treated with 100, 200 and 400 µg/mL unloaded MPs **(A0)**, respectively; **(H, I, J)** HDFs treated with 100, 200 and 400 µg/mL hemin-loaded MPs **(A7)**, respectively; and **(K)** untreated cells. Scale bar: 100 µm.

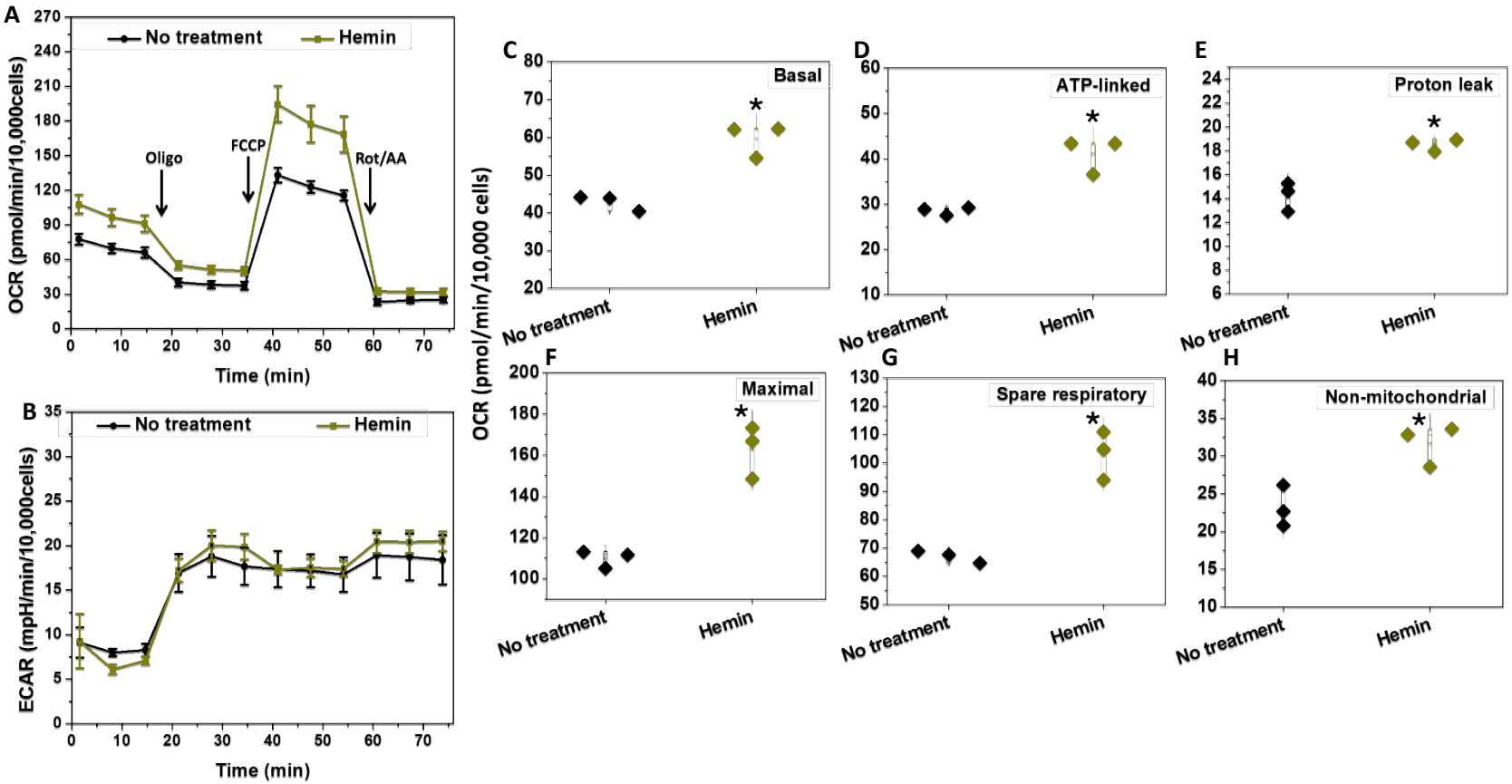


Fig. S13 The impact of hemin on the mitochondrial function of HDFs, assessed using the Mito Stress test. **(A,B)** Kinetic plots displaying the alterations in OCR and ECAR values following 24 hours of treatment with 8 µM hemin. The specific mitochondrial function parameters analysed include: **(C)** basal, **(D)** ATP-linked, **(E)** proton leak, **(F)** maximal respiration, **(G)** spare respiratory capacity, and **(H)** non-mitochondrial OCR. Results are expressed as mean ± S.D; n=3. *$p < 0.05$ compared to the untreated cells using a two-tailed unpaired student's t-test. This experiment was replicated twice.

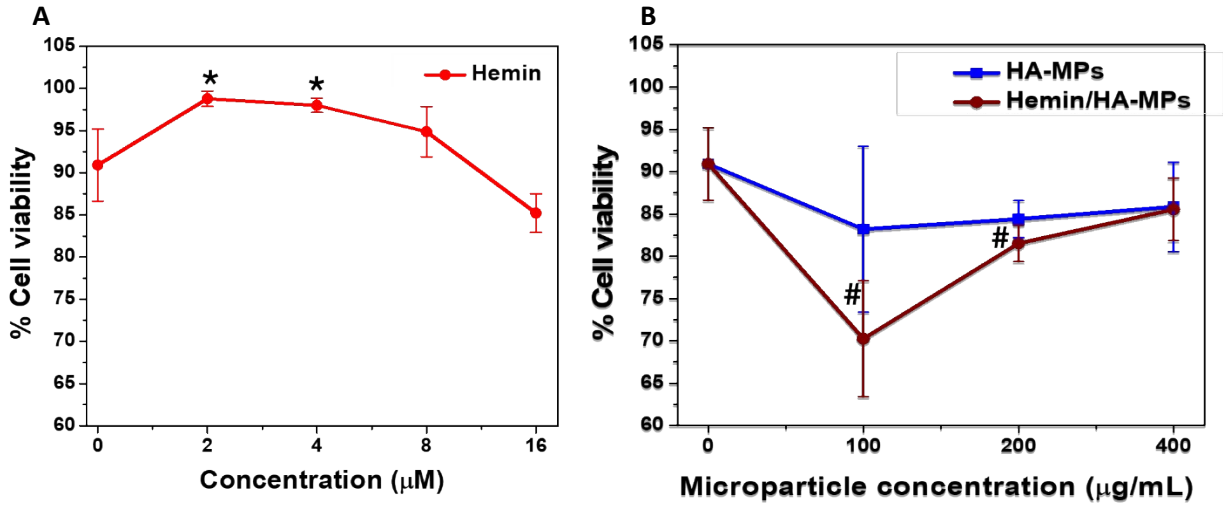


Fig. S14 The influence of UVC irradiation (10 seconds) on viability of HDFs pre-treated with hemin **(A)**, or different MPs, followed by culturing for 24 hours. Results are presented as mean values ± SD, n = 3. *,#, *P* < 0.05 for cells, pre-treated with hemin and hemin/HA-MPs, respectively compared to the UV-only treated cells, using a two-tailed unpaired student's t-test.

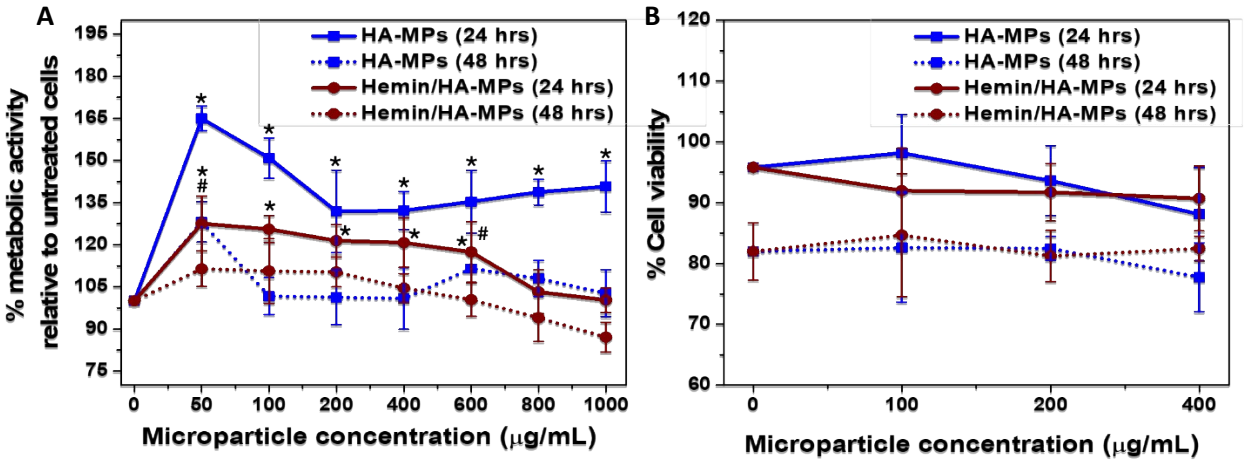


Fig. S15 The influence of microparticles on **(A)** the metabolic activity and **(B)** viability of HDFs after treatment for 24 and 48 hours. Results are presented as mean values ± SD, n = 3. *,#, *P* < 0.05 for cells treated for 24 and 48 hours, respectively versus the untreated cells, using a two-tailed unpaired student's t-test.

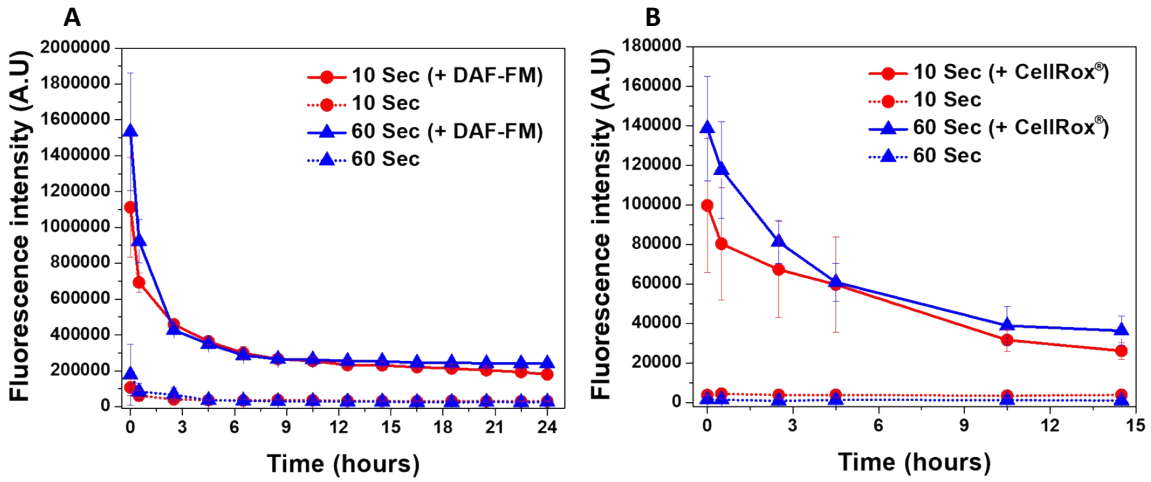


Fig. S16 Kinetics of changes in intracellular •NO and ROS levels. Intracellular •NO levels were assessed with or without the •NO -specific indicator DAF-FM (A), and intracellular ROS levels were assessed with or without the indicator CellROX® Deep Red Reagent (B), following cell treatment with UVC radiation for 10 and 60 seconds. Generally, cells were incubated with DAF-FM-DA for 1 hour or CellROX® Deep Red Reagent for 30 minutes prior to UVC exposure, and subsequently imaged using the real-time Incucyte® imaging system. Results are presented as mean fluorescence intensity ± SD, n = 3.

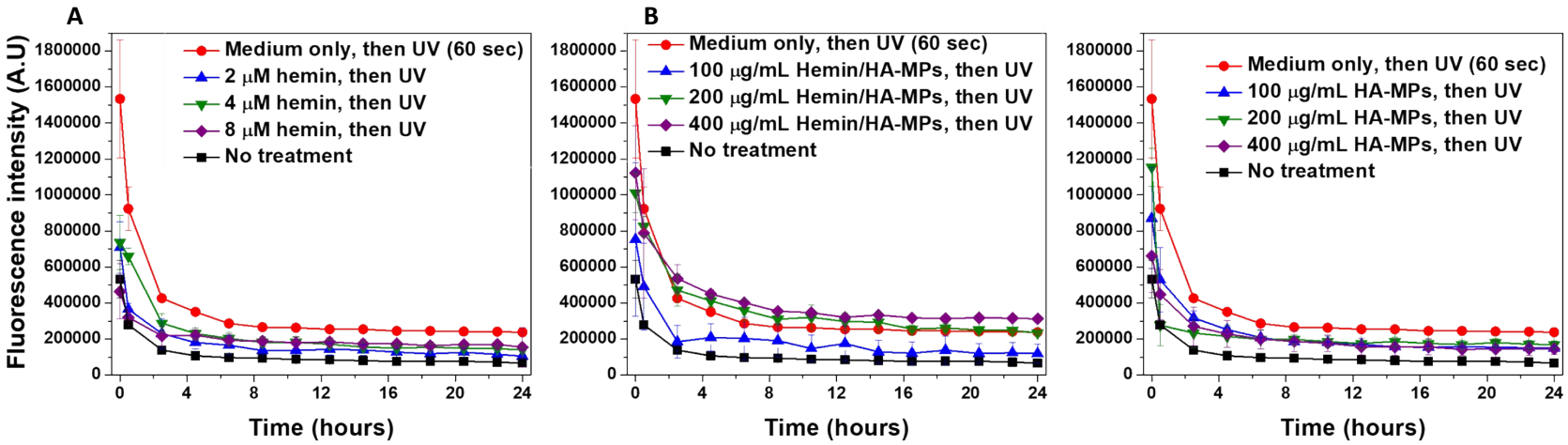


Figure S17 Kinetics of changes in intracellular •NO levels in HDFs as revealed by the •NO-specific indicator DAF-FM. The fluorescence levels were detected following pre-treatment with different concentrations of hemin **(A)**, **A**-MPs **(B)**, or **A7**-MPs **(C)** for 24 hours, followed by UVC treatment for 60 seconds. Generally, after different treatments, cells were incubated with DAF-FM-DA for 1 hour before UVC exposure and subsequently imaged using the real-time Incucyte® imaging system. Results are presented as mean fluorescence intensity ± SD, n = 3.

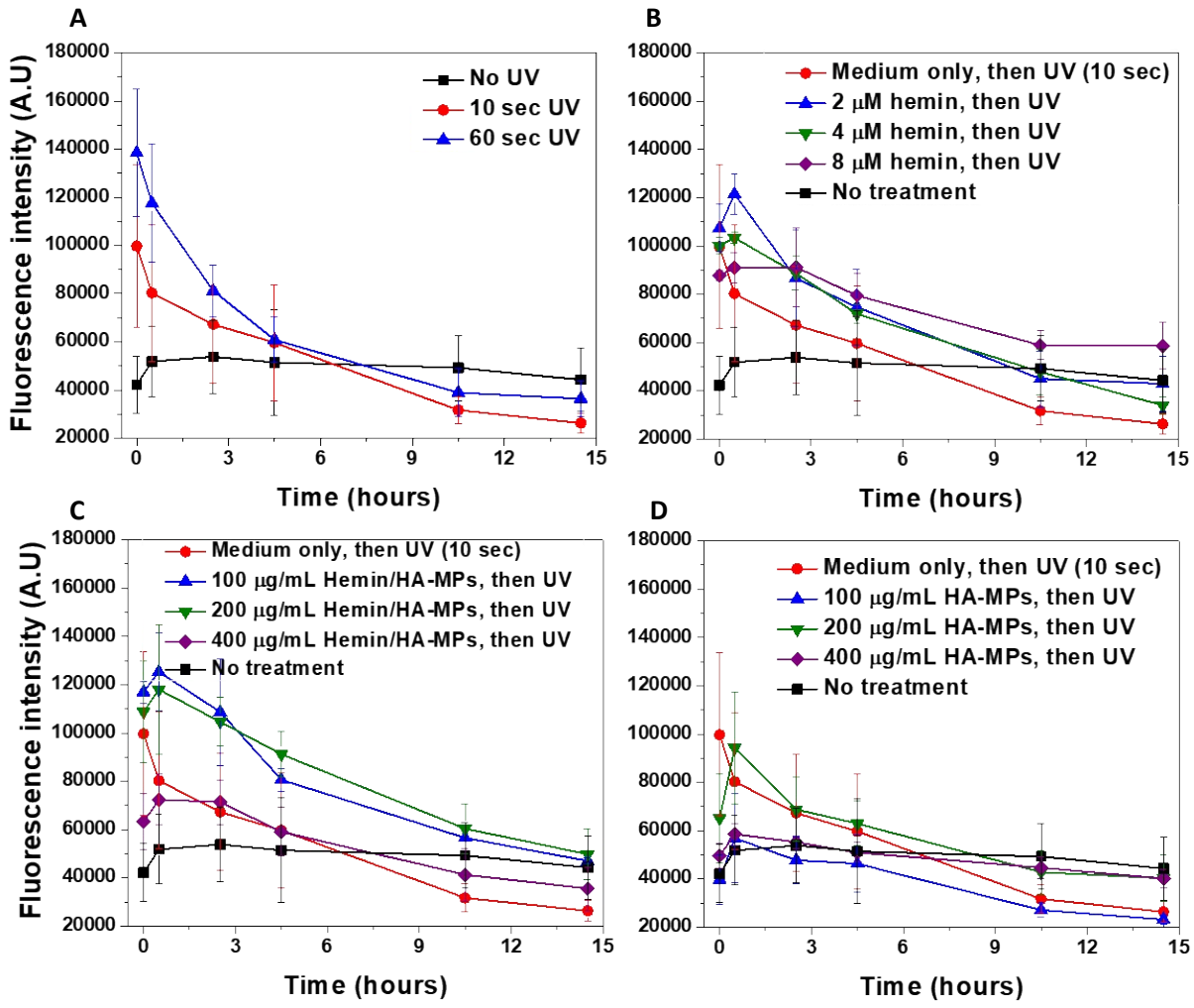
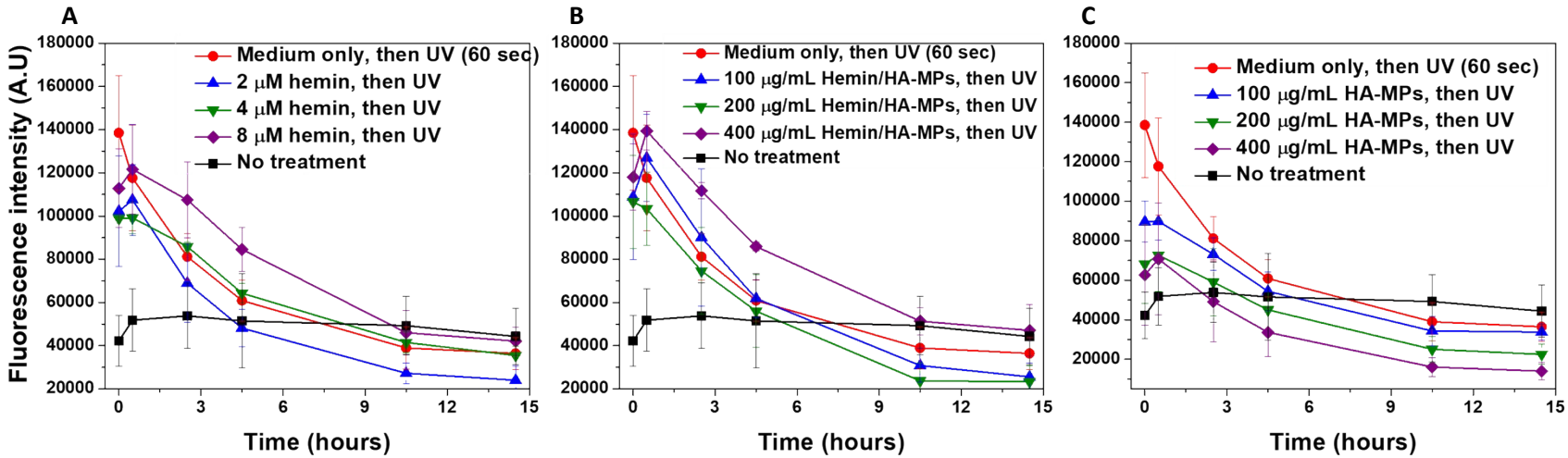


Fig. S18 Kinetics of changes in intracellular ROS levels in HDFs as revealed by the indicator CellROX® Deep Red Reagent. **(A)** Fluorescence levels following cell treatment with UVC radiation for 10 and 60 seconds. **(B,C,D)** Fluorescence levels following pre-treatment with different concentrations of hemin, **A**-MPs, or **A7**-MPs, respectively for 24 hours, followed by UVC treatment for 10 seconds. Generally, after different treatments, cells were incubated with CellROX® Deep Red Reagent for 30 minutes before UVC exposure and subsequently imaged using the real-time Incucyte® imaging system. Results are presented as mean fluorescence intensity ± SD, n = 3.



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ig. S19 Kinetics of changes in intracellular ROS levels in HDFs as revealed by the indicator CellROX® Deep Red Reagent. The fluorescence levels were detected following pre-treatment with different concentrations of hemin **(A)**, **A**-MPs **(B)**, or **A7**-MPs **(C)** for 24 hours, followed by UVC treatment for 60 seconds. Generally, after different treatments, cells were incubated with the indicator CellROX® Deep Red Reagent for 30 minutes before UVC exposure and subsequently imaged using the real-time Incucyte® imaging system. Results are presented as mean fluorescence intensity ± SD, n = 3.

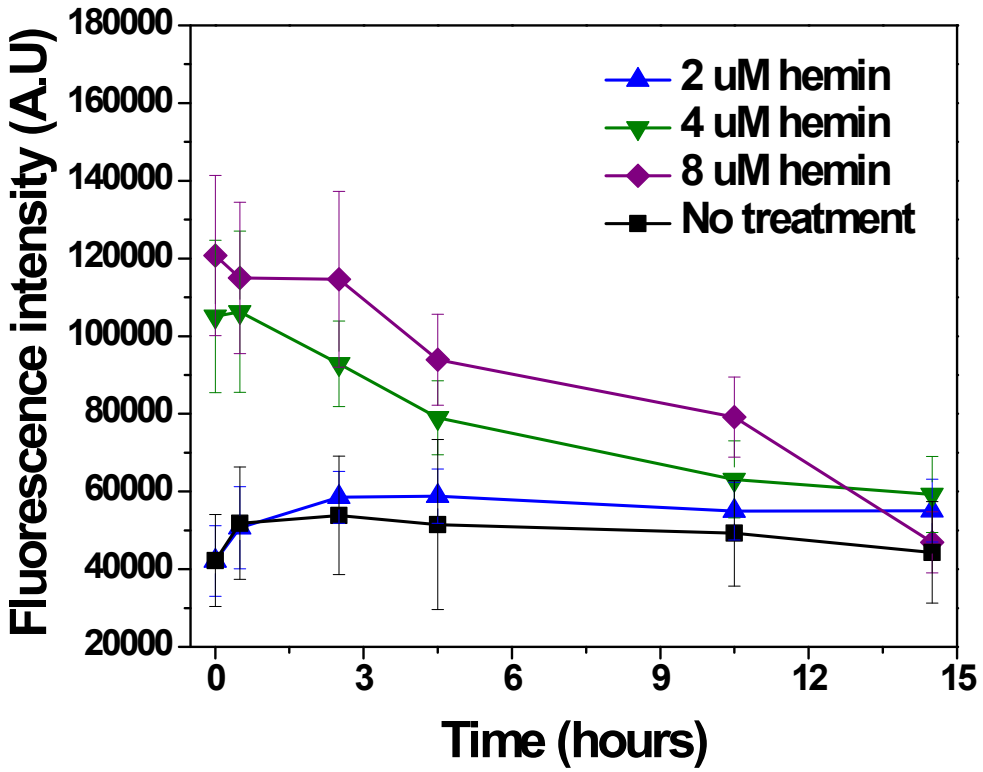


Fig. S20 Kinetics of changes in intracellular ROS levels in HDFs as revealed by the indicator CellROX® Deep Red Reagent, following cell treatment with different concentrations of hemin. Generally, after cell treatment, they were incubated with the indicator CellROX® Deep Red Reagent for 30 minutes, and subsequently imaged using the real-time Incucyte® imaging system. Results are presented as mean fluorescence intensity ± SD, n = 3.

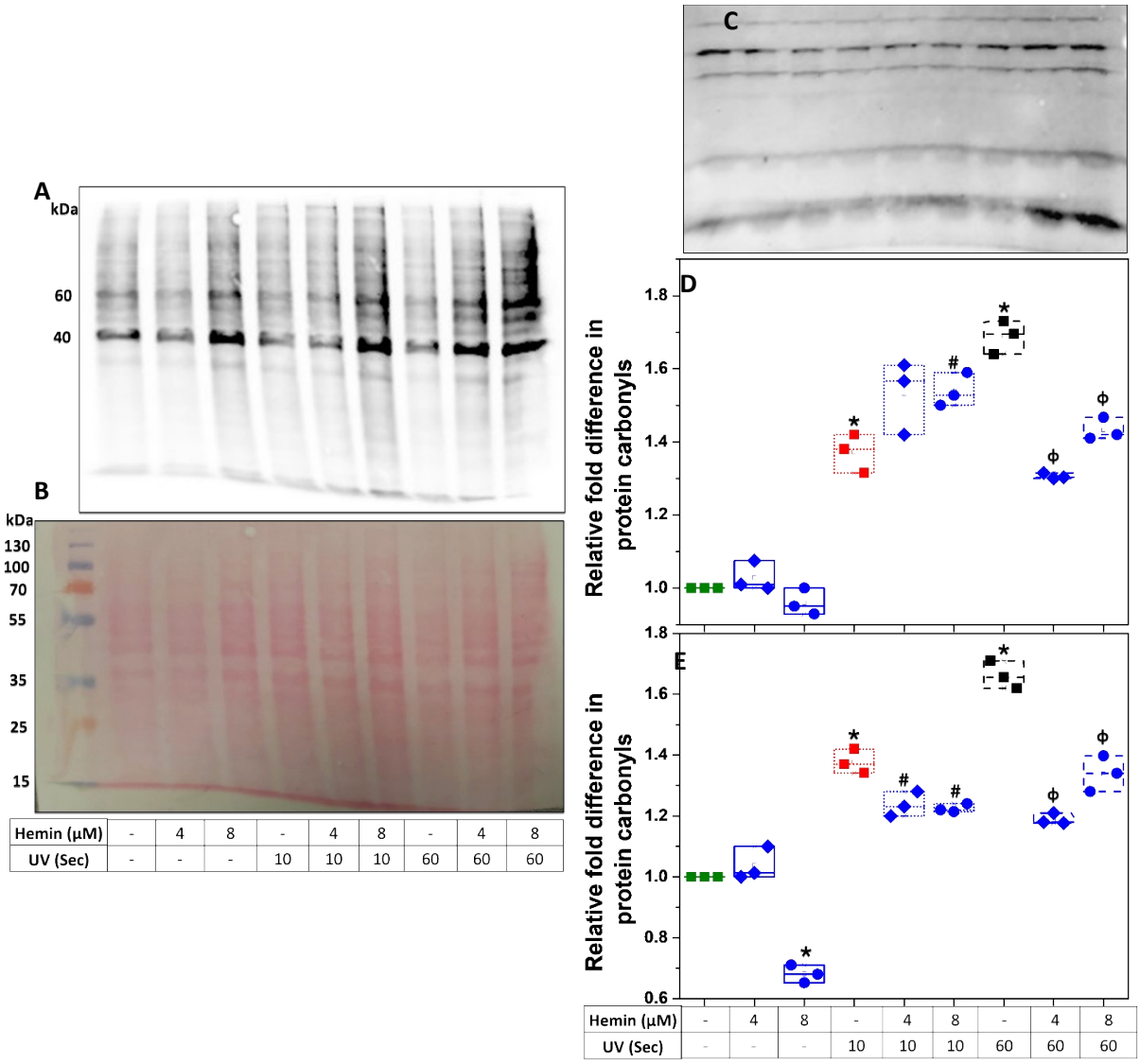
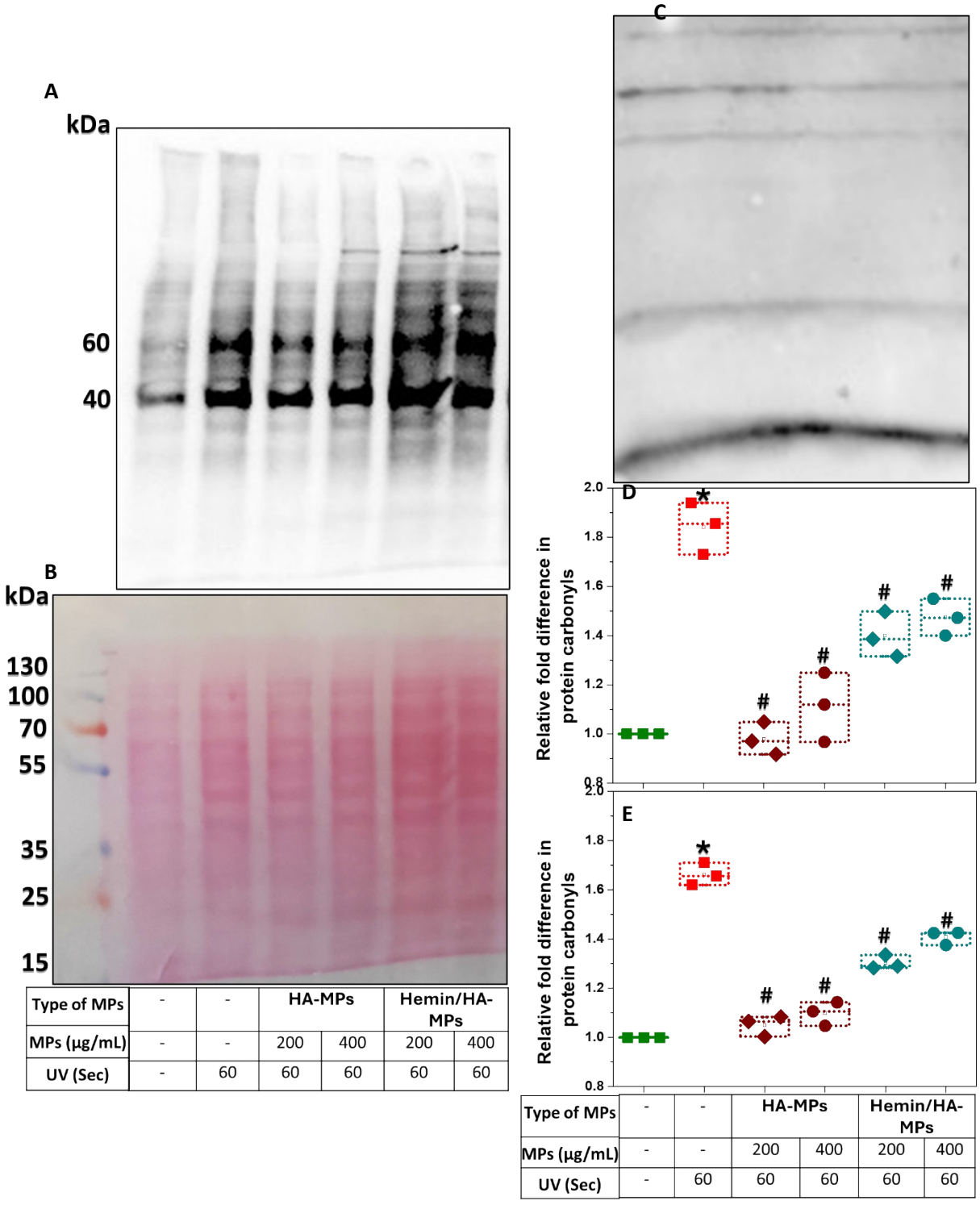


Fig. S21 Protein carbonylation following cell treatment with hemin and UV irradiation. **(A)** Representative oxyblot of total cell proteins (DNPH) after cell treatment with 4 and 8 µM hemin without/with subsequent UV treatment for 10 and 60 seconds, with the corresponding membranes stained with Ponceau S **(B)**. **(C)** The blot of negative controls. **(D, E)** Relative quantification of protein carbonyl levels at the detected MWs of 60 and 40 kDa, respectively, obtained from triplicate samples, measured by scanning densitometry, and normalized to the corresponding densities from the Ponceau S-stained membranes. Following hemin treatment for 24 hours, the cells were cultured in FBS-containing medium for three hours, with/without prior treatment with UV for 10 and 60 seconds, followed by extraction of the intracellular proteins. After quantification using BCA assay, the proteins were derivatized to 2,4-dinitrophenylhydrazone (DNPH), subjected to SDS-PAGE using 12% polyacrylamide gel, followed by membrane transfer, staining with Ponceau S, and probing with primary antibody, specific to the DNP moiety of the proteins. Results are presented as mean values ± SD, n = 3. *P < 0.05 compared to untreated cells. $^{\#}$P < 0.05 compared to UV-only treated cells for 10 seconds. $^{\Phi}$P < 0.05 compared to UV-only treated cells for 60 seconds, using a two-tailed unpaired student's t-test.



Type of MPs	-	-	HA-MPs		Hemin/HA-MPs	
MPs (µg/mL)	-	-	200	400	200	400
UV (Sec)	-	60	60	60	60	60

Type of MPs	-	-	HA-MPs		Hemin/HA-MPs	
MPs (µg/mL)	-	-	200	400	200	400
UV (Sec)	-	60	60	60	60	60

Fig. S22 Protein carbonylation following HDFs treatment with HA-MPs and hemin/HA-MPs and UV irradiation. **(A)** Representative oxyblot of total cell proteins (DNPH) after cell treatment with 200 and 400 µg/mL HA-MPs and hemin/HA-MPs without/with subsequent UV treatment for 60 seconds, with the

corresponding membranes stained with Ponceau S **(B)**. **(C)** The blot of negative controls. **(D, E)** Relative quantification of protein carbonyl levels at the detected MWs of 60 and 40 kDa, respectively, obtained from triplicate samples, measured by scanning densitometry, and normalized to the corresponding densities from the Ponceau S-stained membranes. Following MPs treatment for 24 hours, the cells were cultured in FBS-containing medium for three hours, with/without prior treatment with UV for 60 seconds, followed by extraction of the intracellular proteins. After quantification using BCA assay, the proteins were derivatized to DNPH, subjected to SDS-PAGE using 12% polyacrylamide gel, followed by membrane transfer, staining with Ponceau S, and probing with primary antibody, specific to the DNP moiety of the proteins. Results are presented as mean values ± SD, n = 3. *$P < 0.05$ compared to untreated cells. $^{\#}P < 0.05$ compared to UV-only treated cells, using a two-tailed unpaired student's t-test.