

SUPPLEMENTARY MATERIALS TO

The Effects of Combined Exposure to Simulated Microgravity, Ionizing Radiation, and Cortisol on the *In Vitro* Wound Healing Process

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SM1. X-ray energy spectrum

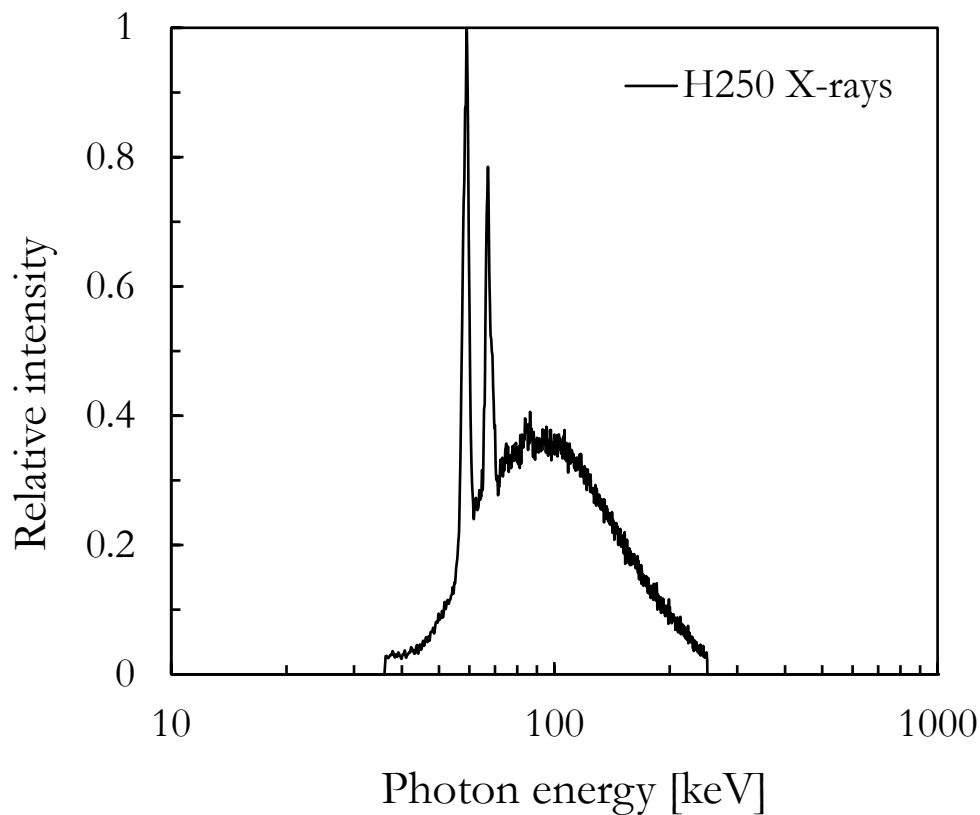


Figure SM1. Normalized energy spectrum of the H250 X-rays used for the cell exposures at SCK CEN, Mol, Belgium.

SM2. Radiation transport computer simulations

Radiation transport simulations were performed using the Particle and Heavy Ion Transport code System (PHITS, [Sato et al 2018](#)) version 3.28. A simplified geometry of the cell holder and of the material between the radiation source (i.e. the exit of the nozzle) and the cell holders (i.e. build up slabs) was implemented in PHITS. The simulated holder was filled with liquid water representing the cells and the medium. The simulated holder was irradiated with monoenergetic beams (energy listed in **Table 1** of the manuscript) impinging perpendicularly its front surface.

The following quantities were assessed in the first 100 μm of water within the holder (as representative of the position of the attached cells): dose-mean unrestricted linear energy transfer (LET) in water of the primary beam, dose-mean unrestricted LET in water of all particles (primary beam and all secondary particles), dose-mean lineal energy within homogeneously distributed liquid water spheres with diameter equal to 0.6 μm . This dimension was chosen as representative of the scale where the accumulation and repair of DNA damage correlates with cell death ([Parisi et al., 2022](#)).

The following physical models and options were used in the PHITS simulations:

- Macroscopic energy loss:
 - Charged particles (except of electrons and positrons): ATIMA.
 - Photons, electrons, and positrons: EGS5.
- Transport cutoffs: 1 keV/n (ions), 1 keV (all other particles but neutrons), 10^{-8} keV (neutrons).
- Angular straggling: Lynch's Coulomb diffusion formula based on Moliere theory.
- Energy straggling: Landau-Vavilov formula.
- Transport and interaction of low energy neutrons: event generator mode v2.
- Mean ionization potential of water = 78 eV.
- Nuclear reactions: default PHITS models.
- Microdosimetry: PHITS microdosimetric function.

More details can be found in [Sato et al 2018](#), the PHITS manual (<https://phits.jaea.go.jp/manual/manualE-phits.pdf>), and references therein.

SM3. Tables of regression model outputs

Supplementary Table 1. Significant estimates for the expression of IL-1RA.

variable	coefficients	standard error	t-value	p-value
Xcort	-0.33	0.13	-2.6	0.009
Xsmg * Xcort * Xd1	-0.5	0.24	-2.1	0.036
Xsmg * XFe * Xd1	-0.76	0.23	-3.3	0.001
Xcort * XC * Xd0.1	-0.74	0.24	-2.9	0.003
Xcort * XFe * Xd1	-0.59	0.24	-2.5	0.013
Xsmg * Xcort * XFe * Xd1	0.99	0.33	3	0.003

Supplementary Table 2. Significant estimates for the expression of IL-6.

variable	coefficients	standard error	t-value	p-value
Xcort	-0.62	0.23	-2.7	0.007
Xsmg * XC	0.99	0.33	3	0.003
Xcort * Xsmg * XC	-0.96	0.46	-2.1	0.038
Xcort * Xsmg * XFe * Xd1	2.04	0.68	2.9	0.003

Supplementary Table 3. Significant estimates for the expression of PDGF- α .

variable	coefficients	standard error	t-value	p-value
XC * Xsmg	4.41	0.75	5.9	< 0.0001
XC * Xsmg * Xcort	-4.39	1.51	-2.9	0.0042

Supplementary Table 4. Significant estimates for the expression of TGF- β .

variable	coefficients	standard error	t-value	p-value
Xsmg	0.36	0.16	2.3	0.0209
Xsmg * Xcort	-1.17	0.22	-5.3	< 0.0001
Xsmg * XFe	-0.035	0.16	-2.2	0.0279
Xsmg * Xcort * Xprot	0.55	0.23	2.4	0.0175
Xsmg * Xcort * XC	0.79	0.23	3.4	0.0007
Xsmg * Xcort * XFe	0.97	0.23	4.3	< 0.0001
Xsmg * Xcort * Xd1	0.55	0.22	2.5	0.0118

Supplementary Table 5. Coefficients of significant estimates for fibroblast migration.

variable	coefficients	standard error	t-value	p-value
Xsmg	-0.24	0.03	-7.76	< 0.0001
Xcort	-0.06	0.03	-2.10	0.0368
Xsmg * Xprot	0.11	0.04	2.85	0.0044
Xprot * Xcort	-0.07	0.04	-2.05	0.0405
Xcort * XFe	-0.19	0.04	-5.06	< 0.0001
Xprot * Xd1	-0.12	0.05	-2.25	0.0249

Supplementary Table 6. Coefficients of significant estimates for actin area after exposure to simulated spaceflight stressors.

variable	coefficients	standard error	t-value	p-value
Xcort	-0.37	0.09	-4.00	< 0.0001
Xd0.1	-0.32	0.09	-3.60	0.0004
Xd0.5	-0.19	0.09	-2.10	0.0356
Xd1	-0.26	0.09	-2.90	0.0044
Xcort * Xsmg	0.28	0.13	2.20	0.0294
Xcort * Xd0.1	0.53	0.14	3.90	<0.0001
Xcort * Xd0.5	0.39	0.14	2.80	0.0047
Xcort * Xd1	0.32	0.13	2.40	0.0167
Xsmg * Xd0.1	0.43	0.13	3.40	0.0008
Xcort * XC	0.33	0.13	2.60	0.0105
Xcort * XFe	0.78	0.13	6.00	<0.0001
Xd0.1 * XFe	0.44	0.13	3.40	0.0006
Xd0.5 * XFe	0.28	0.13	2.20	0.0258
Xd1 * XFe	0.62	0.13	4.70	<0.0001
Xcort * Xsmg * Xd0.1	-0.77	0.19	-4.00	<0.0001
Xcort * Xsmg * XC	-0.41	0.19	-2.20	0.0315
Xcort * Xsmg * XFe	-0.58	0.19	-3.10	0.0018
Xcort * Xd0.1 * XC	-0.43	0.19	-2.30	0.0244
Xcort * Xd0.1 * XFe	-0.72	0.19	-3.80	0.0002
Xcort * Xd0.5 * XFe	-0.60	0.19	-3.50	0.0016
Xcort * Xd1 * XFe	-0.69	0.19	-3.70	0.0002
Xsmg * Xd0.1 * Xprot	-0.36	0.18	-2.00	0.0493
Xsmg * Xd0.1 * XC	-0.70	0.19	-3.60	0.0003
Xsmg * Xd0.1 * XFe	-0.53	0.18	-2.90	0.0042
Xcort * Xsmg * Xd0.1 * Xprot	0.66	0.27	2.50	0.0137
Xcort * Xsmg * Xd0.1 * XC	0.94	0.27	3.50	0.0005
Xcort * Xsmg * Xd0.5 * XC	0.59	0.27	2.20	0.0302
Xcort * Xsmg * Xd0.1 * XFe	1.04	0.27	3.90	0.0001
Xcort * Xsmg * Xd0.5 * XFe	0.65	0.27	2.40	0.0147

Supplementary Table 7. Coefficients of significant estimates for number of stress fibers per cell.

variable	coefficients	standard error	t-value	p-value
actin area	1.63E-04	4.10E-06	39.7	< 0.0001
Xd1	-0.17	0.08	-2.10	0.0336
Xprot	0.16	0.08	2.00	0.0478
XC	0.16	0.08	1.90	0.0519
XFe	0.20	0.08	2.50	0.0129
Xcort	-0.18	0.05	-3.60	0.0004
Xd1 * XC	0.27	0.11	2.60	0.0106
Xd1 * XFe	0.26	0.11	2.40	0.0188
Xsmg * Xd0.1	-0.31	0.11	-2.80	0.0060
Xcort * Xd0.5	0.15	0.06	2.80	0.0052
Xcort * XFe	0.40	0.06	7.10	<0.0001
Xsmg * Xd0.1 * XC	0.48	0.16	3.10	0.0023
Xsmg * Xd1 * XFe	-0.39	0.15	-2.50	0.0111

Supplementary Table 8. Coefficients of significant estimates for the number of vinculin spots.

variable	coefficients	standard error	t-value	p-value
actin area	1.7 E-04	0.00	69.6	< 0.0001
Xcort	0.28	0.05	5.6	< 0.0001
Xprot	0.28	0.06	4.7	< 0.0001
XC	0.23	0.06	3.9	0.0001
XFe	0.21	0.06	3.6	0.0004
Xd1	0.18	0.06	3.1	0.0021
Xcort * Xsmg	-0.12	0.03	-4.8	< 0.0001
Xsmg * Xd0.1	-0.16	0.07	-2.3	0.022
Xsmg * Xd1	-0.18	0.07	-2.7	0.0075
Xprot * Xd1	-0.19	0.09	-2.1	0.027
XC * Xd1	-0.17	0.08	-2.1	0.0357
XFe * Xd1	-0.19	0.09	-2.3	0.0228
Xcort * Xprot	-0.24	0.07	-3.4	0.0007
Xcort * XC	-0.24	0.07	-3.4	0.0008
Xcort * XFe	-0.30	0.07	-4.4	< 0.0001
Xcort * Xd0.1	-0.17	0.07	-2.4	0.0169
Xsmg * XC * Xd1	0.29	0.10	3	0.0032
Xcort * XC * Xd0.1	0.24	0.10	2.4	0.0186
Xcort * XFe * Xd0.1	0.38	0.10	3.8	0.0001
Xcort * XFe * Xd0.5	0.29	0.10	2.9	0.0035
Xcort * XFe * Xd1	0.38	0.10	3.9	0.0001

Supplementary Table 9. Coefficients of significant estimates for the synthesis of fibronectin.

variable	coefficients	standard error	t-value	p-value
Xcort	0.85	0.17	4.8	< 0.0001
Xprot	0.43	0.13	3.4	0.0008
XC	0.29	0.13	2.2	0.0262
Xcort * Xd0.5	-0.46	0.21	-2.2	0.0309
Xcort * Xd1	-0.55	0.21	-2.6	0.0092
Xcort * XFe	-0.32	0.15	-2.2	0.03
Xsmg * XFe	-0.41	0.15	-2.7	0.0063
Xcort * Xsmg * Xd0.5	0.88	0.29	3	0.0032
Xcort * Xsmg * Xd1	1.35	0.3	4.5	<0.0001

Table 10. Coefficients of significant estimates for the expression of type I $\alpha 1$ procollagen.

variable	coefficients	standard error	t-value	p-value
XFe	0.48	0.17	2.9	0.0044
Xsmg	-0.48	0.15	-3.1	0.0020
Xsmg * Xcort	0.37	0.15	2.4	0.0152
Xcort * XFe	0.90	0.20	4.5	< 0.0001
Xsmg * XFe	0.47	0.20	2.4	0.0181

Supplementary Table 11. Coefficients of significant estimates for the expression of type I $\alpha 2$ procollagen.

variable	coefficients	standard error	t-value	p-value
Xprot	0.31	0.12	2.6	0.0096
XC	-0.29	0.14	-2.2	0.035
XFe	0.23	0.11	2.1	0.0363
Xsmg	-0.21	0.06	-3.43	0.0007
Xcort * Xprot	-0.37	0.17	-2.2	0.0282

References

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