Supplementary Information for Wear resistant antibacterial UHMWPE-based implant materials by radiation crosslinking

*Nicoletta Inverardi[a,b], Maria F. Serafim[a], Amita Sekar[a,b], Keita Fujino[a], Matheus Ferreira[a], Anthony Marzouca[a], Emma Nagler[a], Orhun K. Muratoglu[a,b], Ebru Oral[a,b,]**

[a]Harris Orthopaedic Laboratory, Massachusetts General Hospital, Boston, Massachusetts 02114, USA

[b]Department of Orthopaedic Surgery, Harvard Medical School, Boston, Massachusetts 02114, USA

Corresponding author:

Ebru Oral

Department of Orthopaedic Surgery, Harvard Medical School

55 Fruit St., GRJ 1231, Boston, MA, 02114, USA

+1 (617) 726-0657

eoral@mgh.harvard.edu

Drug loading evaluation by thermogravimetric analysis (TGA)

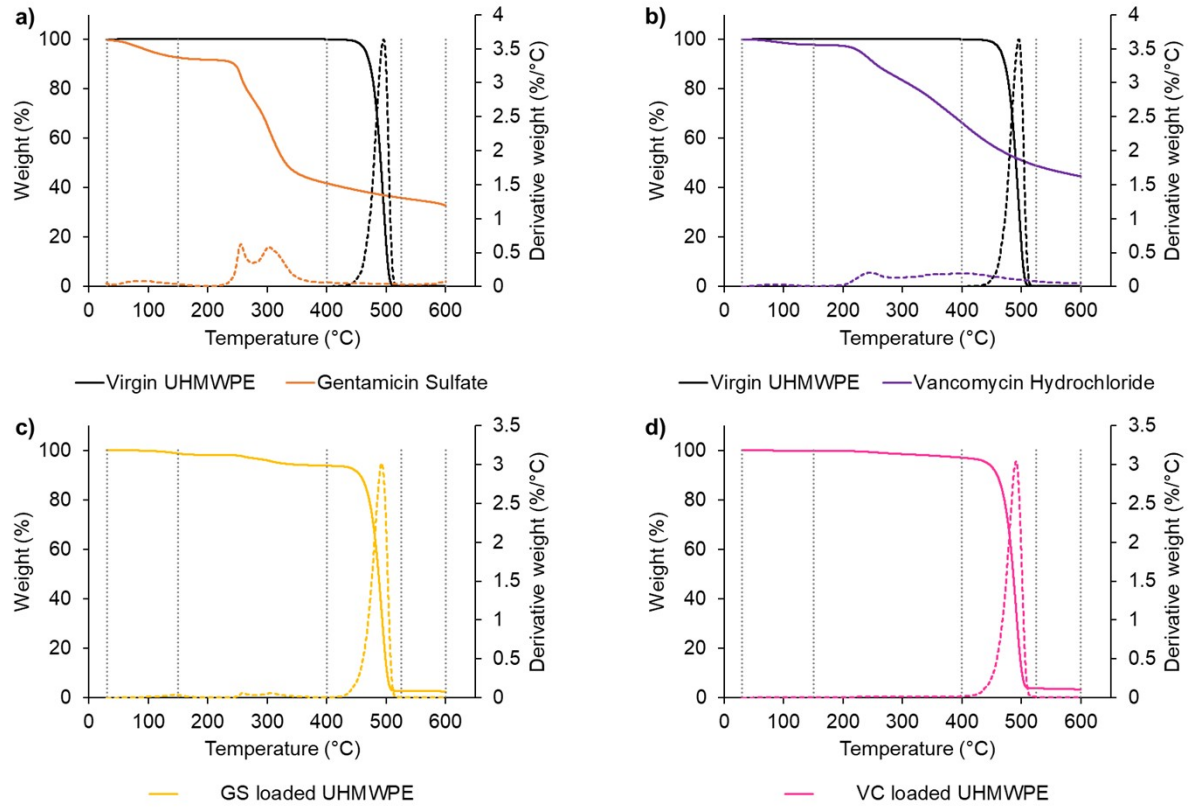

Fig. S1. Composition analysis by TGA. Weight loss (solid line, left Y axis) and derivative weight with respect to the temperature (dashed line, right Y axis) for virgin UHMWPE and for a) Gentamicin sulfate (GS), free drug and for b) Vancomycin hydrochloride (VC), free drug. Examples of TGA scans of antibiotic loaded UHMWPE for c) GS loaded UHMWPE and d) VC loaded UHMWPE. Vertical grey dashed lines indicate the temperature intervals where the weight evaluations were done for the calculation of the drug loading (150, 400, 550 and 600 °C).

An estimation of the drug loading is provided with Eq. S1 and S2 for GS and VC, respectively:

$$X_{GS}(\%) = w_{400-150} + \left(w_{525} - Residue_{525,UHMWPE}\right) + w_{GS,400-525}\left(100 - w_{400-525}\right) \quad \text{(S1)}$$

$$X_{VC}(\%) = w_{400-150} + \left(w_{525} - Residue_{525,UHMWPE}\right) + w_{VC,400-525}\left(100 - w_{400-525}\right) \quad \text{(S2)}$$

$w_{400-150}$ is the weight loss measured between 150 °C and 400 °C, where only the drug loses weight and virgin UHMWPE is stable; $(w_{525} - Residue_{525,UHMWPE})$ is the residue at 525°C corrected by the small residue left for virgin UHMWPE (<0.3 wt.%); $w_{400-525}$ is the weight loss measured between 400 °C and 525 °C and $w_{GS,400-525}$ and $w_{VC,400-525}$ are coefficients that take into account the fraction of weight loss for the free drug in the 400 °C – 500 °C temperature interval. These coefficients are measured on the weight loss curves of the free drugs (orange line in Fig. S1a and purple line in Fig. S1b).

The weight loss measured between room temperature and 150 °C is ascribed to water evaporation for these highly hydrophilic drugs, thus it is not included in the estimation of the drug loading.

^{1}H NMR

Gentamicin sulfate (GS) stock solution, unprocessed drug

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 5.76, 5.75, 5.73, 5.72, 5.71, 5.71, 5.70, 5.69, 4.97, 4.96, 4.07, 4.06, 4.04, 4.04, 4.01, 3.99, 3.98, 3.98, 3.96, 3.93, 3.91, 3.89, 3.87, 3.84, 3.72, 3.70, 3.69, 3.68, 3.67, 3.67, 3.66, 3.65, 3.63, 3.63, 3.46, 3.45, 3.45, 3.44, 3.42, 3.39, 3.37, 3.37, 3.35, 3.34, 3.33, 3.32, 3.29, 3.29, 3.28, 3.27, 3.21, 3.20, 3.18, 3.17, 3.15, 3.09, 3.09, 3.07, 3.06, 2.93, 2.91, 2.90, 2.89, 2.75, 2.64, 2.60, 2.59, 2.58, 2.41, 2.36, 2.34, 1.93, 1.88, 1.88, 1.87, 1.86, 1.85, 1.78, 1.75, 1.72, 1.49, 1.46, 1.44, 1.42, 1.40, 1.38, 1.30, 1.18, 1.16, 1.14, 1.14, 1.13, 1.12, 1.11, 1.05.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 5.77 – 5.67 (m, 1H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.05 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.02 – 3.88 (m, 1H), 3.85 (d, *J* = 12.8 Hz, 1H), 3.74 – 3.62 (m, 2H), 3.48 – 3.25 (m, 6H), 3.18 (p, *J* = 6.9 Hz, 0H), 3.08 (dd, *J* = 13.4, 3.2 Hz, 0H), 2.91 (dd, *J* = 13.4, 8.5 Hz, 0H), 2.75 (s, 3H), 2.64 (s, 0H), 2.58 (s, 1H), 2.41 (s, 0H), 2.35 (d, *J* = 12.5 Hz, 1H), 1.95 – 1.80 (m, 3H), 1.75 (t, *J* = 13.8 Hz, 0H), 1.43 (dq, *J* = 23.9, 12.4 Hz, 1H), 1.30 (s, 0H), 1.22 – 1.07 (m, 5H), 1.06 – 1.03 (m, 0H).

GS 0kGy eluent

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 5.74, 5.73, 5.68, 5.67, 4.96, 4.96, 4.06, 4.06, 4.04, 4.03, 4.02, 3.99, 3.98, 3.97, 3.96, 3.94, 3.87, 3.84, 3.80, 3.73, 3.71, 3.66, 3.65, 3.63, 3.62, 3.45, 3.45, 3.44, 3.43, 3.42, 3.39, 3.39, 3.37, 3.36, 3.34, 3.32, 3.32, 3.29, 3.28, 3.28, 3.27, 3.21, 3.20, 3.18, 3.17, 3.15, 3.09, 3.08, 3.06, 3.06, 2.93, 2.91, 2.90, 2.89, 2.75, 2.60, 2.59, 2.58, 2.41, 2.32, 1.89, 1.88, 1.87, 1.86, 1.85, 1.77, 1.75, 1.72, 1.48, 1.46, 1.45, 1.44, 1.42, 1.41, 1.40, 1.38, 1.30, 1.18, 1.16, 1.14, 1.14, 1.13, 1.12, 1.11, 1.07.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 5.76 – 5.64 (m, 1H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.05 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.02 – 3.92 (m, 1H), 3.85 (d, *J* = 12.8 Hz, 2H), 3.64 (q, *J* = 7.5 Hz, 3H), 3.47 – 3.23 (m, 4H), 3.18 (p, *J* = 6.8 Hz, 0H), 3.07 (dd, *J* = 13.4, 3.2 Hz, 0H), 2.91 (dd, *J* = 13.5, 8.4 Hz, 0H), 2.75 (s, 4H), 2.58 (s, 1H), 2.41 (s, 0H), 2.32 (s, 1H), 2.00 – 1.80 (m, 5H), 1.75 (t, 1H), 1.43 (dq, *J* = 16.2, 11.0 Hz, 2H), 1.30 (s, 0H), 1.22 – 1.10 (m, 7H), 1.07 (s, 0H).

GS 25kGy eluent

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 5.74, 5.73, 5.70, 5.69, 5.68, 5.68, 4.96, 4.96, 4.06, 4.06, 4.04, 4.03, 4.01, 3.99, 3.98, 3.96, 3.94, 3.87, 3.84, 3.80, 3.73, 3.71, 3.69, 3.67, 3.66, 3.65, 3.63, 3.62, 3.47, 3.46, 3.45, 3.44, 3.43, 3.39, 3.37, 3.34, 3.32, 3.32, 3.29, 3.28, 3.28, 3.27, 3.20, 3.18, 3.17, 3.15, 3.09, 3.08, 3.06, 3.06, 2.93, 2.91, 2.90, 2.89, 2.75, 2.60, 2.59, 2.59, 2.58, 2.41, 2.32, 1.88, 1.87, 1.85, 1.78, 1.77, 1.75, 1.72, 1.48, 1.46, 1.45, 1.43, 1.42, 1.41, 1.40, 1.30, 1.18, 1.16, 1.14, 1.14, 1.13, 1.12, 1.11, 1.07.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 5.75 – 5.66 (m, 1H), 4.96 (d, *J* = 3.8 Hz, 1H), 4.05 (dd, *J* = 10.9, 3.7 Hz, 1H), 3.98 (dd, *J* = 27.2, 11.9 Hz, 1H), 3.85 (d, *J* = 12.8 Hz, 1H), 3.75 – 3.58 (m, 2H), 3.48 – 3.24 (m, 6H), 3.22 – 3.12 (m, 0H), 3.07 (dd, *J* = 13.4, 3.2 Hz, 0H), 2.91 (dd, *J* = 13.5, 8.4 Hz, 0H), 2.75 (s, 3H), 2.62 – 2.59 (m, 0H), 2.58 (s, 1H), 2.41 (s, 0H), 2.32 (s, 1H), 1.86 (d, *J* = 6.5 Hz, 0H), 1.79 – 1.70 (m, 1H), 1.53 – 1.33 (m, 1H), 1.30 (s, 0H), 1.21 – 1.09 (m, 6H), 1.07 (s, 0H).

GS 100kGy eluent

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 5.76, 5.75, 5.73, 5.73, 5.72, 5.71, 5.70, 5.69, 4.97, 4.96, 4.07, 4.06, 4.04, 4.04, 4.00, 4.00, 3.99, 3.99, 3.98, 3.95, 3.93, 3.92, 3.91, 3.90, 3.87, 3.85, 3.84, 3.69, 3.69, 3.68, 3.67, 3.66, 3.66, 3.65, 3.65, 3.63, 3.63, 3.52, 3.46, 3.45, 3.45, 3.44, 3.42, 3.42, 3.40, 3.40, 3.39, 3.38, 3.37, 3.35, 3.34, 3.33, 3.32, 3.30, 3.29, 3.29, 3.28, 3.27, 3.27, 3.26, 3.21, 3.20, 3.18, 3.18, 3.16, 3.09, 3.09, 3.07, 3.06, 2.93, 2.91, 2.90, 2.89, 2.75, 2.65, 2.60, 2.59, 2.58, 2.41, 2.37, 2.36, 2.34, 1.95, 1.92, 1.90, 1.89, 1.87, 1.86, 1.78, 1.78, 1.75, 1.74, 1.72, 1.72, 1.49, 1.46, 1.44, 1.43, 1.40, 1.30, 1.18, 1.16, 1.14, 1.14, 1.13, 1.12, 1.11, 1.07.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 5.77 – 5.68 (m, 1H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.05 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.02 – 3.88 (m, 1H), 3.85 (d, *J* = 12.9 Hz, 1H), 3.74 – 3.61 (m, 2H), 3.53 – 3.23 (m, 6H), 3.21 – 3.14 (m, 0H), 3.08 (dd, *J* = 13.5, 3.2 Hz, 0H), 2.91 (dd, *J* = 13.4, 8.5 Hz, 0H), 2.75 (s, 3H), 2.65 (s, 0H), 2.58 (s, 1H), 2.41 (s, 0H), 2.36 (d, *J* = 13.5 Hz, 1H), 2.00 – 1.81 (m, 4H), 1.81 – 1.69 (m, 1H), 1.43 (dq, *J* = 21.5, 10.8 Hz, 1H), 1.30 (s, 0H), 1.25 – 1.09 (m, 5H), 1.07 (s, 0H).

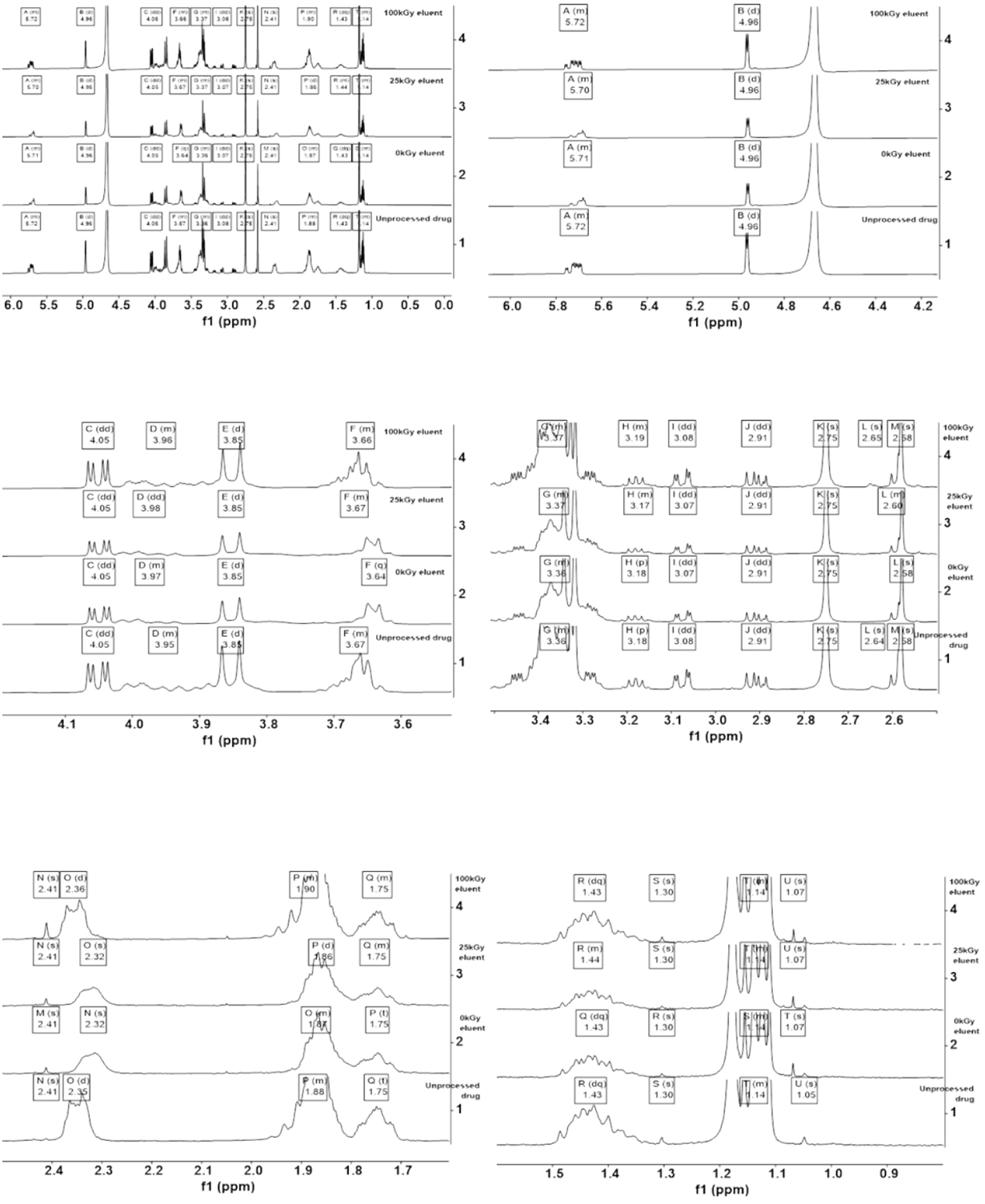

Fig. S2. Comparison of ^{1}H NMR spectra for gentamicin sulfate – stock solution and eluents from the 0, 25 and 100kGy irradiated UHMWPEs. Comparison of the full spectra and of enlarged spectral regions (Solvent peak: D_20 at 4.67 ppm).

Vancomycin Hydrochloride (VC) stock solution, unprocessed drug

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 7.57, 7.47, 7.42, 7.41, 7.13, 6.95, 6.87, 6.79, 6.38, 6.38, 6.33, 5.55, 5.39, 5.29, 5.25, 5.17, 5.16, 4.51, 4.41, 4.36, 4.08, 3.92, 3.63, 3.45, 3.36, 3.30, 2.65, 2.62, 2.59, 1.94, 1.91, 1.88, 1.86, 1.65, 1.63, 1.58, 1.56, 1.25, 1.02, 1.01, 0.77, 0.76, 0.73, 0.72.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 7.57 (s, 1H), 7.47 (s, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.13 (s, 1H), 6.95 (s, 1H), 6.87 (s, 1H), 6.79 (s, 1H), 6.38 (d, *J* = 2.3 Hz, 1H), 6.33 (s, 1H), 5.55 (s, 1H), 5.39 (s, 1H), 5.29 (s, 1H), 5.25 (s, 1H), 5.17 (d, *J* = 4.3 Hz, 1H), 4.51 (s, 1H), 4.41 (s, 1H), 4.36 (s, 1H), 4.08 (s, 1H), 3.92 (s, 1H), 3.63 (s, 4H), 3.45 (s, 1H), 3.36 (s, 1H), 3.30 (s, 1H), 2.59 (t, 4H), 1.90 (q, *J* = 13.1 Hz, 2H), 1.64 (d, *J* = 7.2 Hz, 0H), 1.57 (d, *J* = 7.0 Hz, 0H), 1.25 (s, 3H), 1.02 (d, *J* = 6.4 Hz, 3H), 0.75 (dd, *J* = 18.6, 6.3 Hz, 6H).

VC 0kGy eluent

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 7.61, 7.56, 7.49, 7.41, 7.40, 7.35, 7.11, 6.97, 6.77, 6.48, 6.37, 6.33, 5.39, 5.31, 5.25, 5.21, 5.17, 4.54, 4.39, 4.32, 4.09, 3.92, 3.62, 3.47, 3.36, 3.31, 2.66, 2.62, 2.60, 2.57, 1.94, 1.92, 1.89, 1.86, 1.64, 1.53, 1.30, 1.26, 1.03, 0.74, 0.70.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 7.56 (s, 1H), 7.49 (s, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.11 (s, 2H), 6.97 (s, 1H), 6.77 (s, 3H), 6.37 (s, 1H), 6.33 (s, 1H), 5.39 (s, 1H), 5.31 (s, 1H), 5.25 (s, 1H), 5.17 (s, 1H), 4.54 (s, 0H), 4.39 (s, 1H), 4.32 (s, 1H), 4.09 (s, 1H), 3.92 (s, 1H), 3.62 (s, 6H), 3.47 (s, 1H), 3.36 (s, 2H), 3.31 (s, 1H), 2.61 (d, *J* = 7.9 Hz, 3H), 1.96 – 1.83 (m, 1H), 1.64 (s, 1H), 1.53 (s, 4H), 1.26 (s, 2H), 1.03 (s, 3H), 0.72 (d, *J* = 20.5 Hz, 8H).

VC 25kGy eluent

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 7.56, 7.49, 7.40, 7.11, 6.95, 6.78, 6.37, 6.33, 5.52, 5.39, 5.31, 5.27, 5.24, 5.17, 4.52, 4.40, 4.08, 3.87, 3.71, 3.61, 3.45, 3.36, 3.31, 2.66, 2.62, 2.60, 2.57, 1.94, 1.91, 1.88, 1.85, 1.62, 1.52, 1.25, 1.03, 1.02, 0.75, 0.71.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 7.56 (s, 1H), 7.49 (s, 1H), 7.40 (s, 1H), 7.11 (s, 1H), 6.95 (s, 1H), 6.78 (s, 2H), 6.37 (s, 1H), 6.33 (s, 1H), 5.52 (s, 1H), 5.39 (s, 1H), 5.31 (s, 1H), 5.27 (s, 1H), 5.24 (s, 1H), 5.17 (s, 1H), 4.52 (s, 1H), 4.40 (s, 1H), 4.08 (s, 1H), 3.87 (s, 1H), 3.71 (s, 1H), 3.61 (s, 3H), 3.45 (s, 1H), 3.36 (s, 1H), 3.31 (s, 1H), 2.79 – 2.35 (m, 3H), 1.89 (q, J = 13.7 Hz, 2H), 1.62 (s, 1H), 1.52 (s, 2H), 1.25 (s, 2H), 1.03 (d, J = 6.6 Hz, 3H), 0.73 (d, J = 20.6 Hz, 8H).

VC 100kGy eluent

List of peaks:

^{1}H NMR (500 MHz, D_2O) δ 7.56, 7.49, 7.40, 7.11, 6.96, 6.78, 6.37, 6.33, 5.39, 5.32, 5.27, 5.24, 5.17, 4.53, 4.40, 4.33, 4.08, 3.92, 3.70, 3.60, 3.45, 3.41, 3.36, 3.31, 2.66, 2.61, 2.60, 2.57, 1.91, 1.88, 1.85, 1.64, 1.53, 1.26, 1.03, 0.75, 0.71.

List of multiplets:

^{1}H NMR (500 MHz, D_2O) δ 7.56 (s, 1H), 7.49 (s, 1H), 7.40 (s, 2H), 7.11 (s, 1H), 6.96 (s, 1H), 6.78 (s, 2H), 6.37 (s, 1H), 6.33 (s, 1H), 5.39 (s, 1H), 5.32 (s, 1H), 5.27 (s, 1H), 5.24 (s, 1H), 5.17 (s, 1H), 4.53 (s, 0H), 4.40 (s, 1H), 4.33 (s, 1H), 4.08 (s, 1H), 3.92 (s, 1H), 3.70 (s, 2H), 3.60 (s, 3H), 3.43 (d, J = 21.7 Hz, 1H), 3.36 (s, 1H), 3.31 (s, 1H), 2.61 (d, J = 6.9 Hz, 3H), 1.88 (t, J = 15.7 Hz, 2H), 1.64 (s, 1H), 1.53 (s, 4H), 1.26 (s, 2H), 1.03 (s, 3H), 0.73 (d, J = 20.8 Hz, 8H).

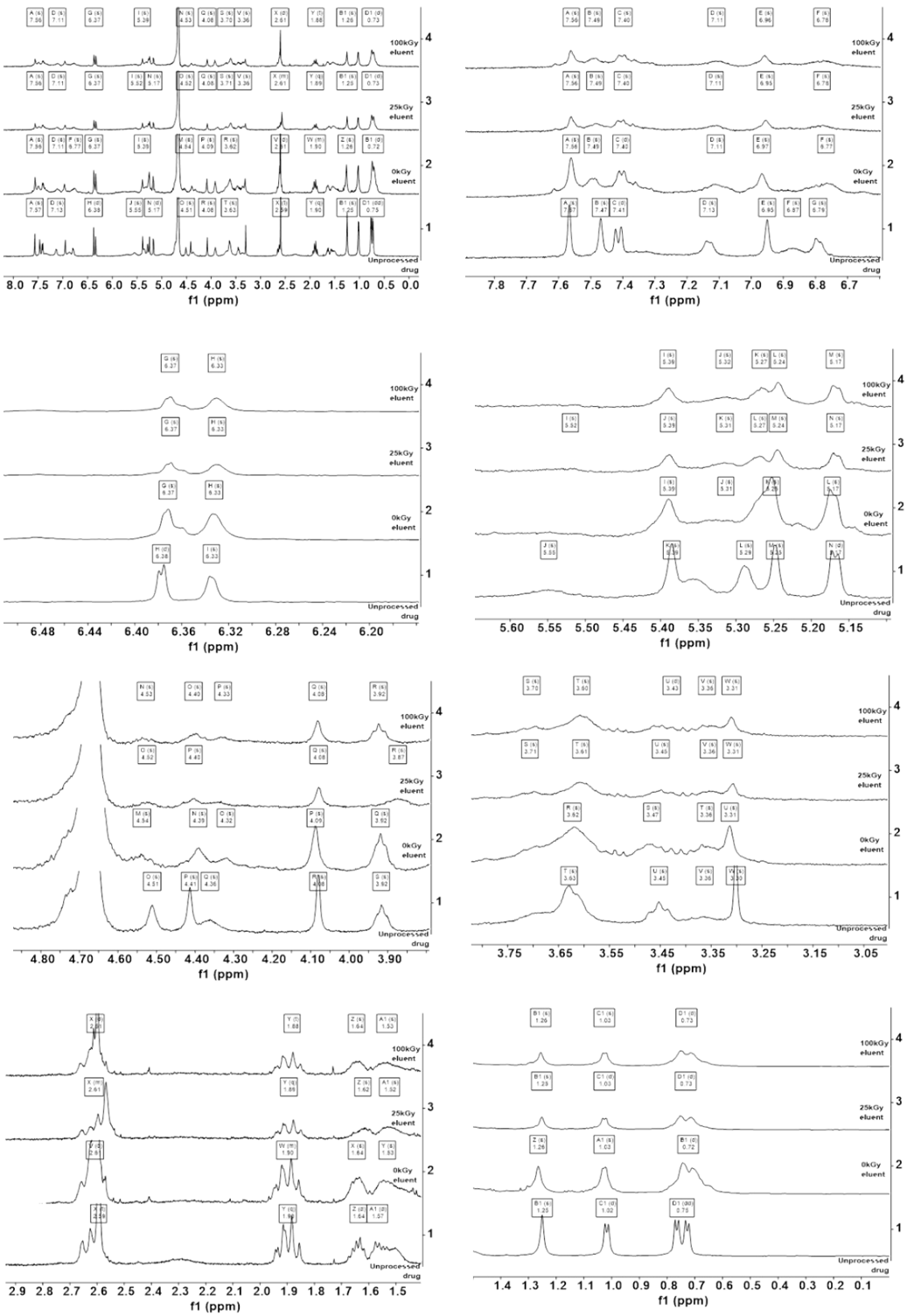

Fig. S3. Comparison of ^{1}H NMR spectra for vancomycin hydrochloride – stock solution and eluents from the 0, 25 and 100kGy irradiated UHMWPEs. Comparison of the full spectra and of enlarged spectral regions (Solvent peak: D_20 at 4.67 ppm).

Morphology of the worn surfaces after pin-on-disk wear testing

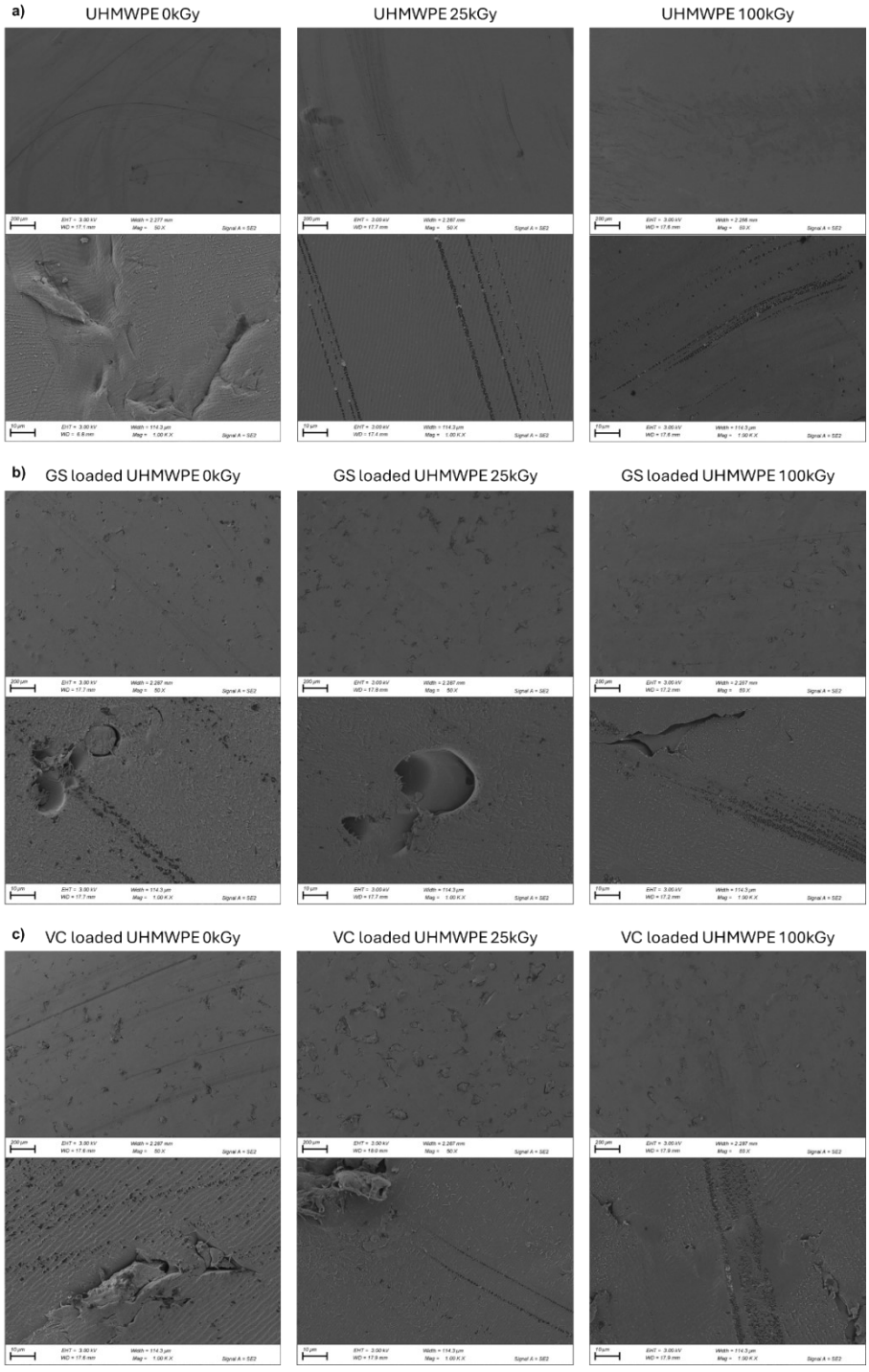

Fig. S4. Morphology of the worn surfaces by SEM. Low magnification images (top row, scale bar: 200µm) and high magnification images (bottom row, scale bar: 10 µm) for a) virgin UHMWPE, b) GS loaded UHMWPE, c) VC loaded UHMWPE. First column: 0kGy; second column: 25kGy; third column: 100kGy.

Pharmacokinetic modeling: fitting for the Korsmeyer-Peppas model

Table S1. Korsmeyer-Peppas fitting of the cumulative drug mass release profiles. Release rate constant, K, release exponent, n, and R^2 value.

Composition	Irradiation dose (kGy)	K	n	R^2
GS loaded UHMWPE	0	2.05	0.11	0.977
	25	1.20	0.12	0.970
	100	2.32	0.10	0.969
VC loaded UHMWPE	0	1.66	0.08	0.995
	25	1.78	0.07	0.995
	100	2.21	0.08	0.988

Antibacterial efficacy of the antibiotic loaded UHMWPE (1)

Plating results for the sonicate

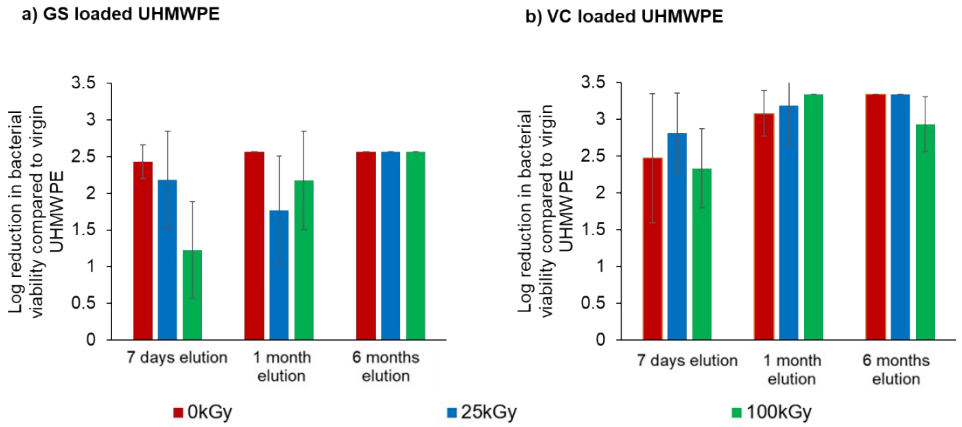

Fig. S5. Antibacterial activity of the antibiotic loaded UHMWPE after drug elution for various durations (7 days, 1 month, 6 months), as measured by plating after sonication of the material. The log reduction in bacterial viability compared to virgin UHMWPE is shown for a) GS loaded UHMWPE and b) VC loaded UHMWPE at various irradiation doses (0kGy: dark red, 25kGy: dark blue, and 100kGy: green).

Morphology of the irradiated antibiotic loaded UHMWPEs after elution and after 24h-incubation with *S. aureus* by SEM

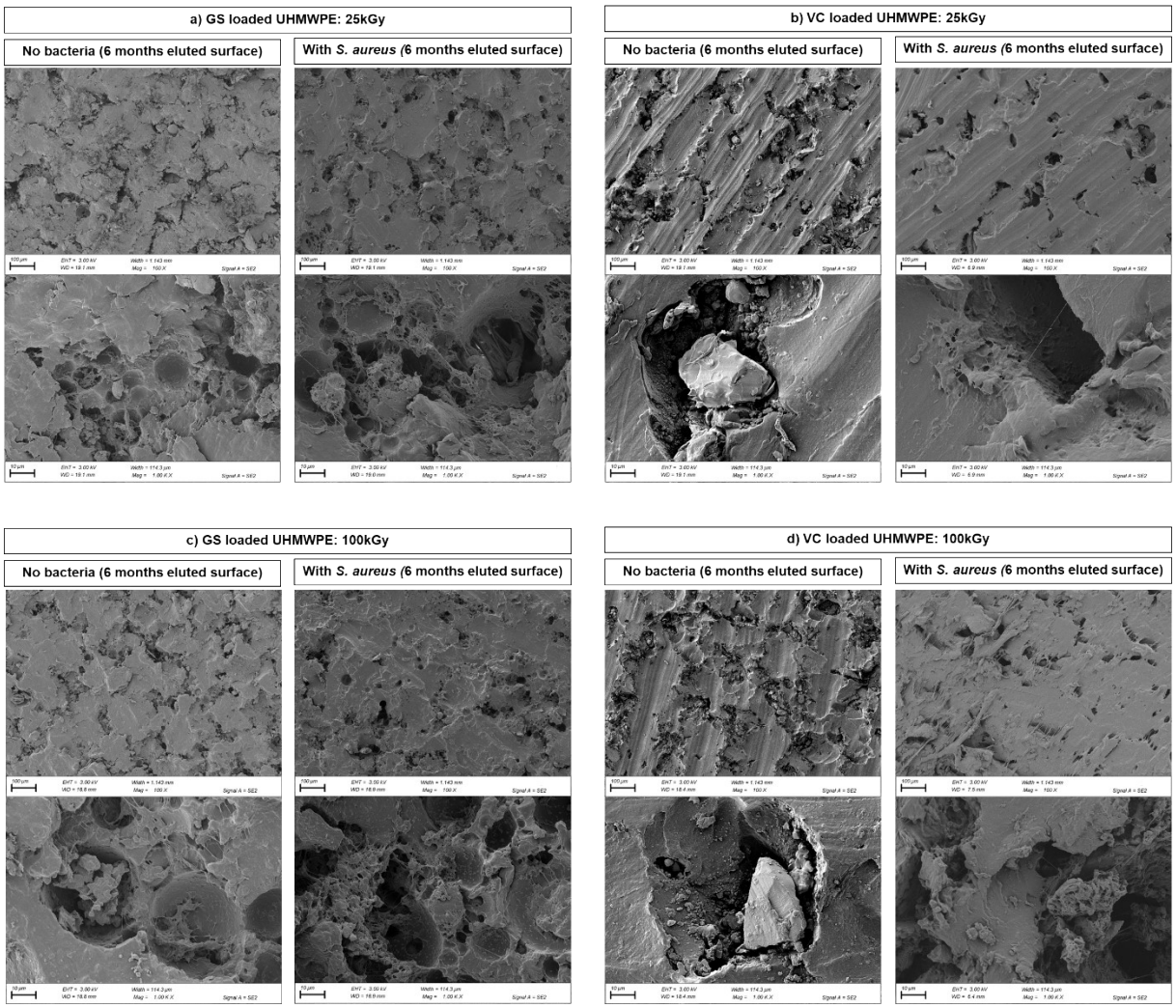

Fig. S6. Comparison of the morphology of the irradiated antibiotic loaded UHMWPE surfaces by SEM after drug elution and after 24h-incubation with *S. aureus*. The morphology of the surface of the antibiotic loaded UHMWPEs is shown after 6 month-elution and after 24h-incubation of the eluted surfaces with *S. aureus* for a) GS loaded UHMWPE - radiation dose: 25kGy; for c) GS loaded UHMWPE - radiation dose: 100kGy; for b) VC loaded UHMWPE radiation dose: 25kGy; for d) VC loaded UHMWPE – radiation dose: 100kGy. Scale bar in the top rows: 100 µm; scale bar in the bottom rows: 10 µm.

No bacteria colonies are visible on any of surfaces of the antibiotic loaded UHMWPEs which were eluted for 6 months and incubated for 24h with *S. aureus*.

Antibacterial efficacy of the antibiotic loaded UHMWPE (2)

Antibacterial efficacy of the eluted drugs by optical density measurements

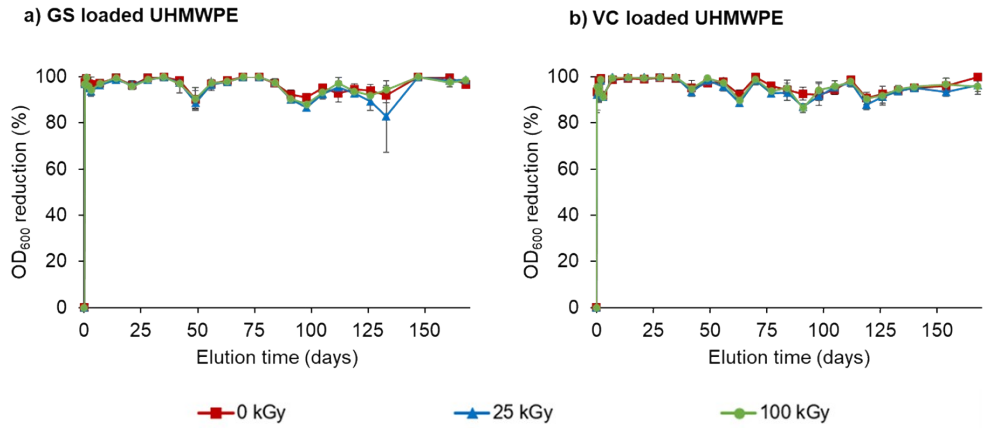

Fig. S7. Antibacterial activity of the drug eluent at various elution timepoints up to 6 months, as measured by optical density (OD_{600}) readings. The percentage reduction with respect to the positive control is shown for the drugs eluting from a) GS loaded UHMWPE and b) VC loaded UHMWPE at various irradiation doses (0kGy: dark red square, 25kGy: dark blue triangle, 100kGy: green circle).