1 Supplementary materials

Silica-Gentamicin Nanohybrids: Combating Antibiotic Resistance, Bacterial Biofilms, and *In Vivo* Toxicity

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36 Materials and methods

Semi-quantitative toxicity profile of zebrafish embryos/larvae exposed to SiO2-G nanohybrids

39 Average semi-quantitative toxicity profiles were developed by using the scoring spectrum (1, 2, and 3), 40 which defines the various degrees of malformations of embryos/larvae exposed to the SiO2-G nanohybrids (500 and 1000 µg/mL). Scoring spectrum values were determined through dividing the 41 number of embryos/larvae developing a specific score at each time point [24 hours post fertilization 42 (hpf), 48 hpf, 72 hpf, 96 hpf, and 120 hpf] by the total number of malformed embryos/larvae in the group 43 44 × 100. The score values were then accumulated for all the time points (representing different 45 developmental stages), yielding an average semi-quantitative cumulative toxicity profile for each exposure group. 46

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48 **Results**



51 **Figure S1.** Histograms of the size distribution of the materials.

52 Notes: (A) Pristine SiO₂ NPs and (B) SiO₂-G nanohybrids analyzed on the obtained TEM, and SEM

and TEM images, respectively. The number of particles analyzed for each histogram is 35.

54 **Abbreviations:** SiO₂ NPs, silica nanoparticles; SiO₂-G, silica-gentamicin; TEM, transmission electron

55 microscope; SEM, scanning electron microscope.

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57 ATR-FTIR spectra of Pristine SiO₂ NPs, pristine gentamicin, and SiO₂-G 58 nanohybrids

FTIR is a technique for identifying drugs.¹ Figure S2 shows the FTIR spectra of pristine SiO₂ NPs, 59 pristine gentamicin, and SiO₂-G nanohybrids. The pristine SiO₂ NPs demonstrated peaks at 1628, 60 representing the deformation vibration of adsorbed water molecules,² and a peak at 1460, representing 61 nanosilica.³ A sharp peak at 1049 cm⁻¹ was also demonstrated, representing the asymmetric Si–O–Si 62 stretching. More peaks were also recorded at 955, representing the Si-OH stretching, and at 791 and 63 552 cm⁻¹, representing the Si-O-Si stretching.⁴⁻⁶ The pristine gentamicin demonstrated two bands at 64 1525 and 1620 cm⁻¹, representing the bending vibrations of N–H (1650 to 1400 cm⁻¹), and a sharp peak 65 at 1034 cm⁻¹, representing the C-N and C-O stretching vibrations (1300 to 900 cm⁻¹).⁷ A peak at 606 66 cm⁻¹ was also recorded, representing a major band for gentamicin.¹ The spectrum of SiO₂-gentamicin 67 68 nanohybrids demonstrated peaks at 1630, representing both the loaded gentamicin and adsorbed water, and 1459, representing nanosilica. A peak at 1045 was recorded representing the same stretching of 69 70 both the asymmetric Si–O–Si of the SiO₂, and the C-N and C-O stretching of gentamicin. Furthermore, 71 a new small peak was also recorded at 666 cm⁻¹, representing the successful loading of gentamicin. 72 The bands of gentamicin were mostly shifted to higher frequencies after the loading, whereas the bands 73 of SiO₂ were mostly shifted to lower frequencies.

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76 Figure S2. ATR-FTIR spectra of the materials

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Notes: (A and B) The determination of the MBC of the SiO₂-G nanohybrids and G against planktonic
 MRSA cells, performed in duplicate on different days. Black lines separate the materials (SiO₂-G and

6) tested. The dashed violet circle (B, pristine gentamicin at 250 μ g/mL) shows a sum of 19 colonies, which is less than the rejection values described by the CLSI.⁸ The MBCs were determined as 500 and

 $125 \,\mu$ g/mL for the SiO₂-G nanohybrids and pristine gentamicin, respectively.

Abbreviations: SiO₂-G, silica-gentamicin; G, pristine gentamicin. MRSA, Methicillin-resistant
 Staphylococcus aureus; CLSI, Clinical and Laboratory Standards Institute; MBCs, Minimum
 Bactericidal Concentrations.

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- 90 Table S1. Log₁₀ numbers of the viable cells (CFU/peg) of MRSA and *E. coli* cells in the biofilms on the
- 91 Calgary biofilm device (CBD) pegs

	Log₁₀ CFU/peg													
	MRSA biofilms							<i>E. coli</i> biofilms						
			SiO ₂ -G		G				SiO ₂ -G		G			
Concentration (µg/mL)	0	500	1000	2000	125	250	500	0	250	500	1000	7.8	15.6	31.3
Mean	8.17	7.86	7.93	8	8	7.97	8.01	7.26	-	-	-	0.85	0.85	-
SD	0.03	0.23	0.04	0.22	0.43	0.39	0.33	0.03	-	-	-	1.21	1.21	-

92 **Abbreviations:** SiO₂-G, silica-gentamicin; G, pristine gentamicin.



- **Figure S4.** SEM images of the negative control pegs, broken from the CBD.
- 96 Notes: Magnifications of 1 000x (A), 5 000x (B), and 10 000x (C).
- **Abbreviations:** SEM, scanning electron microscope; CBD, Calgary biofilm device.



- **Figure S5.** SEM images of MRSA (A) and *E. coli* (B) biofilms formed on the CBD pegs.
- **Note:** Magnification, 1 000×.
- Abbreviations: SEM, scanning electron microscope; MRSA, Methicillin-resistant *Staphylococcus aureus*; *E. coli, Escherichia coli*; CBD, Calgary biofilm device.

105	Table S2.	Cardiac rates o	f zebrafish	embryos/larvae	exposed to th	e SiO ₂ -G nai	nohybrids

	Cardiac rates (beats/min) Exposure							
	C	SiO ₂ -G	SiO ₂ -G					
Developmental stage		500 µg/mL	1000 µg/mL					
24 hpf	157 ± 35	178 ± 18	173 ± 25					
48 hpf	178 ± 4	195 ± 42	180 ± 42					
72 hpf	170	181 ± 1	185 ± 28					
96 hpf	165 ± 49	203 ± 3	205 ± 7					
120 hpf	175 ± 64	172 ± 3	193 ± 11					

Abbreviations: C, no exposure; SiO₂-G, silica-gentamicin.



Figure S6. Normal and malformed zebrafish embryos/larvae.

Notes: (A) Microscopic images (3x objective) of normal zebrafish embryos in the control group at 4 hpf and (B) malformed larva in the control group, showing PE, at 48 hpf (B). Representative images of malformed larvae treated with 500 μ g/mL (C) and 1000 μ g/mL (D) of the SiO₂-G nanohybrids, showing a SD, reaching almost 90° flexion; and a BT, respectively, at 72 hpf. Malformed larva treated with the

SiO₂-G nanohybrids (1000 μ g/mL), showing PE and a YND-type of malformation, at 96 hpf (E).

Abbreviations: PE, pericardial edema; SiO₂-G, silica-gentamicin; SD, spinal deformity, BT, broken tail,
 BT; YND, yolk not depleted.

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Table S3. Scoring spectrum values developed by zebrafish embryos/larvae exposed to the SiO₂-G
 nanohybrids (500 and 1000 μg/mL) at different time points and the cumulative values

		SiO ₂ -G		SiO ₂ -G				
		500 µg/mL		1000 μg/mL				
Developmental stage	Score: 1	Score: 2	Score: 3	Score: 1	Score: 2	Score: 3		
24 hpf	25 ± 35.4	0	0	26.7 ± 9.4	0	0		
48 hpf	25 ± 35.4	29.2 ± 5.9	29.2 ± 5.9	16.7 ± 23.5	26.7 ± 9.4	10 ± 14.1		
72 hpf	25 ± 35.4	33.4 ± 37.2	41.7 ± 11.9	26.7 ± 9.4	36.7 ± 4.7	10 ± 14.1		
96 hpf	25 ± 35.4	33.4 ± 47.2	41.7 ± 11.8	26.7 ± 9.4	36.7 ± 4.7	20 ± 28.3		
120 hpf	25 ± 35.4	33.4 ± 47.2	41.7 ± 11.8	43.4 ± 33	36.7 ± 4.7	20 ± 28.3		
Cumulative value	25	25.9 ± 14.6	30.9 ± 18.1	28 ± 9.6	27.4 ± 15.9	12 ± 8.4		

120 **Abbreviation:** SiO₂-G, silica-gentamicin.

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Figure S7. The average cumulative semi-quantitative toxicity profiles of zebrafish embryos/larvae exposed to the SiO₂-G nanohybrids (500 and 1000 μ g/mL).

125 **Abbreviation:** SiO₂-G, silica-gentamicin.





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129 Notes: SiO₂-G nanohybrids (500 and 1000 µg/mL) induced frequencies of common malformations. The

error bars represent the standard errors of the means of two separate experiments, using 30 embryosin each group.

- 132 **Abbreviations:** SiO₂-G, silica-gentamicin; C, control group.
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