SUPPLEMENTARY INFORMATION for:

Designing Ga(III)-Containing Hydroxyapatite with Antibacterial Activity

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Experimental details

^{1}H magic-angle spinning (MAS) NMR spectra were recorded on a 600 MHz (14.1 T) Varian NMR system using a 3.2 mm Varian HX MAS probe. A ^{1}H Larmor frequency of 599.54 MHz was reported relative to the proton signal of tetramethlysilane. A rotation-synchronized Hahn-echo pulse sequence was used with 90° and 180° pulse widths of 2.3 µs and 4.6 µs, respectively. The sample rotation frequency was 20 kHz, the repetition delay between consecutive scans was 30 s, and the number of scans was 4.

HAp(Ga)IE with 6 wt % was prepared in a similar way as other HAp(Ga)IE materials, but using a 0.85 mM Ga^{3+} concentration and 0.0844 Ga/Ca ratio.

Amorphous gallium phosphate hydrate was prepared by mixing $Ga(NO_3)_3$ with disodium phosphate buffer.[1] 1 phosphate buffered saline tablet was dissolved in 200 mL of water and 25 mL of the resulting solution were mixed with 25 mg of $Ga(NO_3)_3 \cdot xH_2O$ for 1 day at 37 °C. The XRD pattern of the washed precipitate confirmed its amorphous nature and was very similar to the XRD pattern of the amorphous gallium phosphate as determined by Mellier *et al*.[1]

Antibacterial tests and cytotoxicity tests, presented here, were done in the same way as the tests presented in the article, except that in case of gallium nitrate we were dealing with solutions instead of suspensions.

The material with mixed octacalcium phosphate (OCP) and hydroxyapatite phase was prepared in the same way as the hydroxyapatite reference but using two times higher concentrations of all the reagents. An aqueous suspension of this OCP/HAp (which was washed but not dried after the synthesis and re-suspended in water) was then mixed with an aqueous solution of $Ga(NO_3)_3$ (to obtain 1.4 mM Ga^{3+} and 0.139 Ga/Ca ratio) for 24 hours.

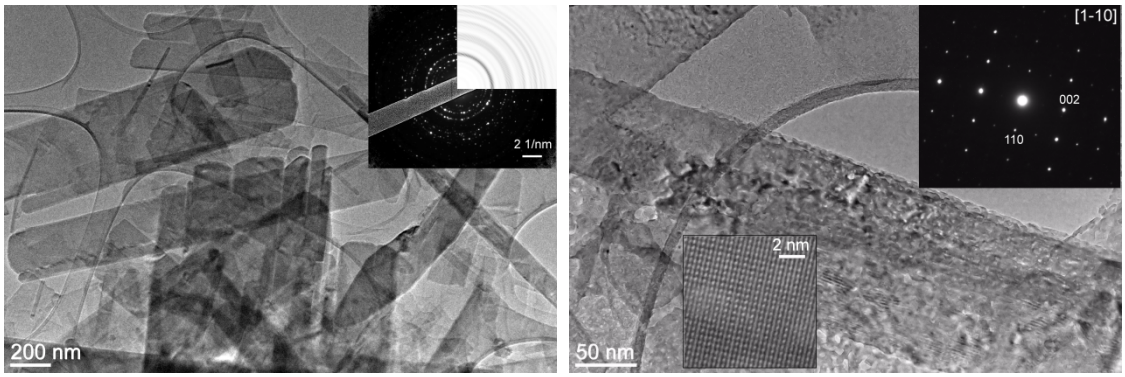

Figure S1: Structural characteristics of the hydroxyapatite reference. TEM images with SAED (ring pattern and simulated pattern on the left and spot pattern on the right) and high magnification (right) insets.

Table S1. Crystallinity determined from the XRD patterns.

sample	X_c [%][2]	Crystallinity index[3]	% crystallinity
HAp	60	0.8	24
HAp(Ga)TR	58	0.8	22
HAp(Ga)IE16	62	1.0	18

The degree of crystallinity X_c was determined from the height of the (300) peak and the valley between the (300) and (112) peaks,[2] while the crystallinity index was determined from the heights of the peaks (211), (300), (112) and (202), and the valleys in between.[3] Both quantities are roughly the same for HAp, HAp(Ga)TR and HAp(Ga)IE. By contrast, the percentage of crystallinity, determined from the ratio of the crystalline peak areas and the whole area between 25° and 36° (rather than the amorphous hump at 31°),[4] exhibits a decreasing trend in the order HAp, HAp(Ga)TR, HAp(Ga)IE.

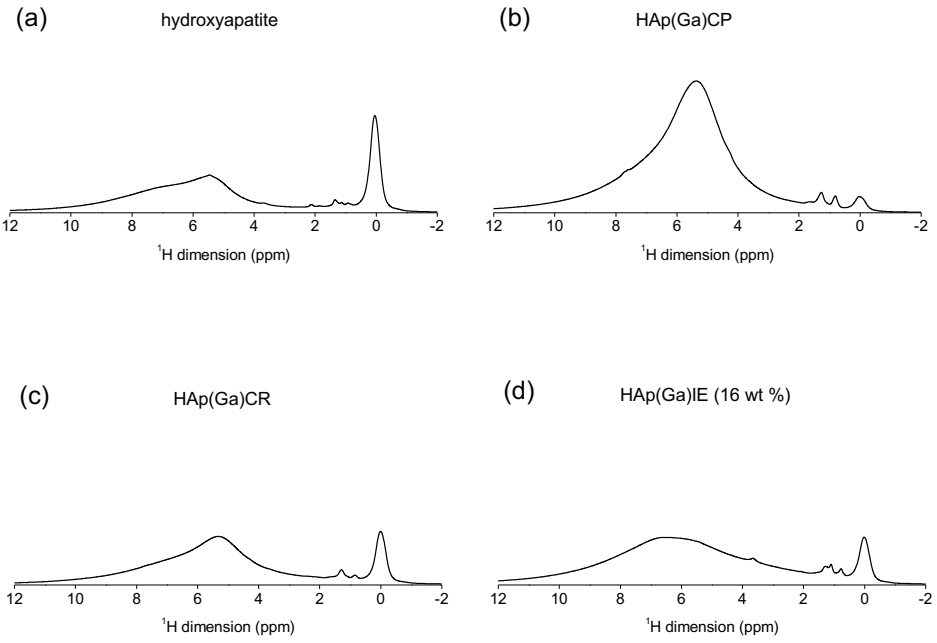

Figure S2: ^{1}H MAS NMR spectra of (a) HAp and HAp(Ga): (b) co-precipitation, (c) transformation, (d) ion exchange.

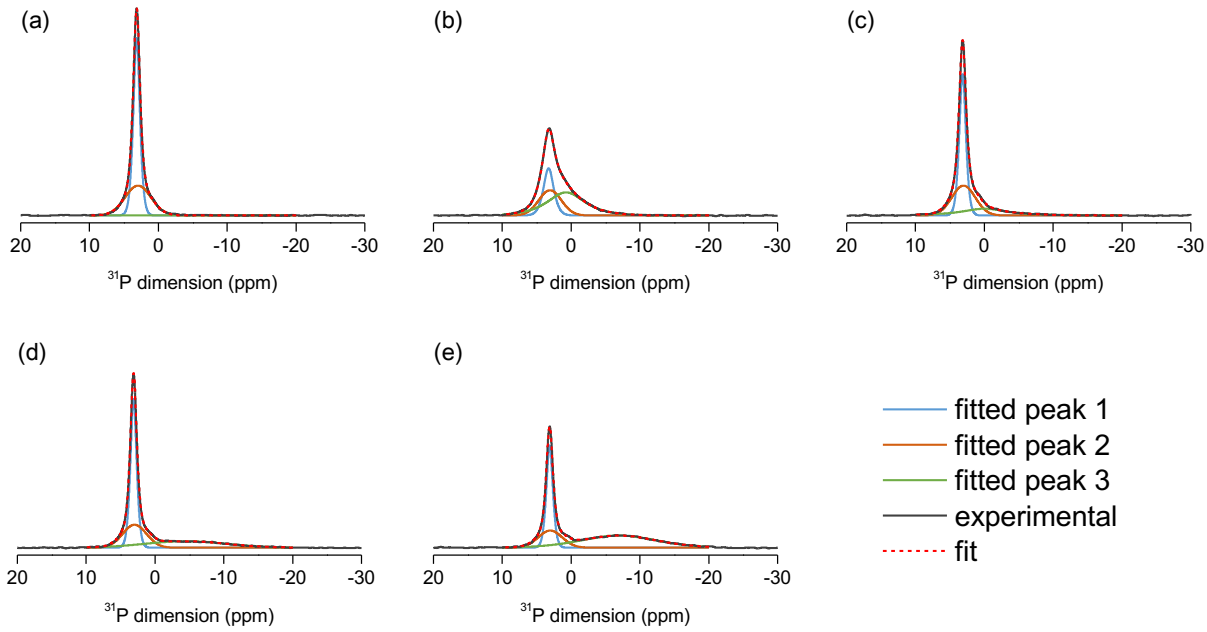

Figure S3: ^{31}P MAS NMR spectra with fitted Voigt profiles. (a) HAp, (b) HAp(Ga) by co-precipitation, (c) HAp(Ga) by transformation, (d) HAp(Ga) by ion exchange (8 wt % Ga), (e) HAp(Ga) by ion exchange (16 wt % Ga).

Table S2. Peaks determined by fitting the ^{31}P NMR spectra.

	Peak 1			Peak 2			Peak 3		
sample	Position [ppm]	Area [%]	FWHM [ppm]	Position [ppm]	Area [%]	FWHM [ppm]	Position [ppm]	Area [%]	FWHM [ppm]
HAp	3.2	61.3	1.1	3.0	38.7	4.5	/	/	/
HAp(Ga)CP	3.3	24.8	1.8	3.1	27.9	3.9	0.73	47.3	6.6
HAp(Ga)TR	3.2	47.8	1.1	3.0	34.1	4.0	-0.036	18.1	7.8
HAp(Ga)IE8	3.1	47.5	1.1	3.0	26.7	4.1	-4.4	25.8	14
HAp(Ga)IE16	3.1	33.7	1.1	3.0	20.1	4.1	-6.9	46.2	13

From the values in the table, the ratio of fractions of the phosphori in the amorphous surface layer (by summing the areas of peak 2+peak3) can be calculated. This yields
HAp(Ga)CP:HAp(Ga)TR:HAp(Ga)IE8:HAp(Ga)IE16 = 1.44:1:1.01:1.27.
By considering also the total Ga(III) amounts in the samples (HAp(Ga)CP:HAp(Ga)TR:HAp(Ga)IE8:HAp(Ga)IE16 = 3:4:8:16) we can calculate the relative fraction of Ga(III) in the amorphous surface layer (Ga/P(amorf)).
HAp(Ga)CP:HAp(Ga)TR:HAp(Ga)IE8:HAp(Ga)IE16 = 1:1.92:3.82:6.05
It is clearly seen that the Ga(III) content in the amorphous surface layer is increasing in the order HAp(Ga)CP<HAp(Ga)TR<HAp(Ga)IE8<HAp(Ga)IE16, which is the same as the order of increasing antibacterial action (MIC) and cytotoxicity.
Furthermore, from the fraction of the gallium-affected phosphori (peak 3) and the overall Ga(III) content we can estimate the composition of the amorphous surface layer and interpret the shifting of the third peak. If we assume an OCP-like Ca/P (8:6) ratio and that peak 3 for the HAp(Ga)IE16 sample corresponds to Ga/P=1 (as in $GaPO_4$), we get a composition of roughly 1 Ga and 7 Ca among 6 P in the amorphous surface layer of the HAp(Ga)CP, 4 Ca and 4 Ga in the amorphous surface layer of the HAp(Ga)TR, 3 Ca and 5 Ga for the HAp(Ga)IE8 and 2 Ca and 6 Ga for the HAp(Ga)IE16 sample. As the composition changes from mainly calcium to mainly gallium phosphate the third peak is shifted towards lower frequencies (upfield).

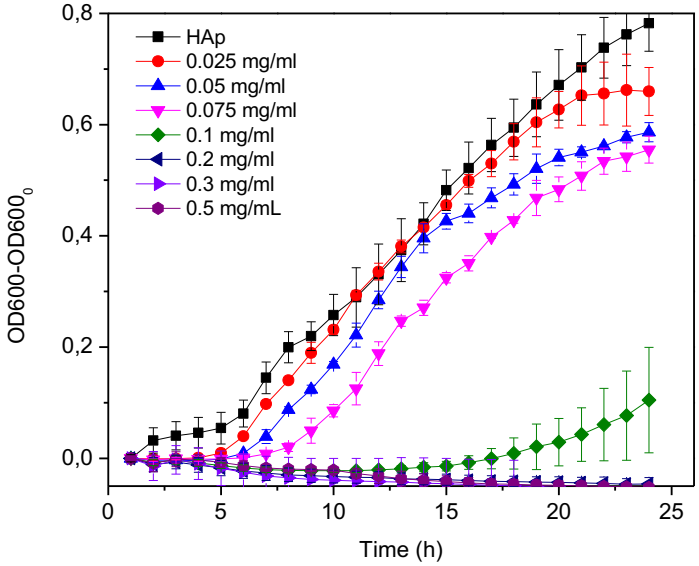

Figure S4: Microdilution antibiogram for the HAp(Ga)IE sample that contains 6 wt % of Ga.

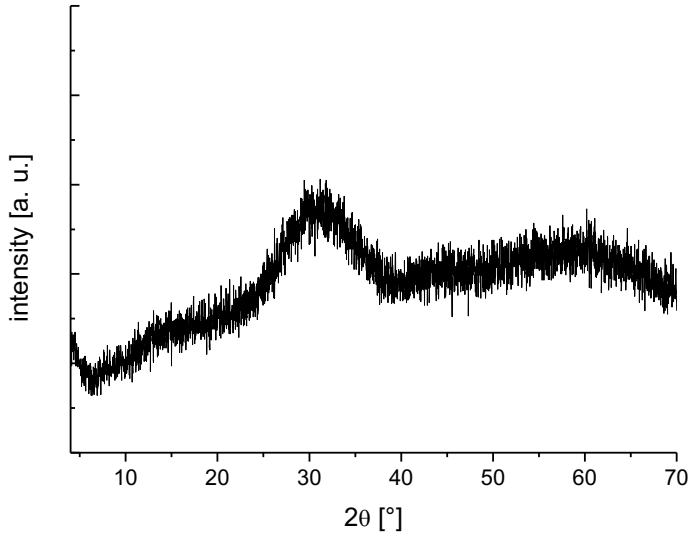

Figure S5: X-ray diffraction pattern of the amorphous gallium phosphate hydrate.

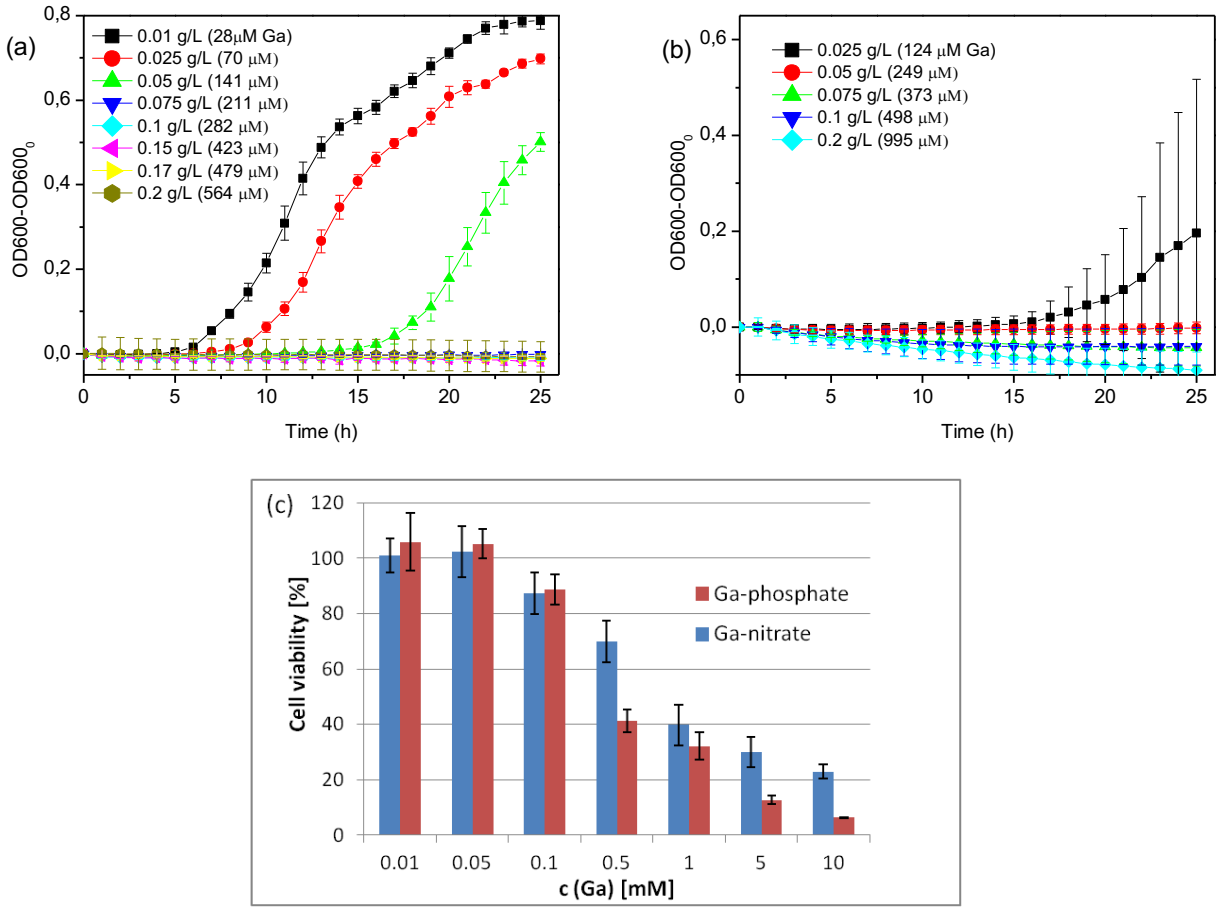

Figure S6: Antibacterial activity of (a) $Ga(NO_3)_3$ and (b) amorphous $GaPO_4$ hydrate against *P. aeruginosa* MW1; (c) cytotoxicity of $Ga(NO_3)_3$ and amorphous $GaPO_4$ hydrate against L929 mouse fibroblasts.

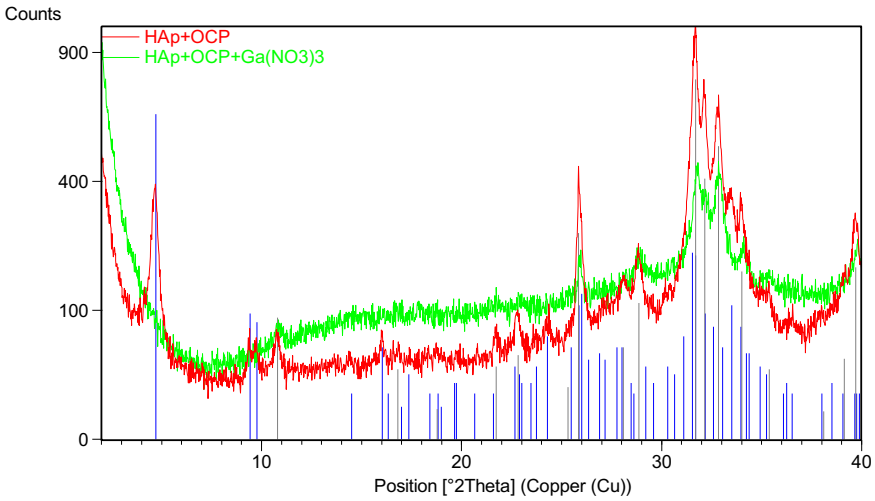

Figure S7: Disappearance of the OCP phase after mixing with $Ga(NO_3)_3$. Blue lines represent OCP reference pattern (PDF number 00-026-1056) and grey lines represent HAp reference pattern (01-089-6438).

References

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