

## Electronic Supplementary Information

### Hybrid particles derived from alendronate and bioactive glass for treatment of osteoporotic bone defects

Mani Diba<sup>1,‡</sup>, Winston A. Camargo<sup>1,†</sup>, Tatiana Zinkevich<sup>2,†</sup>, Alina Grünewald<sup>3</sup>, Rainer Detsch<sup>3</sup>, Yoones Kabiri<sup>4</sup>, Arno P.M. Kentgens<sup>2</sup>, Aldo R. Boccaccini<sup>3</sup>, Jeroen J.J.P. van den Beucken<sup>1</sup> and Sander C.G. Leeuwenburgh<sup>1,\*</sup>

<sup>1</sup>Department of Regenerative Biomaterials, Radboud University Medical Center, Philips van Leydenlaan 25, 6525 EX, Nijmegen, The Netherlands

<sup>2</sup>Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

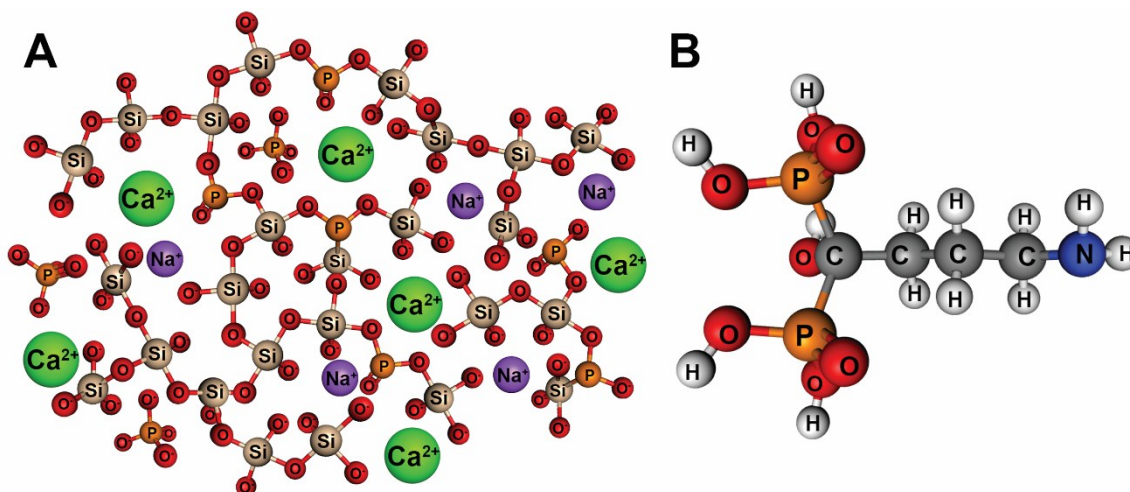
<sup>3</sup>Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Cauerstraße 6, 91058 Erlangen, Germany

<sup>4</sup>Kavli Institute of Nanoscience Delft, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

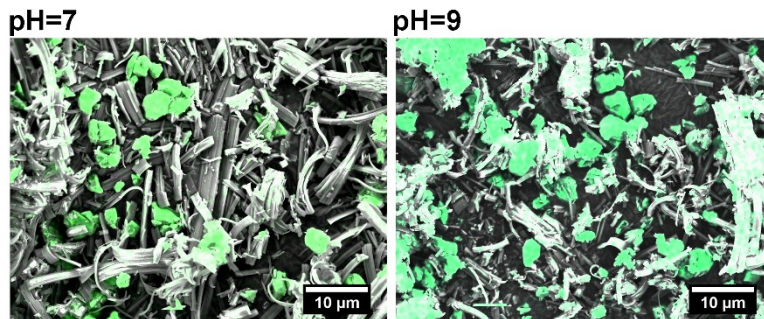
<sup>‡</sup>Current address: Institute for Complex Molecular Systems, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, The Netherlands

<sup>†</sup>These authors contributed equally to this work.

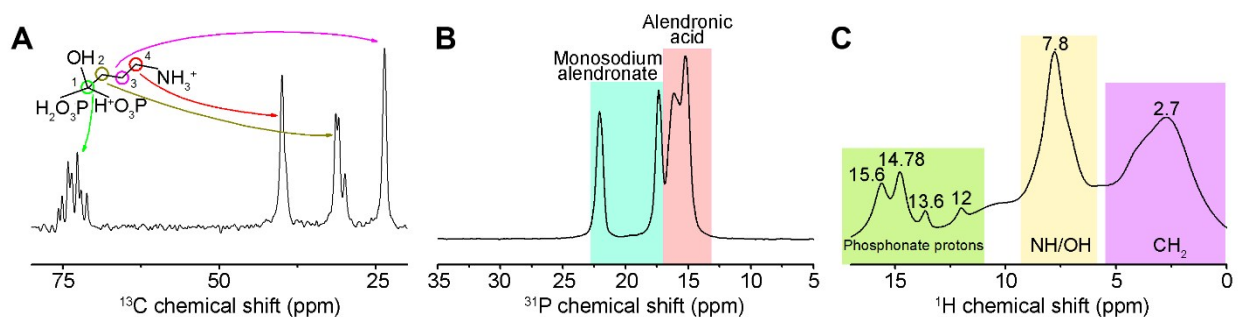
\*Corresponding author. Email address: [Sander.Leeuwenburgh@radboudumc.nl](mailto:Sander.Leeuwenburgh@radboudumc.nl)



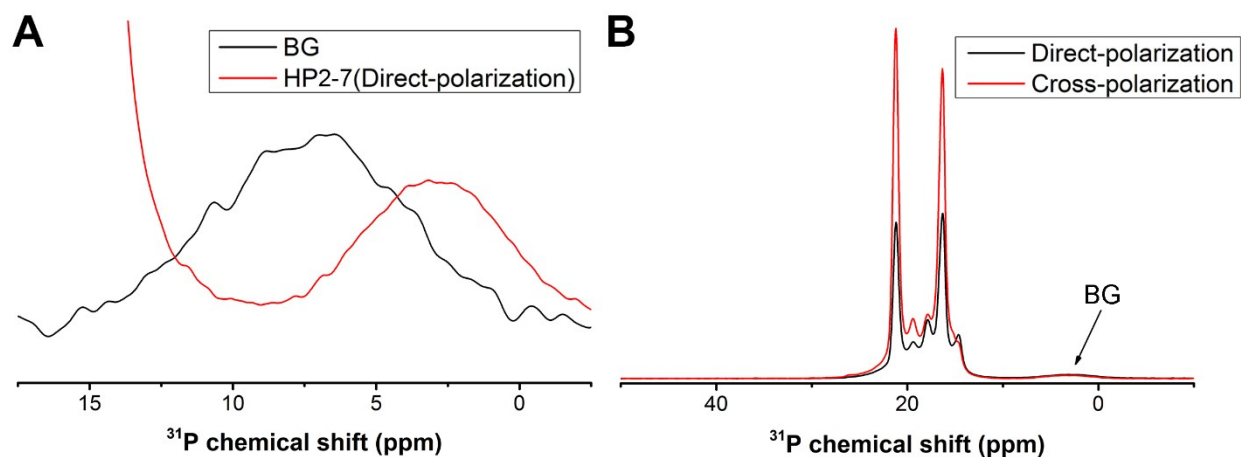
**Figure S1.** Schematic illustrations of the chemical structures of A) bioactive glass and B) alendronic acid.



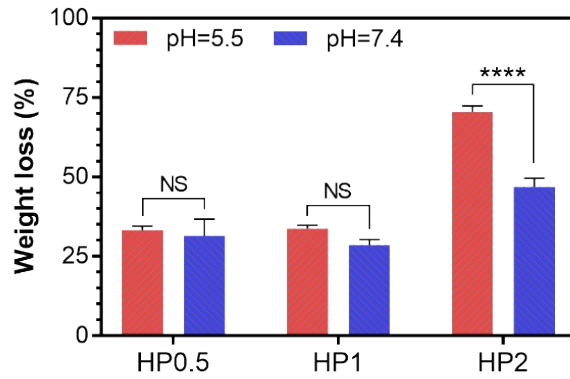
**Figure S2.** SEM micrographs with overlaid EDX maps of HP2-7 and HP2-9 particles. The green color corresponds to the distribution of silicon.



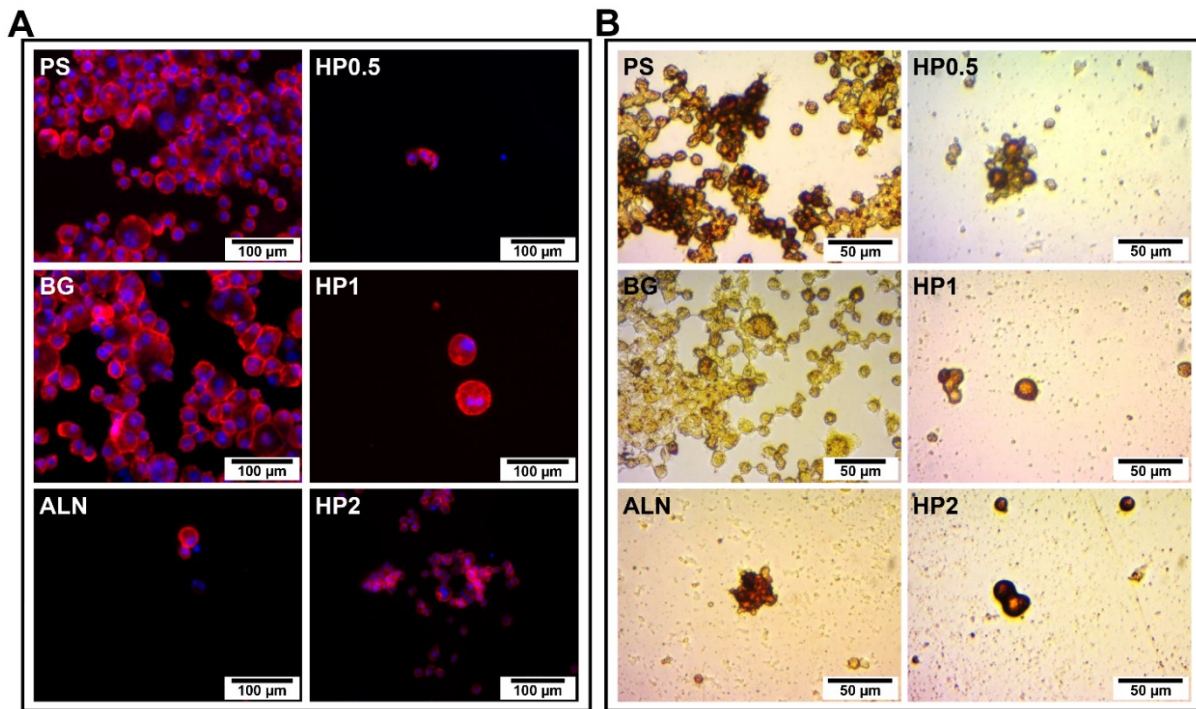
**Figure S3.** A)  $^{13}\text{C}$ , B)  $^{31}\text{P}$  and C)  $^1\text{H}$  solid-state NMR spectra of pure ALN powder.



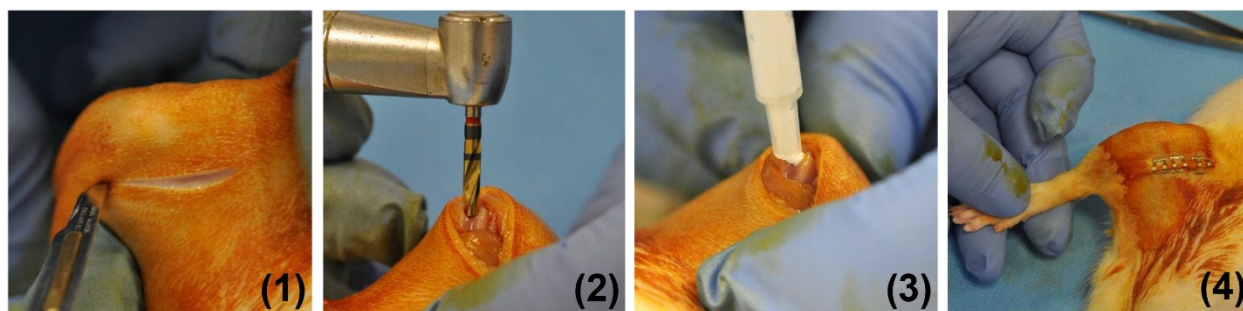
**Figure S4.** A) Zoomed-in region of the 45S5 BG and HP2-7  $^{31}\text{P}$  solid-state NMR spectra. B) Direct- and cross-polarization  $^{31}\text{P}$  solid-state NMR spectra of HP2-7 particles.



**Figure S5.** Weight loss (%) of hybrid particles after 14 days of immersion in solutions with acidic or neutral pH. NS and \*\*\*\* indicate no statistical difference and statistical difference with  $P < 0.0001$ , respectively. All values are presented as average  $\pm$  standard deviation for  $n=3$  per experimental condition.



**Figure S6.** *In vitro* effect of hybrid particles on osteoclastic cells. Representative microscopy images showing A) actin/nucleus or B) TRAP stained cells upon incubation with different materials. All measurements were performed at a particle concentration of 100  $\mu\text{g/mL}$ . PS refers to particle-free polystyrene controls.



**Figure S7.** Digital photographs showing the surgical procedure for implantation of pastes consisting of hybrid particles dispersed in a hyaluronic acid carrier into femoral condyles of osteoporotic rats.

**Table S1.** Randomized scheme for the implantation of the particle pastes.

<b>Rat No.</b>	<b>Left femur</b>	<b>Right femur</b>
<b>1</b>	45S5 BG	HP1-7
<b>2</b>	HP2-7	45S5 BG
<b>3</b>	45S5 BG	HP1-7
<b>4</b>	HP1-7	45S5 BG
<b>5</b>	HP2-7	HP1-7
<b>6</b>	HP1-7	45S5 BG
<b>7</b>	HP1-7	HP2-7
<b>8</b>	45S5 BG	HP2-7
<b>9</b>	HP2-7	HP1-7
<b>10</b>	45S5 BG	HP2-7
<b>11</b>	HP1-7	HP2-7
<b>12</b>	HP2-7	45S5 BG

**Table S2.** Chemical composition of hybrid particles precipitated at different conditions after 3 days of incubation at 37 °C.

Sample abbreviation	Immersion conditions		Final composition (wt%)				
	Ca <sub>BG</sub> /ALN ratio	pH	ALN	Ca	Na	Si	P*
HP0.5-7	0.5	7	69.3±0.5	11.5±0.0	11.9±0.0	<2	<1
HP1-7	1	7	62.3±0.6	11.4±0.0	12.8±0.0	<2	<1
HP2-7	2	7	25.5±9.8	16.7±0.3	34.7±0.0	7.3±0.3	9.9±0.2
HP0.5-9	0.5	9	68.6±0.2	10.5±0.0	10.7±0.0	<2	<1
HP1-9	1	9	55.6±2.5	10.1±0.3	10.8±0.0	<2	<1
HP2-9	2	9	25.6±4.4	11.3±0.4	20.5±0.0	5.8±0.3	4.3±0.2

\* Derived from bioactive glass

**Table S3.** Solid-state DP <sup>31</sup>P NMR chemical shifts of phosphate and phosphonate peaks during the formation of HP1-7 particles.

Time (min)	Phosphate chemical shift (ppm)	Phosphonate chemical shift (ppm)
5	7.7	21.6
50	7.7	20.8
100	6.6	20.4
200	4.2	18.4
1000	Not observable anymore	18.2
4320	Not observable anymore	17.8