Bionics synthesis of magnetic calcite skeletal structure through living foraminifera


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Supporting information
Experimental section

NPs screening
In order to identify the type of particles with the surface functionalization allowing the best occlusion into CaCO$_3$, in vitro precipitation of calcite crystals was performed. Superparamagnetic magnetite nanoparticles with a diameter of 100 nm labelled with a fluorescent dye functionalized with i) Polyethylene glycol (2000 Da) having as a functional group phosphate sodium salt (peg-P-nPs), ii) Polyethylene glycol (20000 Da) having amine as a functional group (peg-Am-nPs) and iii) dextran (mds-nPs) provided by Chemicell. 750 µL of a solution of nPs at the desired concentration in 10 mM CaCl$_2$ • 2H$_2$O (Fulka) were put in a well of a 24-well microplate covered with aluminium foil, one each well was performed a hole with a needles and it was put into a closed desiccator with a beaker containing 3,5 g of crushed (NH$_4$)$_2$CO$_3$ covered with parafilm with 10 holes on it and 16 g of anhydrous CaCl$_2$. After 4 days the wells were washed 3 times with DDW and 1 time with absolute ethanol. Peg-P-nPs and peg-Am-nPs were not stable at the highest concentration used.

Foraminifera culturing
Foraminifera (collected in Eilat-Israel on 7/7/16) with dimension between 250 µm and 350 µm were selected using sieves and A. lessoni specimens were selected. The dead control was prepared washing the forams 2 times with distilled water (DW) and 3 times with double distilled water (DDW), putting them in sodium hypoclorite (NaOCl) (prepared diluting the analytical grade one 1:5 NaOCl:DW) for at least 2 hours and drying them in the oven at 60°C. The solutions were prepared diluting the dyes directly in seawater to obtain calcein 40 µM and mds-nPs 0,01 mg/mL.

40 specimens were put in a jar and the following experiment were performed:

<table>
<thead>
<tr>
<th></th>
<th>Calcein</th>
<th>mds-nPs</th>
<th>Calcein/mds-nPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living specimen</td>
<td>40 specimens in 50 mL</td>
<td>40 specimens in 50 mL</td>
<td>40 specimens in 50 mL</td>
</tr>
<tr>
<td>Dead control</td>
<td>10 specimens in 1 mL</td>
<td>10 specimens in 1 mL</td>
<td>10 specimens in 1 mL</td>
</tr>
</tbody>
</table>

The samples were kept under a lamp with a temperature of 23,5 °C. After 2 days the samples treated with calcein were observed under a binocular and, since the last chamber of all the specimens was labelled, all the incubations were stopped.

Characterization
Optical images were acquired with a Leica light microscope equipped with a Moticam 5.0 camera. X-ray powder diffraction (XPRD) measurements were carried out using a PanAnalytical X'Pert Pro diffractometer equipped with X'Celerator detector with Cu Kα radiation (range 20°-60°, step size 0.05°, time per step 45 s). Scanning electron microscopy (SEM) images were collected with a Phenom G2 Pure when detecting backscattered electron without coating the samples and an Hitachi FEG 6400 microscope after coating the samples with 5 nm of gold. SEM imaging and EDS analysis were performed using Zeiss LEO 1530 on uncoated samples. SOLVER P47H Scanning Probe Microscope (NT-MDT, Zelenograd, Moscow, RU) was employed to acquire topographic images of the skeleton morphology and electrostatic images of mds-nPs embedded into the skeleton. Topographic images were acquired in Tapping Mode through NT-MDT NSG01 cantilevers (resonant frequency $\omega_0 \sim 150$ kHz, elastic constant $k \sim 5$ N/m), whereas
electrostatic images were acquired by second pass technique with NT-MDT NSG01 cantilevers ($\omega_0 \sim 140$ kHz, $k \sim 5$ N/m).
Structural and morphological characterization of the nanoparticles was performed using a 200 kV FEI Tecnai F20 ST Transmission Electron Microscope (TEM). Confocal microscope images were collected using an upright Olympus microscope equipped with laser 488 nm and 561 nm lasers and a spectral detector and analysed using the software imageJ, making a sum of the slides.
Dynamic Light Scattering measurements were performed with a Malvern Nano ZS instrument equipped with a 633 nm laser diode. Samples were diluted in water and then housed in disposable polystyrene cuvettes of 1 cm optical path length. The values were taken averaging three different runs to get the standard deviation.
The concentration of elements was determined by plasma spectrometer (ICP-OES Ametek Spectro, Arcos, Kleve, Germany).
**Supplementary data**

**Table SI1.** Nano-particle loading in synthetic calcite crystals determined by ICP-OAS.

<table>
<thead>
<tr>
<th></th>
<th>Ca (ppm)</th>
<th>Fe (ppm)</th>
<th>Fe loading (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>51.61</td>
<td>0.12</td>
<td>0.22%</td>
</tr>
<tr>
<td>0.1 mg/mL mds-nP</td>
<td>129.02</td>
<td>4.07</td>
<td>3.16%</td>
</tr>
<tr>
<td>0.1 mg/mL peg-P-nPs</td>
<td>180.04</td>
<td>3.41</td>
<td>1.89%</td>
</tr>
<tr>
<td>0.1 mg/mL peg-am-nPs</td>
<td>40.63</td>
<td>0.21</td>
<td>0.52%</td>
</tr>
</tbody>
</table>

**Figure SI1.** (a,b) TEM images of mds-nPs and (c) core size distribution (n=100).

**Figure SI2.** DLS measurement of the hydrodynamic radius of mds-nPs at a concentration of 0.01 mg/ml.
Figure SI3. XRPD pattern of samples prepared (a) without additives and in the presence of (b) 0.1 mg/mL mds-nPs, (c) 0.01 mg/mL mds-nPs, (d) 0.001 mg/mL mds-nPs, (e) 0.1 mg/mL peg-P-nPs, (f) 0.01 mg/mL peg-P-nPs, (g) 0.001 mg/mL peg-P-nPs, (h) 0.1 mg/mL peg-Am-nPs, (i) 0.01 mg/mL peg-Am-nPs and (l) 0.001 mg/mL peg-Am-nPs. Only the diffraction peaks of calcite (JCPDS: 00-005-0586) are present in all the patterns.
**Figure SI4.** SEM images of samples prepared (a) without additives and in the presence of (b) 0.1 mg/mL mds-nPs, (e) 0.01 mg/mL mds-nPs, (h) 0.001 mg/mL mds-nPs, (d) 0.1 mg/mL peg-P-nPs, (g) 0.01 mg/mL peg-P-nPs, (l) 0.001 mg/mL peg-P-nPs, (c) 0.1 mg/mL peg-Am-nPs, (f) 0.01 mg/mL peg-Am-nPs and (i) 0.001 mg/mL peg-Am-nPs. The scale bar is 40 µm in the main image and 10 µm in the inset.

**Figure SI5.** (a) Optical microscopy image of a crystal of calcite grown in the presence of 0.1 mg/mL mds-nPs. (b) The same image under crossed polars. The fact that all crystal is birefringent confirms that it is a single crystal. Scale bar: 40 µm.
Figure S16. Surface of a calcite crystal grown in the presence of 0.1 mg/mL of mds-nPs acquired detecting back-scattered electron. The presence of different shades of grey shows the distribution of calcium (darker grey) and iron (lighter grey) atoms.

Figure S17. Confocal fluorescence microscopy images of sections of a calcite crystal grown in the presence of 0.1 mg/mL mds-nPs. Scale bar: 50 µm.
Figure S18. Confocal fluorescence microscopy images of sections of a bleached and then broken calcite crystal grown in the presence of 0.1 mg/mL mds-nPs. Nanoparticles are homogeneously included inside the crystal. Scale bar: 40 µm.

Figure S19. Crystals prepared in the presence of 0.1 mg/mL mds-nPs respond to an external magnetic field.
Figure SI10. *A. lessoni* skeleton growth in the presence of calcein. The last grown chamber is labelled. It is visible mainly in the left lower part of the skeleton, but it extends along its periphery. Scale bar: 500 µm.

Figure SI11. (a) EDS graph acquired on a dead foraminifera skeleton first incubated in seawater containing nanoparticles and then bleached, no signal due to iron (6.4 KeV) was detected. (b) SEM image of the investigated skeleton, in read perimeter of the area analyzed.
Figure SI12. Optical microscope image of an *A. lessoni* specimen treated with calcein and mds-nPs. Darker spot due to the presence of mds-nPs in the last grown chamber, mainly localized in the periphery of the foraminifer.

Figure SI13. Confocal microscopy images of (a) shell of *A. lessoni* grown with mds-nPs and calcein, (b) shell of dead control. Scale bar: 40 µm.