BIOTECHNOLOGY and BIOENGINEERING

A synthetic biology approach for the fabrication of functional (fluorescent magnetic) bioorganic-inorganic hybrid materials in sponge primmorphs

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18 February 2020

S. Jenifer
29.02.2020

Cell Tissue Res (2010) 339:429–436
DOI 10.1007/s00441-009-0903-x

REGULAR ARTICLE

Silicatein-mediated incorporation of titanium into spicules from the demosponge *Suberites domuncula*

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Angewandte International Edition Chemie

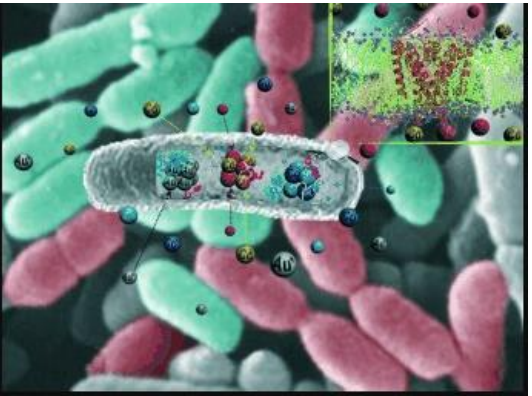
GDCh A Journal of the German Chemical Society

Communication

In Vivo Synthesis of Diverse Metal Nanoparticles by Recombinant *Escherichia coli*†

Dr. Tae Jung Park, Prof. Sang Yup Lee ✉, Nam Su Heo, Prof. Tae Seok Seo

First published:14 September 2010 | https://doi.org/10.1002/anie.201001524 | Citations: 85



A metal-nanoparticle factory: Various metal nanoparticles with tailored optical, electronic, chemical, and magnetic properties were synthesized in vivo in a size-tunable manner in recombinant *E. coli* (see picture) through interaction with the metal-binding protein metallothionein and the metal-binding peptide phytochelatin, which was synthesized by phytochelatin synthase.

Introduction

- **Synthetic biology** is a field of science that involves redesigning organisms for useful purposes by engineering them to have new abilities. **Synthetic biology** researchers and companies around the world are harnessing the power of nature to solve problems in medicine, manufacturing and agriculture.
- During evolution, **sponges** (Porifera) have honed the genetic toolbox and **biosynthetic** mechanisms for fabrication of siliceous skeletal components (spicules).
- Spicules carry **a protein scaffold** embedded within biogenic silica (biosilica) and feature an amazing range of optical, structural, and mechanical properties.
- In this synthetic biology approach, the uptake of multifunctional non-biogenic nanoparticles (fluorescent, superparamagnetic) by spicule-forming cells of bioreactor cultivated sponge primmorphs provides access to spiculogenesis.
- During spiculogenesis, the nanoparticles initially formed **an incomplete layer around juvenile**, intracellular spicules.
- In the mature, extracellular spicules the nanoparticles were densely arranged as a surface layer that rendered the resulting **composite fluorescent and magnetic property**.
- By branching off the conventional route of solid-state materials synthesis under harsh conditions, a new pathway has been opened to a versatile platform that allows adding functionalities to growing spicules as templates in living cells, using non-biogenic nanoscale building blocks with multiple functionalities.

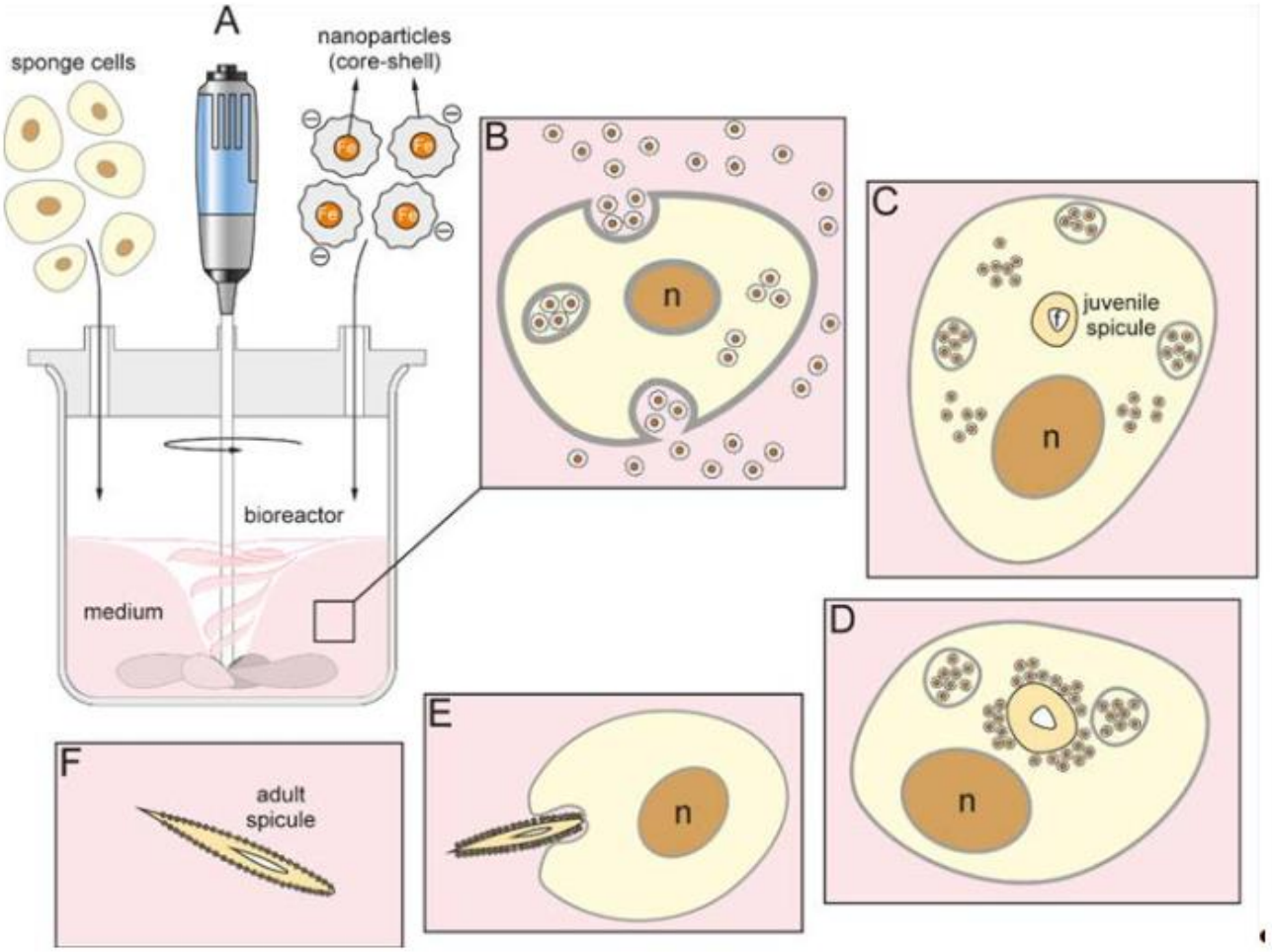


Fig. 1 Synthetic biology approach for the *in vivo* assembly of multifunctional bioorganicinorganic composite materials. (A) Bioreactor-based cultivation of *S. domuncula* primmorphs in the presence of non-biogenic ferrous core-shell nanoparticles (here, fluorescent, superparamagnetic). (B) Within minutes of exposure, the nanoparticles are taken up into sclerocytes (spicule-forming cells) and can be detected either as cytosolic clusters or within intracellular silicasome-like vesicles. (C) In the initial (intracellular) stage of spiculogenesis, the sclerocytes contain juvenile spicules (f, axial filament; n, cell nucleus). (D) Over several days, the ingested nanoparticles assemble around the juvenile spicule, forming an incomplete and inhomogeneous array that is not tightly attached to the spicule surface. (E) At the onset of the terminal (extracellular) stage of spiculogenesis, the coated spicules are released by evagination into the extracellular space. (F) In the adult and extracellular spicule the nanoparticles are arranged in a dense and compact layer, homogeneously surrounding the spicule. The resulting engineered and prototypic multi-component composite combines the multifunctional properties of spicules with those of the coating nanoparticle building blocks (here, fluorescence and magnetism). Scheme not drawn to scale.

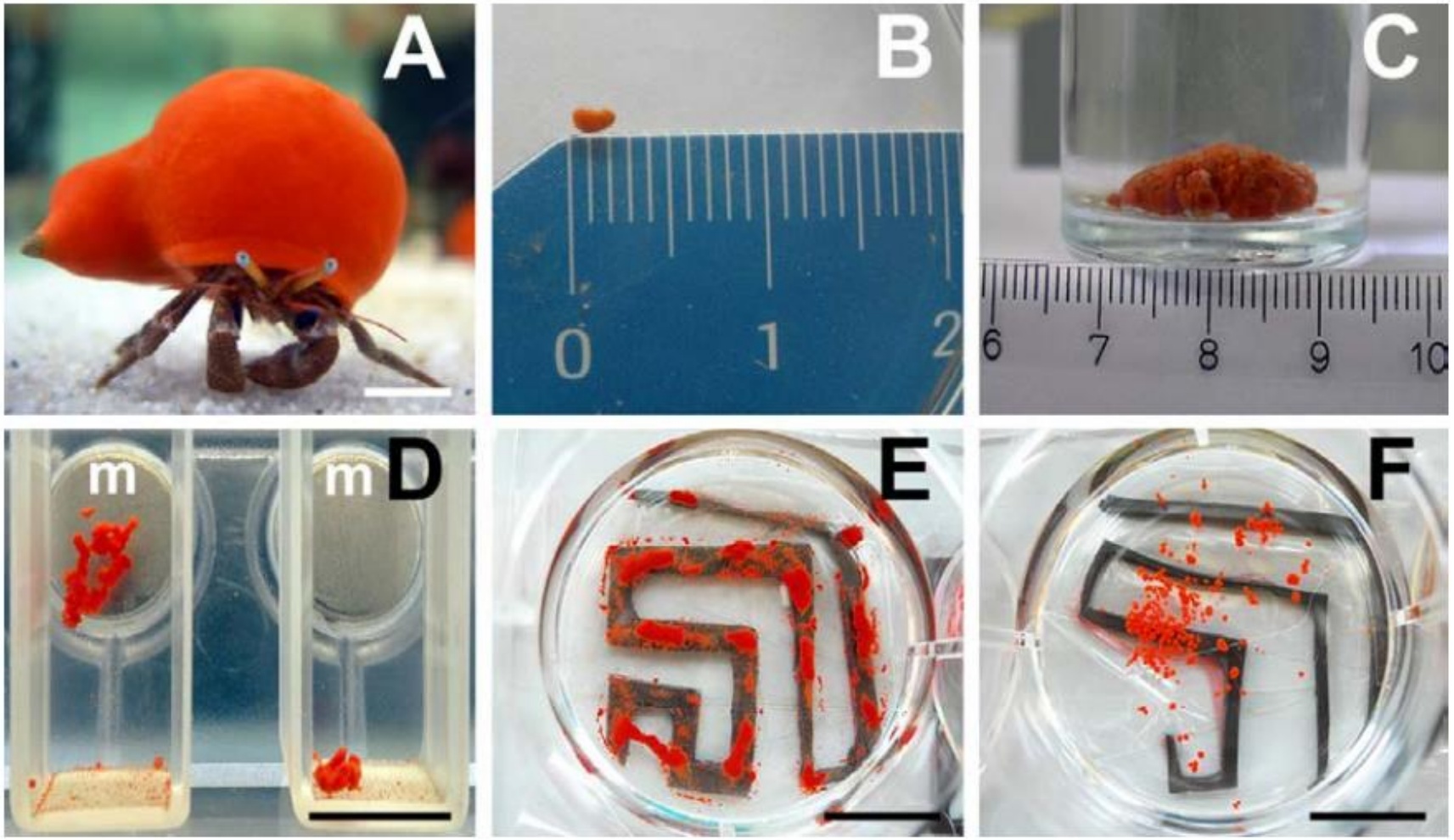


Fig. 2 *Suberites domuncula* conventional primmorphs and primmorphs with magnetic properties. (A) The marine demosponge *S. domuncula* (orange-red) usually grows on gastropod shells in symbiosis with the eye spot hermit crab *Paguristes eremite*. (B) *S. domuncula* primmorphs that are conventionally prepared and maintained in cell culture dishes under dynamic culture conditions rarely reach a diameter beyond 2 mm. (C) *S. domuncula* primmorphs that are cultivated in a stirred-tank vessel with continuous medium exchange (bioreactor) grow to a diameter of up to 20 mm. (D) Primmorphs that had been cultivated under dynamic culture conditions for 3 d in the presence of 0.1 mg/ml FMNP (left vessel) orhad remained untreated (right) were placed in front of a circular ferrite magnet (m) (lateral view). (E, F) Primmorphs were maintained under static conditions in dishes and on top of cutout patterns of magnetic ferrite foil for 3 d in the presence (E) or the absence (F) of FMNP (top view). Scale bars, 1 cm (A, D) and 0.5 cm (E, F).

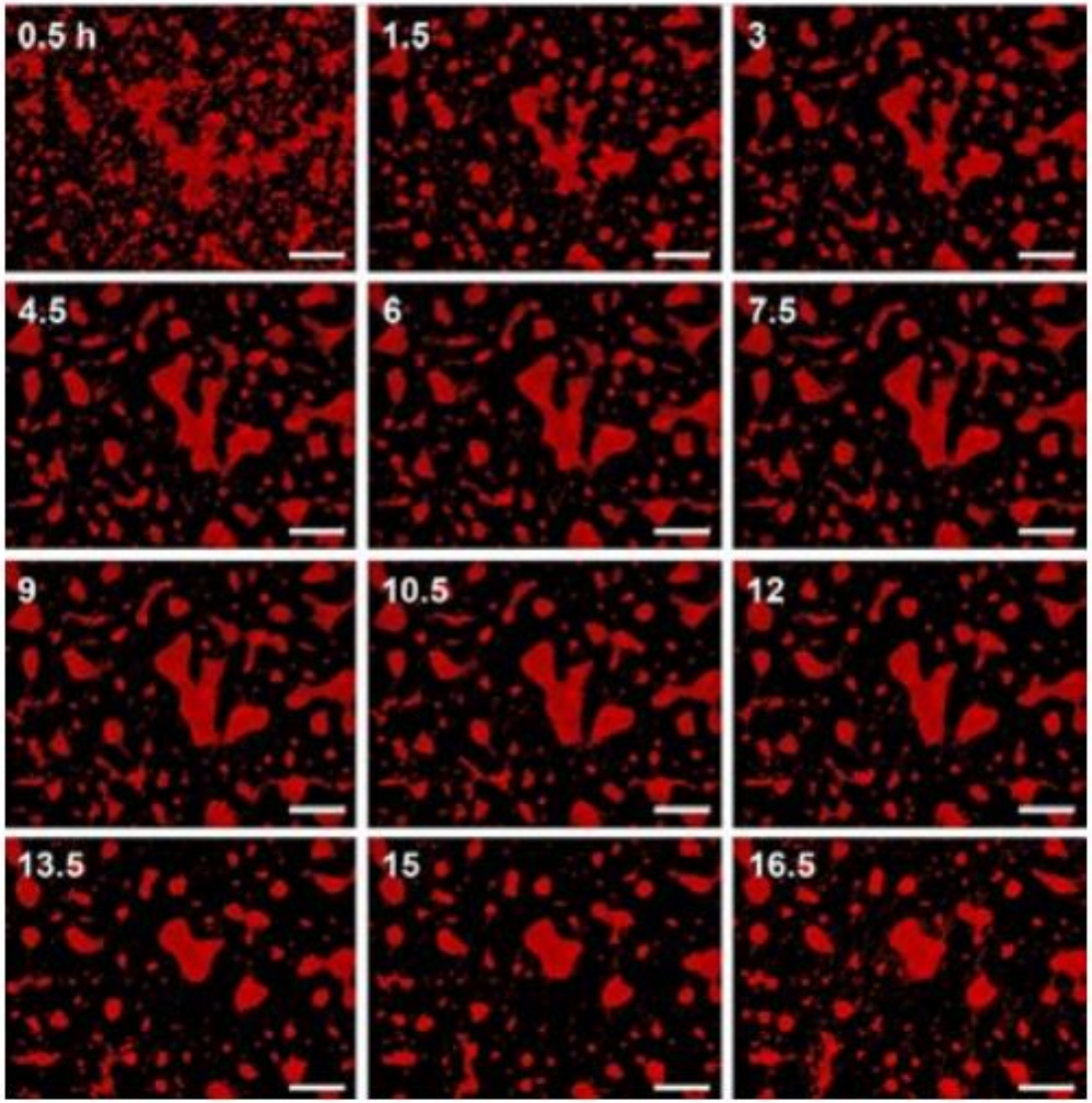


Fig. 3 Time-resolved dynamics of *S. domuncula* primmorph formation in the presence of FMNP – live cell time-lapse fluorescence imaging. Freshly dissociated sponge cells had been supplemented with 0.1 mg/ml red fluorescent FMNP for 0.5 h. Then, fluorescence micrographs were sequentially taken after the indicated incubation time (in hours [h]) under static culture conditions. Scale bars, 400 µm.

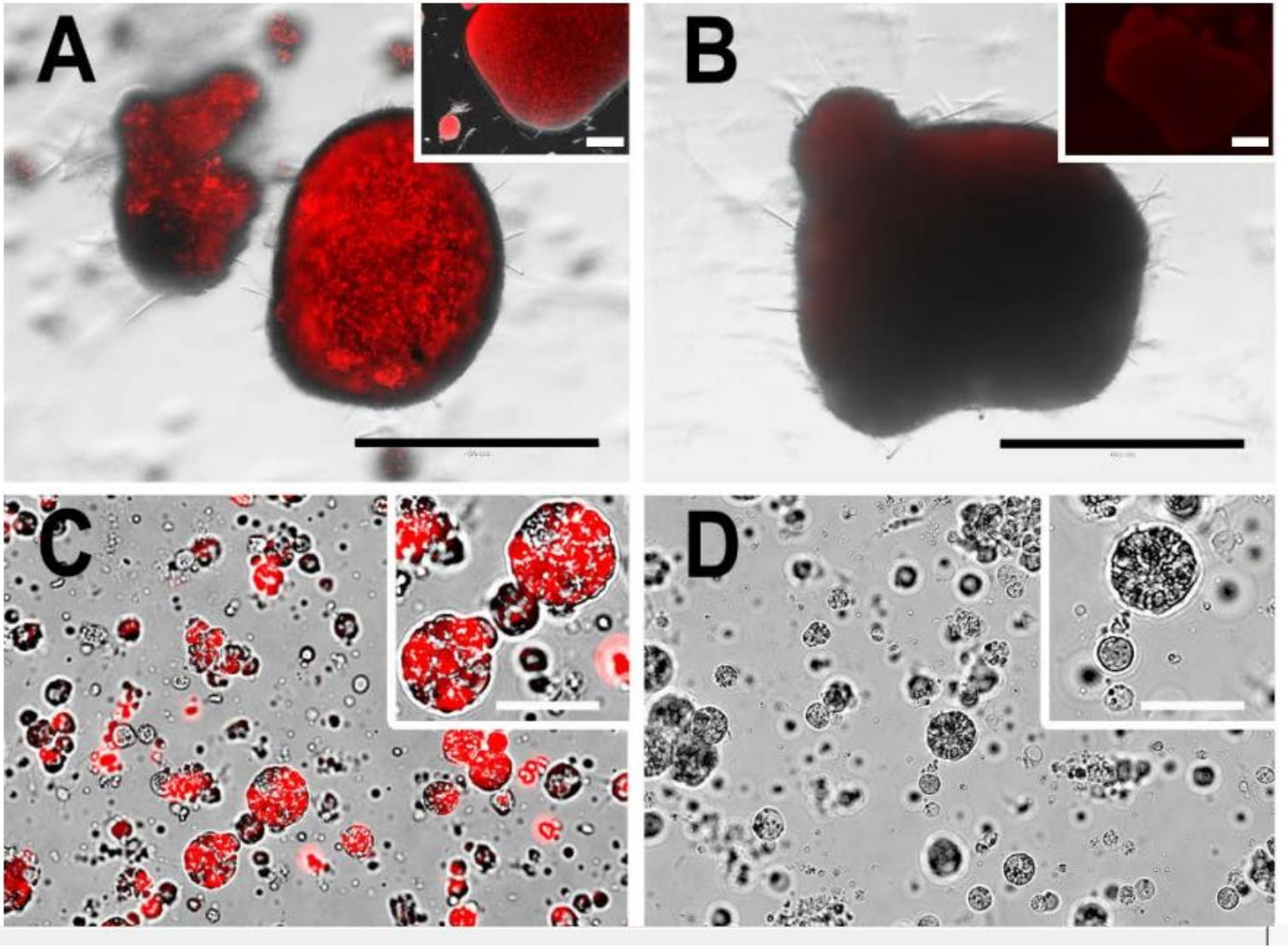


Fig. 4 *S. domuncula* primmorphs with fluorescence properties. Merged fluorescence and transmitted light micrographs of primmorphs that had been supplemented with 0.1 mg/ml red fluorescent FMNP for 9 d (A) or 19 d (A inset) or had remained non-supplemented for the same time (control; B and B inset). Merged micrographs of dissociated cells (in suspension) that were derived from supplemented 19 d-primmorphs (C; C inset, 2.5 x magnified detail) or control primmorphs (D; D inset, 2.5 x). Scale bars, 0.4 mm (A, B, and respective insets); 0.1 mm (C, D) and 40 µm (respective insets).

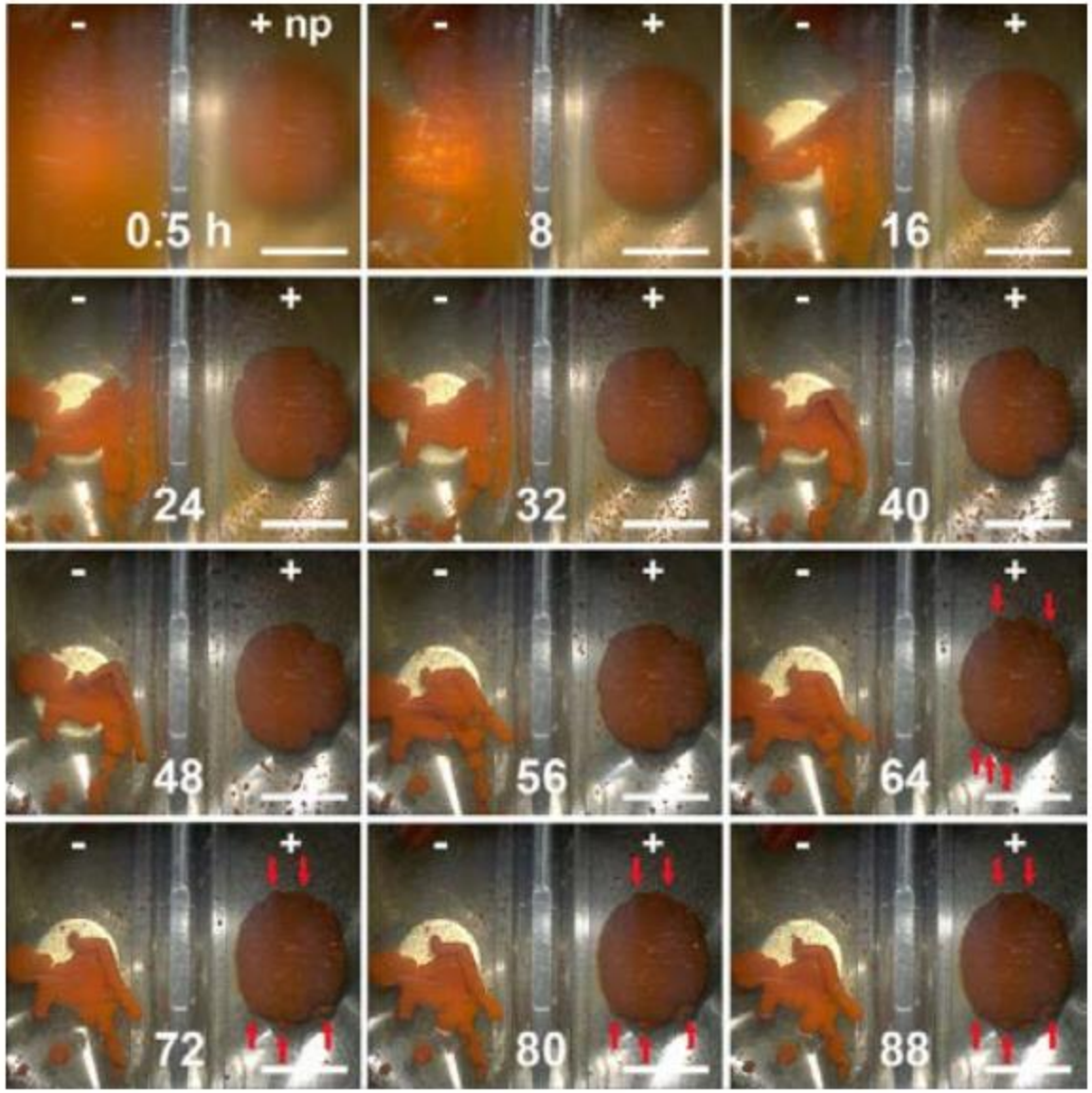


Fig. 5 Time-resolved dynamics of *S. domuncula* primmorph formation in the presence of FMNP – time-laps imaging of magnetic susceptibility. Freshly dissociated sponge cells had been supplemented with 0.1 mg/ml FMNP (+ np) for 0.5 h or had remained untreated as control (–). Then, the culture dishes were placed on top of circular ferrite magnets and digital micrographs were sequentially taken from above after the indicated incubation time under static culture conditions (in hours [h]). Blebbing of the primmorph surface is marked (arrows). Scale bars, 1.5 cm.

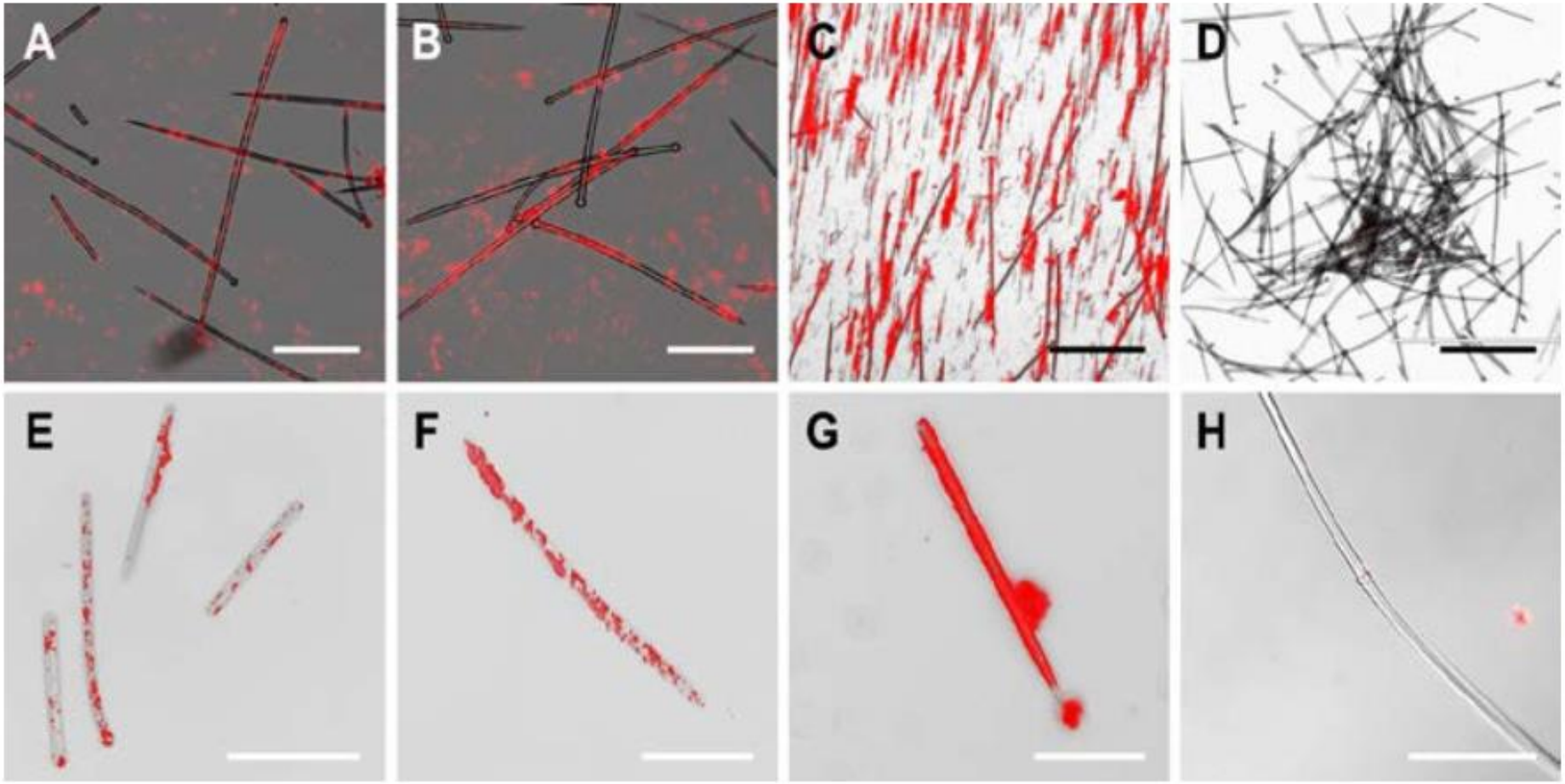


Fig. 6 *In vivo* and *ex vivo* surface immobilization of FMNP on spicules of *S. domuncula* primmorphs. (A – H) Fluorescence micrographs, with (E – H) cLSM 3D projections. Prior spicule extraction, the primmorphs had been incubated with 0.1 mg/ml FMNP for 9 d (A, E), 19 d (B, C, F) or had remained untreated for 19 d as control (D, H). To demonstrate their magnetic properties, spicules isolated of primmorphs that had been incubated for 19 d with FMNP (C) or spicules of untreated control primmorphs (D) were placed within the magnetic field of a subjacent permanent magnet. For comparison purposes, an example of a spicule with chemically cross-linked FMNP is shown (G). These spicules had been isolated from untreated control primmorphs, silanized with APTMS, and reacted with EDCactivated FMNP. (H) Example of an untreated and non-silanized control spicule. Scale bars, 100 (A, B, E), 200 (C, D), and 50 µm (F – H).

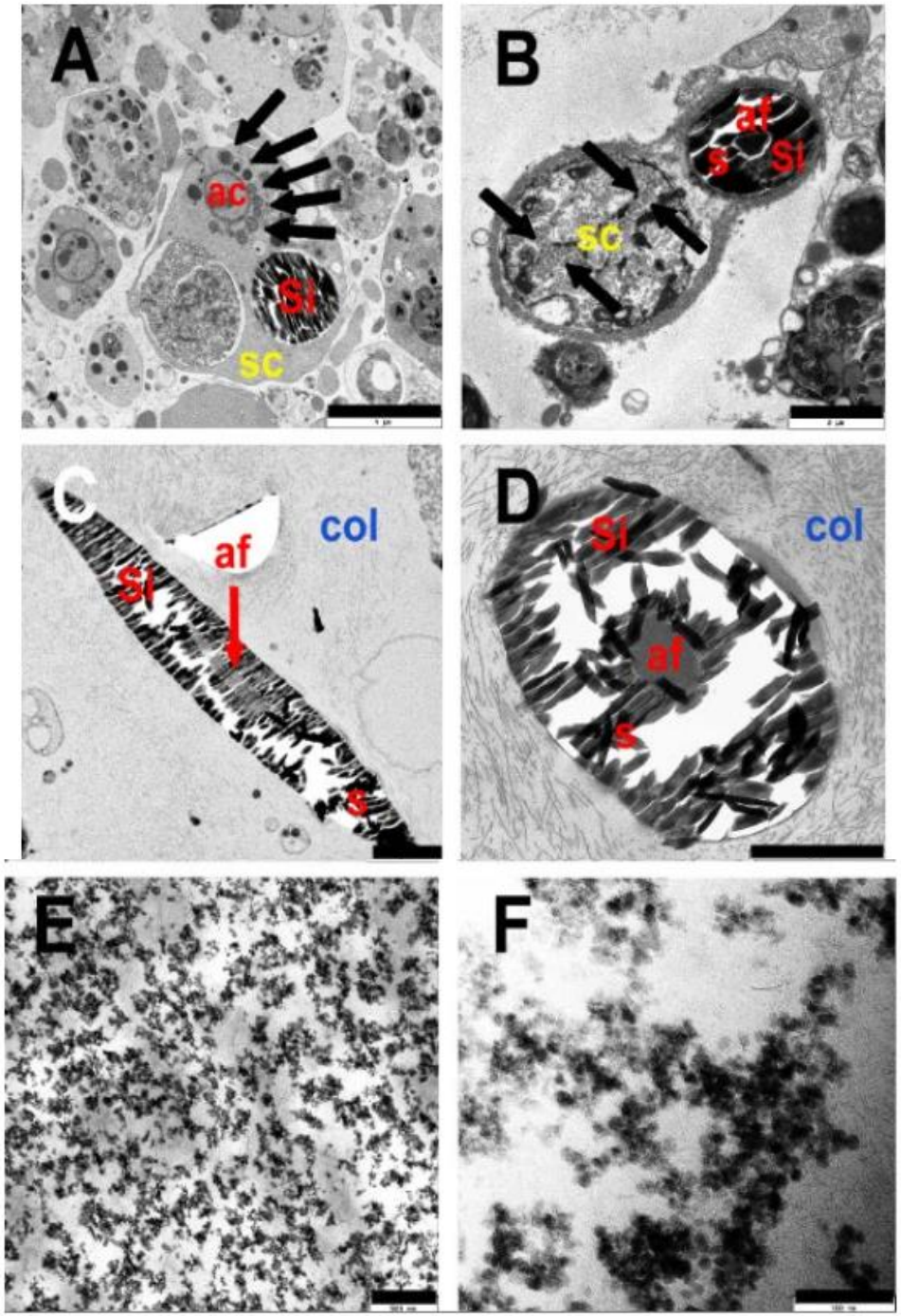


Fig. 7 Intra- and extracellular stages of spiculogenesis in *S. domuncula* primmorphs. TEM of sections of *S. domuncula* primmorph tissue show the three principal stages of spicule formation. While the first and second stage occur in sclerocytes (A, B) the third stage is extracellular (C, D). (A) A broad axial canal (ac) of a primordial spicule in the first stage of spiculogenesis within a sclerocyte (sc) is cross-sectioned. In this canal, proteins selfassemble to the templating axial filament. Surrounding silicasomes (↓) discharge their content into the canal to generate the juvenile spicule. In the second stage, here discernible within the same sclerocyte, the massive silica mantle (Si) of a cross-sectioned juvenile spicule is visible (the brittle silica always fractions during sectioning). (B) Intermediary stage, where the juvenile spicule (s) is progressively extruded in the ES by cell evagination, still surrounded by the sclerocyte's cell membrane. Note the axial filament (af) as well as the numerous smaller silicasomes (↓) that fill the sclerocyte. (C, D) Following their extrusion from the sclerocyte, in the third and final stage of spiculogenesis, mature spicules are embedded in the extracellular matrix and surrounded by collagen (col). Longitudinal (C) and transversal (D) section of a spicule (s) in this third, extracellular stage. Clearly visible is the silica mantle (Si) around the central axial filament (af). Siliceous spicule fragments were partially removed during sectioning. (E, F) TE micrographs of the FMNP applied for coating of spicules. Scale bars, 4 µm (A), 2 µm (B, C, D), 0.5 µm (E), and 0.1 µm (F).

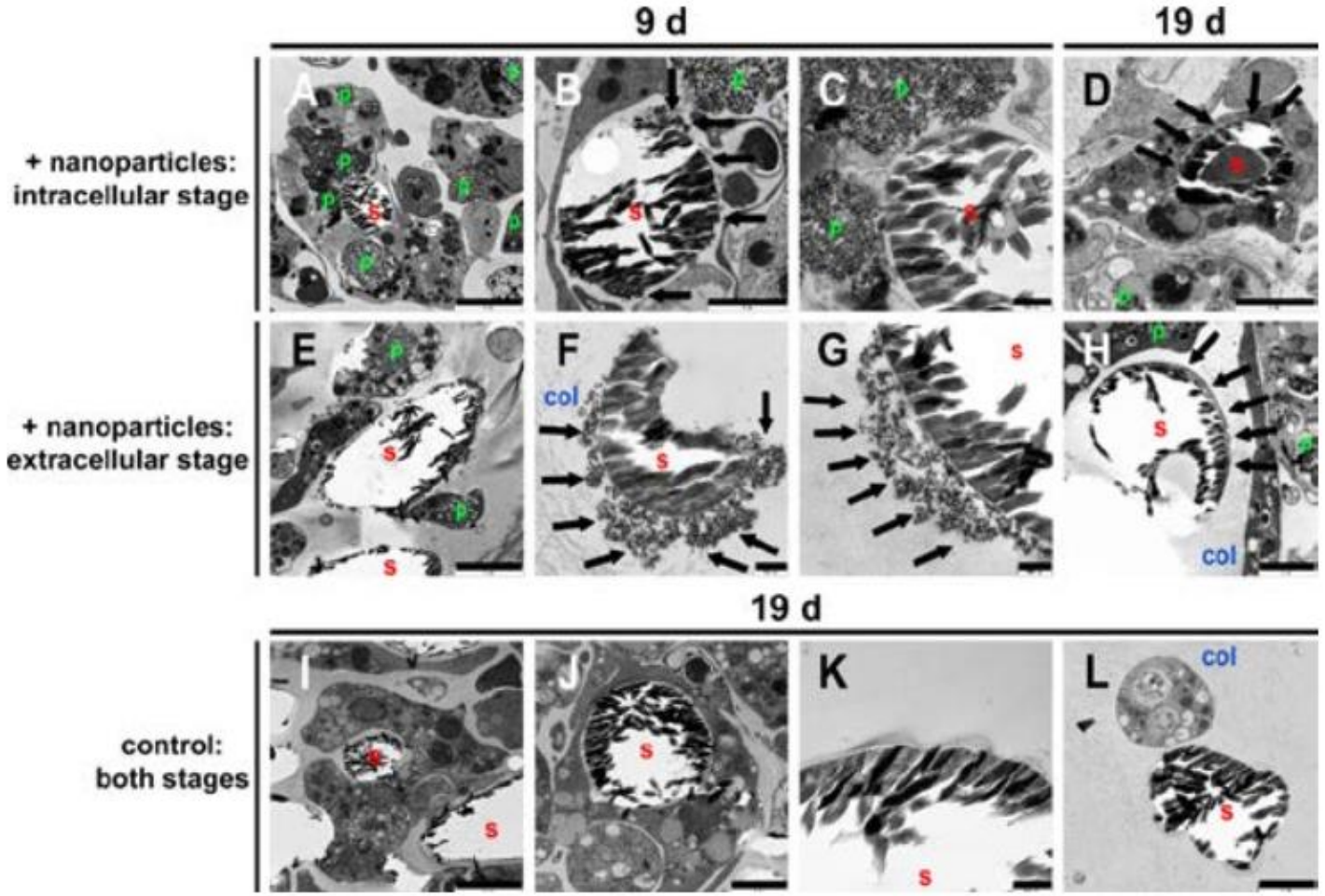


Fig. 8 Cellular uptake of FMNP and their *in vivo* surface immobilization onto spicules of *S. domuncula* primmorphs. Primmorphs had been incubated with 0.1 mg/ml FMNP for 9 d (A – C, E – G), 19 d (D, H), or had remained untreated for 19 d as control (I –L). TE micrographs of primmorph sections depict the respective intracellular (A – D) and extracellular stage (E – H) of spiculogenesis or, in case of the control, a representative selection of both (I, J and K, L respectively). Spicules are marked (s) as well as some representative intra- and extracellular clusters of nanoparticles (p) and collagen fibers (col). Nanoparticles that coated spicules or accumulated at the spicules' surfaces are indicated (↓). Scale bars, 4 µm (A, E, I), 2 µm (B, D, H, J, L), and 0.5 µm (C, F, G, K).

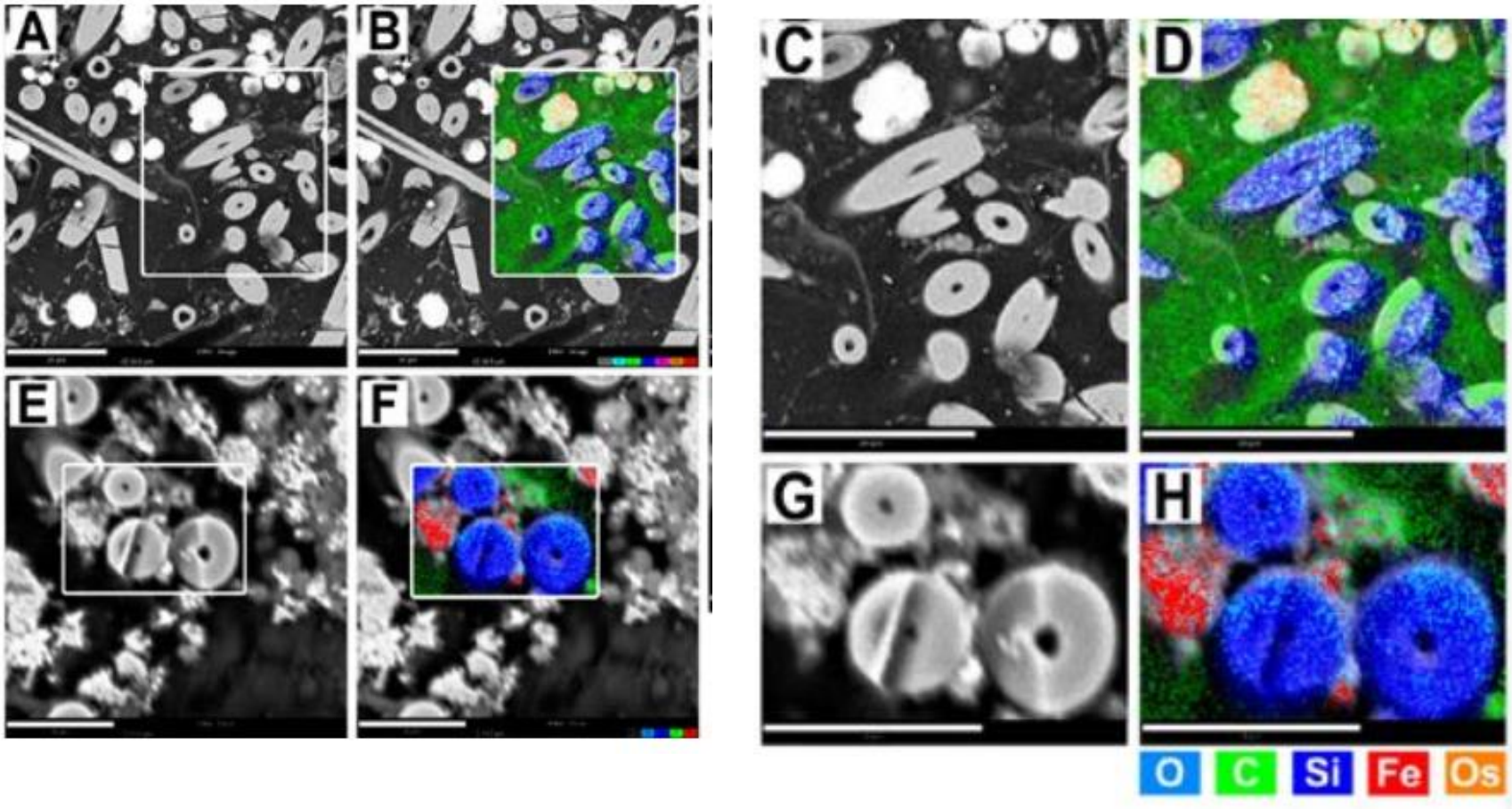


Fig. 9 *In vivo* surface immobilization of FMNP on spicules of *S. domuncula* primmorphs – BSE-SEM/EDX. Backscattered electron scanning electron microscopy (BSE-SEM) with energy-dispersive X-ray spectroscopy (EDX) was applied to map the elemental distribution in cross-sections of primmorphs that had remained untreated as control (A – D) or had been incubated with 0.1 mg/ml FMNP for 19 d (E– H). (A, E) BSE-SE micrographs. The areas that were subject to EDX mapping are marked by white boxes. (B, F) Pseudocolor coded SEM-EDX elemental maps (each pixel represents one EDX measurement). (C, D and G, H) Higher magnification of respective boxed areas. The analyzed elements are: Oxygen (cyan), carbon (green), silicon (dark blue), and iron (red). The signal for osmium (orange) can be attributed to osmium tetroxide that was used during sample preparation for fixing and staining. Scale bars, 20 µm (A – D) and 8 µm (E – H).

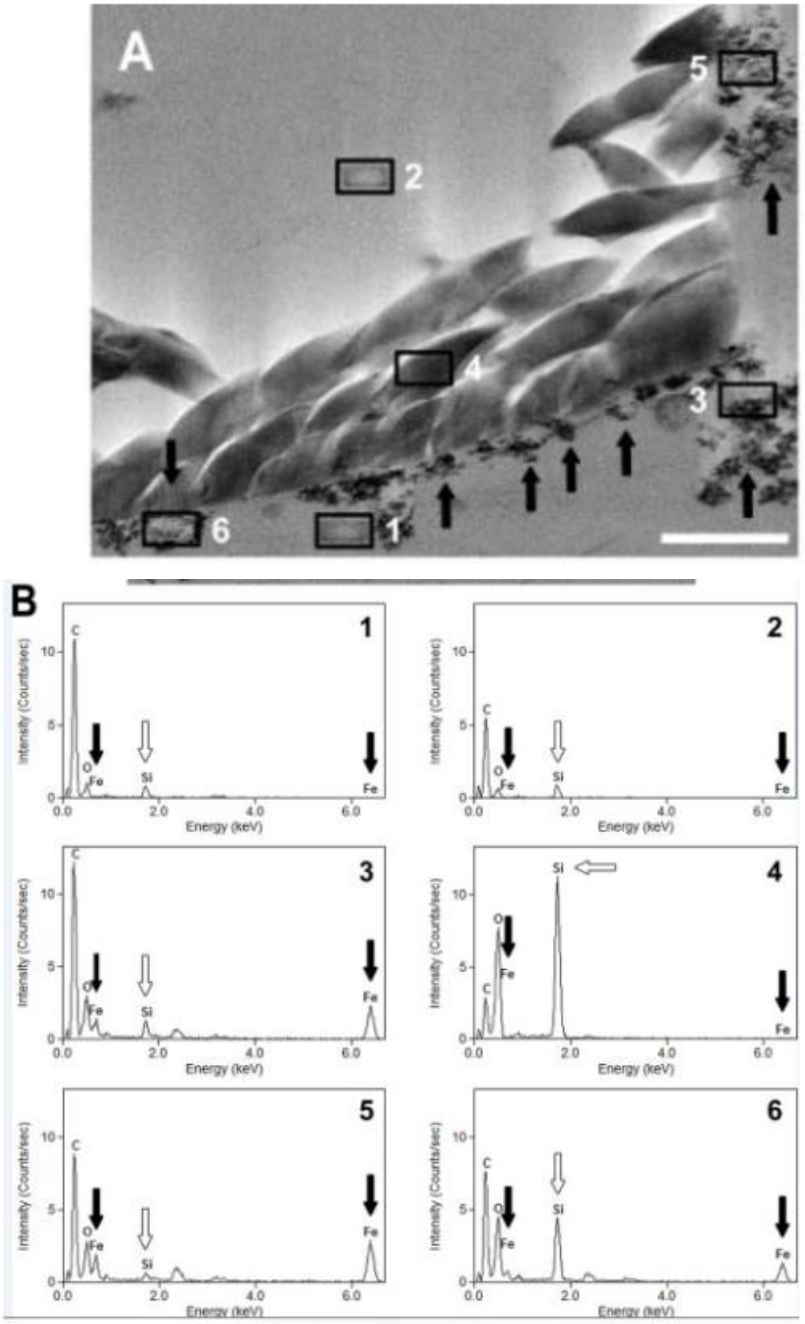


Fig. 10 *In vivo* surface immobilization of FMNP on spicules of *S. domuncula* primmorphs TEM-EDX. TEM-EDX was applied to characterize the elemental composition in the proximity of cross-sectioned extracellular spicules of primmorphs. Before sectioning, the primmorphs had been treated with 0.1 mg/ml FMNP for 19 d. The numbering of the boxed areas in the TE micrograph (A) corresponds to the respectively numbered EDX spectra (1 – 6) in B. In the TE micrograph, several clusters of nanoparticles are marked (↓). In the EDX spectra, peaks for the key elements Fe and Si are marked by closed and open arrows, respectively. Scale bar, 500 nm.