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## Data Article

Data from *in vivo* functionalization of diatom mesoporous biosilica with bisphosphonates

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## ABSTRACT

Diatoms are unicellular photosynthetic microalgae that produce a sophisticated mesoporous biosilica shell called *frustule*. Easy to achieve and extract, diatom frustules represent a low-cost source of mesoporous biocompatible biosilica. In this paper, the possibility to *in vivo* functionalize the diatom biosilica with bisphosphonates (BPs) was investigated. In particular, two BPs were tested: the amino-containing sodium alendronate (ALE) and the amino-lacking sodium etidronate (ETI). According to first SEM-EDX analysis, the presence of the amino-moiety in ALE structure allowed a better incorporation of this BP into living diatom biosilica, compared to ETI. Then, diatom growth was deeply investigated in presence of ALE. After extraction of functionalized frustules, ALE-biosilica was further characterized by XPS and microscopy, and ALE release was evaluated by ferrochelation assay. Moreover, the bone regeneration performances of ALE-functionalized frustules were preliminarily investigated on bone osteoblast-like cells, *via* Comassie staining. Data are related to the

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research article “*In vivo* functionalization of diatom biosilica with sodium alendronate as osteoactive material”.

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#### Specification table

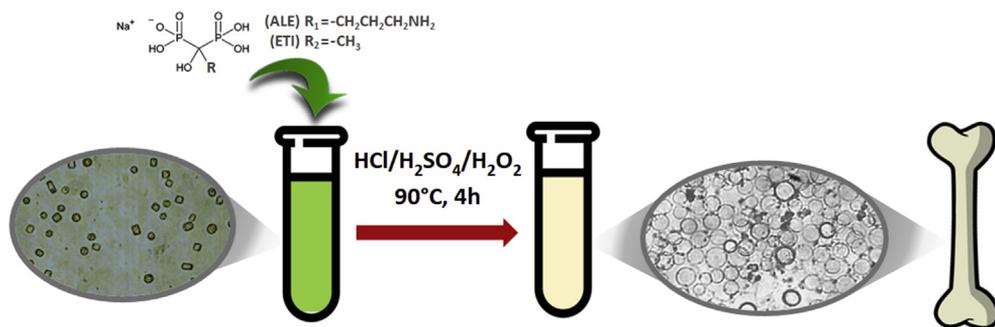
Subject area	Chemistry, biology, biotechnology, tissue engineering.
More specific subject area	Diatoms, biomaterial
Type of data	Graphs, Images, Table
How data was acquired	X-ray photoelectron spectroscopy (XPS) analysis was performed with a ThermoVG Thetaprobe spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA, $10^{-9}$ mbar) equipped with a microspot monochromatized AlK $\alpha$ (1486.6 eV) source (BE range= 0–1200 eV, FAT mode, pass energy = 150 eV (X-ray beam spot 300 $\mu$ m). Spectrometer was calibrated using the Cu2p3/2 (932.7 eV) and Au (84.0 eV). Data were analyzed with Avantage software package (Version 4.75 Thermo Fisher Scientific Inc., Waltham, MA, USA): the experimental points of detailed spectra were fitted using Gaussian–Lorentzian peaks (max error $\pm$ 0.2 eV). SEM analysis was carried out by the field emission gun (FEG) Zeiss SUPRA 40 apparatus (3 KV, tilting angle of 0°–30°). EDX analysis was performed by collecting X-rays at 15kV at a working distance of 8.5 mm, with an INCA energy dispersive x-ray spectrometer from Oxford, mounted onto the SEM FEG apparatus. Contrast phase microscopy analyses were performed by Leica DM ILL, Leica Microsystems Srl, Milan, Italy.
Data format	Analyzed
Experimental factors	Diatom shell functionalization with bisphosphonate in F/2 Guillard enriched seawater medium and purification of the bio-hybrid material for eukaryotic cell tests
Experimental features	Characterization of alendronate doped biosilica via XPS, SEM-EDX and optical microscopy analyses followed by <i>in vitro</i> testing on SaOS-2 cells.
Data source location	University of Bari, Department of chemistry
Data accessibility	Data are provided with this article
Related research article	S. R. Cicco, D. Vona, G. Leone, E. De Giglio, M. A. Bonifacio, S. Cometa, S. Fiore, F. Palumbo, R. Ragni, G. M. Farinola, <i>In vivo</i> functionalization of diatom biosilica with sodium alendronate as osteoactive material, Mater. Sci. Eng. C, Submission status: submitted (no: MSEC_2019_319).

#### Value of the data

- Easily achievable functionalization of the diatom biosilica shell *in vivo* and extraction of the biomaterial via acid-oxidative treatment
- Evaluation of the cell density during the functionalization and bisphosphonates-biosilica characterization
- *In vitro* test of the biomaterial on eukaryotic cells for bone tissue purposes

## 1. Data

This article provides data resulting from the *in vivo* functionalization of *Thalassiosira weissflogii* diatom biosilica with ALE and ETI bisphosphonates (BPs). All the steps required to obtain the BP-biosilica are represented in Fig. 1. Fig. 2 reports the SEM analysis of BP-biosilica and the EDX results are reported in Table 1. Fig. 3 shows the growth curve of diatoms grown with Na alendronate, evaluated at 2 different cell densities. Architecture parameter analysis of ALE-biosilica is evidenced in Fig. 4. Figs. 5 and 6 report respectively the XPS and the ferrochelation assay of ALE-biosilica. Comassie stained SaOS-2 grown on ALE-biosilica and controls are presented in Fig. 7.



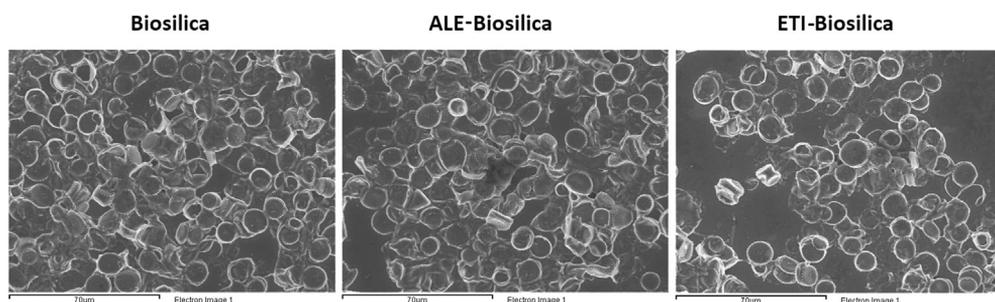
**Fig. 1.** From the living algae to the ALE-biosilica. This general scheme illustrates all the steps required to achieve bisphosphonate-based hybrid material from diatom biosilica.

## 2. Experimental design, materials and methods

*In vivo* functionalization experiment of *T. weissflogii* diatom shells was performed by growing the algae in presence of two different bisphosphonates in the culture medium at a final concentration of 1 mM. The BP-biosilica was then extracted and the most efficient BP incorporation was evaluated by SEM-EDX analysis. The diatom growth in presence of ALE was investigated over time and the ALE-biosilica was then characterized by SEM and XPS analyses. The release of the ALE was evaluated by ferrochelation assay. ALE-biosilica was then deposited on glass slides and SaOS-2 osteoblast-like cells were grown on glass/biosilica *quasi* monolayers. Cell staining was performed and morphology of SaOS-2 was microscopically evaluated. A schematic representation of all the processes is represented in Fig. 1.

### 2.1. *In vivo* functionalization of silica shells with BPs and biosilica extraction

*Thalassiosira weissflogii* diatoms (culture collection of algae and protozoa, *Conticribra weissflogii* CCAP strain 1085/18) were grown in a sterile F/2 Guillard enriched by sea water medium [2] in a vertical bioreactor (18 ± 2 °C, 64% humidity, light:dark cycle of 16:8 h, pump photon flux 70000 lux). For the *in vivo* functionalization [3], Na alendronate (1 mM) and Na etidronate (1 mM) were added in 2 different sets of medium containing cells. For the biosilica functionalization 5 days of incubation with the bisphosphonates are required. Acid oxidative treatment was used to extract the BPs-biosilica by removing the organic matter [4]. For the cleaning, the algal pellet (3 mL, 10<sup>6</sup> cell/mL) was treated with H<sub>2</sub>SO<sub>4</sub> (150 μL, 98% w/w) and HCl (50 μL, 35%) at 80 °C for 30' to completely dehydrate and remove organic matter. Then an oxidation step with hydrogen peroxide (400 μL, 30% w/w) at 90 °C for 4 h ensured the removal the rest of the organic matter. Shells were then washed in H<sub>2</sub>O and in ethanol. At the end of the treatments the whitish frustules were dried by vacuum pump.

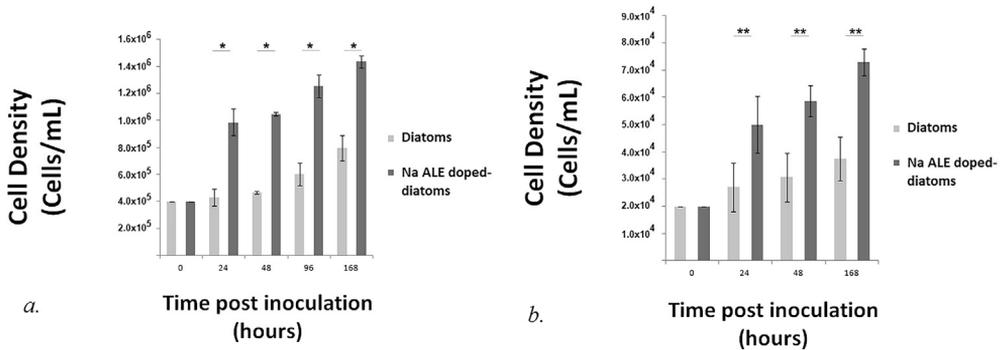


**Fig. 2.** SEM images of extracted bare biosilica, ALE-biosilica and ETI-biosilica.

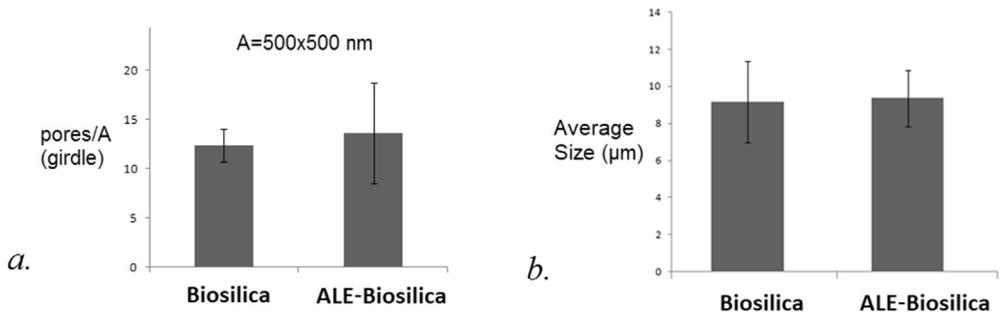
**Table 1**

EDX atom percentages and P/Si signal ratio for extracted bare biosilica, ALE-biosilica and ETI-biosilica.

	Biosilica	ALE-biosilica	ETI-biosilica
P/Si	0	0.006	0.002
% Si	10 ± 3	19 ± 7	19.63 ± 3
% P	0	0.10 ± 0.003	0.05 ± 0.01



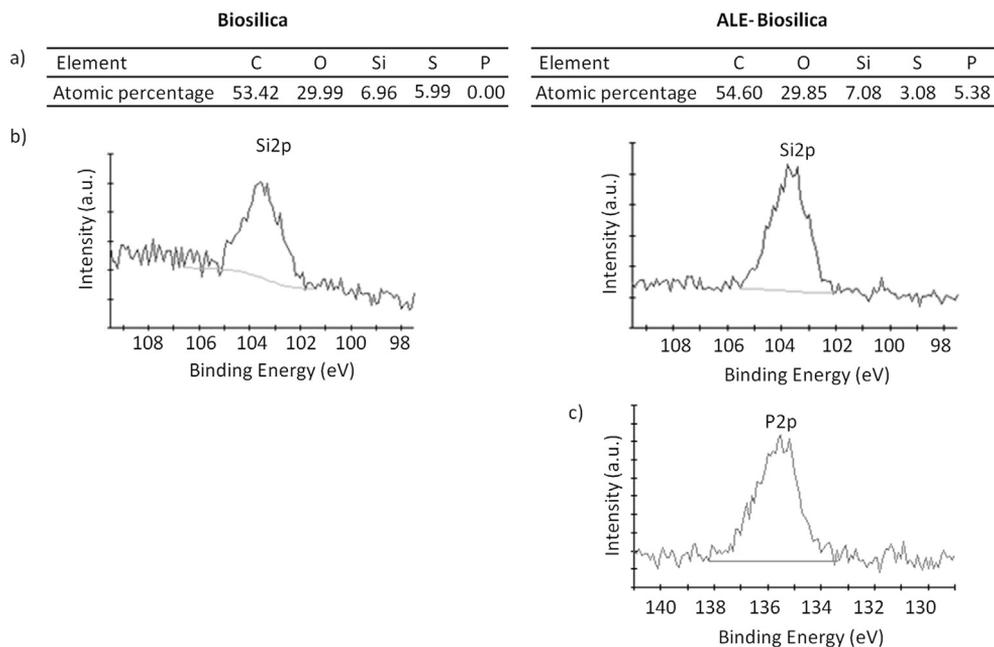
**Fig. 3.** Growth kinetics evaluation of *T. weissflogii* grown without (Diatoms) and with (Na ALE doped-diatoms) sodium alendronate, at 2 different cell-cycle stages (expo phase, from  $10^5$  cells/mL (a); pre-expo phase, from  $10^4$  cells/mL (b)). Measures were statistically evaluated by a two-way ANOVA test within groups (sample X vs sample Y, same time group), followed by a Bonferroni post-test, using the GraphPad Prism version 4.00 for Windows, GraphPad Software (San Diego, CA; [www.graphpad.com](http://www.graphpad.com)). Differences were considered statistically significant for  $p < 0.05$  (\*\*) and  $p < 0.01$  (\*).



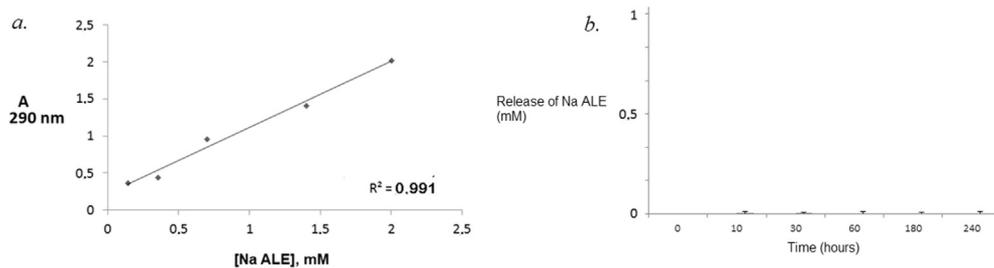
**Fig. 4.** Evaluation of number of pores/A per girdle for biosilica control and ALE-biosilica (a); average size analyses for biosilica and ALE-biosilica shells (b).

## 2.2. SEM-EDX analysis after in vivo functionalization of biosilica with BPs

SEM (Fig. 2) and EDX (Table 1) analyses were carried out on ALE-biosilica, ETI-biosilica and bare frustules after extraction. For the analyses, the diatom extracted biosilica ( $5 \mu\text{L} \times 5$  of a solution containing 1 mg biosilica in 200  $\mu\text{L}$  of distilled water) were drop casted on a silicon wafer pre-treated with acetone, ethanol and isopropanol (using  $10''$  of sonication per washing step); the suspension was then dried by vacuum pump (1 h). For the EDX analysis a set of 10 points per sample was analyzed. SEM investigations revealed that bisphosphonates functionalization did not affect biosilica shell morphology. According to the EDX analyses, the P signal is not present on the pure biosilica. Indeed, biosilica from diatoms grown with the amino BP alendronate exhibits a P signal value higher compared to biosilica from diatoms grown with the non-amino-BP etidronate.



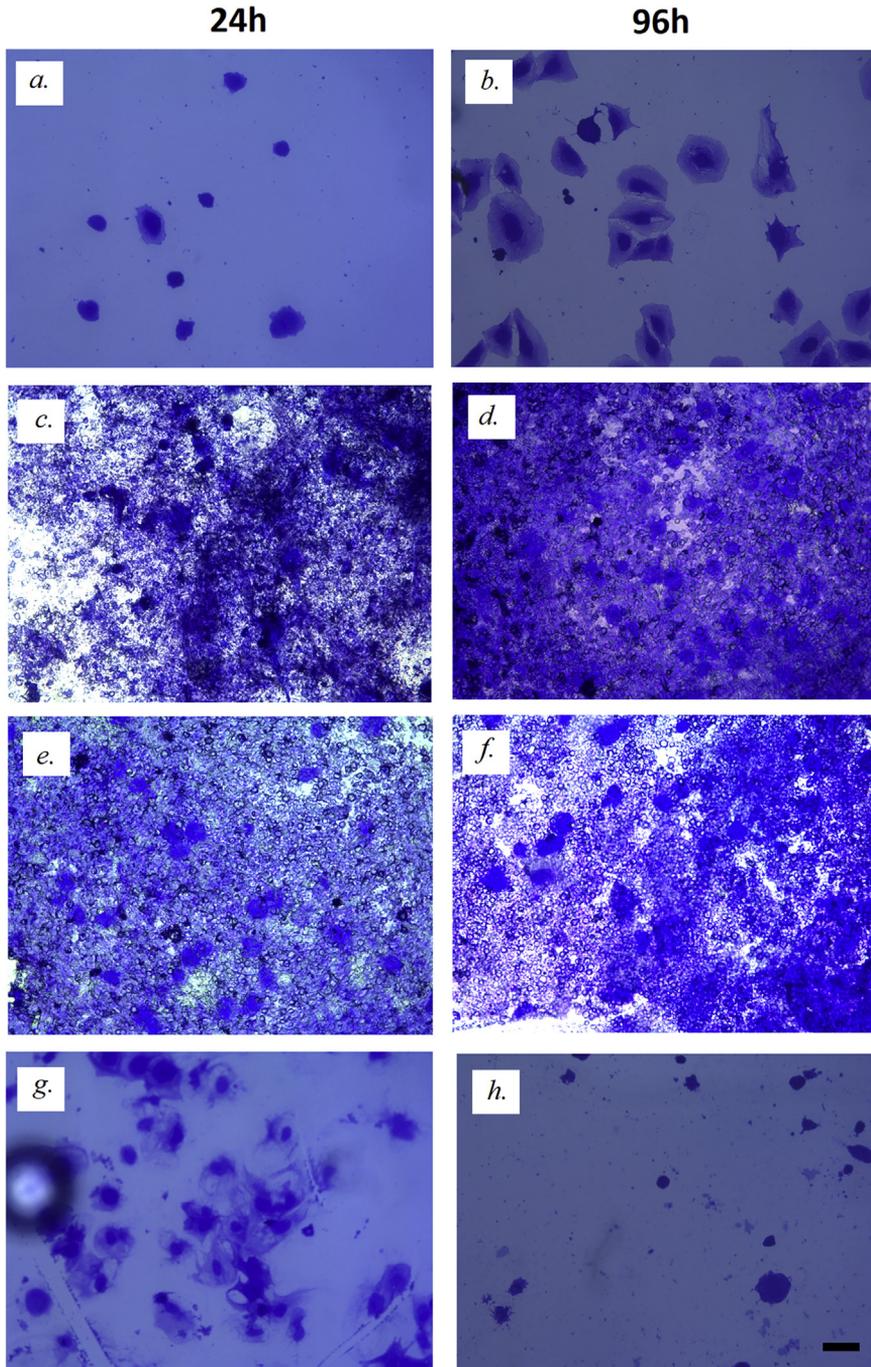
**Fig. 5.** Atomic percentages of bare and ALE-biosilica (a); XPS surveys for Si2p of ALE-biosilica and control (b). The P signal on ALE-biosilica is reported (c).



**Fig. 6.** (a) Na ALE solutions concentrations correlated in a calibration curve with A values at 290 nm; (b) Na ALE release profile by frustules obtained from hard cleaning procedure of *in vivo* doped diatoms.

### 2.3. Growth kinetics evaluation of *T. weissflogii* with sodium alendronate (ALE)

Since the ALE was the most efficiently incorporated BP into the biosilica, a detailed investigation on diatom growth in presence of this amino-BP was performed, together with exhaustive characterization of the derived ALE-biosilica after extraction. For diatoms growth kinetics, samples were prepared in quadruplicates starting from different concentration of diatoms (pre-exponential phase,  $10^4$  cells/mL, and exponential phase,  $10^5$  cells/mL): the growth of diatoms with and without ALE was evaluated by monitoring their density over days via Burkler hemocytometer (Fig. 3). The growth trend of *T. weissflogii* was positively affected by the ALE addition in the culture medium and no cytotoxic influence was observed, both at the 2 cell-cycle stages.



**Fig. 7.** Coomassie staining of SaOS-2 after 2 different times of growth, on glass control (a, 24h; b, 96h), bare biosilica (c, 24h; d, 96h), ALE-biosilica (e, 24h; f, 96h) and Na ALE free drug (g, 24h; h, 96h). Size bar: 50  $\mu$ m.

#### 2.4. Architecture parameters evaluation of ALE-biosilica

Architecture analyses on bare and ALE-biosilica were performed on recorded SEM images of extracted shells. SEM images were used for evaluating the number of pores/Area ( $500 \times 500$  nm) of girdles (central porous structure of the diatom shell). Data elaboration was performed by using Image.J software [5,6]. Monolayers of extracted bare biosilica and ALE-biosilica were produced via SAM procedure from ethanol on glass slides [7]. Images in contrast phase microscopy were collected for calculating the shell average size. According to the results reported in Fig. 4, no significant differences in porosity and size were observed among ALE-biosilica and control.

#### 2.5. XPS analyses

XPS analysis was performed on extracted diatom biosilica derived from diatoms grown with (ALE-biosilica) and without (biosilica) alendronate. Fig. 5 shows XPS surveys and atom percentage from bare biosilica and ALE-biosilica samples. P atoms are present only in the frustules of diatoms grown with ALE, even though the hard cleaning procedure.

Bare frustules XPS survey (Fig. 3): Carbon (C1s BE ~ 285eV), Oxygen (O1s BE ~ 533eV, OKL1 BE ~ 979eV), Silicon (Si2p BE ~ 103.5eV, Si2s BE ~ 150eV), Sulfur (S2p BE ~ 170eV, S2s BE ~ 233eV), Chlorine (Cl2p BE ~ 201eV, Cl2s BE ~ 269eV) and sodium (Na1s BE ~ 1072eV). Au standard support (Au4f ~ 85eV, Au4d5 ~ 335eV, Au4d3 ~ 354eV). ALE-biosilica XPS survey (Fig. 3): Carbon (C1s BE ~ 285eV), Oxygen (O2s BE ~ 26eV, O1s BE ~ 533.5eV, OKL1 BE ~ 979eV), Silicon (Si2p BE ~ 103.7eV, Si2s BE ~ 155eV), Phosphorus (P2s BE ~ 193eV, P2p BE ~ 135.5eV) and Sulfur (S2s BE ~ 234eV, S2p BE ~ 170eV). Au standard (Au4f7 ~ 85eV, Au4f5 ~ 89eV, Au4d5 ~ 337eV, Au4d3 ~ 354eV).

#### 2.6. Evaluation of bisphosphonate release

Extracted biosilica shells from diatoms grown with alendronate were collected for evaluating the content of released alendronate over time. For the investigation, a protocol based on iron complexation was adopted [8]. A set of 5 samples were produced for the construction of a calibration curve. In each sample, an amount of 1998  $\mu$ L of an iron stock solution (13.5 iron (III) chloride, 10 mL of perchloric acid 2 M) was mixed with 2  $\mu$ L of Na ALE standard solutions (0.14 M, 0.35 M, 0.7 M, 1.4 M, 2 M) reaching different final concentration of the bisphosphonate (0.14 M, 0.35 M, 0.7 M, 1.4 M, 2 M). Absorbances (at 290 nm) of these ALE solutions, together with the pure iron stock solution (blank reference), were collected to produce the calibration curve (Fig. 6a). Then, the release of alendronate of the hybrid system was measured on extracted pure biosilica (6 samples) and ALE-biosilica (6 samples). Shells (3.5 mg per sample) were dried, rinsed with 1 mL of physiologic solution (25 °C, stirred 100 rpm) and kept closed during each sampling. The collected supernatant (100  $\mu$ L per biosilica sample, substituted immediately by fresh physiological solution), was rinsed with the fresh iron (III) chloride/perchloric acid solution. Absorbances (290 nm) were measured and an interpolation function on the previous calibration curve was used to quantify the amount of ALE released at different timing points. As shown in Fig. 6b, no significant release of Na ALE has been detected.

#### 2.7. Coomassie staining of SaOS-2 cells grown on ALE-doped biosilica

Human cell viability evaluation was performed on bare and ALE-biosilica samples after deposition on glass slides. Human SaOS-2 osteoblast-like cells were grown on substrata as reported in the reference article [1]. The cell sediment ( $10^5$  cell/mL) was seeded on glass slides uncoated and coated with pure or ALE-biosilica and bare biosilica. An additional control made of cells grown on glass slides in presence of lone ALE solution ( $10^{-6}$  M) was used. After 24 and 96 h of incubation, cells were fixed in 4% of paraformaldehyde/PBS solution (20') and stained for 3' with a dye solution (0.2% Coomassie Brilliant Blue R250 Sigma, 50% methanol, 10% acetic acid) [9]. After PBS washings for removing the excess of dye, cells were microscopically evaluated (Fig. 7). Cell proliferation is confirmed on glass control (Fig. 7a and b), bare (Fig. 7 c and d) and alendronate doped (Fig. 7 e and f) biosilica while the ALE free drug (Fig. 7 g and h) stopped the growth and adhesion over time.

## Transparency document

Transparency document associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2019.103831>.

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