ELECTRONIC SUPPORTING INFORMATION

Surface bioengineering of diatomite based nanovectors for efficient intracellular uptake and drug delivery

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**Experimental**

**Diatomite NPs production and characterization**

5g of crashed diatomite powered was resuspended into 250 mL of absolute EtOH and sonicated for 18 h to break large aggregates. The dispersion was sieved through a nylon net filter with pore size of 41 μm, and then filtered with pore size of 0.45 μm (Millipore, Billerica, MA, USA). The diatomite nanopowder was purified to remove the organic and inorganic impurities. First, the sample was centrifuged and the pellet treated with Piranha solution (2 M H₂SO₄, 10% H₂O₂) for 30 min at 80°C. Then, the NP dispersion was centrifuged for 30 min at 13.500 rpm, washed twice with distilled water, resuspended in 5 M HCl, and incubated overnight at 80°C. DNPs were then centrifuged for 30 min at 13.500 rpm and washed twice with distilled water to eliminate HCl residues. The diatomite powder was morphologically characterized before and after the processing by TEM, SEM, and DLS analysis. Before the processing, diatomite resulted as a mixture of fragments with circular, elliptical, elongated, and squared shape, with dimension distribution ranging from few microns up to ~40 μm, as shown in Fig. S1.

**Cells culturing**

MCF-7 human breast cancer cells were grown in Dulbecco’s modified Eagle’s medium and MDA-MB-231 breast carcinoma cells were grown in Roswell Park Memorial Institute 1640 medium. The media were supplemented with 10% (v/v) fetal bovine serum (FBS), 1% non-essential amino acids (NEAA), 1% L-glutamine, penicillin (100 IU/mL), and streptomycin (100 mg/mL), all from EuroClone Spa (Italy). The cell lines were obtained from the American Type Culture Collection and cultured in 75 cm² culture flasks (Corning Inc. Life Sciences, USA) at 37 °C with humidified atmosphere (95%) containing 5% CO₂ (BB 16 gas incubator, Heraeus Instruments GmbH, Germany). The cells were subcultured at 80% confluence using the growth medium and using
trypsin-PBS-ethylenediaminetetraacetic acid solution to harvest them for passaging and prior to each test.

**Results and discussion**

**Fig. S1.** SEM images of raw diatomite powder (A–C) and particles size histogram (D) calculated from (A). ²

Diatomite powder after the mechanical and chemical treatments, appeared as an heterogeneous population constituted by nanostructures morphologically different in shape with size, ranging from 100 nm up to 300 nm, as shown in **Fig. S2.** ¹

The nanosize of the diatomite particles, obtained after the processing of the raw diatomite powder was further confirmed by TEM and DLS analysis (data not shown). ¹⁻²
Fig. S2. TEM image of DNPs (A), particles’ size distribution (C) calculated from (A), and SEM image of NPs pores (B). $^2$

The pore size of diatomite frustules estimated by SEM images, reported mesopores (10 nm < pores diameter < 50 nm) and macropores (pores diameter > 50 nm) on their surface, preserved also after the reduction from micro to nanoparticles. $^1$-$^2$ The variation in the chemical composition of diatomite powder was evaluated by photoluminescence, FTIR and EDS analyses, confirming the improvement of the silica nanopowder quality due the removal of impurities.$^2$

In Fig. S3-A, the FTIR spectra of untreated (A) and purified (B) diatomite shows, in both samples, the broad band at 1200–1050 cm$^{-1}$ and the peak at 790 cm$^{-1}$ due to the presence of Si–O–Si bond characteristic of the diatomite silica framework. After the chemical treatment in acid solutions, the peak at 680 cm$^{-1}$ and the band at 620–580 cm$^{-1}$ related to Si–O–Fe and Fe–O–Fe bonds, respectively, disappeared due to the removal of metallic impurities from the diatomite amorphous silica matrix.$^2$

Fig. S3-B shows the comparison between the photoluminescence spectra of diatomite before (A) and after (B) treatments. The untreated sample (A) was characterized by a peak at 390 nm of
metallic impurities which became weaker after purification treatment, confirming the removal of impurities.²

Fig. S3. FTIR (A) and photoluminescence (B) spectra of diatomite before (A) and after (B) purification treatments.²

Moreover, EDS spectra (data not shown) revealed changes in the chemical composition of the samples: after the cleaning treatments, the intensities of the peaks corresponding to inorganic oxides (calcium, iron, and aluminium) decreased, whereas the silica peak increased, as reported in detail elsewhere.²

In Fig. S4, the in vitro toxicity studies on MCF-7 (A-B) and MDA-MB 231 (C-D) breast cancer cells after 6 and 24 h of exposure to the bare DNPs at increasing NP concentrations (25, 50, 100, and 200 µg/mL) demonstrated the non-toxic nature of bare NPs. These results further confirmed the safety of the DNPs and their potential applicability as nanocarriers for drug delivery applications, as previously demonstrated by the MTT assay on H1335 epidermoid carcinoma cells.¹
Cell viability of MCF-7 (A and B) and MDA-MB 231 cells (C and D) treated with concentrations of 25, 50, 100, and 200 μg/mL for 6 and 24 h at 37 °C. Data are provided as the mean ± s.d. (n = 3).

The cellular uptake of bare DNPs labeled with TRITC (tetramethylrhodamine) has been also evaluated by confocal analysis. The results showed that an efficient NP uptake into the cytoplasm.
of cancer cells, demonstrating that DNPs could represent a promising tool for the delivery of anticancer molecules, as reported in detail elsewhere.\textsuperscript{1-2}

![Fig. S5. The FTIR bands of the Si-O-Si bond at 1100 and 790 cm\textsuperscript{-1} as the main constituent of diatomite frustules.\textsuperscript{2}}](image)

**Notes and references**

1. I. Ruggiero, M. Terracciano, N. M. Martucci, L. De Stefano, N. Migliaccio, R. Taté, I. Rendina, P. Arcari, A. Lamberti and I. Rea, *Nanoscale Res. Lett.*, 2014, 9, 1–7.
2. I. Rea, N. M. Martucci, L. De Stefano, I. Ruggiero, M. Terracciano, P. Dardano, N. Migliaccio, P. Arcari, R. Taté, I. Rendina, A. Lamberti, *Biochim. Biophys. Acta*, 2014, 1840, 3393–3403.