Electronic Supplementary Information (ESI)

Surface Functionalisation of Diatoms with Dopamine Modified Iron Oxide Nanoparticles: Toward Magneticaly Guided Drug Microcarriers with Biologically Derived Morphologies

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Purification and characterisation of diatom structures

Diatomaceous earth (DE) rocks were obtained from Mount Sylvia Pty. Ltd. diatomite mining company, Queensland, Australia. Diatom samples in the form of ultra-pure silica microcapsules (frustules) were prepared from raw DE material using several purification steps which includes acid treatment (sulphuric acid), filtration and particle size separation.[1-2] A series of characterization techniques were applied to confirm their properties which are essential for drug delivery applications. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDAX)) was performed using Philips XL-30 and FIB Helios Nanolab 600 from FEI Company. SEM images in **Figure S1a-c** show a typical structure and morphology of *Aulacoseira specie,* fresh-water diatom which was a major specie (> 90%) in our samples. Silicon (Si) and oxygen (O) are the main elements present in the purified DE particles (**Figure S1d)** as recourse to energy dispersive spectroscopy (EDAX) analysis showed. Particular attention during purification of DE sample was paid to produce isolated diatom frustules with minor aggregations and without fractured parts. The light microscopy and particle size measurements were used as simple characterization methods to probe the quality of prepared of diatoms from their water suspensions. The particle size distribution graph of diatom solution (1% w/v) obtained by Malvern Mastersizer X, (**Figures S2)** shows only one peak with maximum of 10-20 μm and confirms the presence of isolated frustules with minor aggregation and fractured parts. X-ray diffraction pattern (XRD) is recorded by Panalytical PW 3040 and shows that diatom's silica is amorphous **(Figure S3).** The zeta potentials curves of diatom microparticles (4 wt.% diatoms in KNO_3 solution with concentrations 10^{-3}M, 10^{-2}M, $5x10^{-2}$M and 10^{-1}M) were determined from dynamic mobility of particles by an Acoustosizer II (Colloidal Dynamics Inc., Australia), which measures the electrokinetic sonic amplitude (ESA) of particles in dispersion. Zeta potential curve in 10^{-2}M KNO_3 solution is shown in **Figure S4**. Nitrogen adsorption-desorption isotherms of diatoms were determined using an automatic ASAP 2010 Micromeritcs porosimeter. BET and surface area analysis was performed using analytical software for the adsorption data. A typical nitrogen adsorption-desorption isotherms of diatom sample is shown in **Figure S5**.

Surface modifications and characterisations

Dopamine modified iron oxide nanoparticles (DOPA/ Fe_3O_4) were prepared using a previously described procedure.[3] Briefly, $Fe(acac)_3$ was reacted with benzyl alcohol in a Teflon cup, which was then carefully placed and tightly sealed in a steel container. The reaction was left to proceed in a furnace preheated to 175ºC and kept at 175ºC for 48 h. A ligand exchange/phase transfer procedure with the ene-diol ligand

2-(3,4-dihydroxyphenyl)ethylamine (dopamine) was used to cap the nanoparticles and transfer them to an aqueous phase. The synthesized nanoparticle suspension in benzyl alcohol was mixed with an aqueous dopamine solution. After sonication was applied to the mixture, the nanoparticles transferred from the organic phase to the aqueous layer. The aqueous phase was collected and washed three times using high speed centrifugation to remove excess dopamine. The size of prepared magnetite nanoparticles was about 20 nm as confirmed by particle size measurement (Nanosizer, Malvern), TEM and SEM (XL-30, Philips).[3] The zeta potential of the dopamine caped nanoparticles was measured using a Zetasizer Nano ZS (Malvern Instruments) equipped with a 633nm He-Ne laser. Measurements were performed on dilute nanoparticle suspensions in MilliQ water. Dopamine-capped nanoparticles were cationic with an average zeta potential of 45.2 mV.

The functionalisation of the diatom samples with the dopamine modified iron-oxide (DOPA/ Fe_3O_4/ diatom was performed by incubation of the diatom solution for 24-40 hours under gentle stirring. After wash with water (5 time), a dense layer of cationic magnetic nanoparticles assembled onto the anionic diatoms surface through electrostatic interactions. The magnetic composite were stable in water and physiological buffer for several days. The prepared DOPA / Fe_3O_4 /diatom composite were kept in water or ethanol solution and characterised by light microscopy, SEM, EDAX and particle size measurements.

High resolution SEM images of modified diatom structures are shown in **Figure S6 a-b** which confirms conformal and high density deposition of DOPA/ Fe_3O_4 nanoparticles on diatom surface and inside of pores. SEM image of nanoparticles deposited on silicon wafer used as control is presented in **Figure S6c.** The experiment of controlled moving magnetized diatoms on the surface under magnetic field was conducted using magnetic stirring bars and captured by video camera (data not shown)

Fluorescence labeling was performed by treating the DOPA/ Fe_3O_4 / diatom with 200 mg/mL of fluorescein isothiocyanate (FITC) in phosphate buffered saline (PBS) buffer (pH 7.4) for min 2 h at room temperature. The unbound dye was washed off by rinsing with large amounts of PBS buffer. Series of confocal images (**Figures S7)** were captured with laser scanning confocal microscope C1-Z (Nikon) equipped with three solid lasers, Sapphire 488 nm to reconstruct 3-d image. Samples were imaged with a single channel (PMT tube) using objectives 20X and 40X. Nikon-C1-Z and NIS-Elements AR.3 softwares for advanced post imaging quantification were used to perform z-sectioning, 3-D image analysis and reconstruction.

Drug loading and drug release characterization

The drug (indomethacin) solution (0.012 g/mL in ethanol) was mixed with solution of DOPA/ Fe_3O_4 modified diatoms (concentration 0.01 g/mL in ethanol) over 30 minutes and then ethanol was evaporated under vaccum condition. Unmodified diatom samples were used for control experiment. The release of 60 mg drug loaded DE (containing 16.85 mg of pure drug) was performed in 5 mL 10 mM phosphate buffer solution (PBS) pH = 7.2 at 37 ^{o}C. 0.5 mL of sample was withdrawn and immediately replaced by the same fresh buffer at specific time intervals. For comparison, equivalent amount of the pure drug 16.85 mg was used for the release under the same conditions. 2.5 ml of the same phosphate buffer was added and analysed for the drug content by Cary 5 UV-Vis Spectrophotometer. Drug content was determined using calibration curve of the pure drug solutions in 10 mM phosphate buffer pH= 7.2 at 320 nm. Sampling was performed every 5 to 10 min

during the first 6 h. From the second day onwards, measurement was taken twice per day for a total of two weeks or more until final completion. The amount of loaded drug inside of diatom microcapsules was quantified using thermo-gravimetric analysis (TGA). More information about drug loaded on diatoms was obtained using SEM, EDAX, XRD and nitrogen adsorption (surface area analysis).

To check our hypothesis that the rate of drug release plays an important role in the second slow release phase we investigated the release of the equivalent amount of the pure drug under the same conditions. It was determined that the complete release of the pure drug occurs in 3 h (**Fig. S8**). The initial drug release of pure drug is very similar to the first 3 hours of drug release from diatoms (Fig. 2b). Thus, drug solubilisation as a mechanism for the slow indomethacin release can be ruled out; slow release phase is rather a consequence of encapsulation, i.e. drug released from the pores and internal structures. The release kinetics can be described in two phases for pure and three phases for the encapsulated drug. Release of the pure drug within the first 8 hours (clearly visible in Figure 2b) can be regarded as a burst, whereas the release patterns from 8 h up to completed release, present a controlled release and can be fitted into both first and zero order models[4-5] (**Table 1**). The fitting parameters show that zero-order kinetics accounts best for the experimental data for the slow release phase of the encapsulated drug. Zero order release kinetics is a desirable release pattern, because a uniform amount of drug is released in each time unit.[6] Zero order release kinetics, typical for reservoir transdermal delivery systems, is rarely reported for porous devices.[5] Our results demonstrate that it is possible to achieve zero order release kinetics from diatom silica for implant applications.

References:

1. Y. Yu, J.Addai-Mensah, D. Losic, *Chemeca Proceedings* 2009 (paper 0108) , Perth, Australia
2. Y. Yu, I.R. Neering, J. Addai-Mensah, D. Losic, *16th AINSE Conference on Nuclear and Complementary Techniques of Analysis, Proceedings*, 2009**,** Sydney, Australia, Australia (accepted, in press).
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5. P. Costa, J. M. Sausa-Lobo, *Europ. J. Pharm. Sci.*, 2001, **13**, 123
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Table 1. Controlled release parameters for pure and encapsulated drug (indomethacin)

Sample	**First-order release**		**Zero-order release**	
	r	$k_1 \times 10^2$ (h^{-1})	r	k_0 (h^{-1})
Pure drug - Phase I (up to 0.5 h)	0.90	360	0.98	137.3
Pure drug - Phase II (0.5-3 h)	0.99	15.8	0.99	13.5
Encapsulated drug - Phase I (up to 8 h)	0.90	11.3	0.85	7.7
Encapsulated drug - Phase II (8-144h)	0.98	0.22	0.99	0.17
Encapsulated drug - Phase III (144-288h)	0.91	0.05	0.98	0.04

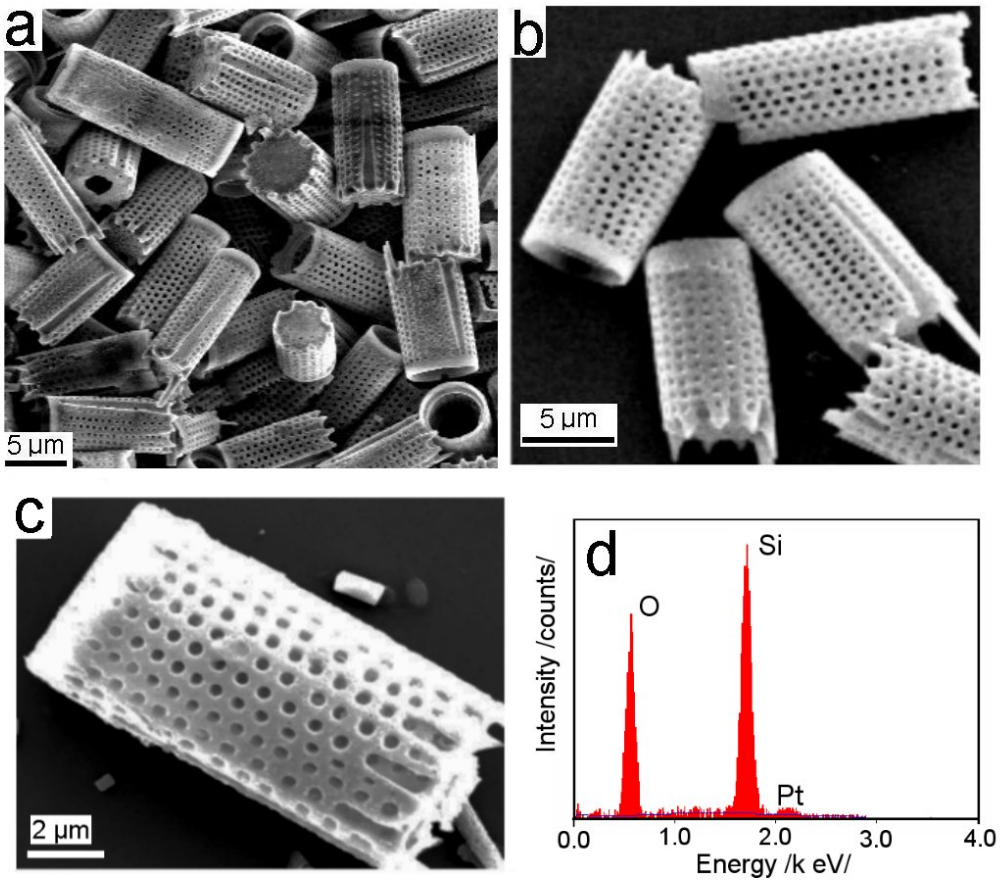

Figure S 1. a-c) SEM images of purified diatom frustules were *Aulacoseira sp.* was identified as major diatom specie. EDAX spectrum confirms that diatom structure is composed from silica.

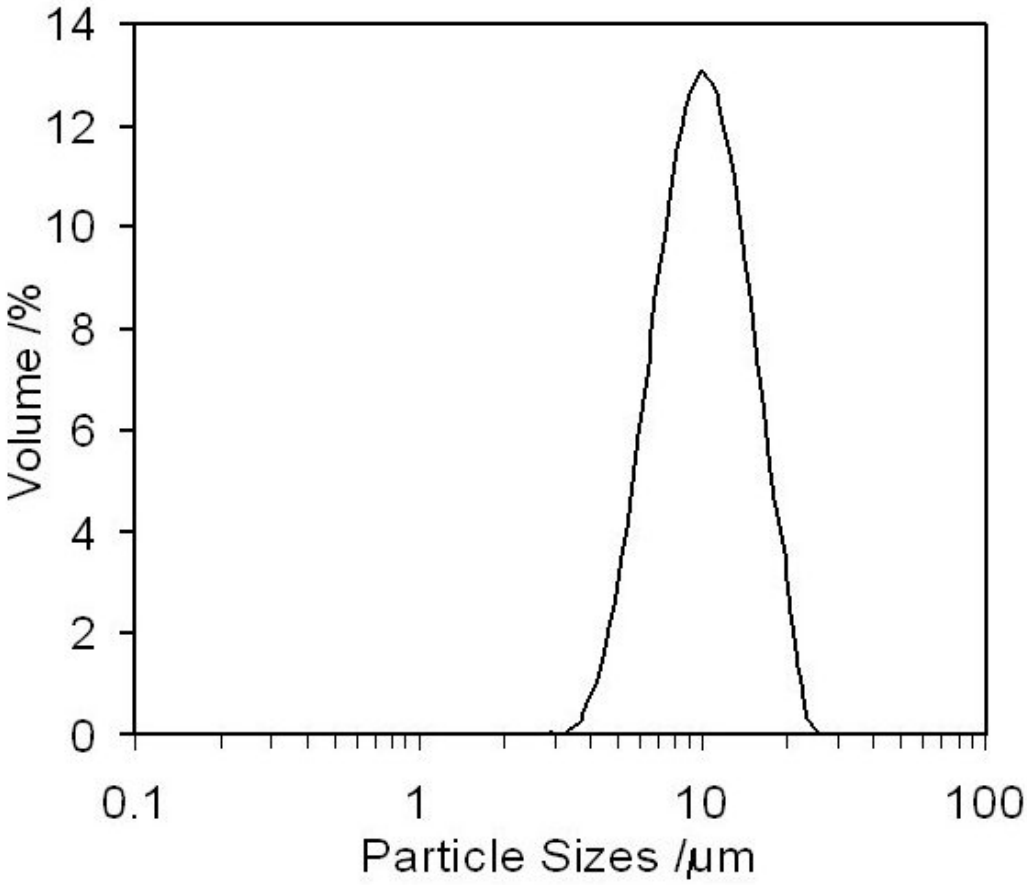

Figure S2. The particle size distribution of diatoms in water suspension showing diatoms as isolated microparticles (10-20 μm) without of diatom fractures and aggregations.

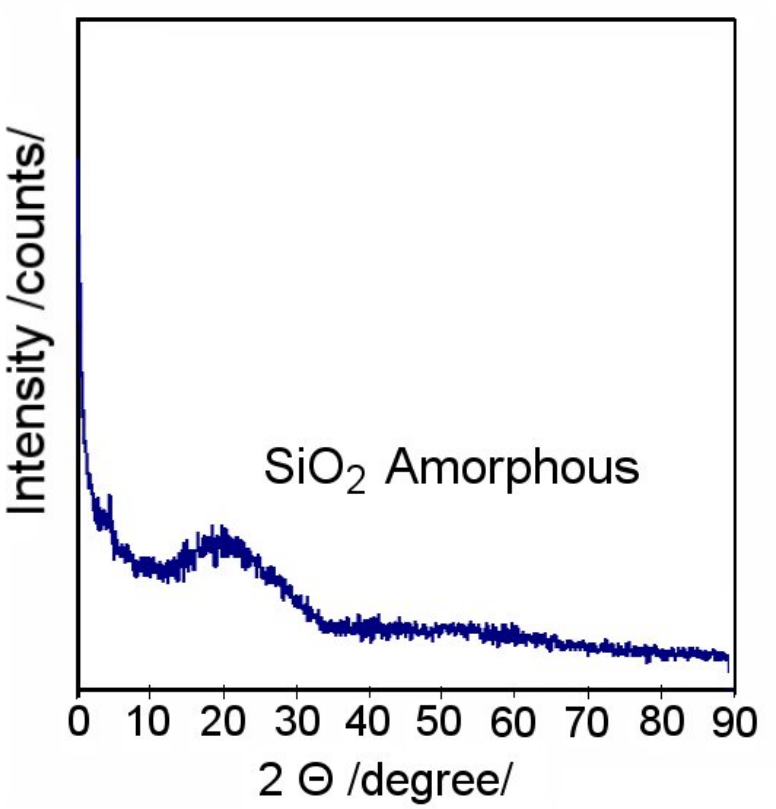

Figure S3. XRD pattern of unmodified diatoms confirms that diatom silica is composed from amorphous silica

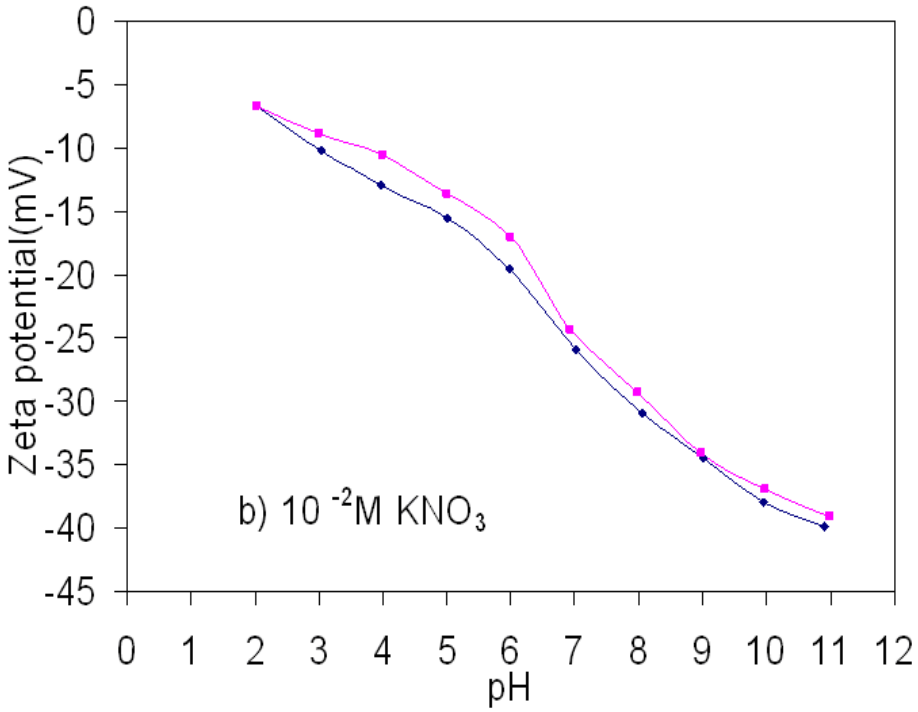

Figure S 4. Zeta potential curve of DE at different pH obtained in 10^{-2} M KNO_3

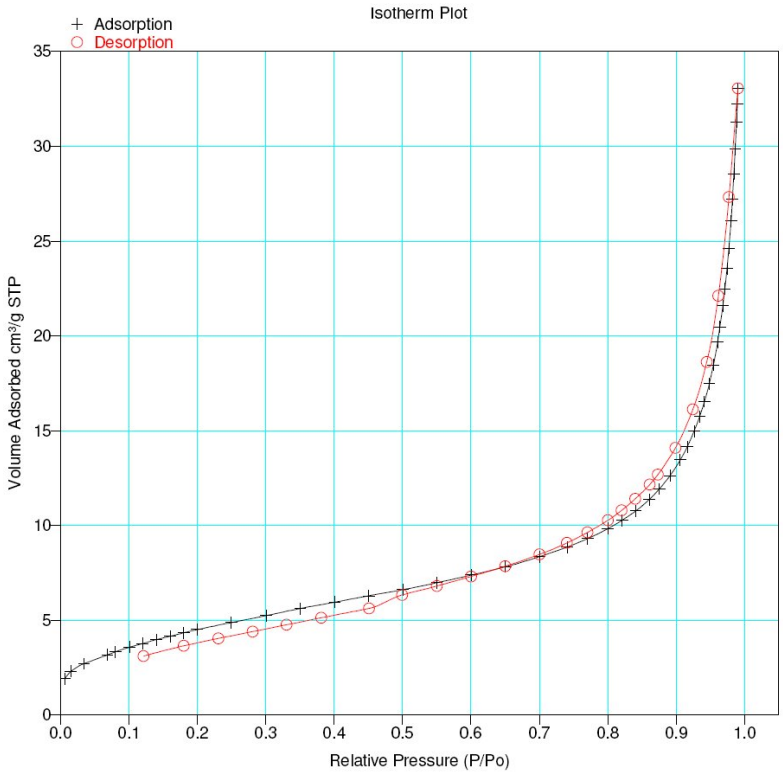

Figure S 5. Adsorption/desorption isotherm of diatom sample

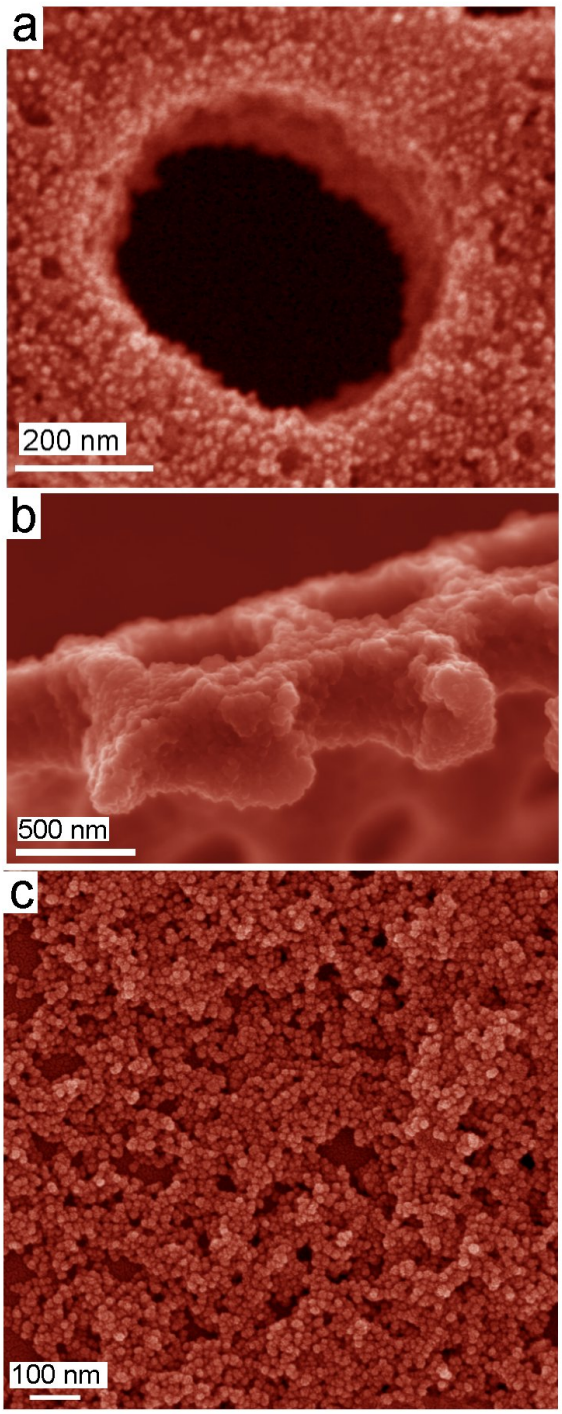

Figure S6. High resolution SEM images of diatom structure modified with dopamine/ iron oxide showing a) intact pore structure (top view) and b) broken pore structures that confirms conformal coating of nanoparticles inside of pores. c) SEM image of dopamine/ iron oxide nanoparticles deposited on silicon waver (control sample)

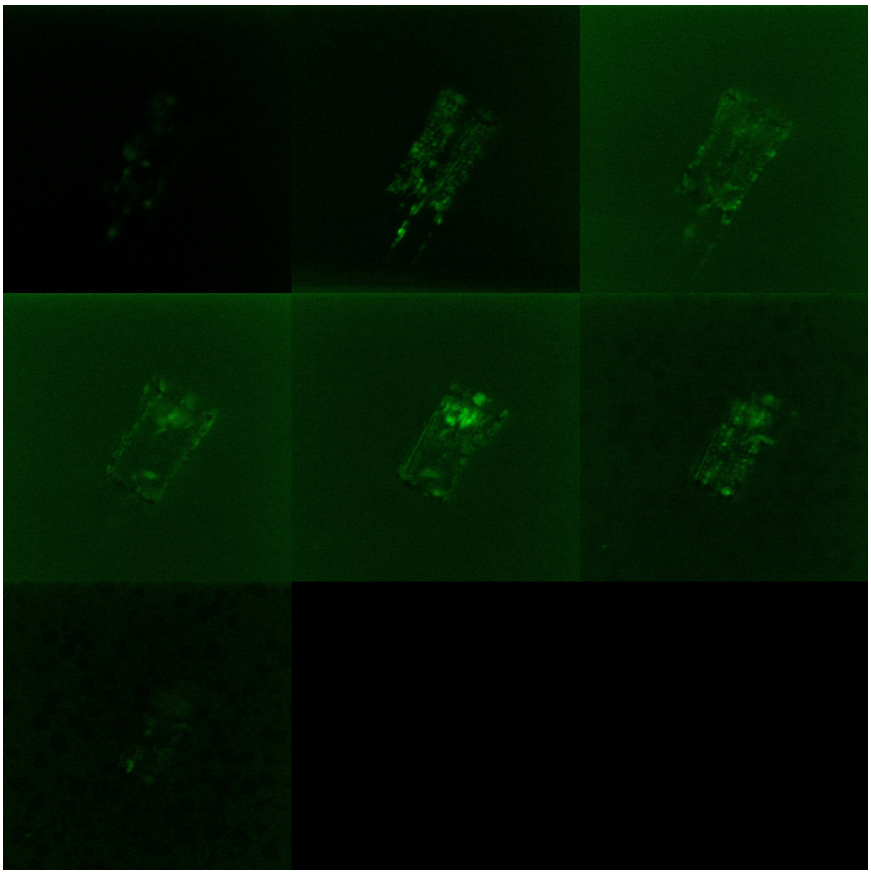

Figure S7. Series of confocal images of fluoro probe labelled diatoms prepared by immobilization of fluorescein isothiocyanate (FITC) by reaction with amino groups of dopamine on DOPA/ Fe_3O_4 nanoparticles modified surface

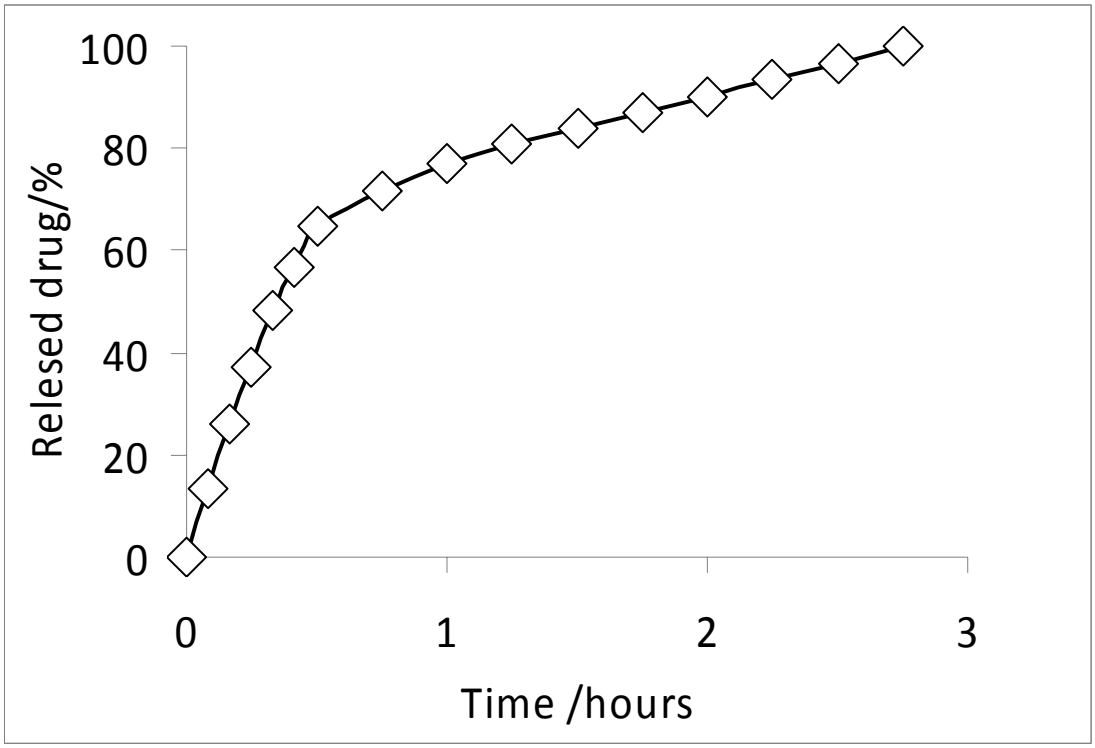

Figure S8. . The drug release of pure drug (indomethacin) from control sample