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A ceramic-based anticancer drug delivery system to treat breast cancer

Ahmed El-Ghannam · Krista Ricci · Ahmed Malkawi · Kiarash Jahed · Kumar Vedantham · Heather Wyan · Lauren D. Allen · Didier Dréau

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Abstract Drug delivery systems offer the advantage of sustained targeted release with minimal side effect. In the present study, the therapeutic efficacy of a porous silica–calcium phosphate nanocomposite (SCPC) as a new delivery system for 5-Fluorouracil (5-FU) was evaluated in vitro and in vivo. In vitro studies showed that two formulations; SCPC50/5-FU and SCPC75/5-FU hybrids were very cytotoxic for 4T1 mammary tumor cells. In contrast, control SCPCs without drug did not show any measurable toxic effect. Release kinetics studies showed that SCPC75/5-FU hybrid provided a burst release of 5-FU in the first 24 h followed by a sustained release of a therapeutic dose (30.7 μg/day) of the drug for up to 32 days. Moreover, subcutaneous implantation of SCPC75/5-FU hybrid disk in an immunocompetent murine model of breast cancer stopped 4T1 tumor growth. Blood analyses showed comparable concentrations of Ca, P and Si in animals implanted with or without SCPC75 disks. These results strongly suggest that SCPC/5-FU hybrids can provide an effective treatment for solid tumors with minimal side effects.

A. El-Ghannam (✉) · K. Vedantham
Department of Mechanical Engineering and Engineering Science, DCH 177, The University of North Carolina at Charlotte, 9201 University City Blvd., Charlotte, NC 28223, USA
e-mail: arelgha@uncc.edu

K. Ricci · H. Wyan · L. D. Allen · D. Dréau
Cellular and Molecular Biology Division, Department of Biology, The University of North Carolina at Charlotte, Charlotte, NC 28223, USA

A. Malkawi
Department of Pharmacology, School of Pharmacy, University of Kentucky, 740 South Limestone Street, Lexington, KY 40536-0001, USA

K. Jahed
Department of Medicine, Medical School, University of Louisville, Louisville, KY 40292, USA

1 Introduction

The standard care for the treatment of solid tumors remains surgery followed by radiation and/or systemic chemotherapy [1]. The systemic administration of the anticancer drug and its circulation within the blood exposes not just tumor cells but all other body organs to the toxicity of the drug. Another limitation of the chemotherapy is the high anticancer drug dose that is selected based on the blood volume in the body. Elevated drug concentration must be injected in order for the drug concentration in the blood to reach a therapeutic level. These limitations of the systemic drug administration compromise the patients' quality of life [1, 2], and result in adverse side effects including severe immune suppression, nephrotoxicity, and cardiotoxicity [3, 4]. Another critical limitation of the effectiveness of systemic drug administration in treating solid-tumor is the inability of the drug to reach and penetrate neoplastic cells distant from tumor vessels [5]. Furthermore, the bioavailability of these drugs is further limited by their short half-life [6].

Drug delivery systems can provide a controlled release of therapeutic doses of the drug directly onto the site of tumor, and therefore, have been tested as an alternative approach to systemic drug administration. The advantages of the drug delivery system are twofold: (1) extended and continuous localized drug delivery and (2) high drug concentrations within the tumor microenvironment and low concentrations of the drug in the blood stream and other organs. The successful application of drug delivery system

in cancer treatment is hindered by multiple material-related factors including the poor control over the drug release and the host immune reaction against the delivery system [7–10]. Various drug delivery systems have been tested including microspheres [11], polymeric micelles [12], as well as pastes loaded with chemotherapeutic drugs including paclitaxel, doxorubicin or cisplatin [13]. These systems were in most cases associated with promising data in vitro and in animal models of solid tumor progression [14]. Liposomes have been used with some success as carriers for anti-neoplastic drugs [15]. More recently, solid lipid nano-particles loaded with tamoxifen demonstrated a strong anti-proliferative activity on human breast cancer MCF7 cells in vitro [16]. Polymeric materials based on polylactic acid have been investigated but the acidic by-products of polylactic acid degradation have been shown to cause local acidity detrimental to wound healing [10]. Similarly, carbon nano-tubes filled with carboplatin showed inhibition of bladder cancer cell growth in vitro [17]. Although anti-tumor efficacy of the drug alone and paclitaxel loaded chitosan micelles was similar, the latter improved the drug bioavailability and significantly reduced the toxicity [18, 19].

Calcium-phosphate ceramics have been tested as a delivery vehicle for steroids, antibiotics, proteins, hormones, anticoagulants and anticancer drugs [8]. The decrease in drug release associated with the formation of a fibrous capsule was compensated by the continuous biodegradation of the ceramic capsule [20]. Porous blocks of hydroxyapatite (HA) and tri-calcium phosphate (TCP) have been evaluated as sustained release system for anticancer drugs including cisplatin and methotrexate [21, 22]. In mice, the diffusion of cisplatin into blood, liver, kidney and brain was less than 10% of that of the implanted site and inhibited tumor growth significantly more than intravenous administration [22]. In vitro, release rates of anti-cancer drug- or hormone-loaded HA ceramic were related to their pore size and shape [21–25]. However, the wide application of HA, ALCAP and TCP as drug delivery system has been limited by either the slow or unpredictable high rate of degradation in tissue fluids. The limitations of the currently available delivery systems emphasize the need for a novel biodegradable drug delivery system.

The silica–calcium phosphate nanocomposite (SCPC) is a porous biodegradable scaffold with physicochemical characteristics that counteract the limitations of traditional calcium phosphate ceramics [26–28]. SCPC is principally composed of nano crystals made of β-$NaCaPO_4$ and α-cristobalite (SiO_2) solid solutions [29, 30]. SCPC has a unique porous structure with pore size range from 2 nm to 650 μm, which provides high surface area available for drug loading and subsequent controlled release [30]. While the nanopores can provide protective pockets for the adsorbed drug molecules, the micro pores facilitate continuous transport of the drug by fluid exchange from the bulk of the SCPC scaffold to the surrounding tissues/cells; securing maximum exposure of cancer cells to the released drug. The surface chemistry and charge of SCPC enhances the adsorption of significant quantities of proteins and drugs [29]. SCPC showed controlled dissolution kinetics mediated by material interaction with cells and tissue fluids [30]. The degradation rate can be controlled through modifications of the material structure and porosity.

In the present study, we demonstrate the ability of porous SCPC to provide a sustained release of therapeutic dose of 5-FU. The toxicity of released 5-FU on mammary cancer cells both in vitro and in vivo is reported.

2 Materials and methods

2.1 Preparation and characterization of the SCPC ceramic particles

SCPC50 and SCPC75 were prepared by thermal treatment at 800°C following procedures previously reported [27]. The physical and chemical characteristics of SCPC50 and SCPC75 are shown in Table 1. Particles in the size range 150–250 μm were obtained by grinding the ceramic in alumina mill and mechanical sifting using ASTM standard sieve set. Particles were immersed in simulated body fluid (SBF) for 24 h. The modification in surface chemistry of the material was analyzed using FTIR as previously reported [31]. Spectra were collected in the diffuse reflectance mode after 200 scans at 4 cm^{-1} resolution using a Thermo Nicolet Nexus 670 spectrometer (WI, USA).

2.2 Preparation of SCPC/5-FU hybrids

A 20 mg/ml solution of 5-Fluorouracil (5-FU) was prepared by direct dissolution of 720 mg of 5-FU powder (Sigma, #F6627—10 g, 097k1352, FW 130.08 (99% TLC)) in 36 ml of de-ionized water in a dry bath incubator at 80°C until complete dissolution of the drug.

To adsorb 5-FU onto various formulations of surface modified SCPC, 0.2 g of each type of SCPC (SCPC50 and

Table 1 Physicochemical features of SCPC50 and SCPC75 including chemical composition (%) and particle size (μm) of the SCPC types

SCPC type	Chemical composition (%)				Particle size (μm)
	SiO_2	P_2O_5	CaO	Na_2O	
SCPC50	19.5	20.3	40.7	19.5	150–250
SCPC75	32.9	11.4	22.8	32.9	150–250

SCPC75) were used as follows: SCPC samples ($n = 5$ for each type) were either immersed separately in 2 ml 5-FU solution or in 2 ml of de-ionized water to serve as a control. The 24-well plates containing the samples were sealed to avoid evaporation and incubated at 37°C on an orbital shaker (75 rpm) for 24 h. The supernatant in different wells were removed and the particles were collected, dried at room temperature for 48 h. The amount of 5-FU adsorbed onto each SCPC formulation was calculated based on the difference between the 5-FU concentrations in the immersing solution before and after ceramic immersion.

2.3 HPLC determination of 5-FU concentrations

Concentrations of 5-FU in the immersion solution as well as that released from 5-FU/SCPC hybrids into PBS were determined using HPLC (Agilent Technologies, Santa Clara CA). The HPLC system consisted of Agilent's 1200 series auto sampler and quaternary pump, a Shodex Asahipak NH2P—50 2D column (5 μm, 150 mm × 2 mm i.d. (Shodex, New York, NY) and operated using an isocratic mobile phase with a column flush. Mobile phase solvent A consisted of 0.1% (v/v) formic acid in ACN while mobile phase B consisted of 0.1% (v/v) formic acid in nano-pure DI water. The initial mobile phase composition was 97% solvent A and 3% solvent B pumped at a flow rate of 0.2 ml/min for 5.0 min. After 5.0–5.1 min, solvent B was increased linearly from 3 to 60%, and the flow rate was increased from 0.2 to 0.4 ml/min. These settings were held for 1.5 min. From 6.6 to 7.6 min, solvent B was decreased to 0.0%. From 7.6 to 9.0 min, solvent B was increased back to 3% and flow rate was reduced to 0.2 ml/min and held for 6 min to establish column equilibration conditions, after which the next sample was injected. The overall run time was 15 min per sample. Briefly, 5 μl solutions were diluted with 795 μl of a solution (v/v/v: 97:3:0.1) of acetonitrile: H_2O: Formic acid. Concentrations of 5-FU were expressed in μg/ml.

2.4 In vitro cytotoxicity of the SCPC/5-FU hybrids

Studies were performed to determine the in vitro toxicity of the formulations of SCPC/5-FU hybrids for tumor cells. 4T1 mammary murine tumor cells (ATCC, Mannassas, VA) were seeded and grown in tissue culture plates (1×10^4 cells/well in 96 well plates; 3×10^5 cells/well in 6 well plates) at 37°C, 5% CO_2 in DMEM media supplemented with 10% fetal calf serum. 24 h following seeding, 4T1 cells were incubated separately with SCPC50 and SCPC75 loaded with or without 5-FU for 96 h. Both SCPC particles and disks loaded with or without 5-FU were tested. Briefly, the cytotoxicity of each of the disk (SCPC50, SCPC75, SCPC50/5-FU, SCPC75/5-FU) against 4T1 cells was tested in triplicate in six well tissue culture plates. The cytotoxicity of SCPC50, SCPC75, SCPC5-/5-FU and SCPC75/5-FU particles against 4T1 cells was tested in 96 well plates in SCPC concentration ranging from 0 to 2 mg/well.

In addition, we analyzed the cytotoxicity of the released drug from SCPC50/5-FU and SCPC75/5-FU. Particles of SCPC50/5-FU and SCPC75/5-FU hybrids as well as of control SCPC50 and SCPC75 without 5-FU were immersed separately in 4 ml protein-free DMEM tissue culture medium at 37°C for 17 h. The supernatant was collected, sterilized by filtration, and used to measure the concentration and toxicity of the released 5-FU on 4T1 mammary tumor cells (ATCC, Mannassas, VA).

In all in vitro assays, the number of viable cells was determined using sulfo-rhodamine B (SRB) stain as described previously [32]. Briefly, the SRB was solubilized in 10 mM Tris and the optical density was measured at 565 nm using a plate reader (μQuant, Biotek, Winooski, VT). The number of viable cells in each well was calculated using a standard curve for 4T1 cells (range: 0–50,000 cells). Average IC90 values were determined using best fitted curve of the growth inhibition percentage versus 5-FU concentrations of at least three independent experiments.

2.5 Long-term release kinetics of 5-FU

SCPC75 samples were immersed in 5-FU aqueous solution under the same experimental conditions as explained earlier. After 24 h, the ceramic particles were removed and dried at 37°C. The supernatant was collected and kept in the freezer before using it to determine the concentration of 5-FU as described above. To analyze the 5-FU release kinetics, SCPC75 particles loaded with and without 5-FU were immersed in 25 ml of PBS and placed on an orbital shaker at 100 rpm at 37°C. At the following time points: 24, 48, 96 and every 48 h thereafter until 192 h, and every 96 h thereafter up to 796 h, 2 ml from the immersing solution was collected. The immersing solution was refreshed by 2 ml of fresh PBS to keep the volume constant at each collection time point. The samples were kept at −20°C until use for the measurement of the concentrations of the released 5-FU.

2.6 SCPC dissolution

Si release kinetics from SCPC75/5-FU into the PBS was taken as a representative of the ceramic dissolution. The

concentrations of Si ions in the PBS incubated with the SCPC75/5-FU samples were determined at the time of evaluating the concentration of the released drug. Inductively coupled plasma-optical emission spectrometer (ICP-OES) (Optima DV 2000, Perkin-Elmer, Wellesley, MA) were used for Si measurements employing the same conditions reported previously [33].

2.7 In vivo effects of SCPC/5-FU hybrids on tumor growth and serum calcium, phosphorus, and silica concentrations

Previous reports published by our group have demonstrated enhanced dissolution of SCPC with the increase in the silica content of the material [28]. In addition, enhanced drug release was associated with SCPC containing higher silica concentration [34]. Therefore, the effects of SCPC75/5-FU hybrids on tumor growth, and calcium, phosphorus, and silica blood concentrations in a modified murine immunocompetent breast tumor model were determined. 4T1 were selected because these mammary tumor cells have an aggressive behavior similar to breast cancer cells developing metastases [35]. Male mice (Balb/c, Jackson Laboratories, Bar Harbor, Maine) following an acclimatization period were injected subcutaneously with the syngeneic 2×10^5 4T1 mammary tumor cells in the mammary fat pad. One week following tumor implantation (tumor masses were palpable in all animals), disks (1.5 mm (thickness) × 5 mm (diameter), ~60 mg) of compacted SCPC75 loaded with no or 5-FU and sterilized by UV exposure (90 min, 302 nm), were implanted subcutaneously 0.1–0.3 mm away from the tumor mass. The SCPC75/5-FU hybrids were loaded with 40.2 ± 0.001 μg/mg of SCPC75. Animal experiments were approved by Institutional Animal Care and Use Committee at the University of North Carolina—Charlotte and supervised by a veterinarian. Tumor volume was monitored twice weekly by measuring the length and the width of the tumor. The tumor volume was calculated as described previously [35, 36]. At euthanasia, tumor mass was dissected and weighed. Blood was also collected and the concentrations of silica, phosphorus and calcium in the plasma were determined using ICP-OES.

2.8 Statistical analyses

All data are reported as means ± SEM. Differences in the parameters measured were assessed by ANOVA. Parameters were further tested using the Fisher–Newman–Keuls post-hoc test. Significance was set a priori to $P < 0.05$.

3 Results

3.1 SCPC drug loading and alteration of SCPC surfaces

The amounts of 5-FU adsorbed onto each SCPC ceramic after a 24 h immersion in 20 mg/ml 5-FU solutions were 37.9 ± 0.001 mg of 5-FU per g of SCPC50 and 40.2 ± 0.001 mg of 5-FU per g of SCPC75, respectively. No change in peak positions between the control SCPCs (no 5-FU), either SCPC50 (Fig. 1a) or SCPC75 (Fig. 1b) and SCPC50/5-FU and SCPC75/5-FU powders were detected on the FTIR spectra. The orthophosphates peak corresponding to P–O stretch appeared at 559, 586, 609, 1,001, and 1,230 cm^{-1}, while the silica peaks corresponding to Si–O–Si, and Si–O appeared at 791, 1,060 and 958 cm^{-1}, respectively. The absence of shift in peak position indicates that the presence of 5-FU in SCPC particles does not alter the surface chemistry of hydroxyapatite, which could have an effect on release kinetics otherwise. The decrease in the magnitude of the peak intensity for 5-FU loaded samples compared to the control could be attributed to the interference of the drug molecule with signal from the SCPC components.

3.2 Bioactivity of SCPC/5-FU hybrids

To test the toxicity and bioactivity of SCPC/5-FU hybrids, the cytotoxic effects of SCPC50 and SCPC75 samples loaded without or with 5-FU on 4T1 mammary murine tumor cells in vitro were determined (Fig. 2). The numbers of 4T1 cells grown in the presence of increasing amounts (0–2 mg) of either SCPC50 (Fig. 2a) or SCPC75 (Fig. 2b) particles remained similar to the number of 4T1 cells grown in media only (n.s., Fig. 2). In contrast, incubations with either SCPC50/5-FU and SCPC75/5-FU hybrid particles (Fig. 2) were associated with drastic significant decreases in cell numbers (Fig. 2, $P < 0.001$ for SCPC50/5-FU and SCPC75/5-FU amounts above 0.02 mg). The bioactivity of SCPC50 and SCPC75 disks coated without or with 5-FU (diameter: 0.5 cm and thickness 0.2 cm, ~60 mg each) was also determined. Growth inhibition associated with the incubation with SCPC50/5-FU or SCPC75/5-FU hybrids were not significantly different (Fig. 2c). The average IC90 values were 10.5 and 7.14 μg/ml for SCPC50/5-FU and SCPC75/5-FU, respectively (n.s.). Although variations were observed in the cytotoxic responses of 4T1 cells after a 4-day incubation period with SCPC50 or SCPC75 without 5-FU coating, the cell numbers indicative of cytotoxicity were not significantly different from the control cell cultures ($793.8 \times 10^3 \pm 14.2 \times 10^3$ cells,

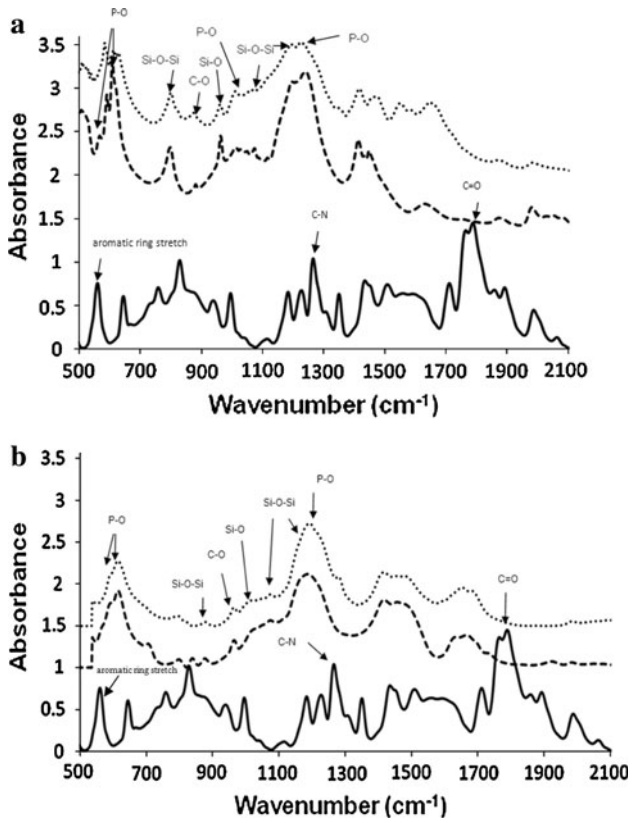

Fig. 1 FTIR spectra for **a** 5-FU (*solid line*), SCPC50 without (*dashed line*) or with 5-FU coating (*dotted line*) and **b** 5-FU (*solid line*), SCPC75 without (*dashed line*) or with 5-FU coating (*dotted line*). The bands at 559, 586, 609, 1,001, and 1,230 cm^{-1} correspond to P–O stretch. The peaks at 791, and 1,060 cm^{-1} represent Si–O–Si bonds, while the peak at 958 cm^{-1} represent Si–O bond, and the peak at 864 cm^{-1} denotes the C–O stretch, respectively. 5-FU reveals peaks at 557 cm^{-1} corresponding to aromatic stretch, 1,260 and 1,760 cm^{-1} representing C–N, and C=O stretch respectively

$419.8 \times 10^3 \pm 1.3 \times 10^3$ cells, and $68.2 \times 10^3 \pm 44.2 \times 10^3$ cells for no SCPC, SCPC50, and SCPC75, respectively, $P > 0.05$, Fig. 3). In contrast, both SCPC50/5-FU and SCPC75/5-FU disks were extremely cytotoxic to 4T1 cells ($1.8 \times 10^3 \pm 0.2 \times 10^3$ cells and $4.4 \times 10^3 \pm 0.6 \times 10^3$ cells compared to $19.8 \times 10^3 \pm 1.3 \times 10^3$ cells, and $68.2 \times 10^3 \pm 44.2 \times 10^3$ cells for SCPC50, and SCPC75, respectively, Fig. 3, $P < 0.01$).

3.3 SCPC dissolution and long-term release of 5-FU from SCPC75/5-FU hybrids

ICP analyses showed that the Si concentration in PBS incubated 24 h with SCPC75/5-FU was 41.4 ± 3.1 mg/l (14.28 ± 1.78%) as shown in Fig. 4a. The Si release from SCPC into the PBS increased from day 1 to day 6 with the Si concentration reaching 122.4 ± 9.1 mg/l (48 ± 3.57%) and the cumulative release of Si remained minimal and stable afterward for up to 32 days (7 ± 3.1 mg/l, Fig. 4a).

Measurements of 5-FU concentrations before and after SCPC75 immersion showed that 27.6 mg/g of 5-FU was adsorbed onto the SCPC75 surface. Release kinetics studies showed that there is an initial burst release of about 1.3 mg or 4.7% of the total amount of adsorbed drug after 24 h of immersion followed by a sustained release of the drug up to 33 days. From day 4 to 32, a steady release of 5-FU was observed (Fig. 4b). On average 30.7 μg/ml of 5-FU was released. The decrease in the 5-FU release followed a slope from 36.5 ± 5.4 μg/ml on day 4 to 24.8 ± 3.2 μg/ml on day 32. A strong inverse correlation between the Si release profile and the 5-FU release kinetics from SCPC75/5-FU was observed ($r = -0.84$, $P = 0.002$).

3.4 In vivo effects of 5-FU released from SCPC75/5-FU hybrids

Subcutaneous implantations of SCPC75/5-FU hybrids pre-immersed in 20 mg 5-FU/ml of PBS lead to decreased breast cancer mass volumes compared to animals implanted with control SCPC75 (no 5-FU) or sham treated animals in an immunocompetent mouse model (Fig. 5a). Growth of tumor in the animal implanted with SCPC75 alone followed an exponential growth curve starting around day 9. Similar tumor growth was observed in animals implanted with SCPC loaded with 5-FU up to day 16. From day 16 to day 27, the effects of 5-FU released by the SCPC75/5-FU hybrid was associated with limited to no growth (Fig. 5a). At euthanasia tumor mass were similar in sham-treated and SCPC without 5-FU implanted animals, whereas mice implanted with SCPC75/5-FU disks had significantly reduced tumor masses (86.5 ± 13.6 mg, 73.4 ± 3.7 mg and 38.6 ± 5.1, respectively, $P < 0.05$, Fig. 5b).

Measurements of the ionic concentrations of calcium, phosphorus and silicon in the plasma of the blood in animals treated with SCPC75/5-FU or SCPC75 were comparable to the corresponding concentrations in control animals without SCPC75 treatment (Fig. 5c). Regardless of the treatment group, plasma calcium and phosphorus concentrations averaged 37 ± 3 and 172 ± 12 mg/l, respectively (n.s., Fig. 5c). The plasma concentration of silica in all blood plasma samples was below the detection level (0.001 mg/l) of the ICP-OES analyzer (Fig. 5c).

4 Discussion

Results of the present study indicate that SCPC/5-FU hybrids can serve as an alternative treatment for breast

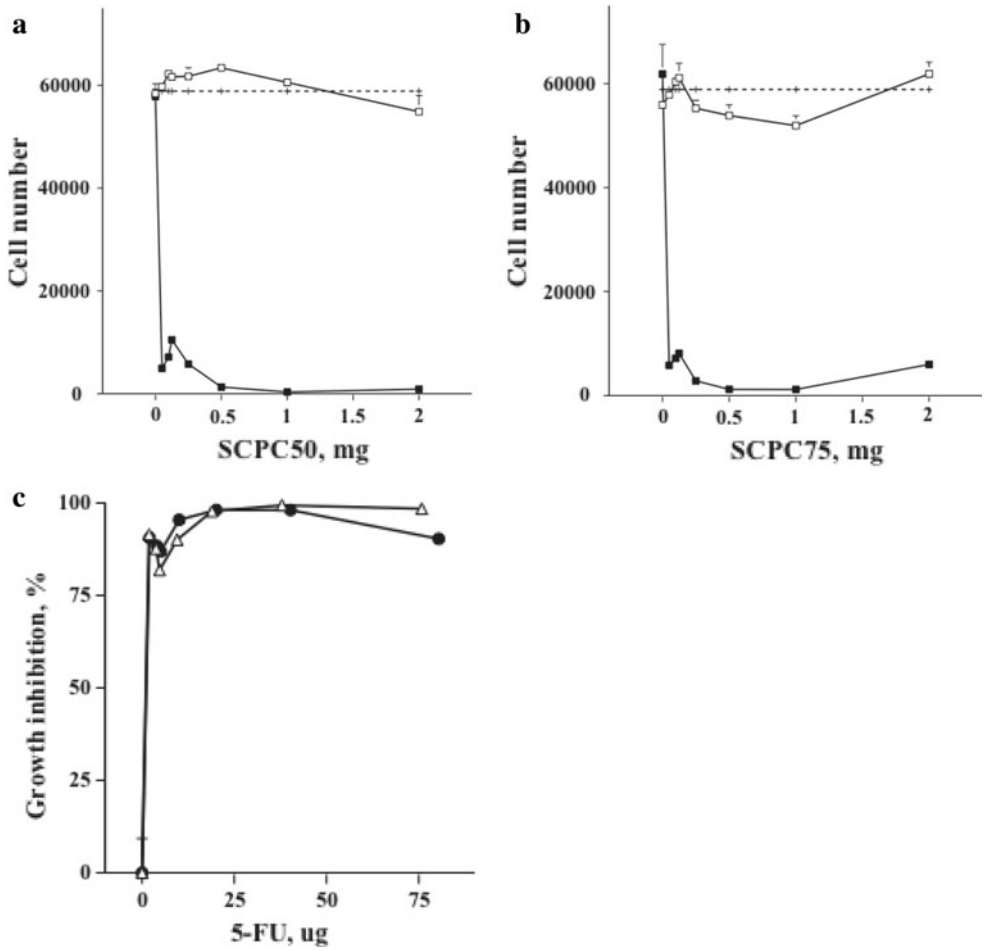

Fig. 2 Bioactivity and toxicity of increasing concentrations of **a** SCPC50, **b** SCPC75 particles loaded with 5-FU (*full squares*) or without 5-FU (*open squares*) for 4T1 mammary murine tumor cells in vitro and **c** 5-FU growth inhibition percentage of 4T1 cells. For **a** and **b**, 4T1 cells grown without SCPC or 5-FU (*dashed line*) served as control. Toxicity was measured by the number of viable 4T1 cells as determined by sulforhodamine B assays. 4T1 tumor cell numbers were significantly decreased in the presence of SCPC50/5-FU (**a**, $P < 0.001$) and SCPC75/5-FU (**b**, $P < 0.001$) hybrids regardless of the concentration range (0.02–2 mg) when compared to 4T1 cells cultured in media alone or in media with SCPC50 or SCPC75 without 5-FU. **c** Depicts the growth inhibition of 4T1 cells in function of the 5-FU concentration associated with incubation with SCPC50/5-FU (*open triangle*) and SCPC75/5-FU (*closed circle*) were determined. The growth inhibition associated with SCPC50/5-FU and SCPC75/5-FU were not statistically different

cancer. Both formulations SCPC50/5-FU and SCPC75/5-FU demonstrated a significant cytotoxic effect against the aggressive 4T1 mammary tumor cells in vitro. Moreover, when SCPC75/5-FU hybrids were implanted near malignant mammary tumors in mice, more than 75% reduction in the tumor volume was observed. The cytotoxic effect of SCPC/5-FU hybrids on tumor cells correlated well with the sustained release of therapeutic dose of 5-FU from the material in vitro. Although the drug release was associated with controlled dissolution of the SCPC ceramic components, the ICP analyses showed comparable values of Ca and P concentrations as well as complete absence of Si in the blood of animals injected with or without SCPC. These results strongly indicate that the use of SCPC/5-FU hybrids (or SCPC coated with other chemotherapy drugs) may greatly improve the treatment of breast cancer.

Effective treatment of cancer depends on adequate exposure of the tumor cells to the therapeutic agent for a duration long enough to achieve tumor cell eradication. In this regard, systemic drug administration is limited by the fluctuation of the blood flow and hence the drug concentration within the tumor [13, 37–39]. Moreover, the decrease in blood flow in tumor hypoxia and low rates of cell proliferation diminish the drug penetration into the tumor mass. This is particularly true when chemotherapeutic drugs targeting the cell division is used [40]. This incomplete distribution of many anticancer drugs in tumor tissue is currently compensated through the administration of higher doses of drugs [41]. The higher the drug concentration, the more severe the side effects associated with the drug toxicity. One approach to prevent the toxicity is to develop long-term local delivery device distributing the

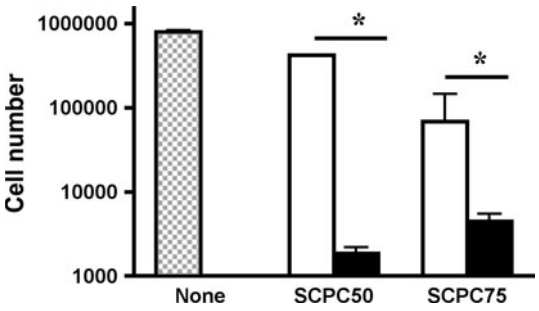

Fig. 3 Bioactivity and toxicity of SCPC50, and SCPC75 disks loaded without 5-FU (*open bars*) or with 5-FU (*black bars*) for 4T1 mammary murine tumor cells, in vitro. 4T1 cells grown without SCPC served as control (*dotted bar*). Toxicity was measured by the number of viable 4T1 cells as determined by sulforhodamine B assays. 4T1 tumor cell numbers were not significantly different in the control conditions, in the presence of SCPC50 or SCPC75 without 5-FU ($P > 0.05$). In contrast, the addition of SCPC50/5-FU or SCPC75/5-FU hybrids was associated with a drastic and significant decrease in cell numbers compared to SCPC50 and SCPC75, respectively ($P < 0.01$). The decrease in cell numbers following the addition of SCPC50/5-FU and SCPC75/5-FU hybrids were not statistically different

drug at high dose near the tumor with limited systemic drug circulation. Alternatively to local implantations, other targeting strategies have been studied. For example, the intravenous injection of iron-based nanoparticles loaded with doxorubicin was successfully magnetically guided to an ectopic hepatocellular carcinoma tumor mass in a mouse model [42]. The data from our study suggest that the SCPC75/5-FU hybrids provide a long-term local delivery of 5-FU in concentration sufficient to halt tumor progression.

The SCPC represents a new category of drug delivery vehicle with excellent ability to tailor its dissolution rate, porosity and physical form. Previous studies have showed that the SCPC is bioactive material that has minimal immunogenicity and controlled bio resorbability [43–45]. We have also demonstrated that SCPC can serve as a delivery system for vancomycin, gentamicin, and bone morphogenetic protein 2 [34, 43]. In the present study the adsorption of 5-FU was dependent on the Si/CaP ratios in SCPC with SCPC75 demonstrating a slightly higher drug loading capacity. The enhanced adsorption of 5-FU to SCPC75 is attributed to the high surface area of the silica-rich sample. Indeed, we have shown that, with increasing Si-contents in SCPC, the proportion of nano-pores (3 nm to 1 μm) increased thereby increasing adsorption sites by increasing the surface area [27, 29]. The pore size and shape were related to the increased release rates of anti-cancer drug- or hormone-loaded on hydroxyapatite ceramics [21–25].

Although the amount of 5-FU bound to SCPC50 in SCPC50/5-FU hybrids was marginally lower than the

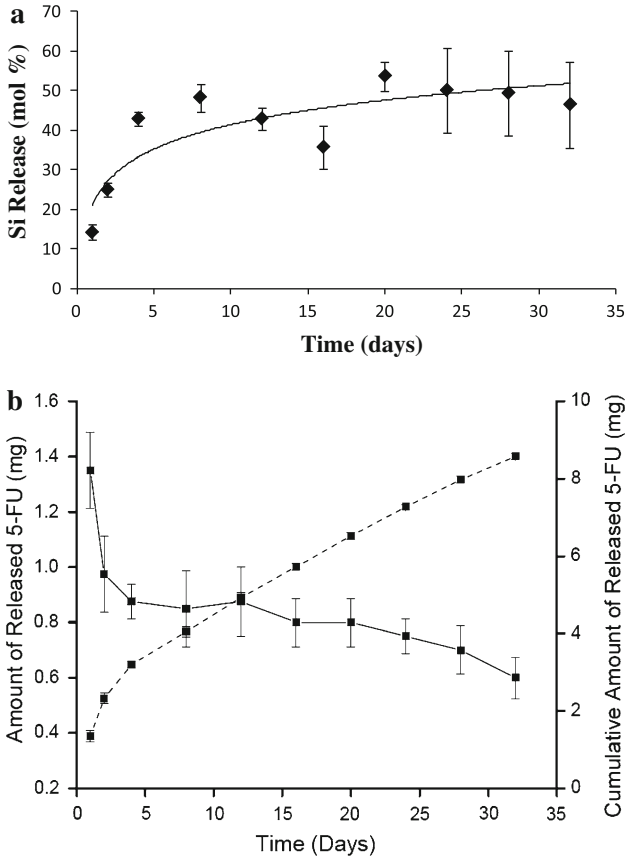

Fig. 4 Release of silicon and 5-FU from SCPC75/5-FU hybrids in vitro. **a** The release of Si was measured through ICP analyses and is presented as the Mole percent of total Si content in SCPC75 released in PBS from SCPC75/5-FU hybrid particles overtime. **b** The release of 5-FU was monitored overtime by HPLC measurements of 5-FU concentration in PBS incubated with SCPC75/5-FU hybrid particles. The 5-FU release obtained at each time point (*solid line*) and cumulative release (*dashed line*) are shown over a 33-day period

amount of 5-FU bound to SCPC75 in SCPC75/5-FU hybrids, both hybrids demonstrated significant inhibitory effects on the viability of 4T1 cells. These results suggest that regardless of the two SCPC formulations, SCPC/5-FU hybrids were able to release therapeutic dose of functionally active 5-FU. Indeed, when the 5-FU released from SCPC75/5-FU hybrid was incubated with 4T1 cells significant dose-dependent cytotoxic effect was observed [46]. Furthermore, HPLC analyses of in vitro release study showed that SCPC75/5-FU hybrid provided a long-term sustained release of 5-FU up to 32 days. In conjunction with the sustained release kinetics of 5-FU, the ICP analyses showed a controlled dissolution rate of the SCPC material. We have also shown that the dissolution of SCPC is followed by a simultaneous precipitation of a calcium phosphate layer onto the material surface [28]. After the precipitation of the calcium phosphate layer the sustained drug release is motivated by the concentration gradient from the SCPC75/5-FU hybrid to the physiological fluid.

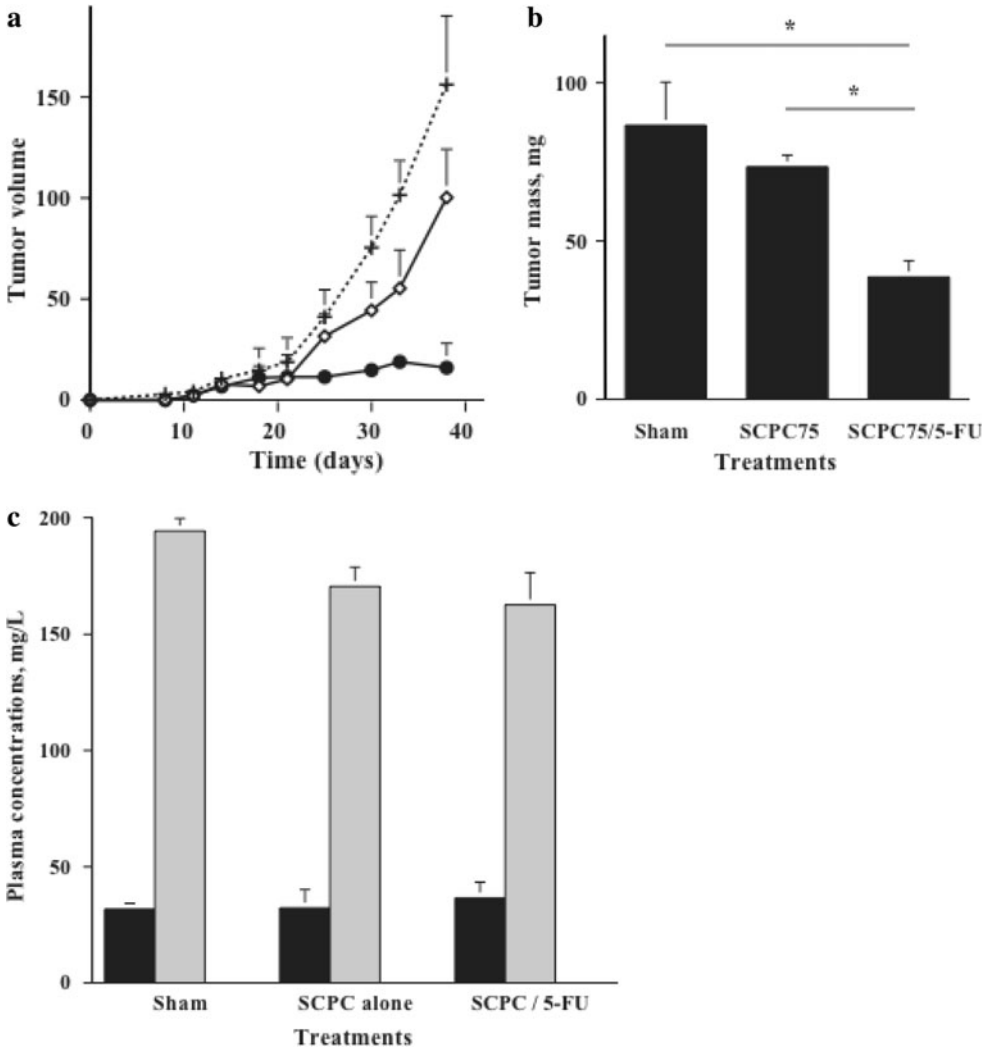

Fig. 5 In vivo bioactivity of SCPC75/5-FU hybrid disks. Following the injection of 4T1 murine mammary tumor cell injection in the mammary fat pad of Balb/c mice, SCPC75 or SCPC75/5-FU hybrid disks were implanted near the 4T1 cell injection site. **a** 4T1 cell tumor volume was evaluated overtime in sham treated animals (*dashed line*), animals implanted with SCPC75 alone (*open diamonds*) or SCPC75/5-FU hybrid (*dark circle*) disks. SCPC75-5-FU hybrid disks inhibited tumor volume following an initial growth period, while control SCPC75 without drug did not affect the normal exponential growth of the tumor observed in sham treated animals. **b** Tumor masses were measured at euthanasia for each group. Only the treatment with SCPC75/5-FU hybrid disks was associated with a significant decrease in the tumor mass (* $P < 0.05$). **c** The concentrations of the main components of SCPC75 i.e., calcium (*black bars*), phosphorus (*grey bars*) and silica (*white bars*) present in the plasma of the mice were analysed by ICP. No difference was found in the concentrations of calcium or phosphorus regardless of the treatment group. Silica concentrations regardless of the treatment were below detection limit of the ICP machine (0.001 mg/l)

Significant inhibition in tumor volume was documented in 4T1 injected mice implanted with SCPC75/5-FU hybrid disks, while control animals implanted with SCPC disks without drug showed exponential tumor growth similar to mice implanted with the aggressive 4T1 cells only. Therefore, the tumor reduction in animals injected with SCPC75/5-FU can be mainly attributed to the release of bioactive 5-FU localized near the tumor mass. Moreover, the reduction in tumor growth started 1 week post-implantation of SCPC75/5-FU disks. This timeline of in vivo drug activity is in accordance with the known effects of 5-FU previously described both in vitro and in vivo [47].

The in vivo toxicity of SCPC75/5-FU correlates well with the long-term release (up to 33 days) of therapeutic dose of 5-FU in vitro. Initial burst release of 4.6% of the loaded drug was observed followed by a sustained release for up to 33 days at a therapeutic dose (>30 μg/day) [48]. This drug release profile could benefit cancer patients post-surgery. Indeed, the implantation of the biodegradable SCPC/drug hybrid in place of the resected tumor

could, through the initial burst of drug, kill the tumor cells present post-surgery. In addition, the long term sustained release of drug could potentially eliminate any recurrence of the tumor in the treated area. Obviously, the SCPC/drug hybrids may be implanted before surgery and/or following surgery, as an adjuvant [1]. It should be noted that while SCPC75/5-FU was able to provide a sustained drug release for a period of 33 days, such long term release from other drug delivery devices was hindered by multiple factors including the delivery rate and strong host immune reactions [7–10]. One injection into the tail vein of melanoma tumor-bearing mice of hydrophobically modified glycol chitosan (HGC) nanoparticles loaded with paclitaxel halted tumor growth for 1-week [49]. However, the injection of bare HGC nanoparticles lead to the death of most of mice within 3 weeks [49]. In contrast, animals injected with SCPC remained alive during the 4-week experiment. The combination of paclitaxel in micelles or hyaluronic acid nanoparticles maintained the functional cytotoxic activities of the drug to cancer cells in vitro [50, 51]. To minimize the initial high burst release of the drug from the cyanoacrylate nanoparticles, He et al. coated the drug loaded particles with hyaluronic acid and reported a lower initial burst release followed by sustained release for the 188 h [52]. These results were comparable to the burst release of 5-FU from SCPC75 demonstrated here. However, the sustained drug release from SCPC75 particles continued for an extended period of 33 days. In vivo, the coating with hyaluronic acid was associated with a low cytotoxicity of the cyanoacrylate nanoparticles. When the particles were loaded with paclitaxel a significant decrease in sarcoma growth was observed in mice [52]. In a separate report, paclitaxel-loaded hyaluronan nanoparticles implanted intratumorally also inhibited tumor growth in DMBA-induced rat mammary cancer model [50]. These results were comparable to the decrease in tumor growth observed with 5-FU/SCPC hybrid described in our study. Given the composition of the SCPC rich in calcium, phosphorus and silica, we analyzed whether the dissolution of SCPC in vivo had any effect on blood ion balance. Blood analyses of animals injected with or without SCPC75/5-FU did not show any difference in serum concentration of Ca, P, or Si indicating that the SCPC dissolution is slow and probably excreted normally. This observation is in agreement with other studies, which showed normal histopathology of the liver, spleen, kidney and lungs of rabbits grafted with resorbable SCPC for 3 months [45].

The results of the present study indicate that the SCPC50 and SCPC75 bioceramics loaded with anti-cancer drug (5-FU) were able to generate a sustained release of functional 5-FU capable of generating an extensive cytotoxic chemotherapeutic response both in vitro and in vivo. These observations further demonstrate the potential of SCPC/drug hybrids for the sustained delivery of chemotherapeutic agents alone or in combination for the treatment of breast cancer and other solid tumors.

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