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Redox-active Microcapsules as Drug Delivery System in Breast Cancer Cells and Spheroids

Marisa Colone[1], **Subramanian Kaliappan**[2], **Annarica Calcabrini**[1], **Mariarosaria Tortora**[2], **Francesca Cavalieri**[2] **and Annarita Stringaro**[1*]

[1]*Department of Technology and Health, Istituto Superiore di Sanità, 00161 Rome, Italy*

[2]*Department of Chemical Science and Technology, University of Tor Vergata, 00133 Rome, Italy*

[*]**Corresponding author:** Annarita Stringaro, Department of Technology and Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy, Tel: 390649902917; Fax: 390649902137; E-mail: annarita.stringaro@iss.it

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Abstract

The purpose of our study was to develop new delivery systems for drugs effective against breast cancer by using biodegradable and biocompatible capsules. Redox-active microcapsules based on thiolated polymethacrylic acid (PMA) were employed. The interaction of these PMA_{SH} capsules with breast cancer cells and the mechanism of their internalization was investigated. PMA_{SH} biocompatibility was evaluated by MTT assay. To analyze their potential as drug carrier, we incorporated doxorubicin into the capsules. Confocal microscopy observations showed the presence of capsules inside the cells. Although some drug molecules still appeared co-localized with PMA_{SH} capsules, strong doxorubicin fluorescence was observed both in the cytoplasm and nucleus, indicating the disassembling of the capsule into PMA_{SH}-drug conjugate after internalization. These results were confirmed by both flow cytometry (time course of capsule uptake) and scanning electron microscopy. PMA_{SH} capsules were also internalized in 3D cell structures (spheroids) suggesting their potential use as drug delivery system for treatment of human diseases.

Keywords: Breast cancer; Spheroids; PMA_{SH} capsules; Drug delivery; Scanning electron microscopy; Confocal microscopy

Abbreviations

CLSM: Confocal Laser Scanning Microscopy; DOX: Doxorubicin; ECM: Extracellular Matrix; GSH: Glutathione; HBSS: Ice-cold Hank's Balanced Salt Solution; MCB: Monochlorobimane; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS: Phosphate Buffer Saline; PMA_{SH}: Thiolated Poly (methacrylic acid) Capsules; SEM: Scanning Electron Microscopy

Introduction

Breast cancer is the most frequent type of malignancy and the leading cause of cancer deaths of women in developed countries; it constitutes 23% of total cancer cases and 14% of the cancer deaths [1]. Approximately up to 7% of breast cancers are being diagnosed in women less than 40 years old years and less than 4% in women below the age of 35 years [2]. Breast cancer is a heterogeneous disease that can be classified into several subtypes, having different histological, molecular expression signature and clinical outcomes [3].

Hormonal therapy, surgery, radiation and chemotherapy represent the treatment options for breast cancer patients. Chemotherapeutic agents currently approved for breast cancer treatment include several drugs (anthracyclines, alkylating agents, platinum drugs, taxanes, vinca alkaloids) (NIH, National Cancer Institute, http://www.cancer.gov/about-cancer/treatment/drugs/breast). Unfortunately, they often show lack of selectivity or specificity toward cancer cells, resulting in several side effects [4].

Recently, nanomedicine has received increasing attention for its ability to improve the efficacy of cancer therapeutics. Nanosized polymer therapeutic agents offer the advantage of prolonged circulation in the blood stream, targeting to specific sites, improved efficacy and reduced side effects. Various drug delivery systems such as nano/microparticles, liposomes and implants have been demonstrated to significantly enhance the preventive/therapeutic efficacy of many drugs by increasing their bioavailability and targetability. Furthermore, their administration would become less expensive in the near future [5]. The achievement of a local, controlled delivery of the drug at the target site would allow keeping the systemic concentration of the drug low. In this regard, other Authors reported that "ideal smart nanoparticles" for redox and pH-responsive drug delivery system (DDS) should enhance therapeutic efficacy thus reducing side effects due to bioaccumulation [6,7].

The purpose of our study was to verify a drug delivery system for breast cancer cells using a microcarrier for doxorubicin (DOX) which disassembles into a polymer-DOX conjugate after internalization. To this aim, we decided to use redox sensitive degradable microcapsules. It is well known that in tumor tissues and in cancer cell cytosol, biological reducing agents have a 4-fold higher concentration than that in the normal tissues [8]. Based on this finding, redox shell-sheddable capsules possess great prospective for anticancer drug release because they are stable in the extracellular environment but able to rapidly respond to the intracellular GSH, releasing drugs into the cytosol and nuclei.

Redox-active microcapsules based on thiolated poly (methacrylic acid) (PMA_{SH}) have been extensively studied over the past few years. PMA_{SH} are assembled by the alternate deposition of PMA_{SH} and poly(vinylpyrrolidone) (PVPON) onto silica particles, followed by subsequent cross-linking of the thiol groups in the PMA_{SH} to form stable disulfide bonds and dissolution of the sacrificial silica core. The disulfide-bonded capsules are designed to be stable in oxidizing

conditions (such as the bloodstream) but to disassemble in reducing environments such as the cytoplasm of the cell [9-12]. The potential of PMA_{SH} as an *in vitro* therapeutic delivery system of encapsulated proteins and peptides, oligonucleotides as well as small molecule drugs, has been demonstrated [13,14]. Previous studies reported the cytotoxic effect induced in human colorectal cells by DOX loaded into PMA_{SH} capsules by an oil phase or by pH labile hydrazone linkages [9,15]. Herein we report the loading of PMA_{SH} microcapsules with DOX using no hydrolysable amide bonds. The interaction of these PMA_{SH} capsules with a cancer cell model (SKBR3, human breast cancer cell line) as well as the mechanism of their internalization were then investigated. In addition, in order to predict more accurately *in vivo* results, PMA_{SH} capsules were assessed as delivery system in SKBR3 cells grown as spheroids in 3D culture model to analyse their efficacy in a system that mimics *in vivo* condition.

Materials and methods

Cell culture

The human breast cancer cell line SKBR3 was from American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in DMEM (EuroClone S.p.A., Milan, Italy) supplemented with 10% FBS at 37°C in a humidified atmosphere containing 5% CO_2 and sub-cultured prior to confluence using trypsin/EDTA.

Three-dimensional (3D) cell culture

SKBR3 cells were also cultured as multicellular spheroids. To this aim, 3D ON-TOP assay was performed [16]. Eight-well glass chamber slides were pre-coated with undiluted growth factor-reduced Matrigel^{TM} (10 mg/ml) (BD Biosciences, Franklin Lakes, NJ, USA). After Matrigel solidification, 3×10^3 SKBR3 cells (resuspended in 100 µl growth medium) were seeded on each well and incubated for 10-30 min at 37°C. A mixture of complete medium containing 10% Matrigel was added to the plated culture (100 µl/well). Cultures were maintained for 12-15 days, replacing Matrigel-medium mixture every 2 days. To analyse the effects of DOX-loaded PMA_{SH}, once spheroids were formed (10 days), the particles (1:25) were added to this mixture (3D cultures) and their effects analysed after 72 h.

Preparation of PMA_{SH} microcapsules and PMA_{SH} capsules loaded with doxorubicin

PMA_{SH} and PMA_{SH} capsules, 1 µm in diameter, were prepared as previously reported [17]. DOX was covalently linked with the carboxyl group of PMA_{SH} capsules using EDC chemistry procedure. The carboxyl groups were activated when 100 µl PMA_{SH} capsules were incubated with 0.010 mg of EDC (5.3×10^{-5} mmol) at pH 7 in PBS (phosphate buffer saline) for 30 minutes. Subsequently the capsules were incubated with DOX (5.3×10^{-5} mmol) for 30 minutes at pH 7 in PBS buffer and washed to remove free drug. The concentration of (PMA_{SH}) microcapsules was determined by using a counting chamber: 0.5 µl of PMA_{SH} capsules were diluted 10 times with PBS buffer at pH 7 and 1 µl of the diluted PMA_{SH} capsules was placed on a glass slide. Then, capsules were counted from the micrographs obtained by optical microscopy.

Determination of DOX loaded into PMA_{SH} capsules

The amount of DOX loaded into PMA_{SH} capsules was determined using UV spectroscopy. At first, the concentration of DOX in the medium (initial concentration) was measured. After loading, the PMA_{SH} capsules were centrifuged and the absorbance of DOX present in the supernatant was measured. The amount of doxorubicin loaded into PMA_{SH} capsules was calculated using the formula: % uptake=Initial DOX Concentration – Concentration of free DOX in the supernatant (after reaction)/ Initial DOX Concentration. Doxorubicin loaded onto PMA_{SH} microcapsules was also quantified after microcapsule disassembly. As DOX is covalently bonded with the carboxylic group of microcapsules by amide linkage and the amide linkage is not hydrolyzed, doxorubicin release was performed in the presence of the reducing agent DTT. Under this condition, dissolution of microcapsules caused PMA_{SH}-DOX release. In brief, 50 mM of freshly DTT in PBS buffer (pH 7) was added to DOX loaded capsules. Subsequently the capsules were centrifuged at 4.5 rcf for 10 minutes. The absorbance of the supernatant was measured by UV spectroscopy. From the absorbance value the amount of DOX released was calculated. The loading of drug into the microcapsule suspension was 3.4×10^{-10} mg of DOX per microcapsule.

MTT assay

Briefly, SKBR3 cells were seeded into 96-well microtiter plates (NunclonTM, Nunc, Germany) at a density of 1.5×10^4 cells/well. After 24 h cells were treated with polymer, empty PMA_{SH} and PMA_{SH} loaded with DOX at concentration of 25, 50, 75 or 100 particles/cell. After an incubation period of 24, 48, 72 h, medium was replaced by fresh medium containing 0.5 mg/ml MTT (Sigma, Deisenhofen, Germany). After 2 h at 37°C, unreacted dye was removed and the purple formazan product was dissolved in 200 µl/well dimethylsulfoxide (Merck, Darmstadt, Germany). The absorbance was read at 570 nm on a scanning microtiter spectrophotometer after agitating the plate for 5 min on a shaker. Data were expressed as absorbance values relative to untreated cells considered as 100%. Assays were performed in triplicate and repeated 2-3 times [18].

Scanning electron microscopy

The effects of PMA_{SH} on cell morphology were visualized by scanning electron microscopy (SEM). Cells were grown on glass coverslips (12 mm-diameter) and treated with particles for 24 and 48 h (1:25). After incubation, samples were washed to remove unbound PMA_{SH} and fixed in 2.5% glutaraldehyde in 0.2 M Na-cacodylate buffer (pH 7.4) for 2 h at RT. After 3 washes, cells were post-fixed with 1% (w/v) OsO_4 for 1 h, dehydrated in an ethanol gradient, critical point dried in CO_2. The coverslips were attached to alluminium stubs, mounted with silver print and coated with gold in a sputter coater. The samples were examined with a Cambridge Stereoscan 360 scanning electron microscope (Cambridge Instruments, Cambridge, UK).

Flow cytometry

To quantitative analyze intracellular uptake of doxorubicin-loaded PMA_{SH} capsules, SKBR3 cells (5×10^5 cells/well) were seeded in 6-well plates and incubated at 37°C in a 5% CO_2 incubator overnight. DOX-loaded PMA_{SH} capsules were added to the culture medium at the final concentrations of 1:25 cell/DOX-particles at 37°C from 24 h to 48 h. At the end of each treatment, cells were washed with ice-cold Hank's balanced salt solution (HBSS; Sigma Chemical Company, St. Louis,

MO, USA), detached with EDTA and 0.25% trypsin, resuspended in ice-cold PBS and immediately examined for a time course analysis of the uptake by LRSII flow cytometer (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Moreover, to determine cellular glutathione (GSH) content, SKBR3 cells (3×10^5 cells/well) were seeded in 6-well plates and incubated at 37° C in a 5% CO_2 incubator overnight. GSH level was quantified using monochlorobimane (MCB, Molecular Probes, Eugene, OR, USA), a non-fluorescent compound that reacts with GSH to form a highly fluorescent derivative. Cells were treated with DOX-PMA_{SH}, at 1:25 cell/capsules, at 37°C from 24 h to 72 h, then incubated with MCB solution (25 μg/ml) for 15 min at 37°C. Samples were washed with HBSS and detached as described above, then analyzed by flow cytometry. Fluorescence emissions from DOX-loaded capsules and from MCB were collected through a 570 and 530 nm band-pass filters, respectively, and acquired in log mode. Capsule uptake and GSH level were evaluated as fluorescence intensity values, expressed as mean fluorescence channel and calculated by DIVA software (Becton Dickinson and Company).

Confocal laser scanning microscopy

The analysis of the internalization process of DOX-loaded PMA_{SH} capsules was performed by confocal laser scanning microscopy (CLSM) on both cell cultures and multicellular spheroids. SKBR3 cells were grown on coverslips and treated with capsules (1:25) for 24, 48, 72 h. 3D cultures grown for 10 days, as previously described, were treated for 72 h with PMA_{SH} at the same concentration. Subsequently, monolayer cultures and spheroids were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 30 min at room temperature (RT), washed in the same buffer and permeabilized with 0.5% Triton X-100 (Sigma Chemicals Co.) in PBS (5 min for cells, 20 min for spheroids) at RT. For nuclei detection, cells and spheroids were stained with DAPI (150 nM Sigma) at 37°C for 30 min.

For Golgi apparatus detection, cells were incubated with IgG1 monoclonal antibody (Purified Mouse Anti-GM130, diluted 1:100, BD Transduction Laboratories™), 1 h at RT, followed by detection with Alexa Fluor 488-labeled goat anti-mouse IgG for 1 h at RT. After washing with PBS, coverslips were mounted with glycerol-PBS (2:1) and analyzed by intensified charge-coupled device video microscopy (IVM) with a Nikon Microphot fluorescence microscope (Nikon Europe B.V., Amsterdam, The Netherlands) equipped with a Zeiss CCD camera (Carl Zeiss, Oberkochen, Germany). Confocal microscopy experiments were performed on a Olympus IX81 confocal laser scanning microscope (Olympus, Tokyo, Japan) equipped with Ar laser 488 nm.

Statistical analysis

One-way ANOVA test was used to perform statistical analysis of the data. Differences between two groups were considered significant at $p<0.05$.

Results and Discussion

To evaluate the potential use of PMA_{SH}-DOX capsules as *in vitro* delivery system for breast cancer cells, the effect of thiolated poly-(methacrylic acid) and PMA_{SH} capsules on cell growth and viability was analyzed by MTT assay. This test is based on the reduction ability of tetrazolium salts to formazan by metabolically active cells, which correlates with the number of viable cells. As shown in Figure 1A, cell viability was not affected after incubation with different polymer solutions at increasing concentration (0.001, 0.01 and 0.1 mg/ml). Only after 72 h of treatment with 0.1 mg/ml the polymer induced a low cytotoxic effect. Furthermore, treatments with PMA_{SH} capsules (25, 50, 75 and 100 PMA_{SH} capsules/cell ratio) up to 72 h of incubation did not alter substantially the number of viable cells (Figure 1B). On the basis of this evidence, in the studied concentration range, both polymer and PMA_{SH} capsules resulted to be not toxic for SKBR3 cells.

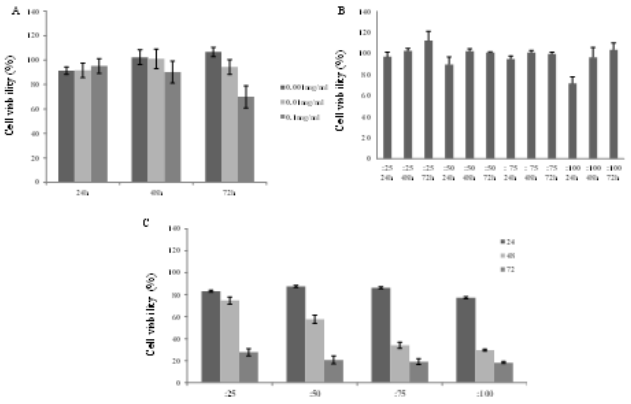

Figure 1: Cell viability (% respect to untreated cells considered 100%) by MTT test. A) SKBR3 cells were treated with different concentrations of PMA_{SH} polymer for 24, 48 and 72 h. B) Cells were incubated with PMA_{SH} capsules at different capsule/cell ratio (ranging from 25 to 100) for 24, 48 and 72 h. C) SKBR3 cells were treated with DOX-loaded PMA_{SH} capsules at different concentration and incubation times.

In order to evaluate the potential of PMA_{SH} capsules as a carrier to deliver anticancer drugs, DOX was covalently linked into the capsules. DOX has an intrinsic fluorescence (excitation at 480 nm, emission at 550-650 nm) that can be exploited to monitor the intracellular accumulation of the drug. SKBR3 cells were treated with different capsules/cell ratios (Figure 1C). The effect induced by these particles was time- and dose-dependent: an evident reduction of viable cell number was observed after incubation for 72 h with all tested capsules/cell ratios (1:50, 1:75, 1:100). These studies indicated 25 capsules/cell as an optimal ratio to study the efficiency and the kinetics of the uptake. Next, SKBR3 cells were incubated with PMA_{SH}-DOX at the same capsules/cell ratio, washed and analysed by flow cytometry.

The increase of DOX-related fluorescence signal was detected as a function of time. Figure 2 shows that the cellular uptake process of PMA_{SH}-DOX capsules was already complete after 48 h incubation as the fluorescent signal did not change at longer incubation times (data not shown).

Scanning electron microscopy (SEM) was used to gain an understanding of the mechanism of interaction between cells and capsules during the uptake process. SEM micrographs revealed the adhesion of PMA_{SH}-DOX capsules on cell surface. Some PMA_{SH}-DOX particles could be observed on cell membranes after 24 h incubation (Figure 3A, arrows). After 48 h, numerous membrane protrusions and ruffles could be observed on cell surface (Figure 3B, arrow) suggesting that the internalization process (Figure 3C, arrow) might involve these structures related to cell cytoskeleton. No evidence of cell damage could be detected after 48 h of incubation (Figure 3B and 3C). In

Figure 3D (72 h of incubation) arrows indicate the PMA_{SH}-DOX capsules inside the cytoplasm underlying the plasma membrane as demonstration of capsules uptake.

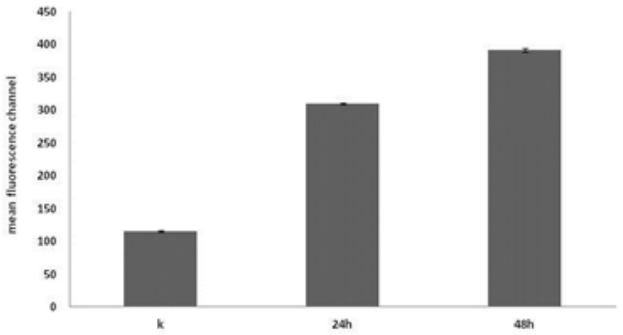

Figure 2: Flow cytometric study of the uptake process of DOX-loaded PMA_{SH} capsules by SKBR3 cells. After 24 and 48 h-incubation, fluorescence signals arising from capsules that entered the cells were analyzed and represented as mean fluorescence channel. Data show the mean value ± the standard deviation of three independent experiments (k, untreated cells).

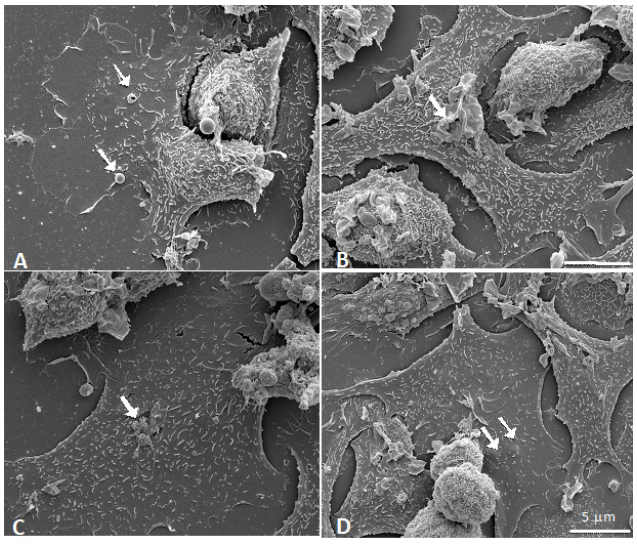

Figure 3: SEM images of SKBR3 cells treated for 24 h (A), 48 h (B and C) and 72 h (D) with PMA_{SH}-DOX at 1:25 ratio.

Further insight into the internalization and intracellular distribution of PMA_{SH}-DOX capsules was gained by CLSM observations (Figure 4) after an incubation period of 48 h. Red, green and blue colours indicate PMA_{SH}-DOX, actin filaments and nuclei stained with Hoechst 33342, respectively. The arrows in the micrographs indicate the internalization of PMA_{SH}-DOX capsules and the localization of DOX inside the nucleus after microcapsules disassembling (Figures 4A and 4B, respectively). These observations suggested that PMA_{SH}-DOX conjugate was able to migrate into the nucleus crossing the nuclear membranes. In order to investigate if other cellular compartments could represent localization sites for PMA_{SH}-DOX capsules, Golgi apparatus was stained with the monoclonal antibody anti-GM130. After 48 h incubation with PMA_{SH}-DOX capsules, shift was observed after intact capsules near Golgi apparatus (Figure 4C, arrows). However, some SKBR3 cells showed evidence of yellow merged fluorescence signal arising from DOX conjugate polymer co-localized near the nucleus, in Golgi area (Figure 4C, inset). Other studies reported that free doxorubicin shows a time-dependent intracellular shift from the nucleus to the mitochondria and Golgi apparatus [19]. In particular, Golgi apparatus plays a pivotal role in the intracellular distribution of DOX [20]. These results indicate that other organelles in addition to nuclei are important sites of accumulation of PMA_{SH}-DOX polymer. In order to investigate intracellular PMA_{SH}-DOX capsule fate in suffering and/or dead SKBR3 cells after 72 h of incubation, detached cells were collected from the supernatant medium and placed on glass coverslips, previously covered with polylysine, then processed for CLSM analysis. The induction of apoptotic death by PMA_{SH}-DOX capsules was confirmed by the observation of nuclei with typical areas of condensed chromatin (Figure 4D). The inset shows a 3D reconstruction image of an apoptotic cell.

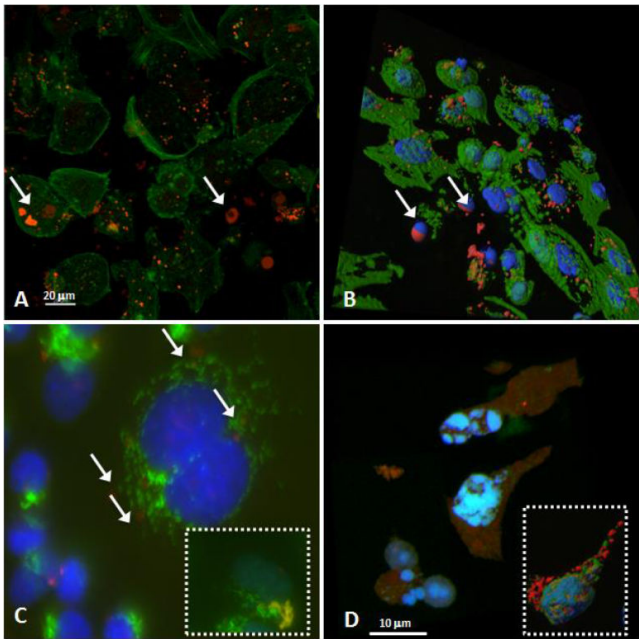

Figure 4: CLSM images of SKBR3 cells incubated with PMA_{SH}-DOX capsules. (A) SKBR3 cells at 48 h of incubation with capsules. (B) A three-dimensional CLSM reconstruction of the same samples (CLSM 3D mode image). (C) CLSM images of adherent SKBR3 cells after 48 h of incubation with PMA_{SH}-DOX. Golgi areas are green while PMA_{SH}-DOX capsules are red. Inset shows open PMA_{SH}-DOX capsules in Golgi area at 72 h of incubation (yellow signal from merged image). (D) Apoptotic SKBR3 cells present in the supernatant after 72 h of PMA_{SH}-DOX incubation. The inset shows a CLSM 3D mode image. Red, green and blue indicate PMA_{SH}-DOX, actin filaments and nuclei stained with Hoechst 33342, respectively.

As previously described, PMA_{SH} capsules are assembled by subsequent cross-linking of the thiol groups [11]. The disulfide-bonded

capsules are stable in oxidizing conditions (such as the bloodstream) but disassemble in reducing environments such as the cytoplasm of the cell [21]. The intracellular reducing condition promotes PMA_{SH}-DOX capsules dissolution into polymer-DOX soluble conjugate. As DOX is linked to PMA_{SH} polymer chains via amide linkages, the DOX intercalation into DNA grooves is likely sterically hindered by the polymer chain. We speculate that PMA_{SH}-DOX caused anti-proliferative and cytotoxic effect in SKBR3 cells through different mechanisms including generation of free radicals and direct membrane alterations.

To demonstrate the intracellular redox-induced rupture of PMA_{SH}-DOX capsules, we evaluated GSH cytoplasmatic concentration during PMA_{SH} capsules trafficking in SKBR3 cells. The modulation of GSH level during PMA_{SH}-DOX particles uptake was evaluated by flow cytometry analysis after labelling of GSH with MCB (Figure 5). The lowest fluorescent signal, corresponding to a lowest concentration of GSH, was observed after 48 h incubation at the ratio 25 capsules/cell. This result suggested that the decrease of reduced GSH could be associated with the dissolution of PMA_{SH} particles and consequently with the release of PMA_{SH}-DOX in the intracellular space. Evidence of PMA_{SH}-DOX capsules dissolution was also provided by the homogeneously distributed red emission observed by CLSM analysis (Figure 4A, inset Figure 4D). From these observations we could hypothesize that PMA_{SH}-DOX capsules exerted their apoptotic activity in SKBR3 cells within 48 h by disassembling into cytotoxic PMA_{SH}-DOX polymer.

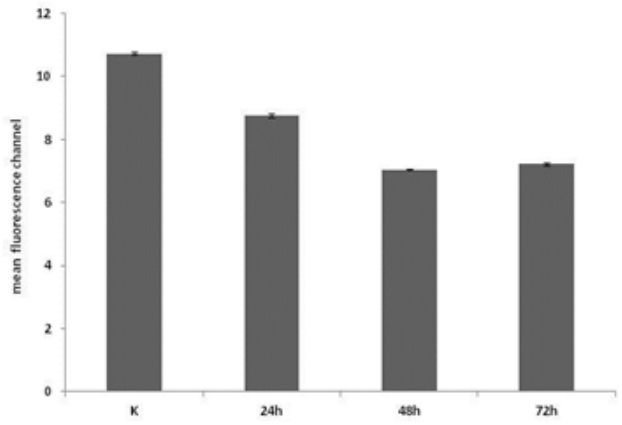

Figure 5: Flow cytometric analysis of GSH levels by using MCB. SKBR3 cells were treated with PMA_{SH}-DOX capsules for 24, 48 and 72 h. Fluorescence signals were represented as mean fluorescence channel. Data show the mean value ± the standard deviation of three independent experiments (k, untreated cells).

To evaluate micro-nanocarriers biological efficacy, monolayer (2D) cell cultures are typically used as *in vitro* testing models. However, these studies often are unable to predict *in vivo* results. 2D-cultures do not reproduce the complex cell-cell and cell-matrix interactions, which significantly limit their ability to recapitulate the appropriate level of *in vivo* cellular response. I*n vitro* 3D-culture models have been introduced to reduce the gap between *in vitro* 2D-cultures and *in vivo* models [22]. Multicellular spheroid is considered the most effective 3D cell culture model [23]; it is characterized by many features of *in vivo* condition such as the interaction of cells with the extracellular matrix (ECM) components [24]. To assess if 3D organization and ECM presence could affect the entry and interaction of PMA_{SH}-DOX capsules with individual cells, SKBR3 cells were grown following a 3D-dimensional cell culture protocol that employs Matrigel [16]. SKBR3 spheroids appeared as multicellular aggregates characterized by poor cell-cell adhesion (Figures 6A and 6B, optical and scanning electron microscopy, respectively), as previously observed by other Authors [25]. To better analyse internal organization, spheroids were labelled with nuclear stain DAPI and observed by confocal microscopy (Figure 6C). To study the interaction with capsules, SKBR3 spheroids after 10 day-growth were treated for 72 h with PMA_{SH}-DOX capsules (1:25) and observed by confocal microscopy (Figure 6D). PMA_{SH}-DOX capsules and polymer were observed inside the 3D cultures (Figure 6D, arrow). In addition, some nuclei positive for DOX signal could be detected suggesting the presence of drug released from the opened capsules (Figure 6D, arrowhead). These results suggested that the presence of Matrigel (which mimics the ECM structure in *in vivo* growth condition) allowed the entry and diffusion of capsules inside the 3D organized structures.

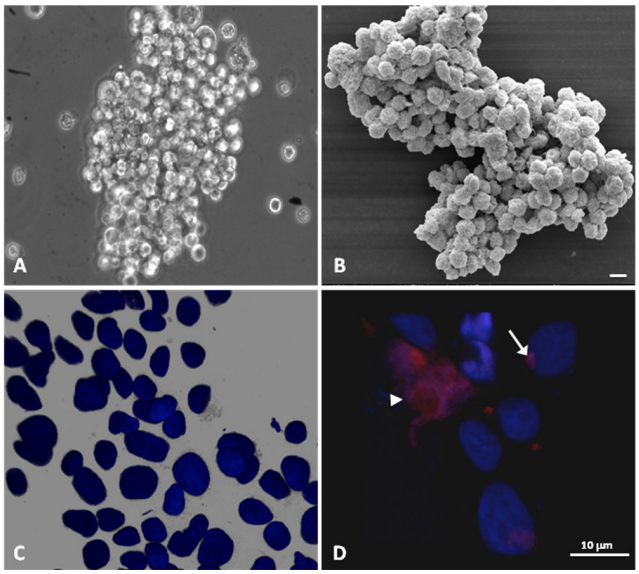

Figure 6: SKBR3 spheroid (10 day-growth) observation by phase-contrast optical (A) and scanning electron microscopy (B). CLSM image of control spheroids labelled with nuclear dye DAPI (C). CLSM micrograph of spheroids after 72h treatment with PMA_{SH}-DOX capsules (D) showing both the presence of capsules near the nuclei (arrow) and some drug-positive nuclei (arrowhead).

Conclusion

We showed that PMA_{SH}-DOX capsules exhibited an efficient intracellular controlled release of PMA_{SH}-DOX conjugate in breast cancer cells and spheroids. *In vitro* cell viability assay indicated that PMA_{SH}-DOX capsules could efficiently inhibit SKBR3 cell viability at long incubation times, suggesting that the antitumoral drug maintained its activity even if conjugated to a polymer by a non-hydrolyzable linkage. Cellular uptake studies showed that PMA_{SH}-DOX conjugate was able to access the nuclei of SKBR3 cells, following PMA_{SH}-DOX capsules dissolution induced by intracellular reducing condition. Results obtained in SKBR3 cells grown as spheroids

indicated that PMA_{SH} capsules were also able to enter and move into these 3D organized cellular structures suggesting that PMA_{SH} capsules could represent a promising vehicle for intracellular controlled drug delivery in nanomedicine.

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